HANDBOOK OF PLANT BREEDING

J.E. Bradshaw
Editor

Root and Tuber Crops

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Root and Tuber Crops

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Preface

This volume in the *Handbook of Plant Breeding* covers ten root and tuber crops in eight chapters: potato (*Solanum tuberosum*), cassava (*Manihot esculenta*), sweet potato (*Ipomoea batatas*), yams (*Dioscorea* spp.), taro (*Colocasia esculenta*) and cocoyam (*Xanthosoma sagittifolium*), sugar beet (*Beta vulgaris*), fodder beet (*Beta vulgaris*), and swedes (*Brassica napus*) and turnip (*Brassica rapa*). Although many of these crops were first domesticated several thousand years ago, none became important on a global scale until after the end of the sixteenth century. This brief introduction is a broad overview and inevitably a simplification of the details which follow in the individual chapters where unresolved issues are discussed.

The potato was domesticated in South America, cassava probably in South America but possibly in Mexico, and sweet potato probably in Mexico but possibly in South America, some 8,000 years ago. All three crops have wild relatives in both Central and South America. Much later, after Columbus discovered the New World in 1492, European sailors introduced the potato to Europe and from there to many other parts of the world, and both cassava and sweet potato to Africa and then Asia. Interestingly, the sweet potato was being grown in Oceania before Columbus, but the routes of introduction are still debated. Today the potato is the third most important food crop in the world, after wheat and rice, and the four largest potato producing countries are China, the Russian Federation, India, and the USA. Cassava is the most important root and tuber crop in the tropics where it is a primary staple food in many of the poorest countries, with the largest production in Nigeria, Brazil, Thailand, and Indonesia. The sweet potato is also a food staple in Asia, Africa, and America, but with production dominated by China, where half of the crop goes for animal feed. Yams are also important staple food crops in tropical and subtropical regions. The four main cultivated yams were independently domesticated on three continents some 7,000 years ago: *Dioscorea rotundata* and *D. cayenensis* in West Africa, *D. alata* in Southeast Asia and the South Pacific, and *D. trifida* in South America. Today, however, the major producers are in West Africa: Nigeria, Ivory Coast, Ghana, and Benin. Although taro and cocoyam are minor crops, they do provide a staple food for poor people in Africa, Asia, and America. Taro was domesticated some 10,000 years ago in Asia, Southeast Asia, and Melanesia, whereas cocoyam was domesticated in South America and subsequently taken in the sixteenth century to Africa and then Asia. Today the main producers of these crops are

Nigeria, Ghana, and China. The relative importance of all these crops can be seen from the 2008 FAO production statistics (http://faostat.fao.org): potato (314 million tonnes), cassava (233), sweet potato (110), yams (52), and taro and cocoyam (12); with sugar beet at 228 million tonnes.

The edible storage organs are underground tubers for potatoes and yams, storage roots for cassava and sweet potato, and corms/cormels for taro and cocoyam. All of these organs store energy as starch and the crops are viewed primarily as sources of carbohydrate energy when used as a food staple. They are, however, also valuable sources of minerals, vitamins, and other antioxidants. In addition, there is a trend toward using them to produce processed products for both human consumption and industrial use. All of the crops are vegetatively propagated: potatoes and yams through their tubers, cassava as stem cuttings, sweet potato as vine cuttings, and taro and cocoyam through side shoots, stolons, or corm heads. These propagation methods are slow, so more rapid ones have been, and are being, developed for the breeding and multiplication of new cultivars.

In contrast, sugar beet, fodder beet, swedes, and turnips are grown from true botanic seed and their above-ground storage organs are swollen hypocotyls with varying amounts of stem above and root below, often simply referred to as 'roots.' Their carbohydrate is primarily in the form of simple sugars, sucrose in beets and glucose and fructose in swedes and turnips. Sugar beet and fodder beet were both derived from the leaf and table beets grown as vegetables by the Greeks and Romans. Fodder beets, with larger roots, were developed during the 'Middle Ages' in Northern Europe for livestock fodder and became an important winter feed for cattle from the 1800s. After the discovery that temperate fodder beets contained the same kind of sugar as tropical sugarcane, the first sugar factory opened in Silesia (Poland) in 1802, and sugar beets were selectively bred from the 1800s. Today sugar beet provides about one-quarter of the world's sugar production from crops grown in temperate climates such as found in Europe and the northern USA.

The turnip, like table beet, was also a vegetable grown by the Romans, and probably the Greeks before. The turnip came to prominence as an agricultural crop as part of the four-field rotation (wheat, turnips, barley, and clover) which originated in Flanders and was introduced into Britain by Lord 'Turnip' Townshend about 1730. This led to the British Agricultural Revolution of the eighteenth and nineteenth centuries, which enabled the population base for the Industrial Revolution to take place. The origin of *B. napus* (swedes, oilseed, and forage rape), the allotetraploid of *B. rapa* (turnips) and *B. oleracea* (kales and cabbages), is uncertain, but it appears to have arisen several times in recent history. Swedes were first recorded in Europe in 1620 and introduced to Britain around 1780, where they were favored over turnips as having better keeping and feeding quality. By around 1870 the area of swedes and turnips in Britain had peaked at almost 1 million hectares. Today fodder beet, swedes, and turnips are minor crops for feeding to livestock in temperate climates such as Northern Europe and New Zealand, with swedes and turnips also grown as vegetables for culinary use. They have been replaced as major crops for animal feed by cereals and silage.

Since domestication, all of the crops have been improved by both conscious and unconscious farmer selection. More modern hybridization and selection by farmers, hobby breeders, and seedsmen occurred for potato, sugar beet, fodder beet, swedes, and turnips during the nineteenth century. These crops were therefore well placed to benefit from the birth of modern genetics in 1900 and the subsequent development of scientific breeding methods. Thus, for example, methods of producing hybrid cultivars to exploit heterosis for yield are available in the four crops grown from true botanic seed. Modern breeding of cassava and sweet potato started in the 1920s, but intensified really only from the 1960s and 1970s when breeding work also started to get underway for yams, taro, and cocoyams. This modern breeding work has been helped by the establishment of International Research Centers aimed at providing food security and eradicating poverty in developing countries; and this will remain important during a period of human population growth and climate change.

The extent to which the crops are benefiting from new biotechnologies reflects both their own economic importance and that of their close relatives. Thus the potato and cassava genomes have already been sequenced and that of sugar beet is due in 2011. Fodder beet breeding will benefit from advances with sugar beet and swedes and turnips from advances in their oilseed relatives. Molecular markers are available in all of the crops and are being used to characterize germplasm as well as resolve issues over domestication. Molecular marker maps have been produced and there are varying degrees of progress in using them for marker-assisted selection. Likewise genetic transformation is either available or becoming available to complement conventional breeding. It should be of particular value in the vegetatively propagated polyploids with complex inheritance patterns such as potato and sweet potato.

The new biotechnologies need to build on and integrate with past progress in breeding. It is still important to appreciate the reproductive biology of the crop, its evolutionary history, and the germplasm available to breeders. All of this is covered in each chapter in this volume, together with breeding methods and objectives, which fall into the broad categories of higher yields, better quality, and improved disease and pest resistance. While all of the harvested products of the crops considered are vegetatively produced storage organs, their breeding involves sexual hybridization and hence a knowledge of flowering, pollination, and seed set. In this respect, sugar and fodder beet, and swedes and turnips, are biennial species with a vernalization requirement for flowering. Swedes, unlike the other crops, are self-fertile and tolerant of inbreeding, despite being insect pollinated and having progenitors with sporophytic self-incompatibity. As expected, natural self-pollination occurs in potatoes as gametophytic self-incompatibility breaks down in polyploids, but the crop is not tolerant of inbreeding. Natural pollination is normally by wind in sugar and fodder beet and by insects in the other crops. Out-crossing is encouraged in some species by separate male and female plants (yams) or flowers (cassava, taro, and cocoyam), as well as by protogyny. In other species self-pollination is prevented by self-incompatibility which is gametophytic in sugar and fodder beet and sporophytic in sweet potato and turnip. Some of the crop species are regarded as diploids (cassava, taro, cocoyam, sugar and fodder

beet, and turnip), although sugar and fodder beet cultivars can be triploid as well as diploid hybrids, whereas other species are clearly polyploids. Swedes are an allotetraploid and the principal cultivated potato is an autotetraploid. Sweet potatoes are hexaploid (probably an allo-autopolyploid as a result of being a hybrid between a diploid and tetraploid species), and yams form a polyploid series, and incidentally are monocotyledonous.

Finally, there are also nine lesser known root and tuber crops native to the Andes of South America and cultivated by indigenous farmers. They have edible underground organs and are used both as subsistence and cash crops. Not enough breeding work has been done on them to justify a chapter in this volume, but further information is available from the International Potato Center (CIP) in Lima, Peru (http://www.cipotato.org), where accessions have been 'Held in Trust' since 1990 in their genebank, and some research has been done. A few brief comments will indicate why they are of interest. Achira (*Canna indica*) has rhizomes which contain large starch granules and hence high-value starch. It is also grown in Vietnam for noodles. Ahipa (*Pachyrhizus ahipa*) is a legume crop which produces carbohydrate-rich (starch and sugars) tuberous roots. Arracacha (*Arracacia xanthorrhiza*) has tuberous storage roots which provide starchy food free from undesirable substances. It has been introduced to Brazil where some breeding work is being done. Maca (*Lepidium meyenii*) is a root crop that can be grown at the upper altitude limits for agriculture and is of interest for its medicinal properties. Mashua (*Tropaeolum tuberosum*) produces yellow-fleshed tubers with a high carbohydrate content, both starch and sugars. Mauka (*Mirabilis expansa*) has fleshy edible storage roots high in carbohydrate and protein. Oca (*Oxalis tuberosa*) is a tuber crop which has also been grown in New Zealand for over a century, and where recent breeding work has led to the release of a new cultivar. Ulluco (*Ullucus tuberosus*) produces starchy tubers and has also been introduced to New Zealand as a new food crop where some research has been done on its yellow and red betalain pigments. Yacon (*Smallanthus sonchifolius*) produces non-starchy roots which contain high levels of sugars and fructooligosaccharides which can be used as sweeteners for diabetics. It was introduced from New Zealand to Japan in 1985 where a new cultivar, Saradaotome, has been bred.

In order to provide uniformity with the other volumes in the *Handbook of Plant Breeding*, each chapter is divided into the following sections: Introduction, Origins and Domestication, Varietal Groups (where appropriate), Genetic Resources, Major Breeding Achievements, Current Goals of Breeding, Breeding Methods and Techniques, Integration of New Biotechnologies in Breeding Programs and Seed (Tuber/Commercial) Production. The length of each section varies with crop, as appropriate, and I tried to give the authors of chapters as much freedom as possible within this overall framework. We hope that the finished product will be of value both to students of plant breeding and professional plant breeders, as well as to anyone interested in this fascinating group of root and tuber crops.

I would like to thank Jaime Prohens for asking me to edit a volume in the *Handbook of Plant Breeding*, the authors of chapters for their contributions and co-operation, and Hannah Schorr of Springer for all of her help and encouragement, it was much appreciated.

Dundee, Scotland John E. Bradshaw

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Chapter 1
Potatoes

John E. Bradshaw and Merideth Bonierbale

1 Introduction

The potato (*Solanum tuberosum*) is the world's third most important food crop after wheat and rice with 309 million tonnes fresh weight of tubers produced in 2007 from 18.5 million hectares of land (http://faostat.fao.org). Half of the potato production in 2007 (150 million tonnes) was in Asia, Africa, and Latin America as a result of steady increases in recent years, particularly in China and India. Indeed, China (56 million tonnes, down from 71 in 2005) is now the number one potato producer in the world and India (22 million tonnes) is third, with the Russian Federation (37 million tonnes) second, and the USA (20 million tonnes) fourth. In contrast, the order for kg/capita/year consumption in 2003 was Russia (125), USA (63), China (35), then India (17) (http://faostat.fao.org), and the provisional figures for 2005 were similar. However, actual rates and impact are highly variable within countries once disaggregated data are reviewed. The increases in production in China have primarily been through increases in the area of potatoes planted (4-fold since 1960), but accompanied by some increases in yield/hectare (1.5-fold since 1960), whereas in India there have been equal contributions (3-fold and 2.5-fold, respectively). As a major food staple the potato is contributing to the United Nation's Millennium Development Goals of providing food security and eradicating poverty, which is helped where the potato provides not only food but also employment and income as a cash crop. In recognition of these important roles, the UN named 2008 as the International Year of the Potato.

More information on potatoes can be found in the following books: *Genetic Improvement of Solanaceous Crops Volume I: Potato* (Razdan and Mattoo, 2005); *Handbook of Potato Production, Improvement, and Postharvest Management* (Gopal and Khurana, 2006); *Potato Biology and Biotechnology, Advances and*

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Perspectives (Vreugdenhil, 2007); *Propitious Esculent, The Potato in World History* (Reader, 2008); and *Advances in Potato Chemistry and Technology* (Singh and Kaur, 2009).

1.1 Nutritional Value

The potato tuber is a subterranean swollen stem which evolved to survive from season to season as a dormant storage organ. The form of energy storage is almost entirely starch. Potatoes are thus a major source of carbohydrate energy in the diets of hundreds of millions of people and are even being considered for human life support in space (Wheeler, 2009). A small but significant portion of potato starch is resistant to digestion by enzymes in the stomach and small intestine and so reaches the large intestine essentially intact. This resistant starch is considered to have similar physiological effects and health benefits as fiber: it provides bulk, offers protection against colon cancer, improves glucose tolerance and insulin sensitivity, lowers plasma cholesterol and triglyceride concentrations, increases satiety, and possibly even reduces fat storage. The amount of resistant starch in potatoes depends much on preparation methods. Cooking and then cooling potatoes have been reported to significantly increase resistant starch. For example, Englyst et al. (1992) showed that about 7% of cooked potato starch is resistant starch, but that this percentage increases to about 13% upon cooling.

While potatoes are commonly perceived as a carbohydrate source, they are also a good source of high-quality protein. Although potatoes contain only about 2% protein on a fresh-weight basis, the value increases to about 10% when examined on a dry-weight basis, equal to that of most cereals such as rice or wheat. Potatoes provide an excellent source of lysine, but low contents of sulfur amino acids limit their nutritive value (Friedman, 1996). They also provide significant amounts of vitamins C, B6 and B1, folate, the minerals potassium, phosphorus, calcium, and magnesium, and the micronutrients iron and zinc. Potassium is the most abundant of the minerals. The concentration of iron and zinc in potato is low compared with the concentration of these micronutrients in cereals and legumes. However, the bioavailability of iron in potato is greater than in cereals and legumes due to the presence of high levels of ascorbic acid (promoter of iron absorption) and low levels of phytic acid (inhibitor of iron absorption) (Fairweather-Tait, 1983).

Potatoes are high in dietary fiber, especially when eaten unpeeled with their skins, and are rich in antioxidants comprising polyphenols, vitamin C, carotenoids, and tocopherols (Storey, 2007). Cooking reduces the concentration of vitamin C in tubers. However, the degree of the reduction depends on the cultivar and on the way of cooking. A recent study using native potatoes from the Peruvian Andes found that the concentration of vitamin C in tubers boiled with their skin was higher than those that had been baked or cooked in a microwave oven (Burgos et al., 2009a). One hundred grams of cooked potatoes can contribute 25–50% of the daily recommendation of ascorbic acid (100–120 mg/day) (Naidu, 2003).

The concentration of carotenoids in potato tubers is related to flesh color. Yellow-fleshed potatoes have high concentrations of total carotenoids, varying up to 1,840 μg/100 g on a fresh-weight basis with zeaxanthin being the principal one (Burgos et al., 2009b). Cream-fleshed potatoes have low concentrations of total carotenoids, with lutein, vioaxanthin, and beta-carotene being the principal ones. Lutein and zeaxanthin, two of the carotenoids of higher concentration in human serum, are localized in significant quantities in the retina and play a part in protecting against macular degeneration. The concentration of zeaxanthin in yellow-fleshed potato tubers reaches 1,290 μg/100 g on a fresh-weight basis. This particular characteristic of yellow-fleshed potatoes is of great importance because dietary sources of zeaxanthin are scarce. It seems that cooking has no negative effect on the lutein and zeaxanthin concentrations of potatoes. One hundred grams of the cooked yellow-fleshed varieties provide a significant amount of zeaxanthin (above 500 μg) to the human diet (Burgos et al., 2009b).

All potatoes contain chlorogenic acid as the principal phenolic acid. Red and purple potatoes also contain anthocyanins. Whole unpeeled potatoes with fully pigmented flesh can have up to 40 mg/100 g FW total anthocyanins. Red-fleshed potatoes contain acylated glucosides of pelargonidin, while purple potatoes contain in addition, acylated glucosides of malvidin, petunidin, peonidin, and delphinin (Brown, 2005). Due to their anti-oxidative properties, phenolic compounds have potential health benefits, possessing antibacterial, antiviral, anticarcinogenic and anti-inflammatory properties, and vasodilatory action (Mattila and Hellstrom, 2006). Potato tubers with red and purple flesh are a rich source of phenolic compounds in the diet (Al-Saikhan et al., 1995). Cooked tubers have shown higher concentrations of phenolic compounds that may be attributable to more efficient extraction from cooked samples (Burgos et al., 2009c).

As a staple food and as a vegetable, potatoes need to be cooked because of the indigestibility of their ungelatinized starch (Burton, 1989). Such cooking is frequently by baking, boiling, steaming, roasting, deep-oil frying, or microwave cooking, although in their native Andes a broad diversity of additional preparation methods are employed. When baked, boiled or mashed and eaten alone, potatoes generally have a high glycemic index (GI) which is a measure of the effect of the consumption of a carbohydrate food on blood glucose levels. Thus the high GI of potatoes became of concern for type 2 diabetics, and more generally for the rising levels of obesity in many countries (Foster-Powell et al., 2002), whereas it was considered beneficial to sports persons after exercise because it produces a rapid supply of muscular glycogen. However, Monro and Mishra (2009) have pointed out that GI is short for GI of available carbohydrate and this is much higher than the relative glycemic impact or potency of potato per se. Indeed, boiled and mashed potatoes are not highly glycemic, being similar to boiled spaghetti and rice on an equal fresh weight basis. Furthermore, potatoes are usually eaten in mixed meals and their nutritional value means that they are generally a very useful and beneficial component of the human diet (McGregor, 2007). Monro and Mishra (2009) concluded from their review that potatoes have an important role as a benign source of moderate-density carbohydrate energy that fits comfortably into a range of

balanced diets. Fresh potatoes are virtually free of fat and cholesterol and have a water content of about 80%.

1.2 Processed Products

The commercial value of potatoes is increased considerably when they are processed into edible products that appeal to consumers on flavor, texture, appearance, and most of all convenience (Kirkman, 2007). Today the major processed products are potato crisps (chips), French fries, and other frozen products, followed by dehydrated products, chilled-peeled potatoes and canned potatoes. Industrial production of crisps (chips) started in the 1920s and French fries in the 1950s, and potato processing has since grown into a global industry which is still expanding. In North America and some European countries between 50 and 60% of the crop is processed (Li et al., 2006; Kirkman, 2007). Furthermore, processors are building factories in countries where the potato is primarily grown as a staple food, and this is a trend that is likely to continue. Kirkman (2007) has estimated that global consumption in processed form will have increased from 13% of total food use in 2002 to nearly 18% by 2020.

Since the first potato starch plant was established in the USA in the 1830s (Treadway, 1959), the industry has developed in North America and Europe, particularly in the Netherlands, Poland, France, and Germany (Burton, 1989). Today potato starch is the starting material for the preparation of more than 500 different commercial products (Davies, 2002). Since the 1990s, potatoes have proved useful for molecular farming whereby plant cells are used to express recombinant genes and produce value-added products such as vaccines (Li et al., 2006). In some countries potatoes are still fed to animals but this use is decreasing.

1.3 Production

Potatoes are grown in 149 countries from latitudes 65°N to 50°S and at altitudes from sea level to 4,000 m (Hijmans, 2001). Potatoes can be grown wherever it is neither too hot (ideally average daily temperature below 21°C) nor too cold (above 5°C), and there is adequate water from rain or irrigation (Govindakrishan and Haverkort, 2006). In practice this means that they are grown as a summer crop in the tropical highlands of Bolivia, Peru, and Mexico; all the year round in parts of China and Brazil and in the equatorial highlands of South America (e.g., Ecuador and Colombia) and East Africa (e.g., Kenya and Uganda); as a winter crop in the lowland subtropics (e.g., northern India and southern China); as spring and autumn crops in the Mediterranean (e.g., North Africa); and in summer in the lowland temperate regions of the world (North America, western and eastern Europe, northern China, and Australia and New Zealand).

The growing season can be as short as 75 days in the lowland subtropics, where 90–120 days is the norm, and as long as 180 days in the high Andes. In the lowland temperate regions where planting is done in spring and harvesting in autumn, crop duration is typically 120–150 days and yields are potentially high. Modern potatoes have a high harvest index of around 0.80 (the proportion of the whole plant's dry weight which is harvestable tuber) and experimentally, tuber fresh-weight yields of 120 tonnes/ha have been achieved in Western Australia with a long growing season in the absence of pests and pathogens and with adequate inputs of water and fertilizers (Mackay, 1996). However, these yields are not achieved in practice. Average fresh-weight yields vary tremendously by country from 2 to 50 tonnes/ha with a global average of 16.7 tonnes/ha in 2007 (http://faostat.fao.org). Within large countries like China, with an average yield of 12.7 tonnes/ha, the variation from region to region can be nearly as great.

As potatoes cannot be grown all of the year round in most parts of the world, it is normal to have to store both seed tubers for planting the next crop and ware tubers for consumption. Hence, post-harvest infrastructure in terms of road transport and cold storage facilities is also an important aspect of successful potato production.

2 Origins and Domestication

2.1 Species Involved

The wild species progenitors of cultivated potatoes have been the subject of much discussion. Recently Spooner et al. (2005a) provided molecular taxonomic evidence for a single domestication in the highlands of southern Peru from the northern group of members of the *S. brevicaule* complex of diploid species. This group contains species such as *S. canasense*, *S. multidissectum*, and *S. bukasovii*, some of which are not always clearly resolved and perhaps could be better reduced to a single species, *S. bukasovii*. Sukhotu and Hosaka (2006) also concluded from chloroplast data that species such as these were first domesticated in Peru with a later spread to Bolivia. The result of domestication was a diploid cultigen *S. tuberosum* group Stenotomum (Dodds, 1962) from which all of the other cultivated potatoes were derived.

Dodds (1962) classified cultivated potatoes into five informal groups within one species *S. tuberosum* in which groups Andigena, Chaucha, Phureja, and Tuberosum were derived from group Stenotomum (Fig. 1.1). He also recognized two additional hybrid species involving wild species (*S.* × *curtilobum* and *S.* × *juzepczukii*) to which subsequent authors added *S.* × *ajanhuiri*, making eight groups in total. These eight groups are used in the rest of this chapter to avoid ambiguity. Hawkes (1990) gave groups Andigena and Tuberosum subspecies status and the other six groups, species status, making seven cultivated species in total. Spooner et al. (2007), using molecular data, have argued for four species. They regard frost-tolerant *S.* × *ajanhuiri* (diploid), frost-resistant *S.* × *juzepczukii* (triploid), and frost-resistant *S.* × *curtilobum* (pentaploid) as separate hybrid species derived from crosses between

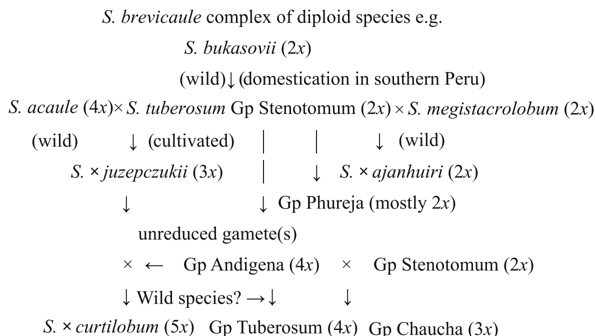

Fig. 1.1 Origin of cultivated groups of *S. tuberosum* (Dodds, 1962) and *S.* × *ajanhuiri*, *S.* × *juzepczukii* and *S.* × *curtilobum*, cultivated species with bitter taste and frost tolerance

domesticates and wild relatives. These 'bitter potatoes' are grown at high altitudes (up to 4,500 m for *S.* × *juzepczukii*) in the central Andes of Peru and Bolivia (Hawkes, 1990). *S.* × *ajanhuiri* is the hybrid of *S. tuberosum* group Stenotomum with the wild frost-resistant diploid species *S. megistacrolobum*, *S.* × *juzepczukii* is the hybrid of *S. tuberosum* group Stenotomum with the wild frost-resistant tetraploid species *S. acaule*, and *S.* × *curtilobum* is the hybrid between an unreduced gamete of triploid *S.* × *juzepczukii* and a normal gamete of *S. tuberosum* group Andigena. Spooner et al. (2007) regard groups Andigena, Chaucha, Phureja, Stenotomum, and Tuberosum (called by them Chilotanum) as a single species *S. tuberosum*, but now divided into two cultivar groups. These are the Andigenum group of upland Andean landraces containing diploids, triploids, and tetraploids, and the Chilotanum group of lowland tetraploid Chilean landraces. It is now useful to return to consider how these landraces arose.

First it is necessary to consider how group Andigena arose from group Stenotomum. Sukhotu and Hosaka (2006) concluded from chloroplast and nuclear DNA markers that group Andigena arose from group Stenotomum through sexual polyploidization from unreduced gametes many times at many places in the fields of group Stenotomum. These tetraploids were subsequently modified by occasional and unintentional selection of natural hybrids with neighboring wild species to give present-day group Andigena. Scurrah et al. (2008) have shown that closely related species growing around farmers' fields can hybridize with group Andigena and that some hybrid progeny would be selected by present-day Andean farmers. These results explain the chromosome behavior and tetrasomic inheritance of tetraploid *S. tuberosum* and why it can be regarded as the autotetraploid of diploid group Stenotomum for practical purposes. Hosaka (2004) suggested that Chilean Tuberosum (T) cytoplasm is derived from the southern wild species *S. tarijense* so that group Tuberosum is not simply group Andigena that has been selected to produce tubers in long days. Spooner et al. (2007) show that the T cytoplasm is also found at low frequency in Andean landraces including some diploids, indicating that the T cytoplasm moved northward as well as becoming predominant in Chilean

germplasm. However, this does not contradict the view that the Chilean landraces are secondarily derived from the Andean ones and that the long-day-adapted landraces of coastal Chile are genetically distinct from the short-day-adapted ones of the Andes (Raker and Spooner, 2002).

Returning to the other landraces, *S.* × *chaucha* is the triploid hybrid between diploid *S. tuberosum* group Stenotomum and tetraploid *S. tuberosum* group Andigena and, like group Stenotomum, is confined to the central Andes of Peru and Bolivia. In contrast, *S. tuberosum* group Phureja (diploid) was selected from group Stenotomum by Andean farmers for lack of tuber dormancy and faster tuber development so that they could grow up to three crops per year in the lower, warmer, eastern valleys of the Andes. Phureja potatoes were therefore able to spread into northern Ecuador, Colombia, and Venezuela and are the second most widely cultivated type in South America, after Andigena which is grown throughout the upland Andes of South America. Interestingly Ghislain et al. (2006) found that 32 out of 102 accessions of Phureja in the International Potato Center (CIP) collection of landraces were triploid or tetraploid, not diploid, in agreement with Hawkes (1990) that not all Phureja are diploid.

2.2 Reproductive Biology

The reproductive biology of potatoes is ideal for creating and maintaining variation. Potatoes, like their ancestral wild species, reproduce by sexual means and also by setting tubers. Potatoes flower and set true seed in berries following natural pollination by insects capable of buzz pollination, such as some bee species, which can release pollen from the poricidal anthers of potatoes (Scurrah et al., 2008). Outcrossing is enforced in cultivated (and most wild) diploid species by a single S-locus, multiallelic, gametophytic self-incompatibility system (Dodds, 1965). While self-incompatibility does not operate in tetraploid *S. tuberosum*, 40% (range 21–74%) natural cross-pollination was estimated to occur in group Andigena in the Andes (Brown, 1993) and 20% (range 14–30%) in an artificially constructed Andigena population (Glendinning, 1976). This sexual reproduction creates an abundance of diversity by recombining the variants of genes that arose by mutation, and potatoes are highly heterozygous individuals that display inbreeding depression on selfing. The genetically unique seedlings that grow from true seeds produce tubers that can be replanted as seed tubers and hence distinct clones can be established and maintained by asexual (vegetative) reproduction. Most potato cultivars are propagated through seed tubers and are genetically uniform. There are, however, circumstances where cultivars based on current methods of true potato seed (TPS) propagation are an attractive proposition despite being genetically variable and inferior to the best genotype that exists within the TPS progeny. These will be discussed in later sections. No doubt domestication occurred by human selection of tubers from naturally occurring variation, and the same was true for the subsequent farmer selection and propagation of landraces of potato.

2.3 History of Crop

The journey from gathering wild tubers to cultivating them and finally domesticating them started early in the human colonization of the Americas. Wild potato remains have been found in a late Pleistocene settlement in south central Chile dated around 12,500 years before present (Ugent et al., 1987; Moseley, 2001). Preserved food plant remains have been found at various excavated sites on the coast of Peru and one site in the high Chilca Canyon, south of Lima (Engel, 1970). The Oxford radiocarbon accelerator dated the tuber remains found by Engel to about 7,000 years before present. Fresh potatoes were most likely baked in the embers of a fire or cooked in an earth oven on hot stones (Hawkes, 1990). Lack of more evidence of cultivation in highland sites where the progenitor species are found probably simply indicates poor preservation conditions in high regions with seasonally wet climates, compared with better conditions in arid environments. Nevertheless, the extensive abandoned cultivation terraces throughout parts of the Andes suggest that potatoes were a very widespread crop in the Andes prior to the discovery of South America by the Spanish in 1533 (Hawkes, 1990) and remain so today. Interestingly, CIP (International Potato Center) are currently doing a project called PAPA ANDINA to develop a market chain for potatoes from small-scale growers in areas of rural poverty in Ecuador, Peru, and Bolivia to urban markets at home and abroad (Anderson, 2009).

2.3.1 Introduction to Europe

It is assumed that Pizarro and his men were the first Europeans to see potatoes being cultivated, in the Andes of Peru in 1533 during the Spanish conquest of the Incas, but there is no written record (Hawkes, 1990). In fact potatoes were first recorded in 1537 in what is now Colombia (Hawkes, 1990). The first record of cultivated potatoes outside of South America is their export in 1567 from Gran Canaria in the Canary Islands to Antwerp in Belgium (Hawkes and Francisco-Ortega, 1993). This was 6 years before they were first recorded in Spain in 1573 in the market archives of the Hospital de La Sangre in Seville (Hawkes and Francisco-Ortega, 1992). Potatoes were therefore probably first introduced from South America into the Canary Islands around 1562 and from there to mainland Europe (Hawkes and Francisco-Ortega, 1993).

The early introductions of potatoes to Europe included the one shown in the first water-color painting of a potato (late maturing, red skinned tubers of irregular shape with deep eyes) dated 1588 and the one shown in the first printed illustration (not as late in maturity, white skinned tubers of irregular shape with deep eyes) of 1597 (Hawkes, 1990). It has often been assumed that these early introductions came as ships' stores from Colombia and were of Columbian, or possibly Peruvian, origin and hence were primarily tetraploid group Andigena potatoes. Then as the growing of potatoes spread north-eastward across Europe, they became adapted to the long summer days of northern Europe and in this respect resembled Chilean potatoes. However, extant Canary Island potatoes comprise both Andean- and Chilean-type

landraces and Rios et al. (2007) have suggested that there were multiple early introductions of both types. Furthermore, they suggest that the early European potato was selected from the Chilean introductions because they were better adapted to European conditions. Potato introductions from South America were reviewed by Glendinning (1983), but one cannot say with certainty how many there were and what their contribution was to the subsequent spread of the potato in and from Europe, as reviewed by Hawkes (1990). It now seems safest to assume that the early introductions of cultivated potatoes to Europe came from both the Andes and coastal Chile (Hosaka et al., 1994; Spooner et al., 2005b; Rios et al., 2007). Analysis of DNA from 49 herbarium specimens has confirmed the presence of Andean potatoes from around 1700 and Chilean potatoes from 1811 in Europe (Ames and Spooner, 2008). Interestingly, molecular analyses of old Japanese cultivars were consistent with them being derived from group Andigena through early European potatoes (Hosaka et al., 1994) while recent molecular analyses have clustered all Indian potato varieties, including putatively remnant Andean populations, with Chilean Tuberosum (Spooner et al., 2005b).

2.3.2 Transition to Major Worldwide Food Crop

After their introduction to Europe, potatoes initially remained a botanic curiosity, being grown and studied in physic gardens for interest and medicinal purposes. Their potential as a food crop was first seen in Ireland at the end of the seventeenth century and throughout the eighteenth century (Burton, 1989). The climate and soil of Ireland proved suitable for potatoes but there were also societal and economic reasons for their increase in importance as a food crop. As a consequence, the population of Ireland increased in size from 2 million in 1700 to 8.5 million in 1845 (Reader, 2008). However, overreliance on the potato meant that the late blight epidemics of 1845 and 1846 resulted in famine in Ireland with profound societal consequences (Zadoks, 2008). Ironically, it was food shortages that proved to be the stimulus to potato cultivation throughout Europe during the eighteenth century because military and economic strength depended upon adequately fed manpower (Burton, 1989; Reader, 2008). Thus the eighteenth century saw potatoes accepted as a food throughout Europe and the nineteenth century saw their ascendancy as a major food crop (Burton, 1989), before a decline in potato production and consumption during the twentieth century. Globally, however, this decline was offset by what happened in the rest of the world.

During the seventeenth and eighteenth centuries many European countries developed widespread political and commercial interests in the rest of the world and the European (British, Dutch, French, Portuguese, and Spanish) colonists and missionaries took with them their common crops including potatoes (Burton, 1989; Pandey and Kaushik, 2003). Potato production expanded worldwide during the nineteenth century, but it was the expansion in China and India during the second half of the twentieth century that led to these countries becoming the first and third most important producers in the world.

3 Varietal Groups

The concept of varietal groups has not been used for potatoes. Nevertheless, some groupings have been found useful. As mentioned earlier, cultivated potatoes can be classified into five informal groups within *S. tuberosum* (groups Andigena, Chaucha, Phureja, Stenotomum, and Tuberosum) and three hybrids with wild species. Group Tuberosum is adapted to tuberization in long days, and this is understood. However, as a result of recent plant breeding, clarity is required when talking about long-day-adapted Andigena (Neotuberosum) and long-day-adapted Phureja potatoes. Otherwise, short-day adaptation will be assumed. It can also be useful to give an indication of adaptation to length of growing season by using maturity groups, a guide to days from planting to maturity and hence harvest date. In Britain there are first early (100–110 days), second early (110–120 days), early maincrop (120–130 days), maincrop (130–140 days), and late maincrop (140–150 days) varieties.

The World Catalogue of Potato Varieties 2009/2010 (Pieterse and Hils, 2009) provides information on the maturity, tuber shape, skin color, depth of eyes, flesh color, disease resistance, and use of varieties. Consumer preferences vary with country regarding skin (e.g., red, white, and russet) and flesh color, and varieties for different uses are also usually recognized. Furthermore, in characterizing Andean potato diversity, de Haan (2008) recognized three groups of cultivars for their roles in farming systems and household economies: native floury, native bitter, and improved or bred potatoes.

4 Genetic Resources

4.1 World Catalogue of Potato Varieties

The World Catalogue of Potato Varieties 2009/2010 (Pieterse and Hils, 2009) lists more than 4,500 varieties from 102 countries worldwide. Furthermore, a number of databases on modern cultivars are available, such as The European Cultivated Potato Database (http://www.europotato.org) which currently has information on 4,136 cultivated varieties. Google searches will find databases for many countries and organizations around the world. These modern cultivars provide breeders with parents adapted to their target environments, growing seasons, and end uses, but which require local evaluation and improvement.

4.2 Cultivated Potatoes in Latin America

Following its creation in Lima, Peru in 1970, The International Potato Center (CIP) (http://www.cipotato.org) assembled a collection of more than 15,000 accessions of potato cultivars (landraces) native to nine countries in Latin America: Argentina, Bolivia, Chile, Colombia, Ecuador, Guatemala, Mexico, Peru, and Venezuela.

Subsequently duplicate accessions were identified and the number of individual cultivars was reduced to 3,527 of which 552 were diploids, 128 triploids, 2,836 tetraploids (2,644 group Andigena, 144 group Tuberosum, and 48 hybrids), and 11 pentaploids (Huaman et al., 1997). By 1997 researchers at CIP had already conducted 46,124 evaluations on the collection for the reactions of cultivars to abiotic and biotic stresses and for other desirable traits. A core set of 306 group Andigena accessions was then established to aid utilization (Huaman et al., 2000a, b). They were chosen to represent the widest morphological diversity and to maximize geographical representation and hence should be valuable for future breeding both in South America and worldwide. The other genebanks mentioned below also have collections of cultivated species, but the one at CIP is recognized as the world collection. The collection is maintained by clonal propagation in the field and in vitro. In the future, cryopreservation may become an important component of maintaining clonal germplasm collections, thus allowing a reduction in the labor required for routine subculture of in vitro stocks. Much research has been done in recent years with some encouraging results that have been reviewed by Veilleux (2005).

4.3 Wild Tuber-Bearing *Solanum* Species

Wild tuber-bearing *Solanum* species are distributed from the southwestern USA (38°N) to central Argentina and adjacent Chile (41°S) (Hawkes, 1990; Spooner and Hijmans, 2001). They are a tremendous resource for potato breeding because of their wide geographical distribution and great range of ecological adaptation (Hawkes, 1994). In the southwestern USA and in Central America wild species generally occur at medium to high altitudes. In South America they are found along the Andes from Venezuela to northwest Argentina and also in the lowlands of Chile, Argentina, Uruguay, Paraguay, and southeastern Brazil. The adaptive range among the different species is very great and includes the high Andean regions from 3,000 m to the vegetational limit at 4,500 m where frosts are common, dry semi-desert conditions and scrub and cactus deserts, cool temperate pine and rain forests, and woodlands and coastal plains. Wild species have also developed resistances to a wide range of pests and diseases, but it is not clear if sources of resistance can be predicted from taxonomic and biogeographical variables. For example, Jansky et al. (2008) were unable to predict sources of early blight resistance.

There have been numerous collecting expeditions, from those pioneered by the Russians in the 1920s (Hawkes, 1990) to the more recent ones of the 1990s (Spooner and Hijmans, 2001). Reference books have been written on the potatoes of Argentina, Brazil, Paraguay and Uruguay (Hawkes and Hjerting, 1969), Bolivia (Hawkes and Hjerting, 1989; Ochoa, 1990), Peru (Ochoa, 2004), and North and Central America (Spooner et al., 2004). The collecting expeditions led to the establishment of a number of potato germplasm collections worldwide. The world collection is held at the CIP in Lima, Peru. The other main germplasm collections are the Commonwealth Potato Collection (CPC, Dundee, Scotland) (Fig. 1.2), the

Fig. 1.2 Part of the Commonwealth Potato Collection and cultivars Vales Everest (QTLs for resistance to white potato cyst nematode from group Andigena CPC 2802), Lady Balfour (QTLs for resistance to cyst nematodes from *S. vernei*), and Vales Sovereign (*H1* gene for resistance to golden potato cyst nematode from group Andigena CPC 1673) (Source: SCRI)

Dutch–German Potato Collection (CGN, Wageningen, The Netherlands), the Groß Lusewitz Potato Collection (GLKS, IPK, Groß Lusewitz, Germany), the Potato Collection of the Vavilov Institute (VIR, St. Petersburg, Russia), the US Potato Genebank (NRSP-6, Sturgeon Bay, USA), and Potato Collections in Argentina, Bolivia, Chile, Colombia, and Peru. Together they comprise the Association for Potato Intergenebank Collaboration and have established an Inter-genebank Potato Database (IPD) (www.potgenebank.org; or individual genebanks through Google). The IPD contains 7,112 different accessions of 188 taxa (species, subspecies, varieties, and forms) out of the 247 tuber-bearing wild potato taxa recognized by Hawkes (Huaman et al., 2000c). Accessions are normally held in true seed form. Data are available through the IPD links to individual collections for more than

33,000 evaluations of wild potato accessions covering 55 traits (dry matter, starch, reducing sugar and glycoalkaloid content, and resistances to fungi, bacteria, viruses, viroids, insects, and environmental stresses, such as frost and heat/drought).

The World Catalogue of Potato Varieties 2009/2010 (Pieterse and Hils, 2009) contains a 'Catalogue of the Global FAO-International Treaty "in trust" Wild Potato Collection at the International Potato Center (CIP).' The collection (CIP-genebank@cgiar.org) has 1,917 accessions and consists of representatives of 141 species with 67 from Peru, 29 from Bolivia, 13 from Mexico, 13 from Ecuador, and 10 from Argentina.

Some of the biological issues involved in the conservation of potato genetic resources have recently been discussed by Bamberg and del Rio (2005) along with the complicated issue of germplasm ownership. The International Treaty on Plant Genetic Resources for Food and Agriculture came into force on June 29, 2004, and was ratified by 55 countries (www.fao.org). It offers a multilateral system for easy access and exchange of germplasm in return for fair and equitable sharing of the benefits. Potatoes (section *Petota*, except *S. tuberosum* group Phureja) are one of the 35 food crops in the initial list covered by the multilateral system. It is too early to assess the impact of the treaty on the utilization of tuber-bearing *Solanum* species in potato breeding.

4.4 Taxonomy of Wild Tuber-Bearing *Solanum* Species

The taxonomy of wild tuber-bearing *Solanum* species is complicated and under continuous revision. Hawkes (1990) recognized 219 wild tuber-bearing species and arranged them into 19 series of subsection *Potatoe* of section *Petota* of subgenus *Potatoe* of genus *Solanum* (Table 1.1). He grouped series I–IX in superseries *Stellata* and series X–XIX in superseries *Rotata.* He considered the sequence of subsections, superseries, and series to reflect an approximate evolutionary one and suggested a possible scenario for the evolution of wild potato species. He postulated that (diploid) wild potatoes originated in Mexico and expanded to South America, from where a newly evolved group returned to North America. However, distributional and ploidy data suggest a South American origin (Hijmans et al., 2007), so the matter remains unresolved. Hawkes also recognized a further nine closely related non-tuber-bearing species that he grouped into two series of subsection *Estolonifera*, but these have been excluded from section *Petota* in more recent taxonomic reviews, leaving a section comprising all tuber-bearing species.

The latest summary by Spooner and Salas (2006) recognizes 188 wild potato species for section *Petota* that are grouped into four clades, based on plastid DNA, rather than 19 series (Table 1.1). Clade 1 comprises the US, Mexican, and Central American diploid species, exclusive of *S. bulbocastanum*, *S. cardiophyllum*, and *S. verrucosum*; Clade 2 comprises *S. bulbocastanum* and *S. cardiophyllum*; Clade 3 comprises all examined members of the South American series *Piurana* and some South American species classified into other series; and Clade 4 comprises

Table 1.1 Classification of wild tuber-bearing *Solanum* species (Section *Petota*) based on Hawkes (1990) and Spooner and Salas (2006) and species mentioned in text

Superseries	Series	Species numbers	Ploidy	EBN	Area[a]	Plastid clade
Superseries *Stellata*						
I	*Morelliformia*	1	2*x*	1	Mex	1
II	*Bulbocastana*	2	2*x*	1	Mex	1
	S. bulbocastanum		2*x*	1	Mex	2
III	*Pinnatisecta*	11	2*x*	1	Mex	1
	S. cardiophyllum		2*x*	1	Mex	2
IV	*Polyadenia*	2	2*x*	1	Mex	1
V	*Commersoniana*	2	2*x*	1	SA	4
VI	*Circaeifolia*	3	2*x*	1	SA	4
VII	*Lignicaulia*	1	2*x*	1	SA	4
VIII	*Olmosiana*	1	2*x*	1	SA	4
IX	*Yungasensa*	9	2*x*	2	SA	4
	S. chacoense		2*x*	2	SA	4
Superseries *Rotata*						
X	*Megistacroloba*	11	2*x*	2	SA	4
	S. megistacrolobum		2*x*	2	SA	4
	S. raphanifolium		2*x*	2	SA	4
XI	*Cuneoalata*	3	2*x*	2	SA	4
XII	*Conicibaccata*	40	2*x*, 4*x*, 6*x*	2,2,4	SA, Mex	4
XIII	*Piurana*	15	2*x*, 4*x*	2	SA	3
XIV	*Ingifolia*	2	2*x*	2	SA	4
XV	*Maglia*	1	2*x*	2	SA	4
XVI	*Tuberosa*	96	2*x*, 4*x*	2	SA	4
	S. brevicaule		2*x*	2	SA	4
	S. bukasovii		2*x*	2	SA	4
	S. canasense		2*x*	2	SA	4
	S. leptophyes		2*x*	2	SA	4
	S. multidissectum		2*x*	2	SA	4
	S. sparsipilum		2*x*	2	SA	4
	S. spegazzinii		2*x*	2	SA	4
	S. vernei		2*x*	2	SA	4
	S. verrucosum		2*x*	2	Mex	4
XVII	*Acaulia*	4	4*x*, 6*x*	2,4	SA	4
	S. acaule		4*x*	2	SA	4
XVIII	*Longipedicellata*	7	4*x*	2	Mex	4
	S. stoloniferum		4*x*	2	Mex	4
XIX	*Demissa*	8	6*x*	4	Mex	4
	S. demissum		6*x*	4	Mex	4

[a]SA = South America; Mex = Southwestern USA, Mexico, and Central America.

all remaining South American species (including cultivated potatoes) and the US, Mexican, and Central American polyploid species and *S. verrucosum*. However, this plastid-based classification splits similar species into different clades and so may not properly represent groupings made on the basis of nuclear DNA. Furthermore, it is not an appropriate means of classifying allopolyploid groups. The number of species

may be further reduced in the future, and clade composition based on chloroplast DNA may change as extensive nuclear DNA sequence data become available. Of more interest to potato breeders is the origin and relatedness of the genomes in wild and cultivated potatoes, including hybrid taxa, and their accessibility for breeding via crossing.

The wild species form a polyploid series from diploid ($2n = 2x = 24$) to hexaploid ($2n = 6x = 72$) in which only diploid cytotypes have been found in 123 species and only polyploids in 43 species (Hijmans et al., 2007). There is some evidence that polyploidy played an important role in the environmental differentiation and range expansion of wild potatoes (Hijmans et al., 2007). The two most widespread species are both tetraploids, *S. stoloniferum* in North and Central America and *S. acaule* in South America. Genomes were classified into five groups A, B, C, D, and P by Matsubayashi (1991), with a sixth group E recognized in closely related non-tuber-bearing species. Spooner et al. (2004) summarized the putative genome compositions of the polyploid species, but it is clear that further research is required to resolve their origins. For example, recent data support *S. bulbocastanum* (Wang et al., 2008) and *S. verrucosum* (Pendinen et al., 2008) as the progenitors of the allotetraploid *S. stoloniferum*. Nearly all of the diploid species are outbreeders, with a single S-locus, multiallelic, gametophytic self-incompatibility system (Dodds, 1965), whereas the tetraploids and hexaploids are mostly self-compatible allopolyploids that display disomic inheritance (Hawkes, 1990). A dominant self-incompatibility inhibitor has been found in *S. chacoense* (Hosaka and Hanneman, 1998) and used in breeding, just as one had previously been found in a dihaploid (see below) of *S. tuberosum* (De Jong and Rowe, 1971).

4.5 Crossability of Species

The crossability of species has been determined through artificial pollinations done across many years (Jansky, 2006). The results can be explained primarily but not exclusively in terms of endosperm balance number (EBN), which can be regarded as the effective rather than the actual ploidy of the species (Johnston et al., 1980). In crosses between species with the same EBN the hybrids have a normal endosperm for nourishing the hybrid embryo, whereas in crosses between species with different EBNs the endosperm degenerates. The endosperm develops following the fusion of a sperm nucleus from the male parent with two polar nuclei from the female parent to give a triple fusion nucleus and it is the genetic composition of this nucleus that is important. Under the EBN hypothesis the endosperm is normal when the three nuclei have the same EBN and hence a 2 maternal to 1 paternal ratio of endosperm balance factors. Attempts have been made to understand the genetic and biological basis of the EBN concept, but EBNs are determined experimentally relative to species assigned an arbitrary value of 1 (Hermsen, 1994). The main groups of species are diploid EBN = 1, diploid EBN = 2, tetraploid EBN = 2, tetraploid EBN = 4 (including *S. tuberosum*), and hexaploid EBN = 4 (Hawkes and Jackson,

1992). Species within these crossability groups have evolved by means of geographical and ecological isolation rather than by genetic incompatibility and hybridizations are usually successful.

Today breeders can usually achieve sexual hybridization between *S. tuberosum* and its wild relatives by manipulation of ploidy with due regard to EBN (Ortiz, 1998, 2001; Jansky, 2006). EBN can be doubled meiotically through the unreduced 2*n* gametes produced from several naturally occurring recessive meiotic mutations that are common in *Solanum* species (Tai, 1994; Jansky, 2009). It can also be doubled mitotically through the use of colchicine for chromosome doubling, something which can also occur naturally during callus culture. In fact, Stupar et al. (2007) were able to produce diploid and tetraploid regenerants when somatic leaf cells from their monoploid were exposed to leaf disk regeneration. EBN can be halved by haploidization using in vitro androgenesis (anther culture) or more commonly in *S. tuberosum* by parthenogenesis using pollinations with particular clones of diploid group *phureja* (Wenzel, 1994; Veilleux, 2005). Furthermore, embryo rescue can be used to secure a hybrid where embryo abortion is due to a defective endosperm (Hermsen, 1994; Jansky, 2006). As the largest compatibility group is EBN = 2, it is now common for potato breeders to secure tetraploid hybrids from 4*x* (*S. tuberosum*) × 2*x* (2*x S. tuberosum* × wild species) crosses in which an unbalanced endosperm prevents the development of triploid embryos. There are, however, other barriers to hybridization, such as interspecific pollen–pistil incompatibility and nuclear-cytoplasmic male sterility, although fertility restorer genes have been found for the latter (Jansky, 2009). Unilateral incompatibility is known to occur when a self-incompatible (SI) species is pollinated by a self-compatible (SC) one so that *S. verrucosum* (SC female) × *S. phureja* (SI male) is successful, but the reciprocal cross fails (Hermsen, 1994; Jansky, 2006). Sometimes incompatible pollen can be helped to achieve fertilization through a second pollination with compatible pollen, a technique known as mentor pollination (Hermsen, 1994; Jansky, 2006). These phenomena have been reviewed by Camadro et al. (2004) in the context of how sympatric species maintain their integrity. From time to time potato breeders have unexpected successes and failures when attempting to overcome barriers to hybridization.

Potato breeders can also use somatic (protoplast) fusion to achieve difficult or impossible sexual hybridizations and in ploidy manipulation to achieve maximum heterozygosity (Wenzel, 1994; Thieme and Thieme, 2005; Veilleux, 2005). Protoplast fusion can be induced chemically by the polycation polyethyleneglycol (PEG) or via electrofusion that is now the preferred method. Somatic hybrids derived from the same fusion combination do show genotypic and phenotypic variation and hence require screening for the desired product. Somatic fusion has allowed the production of fertile hexaploid hybrids between tetraploid *S. tuberosum* (EBN = 4) and diploid EBN = 1 species, such as the non-tuber-bearing species *S. brevidens* that has tuber soft rot and early blight resistances (Tek et al., 2004) and *S. bulbocastanum* that has a major gene for broad spectrum resistance to late blight (Naess et al., 2000). Somatic fusion has also allowed the production of diploid hybrids from monoploids of group Tuberosum and group Phureja (Lightbourn and Veilleux, 2007) and tetraploid hybrids from dihaploids (the haploids produced from 4*x*

S. tuberosum), including male-sterile ones (Thieme and Thieme, 2005). Dominant resistance genes as well as quantitative traits can be combined in the same way as when gametes fuse in sexual hybridization.

5 Major Breeding Achievements

5.1 *In South America Following Domestication*

It is assumed that domestication involved selection for less bitter and hence less toxic tubers, but interestingly Johns and Alonso (1990) found that some genebank accessions of *S. bukasovii* had tuber glycoalkaloid levels which were consistently close to the levels found in many clones of *S. tuberosum* group Stenotomum. They concluded that exploitation and domestication of this species would have required little or no selection for lower glycoalkaloid level, unlike their samples of *S. canasense*, *S. leptophyes*, and *S. sparsipilum*. Nevertheless, it seems fair to credit Andean farmers with making the potato an edible crop.

Andean farmers certainly retained a much wider variety of tuber shapes and skin and flesh colors than is seen in wild species (Simmonds, 1995), and this diversity can only have arisen by mutation under domestication. Furthermore, naturally occurring tetraploid types of potato came to be selected in preference to their diploid ancestors, presumably because farmers found them superior for yield and other traits. Subsequent selection for appropriate maturity and dormancy, higher yields and harvest index, and resistance to abiotic and biotic stresses must have occurred in many environments. Stupar et al. (2007) developed a synthetic autopolyploid series in potato (primarily group Phureja) that included one monoploid (1*x*) clone, two diploid (2*x*) clones, and one tetraploid (4*x*) clone, in order to explore phenotypic and transcriptomic (about 9,000 genes) changes associated with autopolyploidization. Interestingly, the diploid plants were the most vigorous and generated the greatest biomass with the monoploid inferior to both the diploids and the tetraploid. However, the diploid and tetraploid plants had similar gene expression patterns. Therefore the eventual superiority of tetraploid potatoes probably resulted from them exploiting their increased potential for heterozygosity rather than polyploidy per se.

The original CIP collection of more than 15,000 accessions of potato cultivars (landraces) native to nine countries in Latin America (Argentina, Bolivia, Chile, Colombia, Ecuador, Guatemala, Mexico, Peru, and Venezuela) is testament to the achievements of the farmers of these countries.

5.2 *Modern Potato Breeding*

Modern potato breeding began in 1807 in England when Knight made the first recorded hybridizations between varieties by artificial pollination (Knight, 1807),

although named cultivars can be traced to the 1730s (Reader, 2008). It flourished in Europe and North America during the second half of the nineteenth century when exchanges of germplasm started to occur and many new cultivars were produced by farmers, hobby breeders, and seedsmen. Even then, the raising of seedlings from seed of self-set berries remained a common practice which continued into the twentieth century. North America's most popular potato cultivar, Russet Burbank, released in 1914, was descended from Rough Purple Chili through three generations of open pollination (Ortiz, 2001). Modern potato breeding in India and China started later in the 1930s, but with rapid expansion since 1948 and 1978, respectively (Gaur et al., 2000; Jin et al., 2004).

The extent of progress since 1807 can be judged by the latest World Catalogue of Potato Varieties (Pieterse and Hils, 2009) which lists more than 4,500 varieties from 102 countries covering all potato growing regions in the world. Although a much smaller number have been widely grown, this is nevertheless a remarkable achievement for a crop which was unknown outside of South America until almost 500 years ago and which was derived from a narrow genetic base. It represents adaptation of the potato to the wide range of environments and end uses mentioned in the Introduction. The initial adaptation of potatoes to growing in long days must have resulted in dramatic increases in yield. Then during the twentieth century in Europe and North America, yields more than doubled in some countries with increases in Great Britain (GB) from 22 to 45 tonnes/ha over the period 1960–2003 (British Potato Council statistics) and likewise in the USA from 5.6 to 33.6 tonnes/ha over the period 1920–1989 (Lucier et al., 1990). However, the contribution of new cultivars to the yield increases appears to have differed in GB and the USA. Simmonds (1981) provided evidence of a 5.5 tonnes/ha increase in GB from the near replacement of cultivars 'King Edward' and 'Majestic' with three more modern cultivars 'Pentland Crown,' 'Maris Piper,' and 'Desiree.' In contrast, Douches et al. (1996) in the USA found a lack of improvement in yield and specific gravity when cultivars released over the period 1861–1991 were compared for 3 years in a high-yielding environment (50 tonnes/ha), but they did find trends to earlier maturity and improved tuber appearance. Furthermore, Love et al. (1998) provided evidence of significant progress since 1960 in North America in developing cultivars with good processing quality.

5.3 Introgression of Genes from Wild and Cultivated Species

In 1908 in Germany and 1909 in GB, recognition of *S. demissum* as a source of genes for resistance to late blight marked the start of introgression breeding (Muller and Black, 1951). Resistance to late blight was introgressed from *S. demissum* and *S. stoloniferum*, resistance to viruses from these species together with *S. chacoense* and *S. acaule*, and resistance to potato cyst nematodes from *S. vernei* and *S. spegazzinii*. By the end of the 1980s these wild species, together

with cultivated *S. tuberosum* groups Andigena and Phureja, had been used extensively in the breeding of successful cultivars in Europe (Ross, 1986). Likewise the species *S. demissum*, *S. chacoense*, and *S. acaule* were used extensively in North America (Plaisted and Hoopes, 1989), and one of the most successful cultivars to be introduced into China by CIP, CIP-24, or Achirana-INTA, bred by the Instituto Nacional de Tecnología Agropecuaria, Argentina, had *S. acaule*, *S. demissum*, and *S. stoloniferum* in its pedigree (Ortiz, 2001). Nevertheless, the introgression of genes from wild and cultivated species has been fairly limited in number but is expected to increase.

The resistances to viruses and cyst nematodes (Fig. 1.2) proved valuable in crop production, whereas the *S. demissum*-derived R genes failed to provide durable resistance to late blight either singly or in combination due to the evolution of new races of *Phytophthora infestans* (Malcolmson, 1969). Breeders therefore switched to selection for high levels of quantitative field resistance, either by using races of *P. infestans* compatible with the R genes present in their material or by creating R gene-free germplasm so that screening could be done with any race (Toxopeus, 1964; Black, 1970; Wastie, 1991; Ortiz, 2001). Results have been mixed and the most widely grown cultivars today are still usually susceptible to late blight (Forbes et al., 2009). Currently there is much debate over whether or not the R genes for blight resistance being found in other wild species will be more durable per se or can be deployed in a more durable way (Goverse and Struik, 2009).

5.4 Introgression and Base Broadening

Peloquin and his coworkers (Hermundstad and Peloquin, 1987; Jansky et al., 1990) developed a novel breeding strategy to introgress specific characteristics and to broaden the genetic base of potato in a way similar to that envisaged by Chase (1963) in his analytic breeding scheme. This strategy was made possible by the production of haploids (also called dihaploids) of *S. tuberosum* from 1958 onward (Hougas et al., 1958). The hybrids between haploids of *S. tuberosum* and diploid wild species with an EBN of 2 will often form tubers in long-day growing conditions (Jansky et al., 2004; Jansky, 2009). When they also produce $2n$ gametes by FDR (first division restitution), much of the genetic diversity of the wild species can be efficiently transferred to the tetraploid offspring from $4x \times 2x$ crosses and results in about 25% of the wild species genes in the final product (Tai, 1994; Jansky, 2009). Tai (1994) concluded, however, that haploid-wild species hybrids need to be improved before they are used in $4x \times 2x$ crosses, for example, through population improvement by recurrent selection (Rousselle-Bourgeois and Rousselle, 1992). In contrast, diploid hybrids with *S. raphanifolium* with resistance to cold-sweetening after storage for 3 months at 2°C have been used by Hamernik et al. (2009) to produce commercially acceptable tetraploid offspring without extensive backcrossing to cultivated germplasm. These should be valuable parents for producing new cultivars suitable for processing.

5.5 Base Broadening

During the second half of the twentieth century recognition was given to the value of the cultivated species of South America for broadening the genetic base of European and North American breeding programs. Breeding experiments in Europe and North America demonstrated that through simple mass selection under northern latitude, long-day summer conditions, group Andigena will adapt and produce parents suitable for direct incorporation into European and North American potato breeding programs (Glendinning, 1975; Munoz and Plaisted, 1981). Neotuberosum germplasm provided adapted sources of resistance to important pathogens including *Globodera rostochiensis* (golden potato cyst nematode) and *Potato virus Y* (PVY) (Plaisted, 1987). Likewise, Carroll (1982) (Fig. 1.3) and Haynes (Haynes and Lu, 2005) produced populations of group Phureja/group Stenotomum adapted to long-day conditions in Europe and North America, respectively. Direct hybridization of Carroll's improved diploid population with tetraploid potato cultivars via unreduced pollen grains ($4x \times 2x$ crosses) resulted in tetraploid hybrids, some of which were superior to standard tetraploid cultivars in both total and marketable yields (Carroll and De Maine, 1989). Furthermore, diploid cultivars, such as Mayan Gold, have now been produced from this population, but they are targeted at niche markets

Fig. 1.3 Long-day-adapted group Phureja potatoes from the population of Carroll (1982) (Source: SCRI)

because their yield is only two-thirds that of Tuberosum potatoes. Despite these breeding efforts, relatively few clones of Neotuberosum (long-day group Andigena) and long-day group Phureja/group Stenotomum have been used to any extent in the breeding of modern cultivars. Part of the reason for this is that while adaptation to tuberization in long days was quickly achieved, other problems remained. Neotuberosum clones lacked the regularity of tuber shape of intensively selected group Tuberosum clones and long-day-adapted group Phureja clones lacked tuber dormancy. Hence these populations need to be selected for further improvements to achieve the original goal of their direct use as parents in breeding finished cultivars for European and North American markets.

CIP on its establishment recognized the need to make broad-based germplasm and candidate varieties from the world collection available to National Programs in developing countries, particularly germplasm with durable resistance to late blight. Probably the best-known broad-based germplasm from CIP's breeders is Population B with quantitative resistance to late blight. The aim was to help with the development of improved cultivars, for a wide range of environments, which possessed high and stable levels of resistance to late blight in combination with resistances to viruses and suitable tuber type, and culinary and processing quality (Trognitz et al., 2001; Landeo, 2002). The population improvement involved recurrent selection for quantitative resistance to late blight and other desirable traits under high endemic disease pressure in the Andean highlands together with selection in those geographical areas where the new cultivars were to be grown, for example, in short days in East Africa (Mulema et al., 2004). Evaluation under long days in Argentina (Trognitz et al., 2001) and in South Korea was included to broaden the range of adaptation of the largely short-day-adapted population. Clones with good general combining ability have been identified for use as parents in the local breeding programs of the National Agricultural Research Systems in developing countries. Landeo (2002) and Landeo et al. (2007) reported good progress in selecting for resistance and other traits in Population B, large additive genetic variances and high heritability estimates for resistance, and stability of resistance across diverse environments and pathogen populations in tropical environments.

Parallel efforts at CIP have developed the lowland tropic virus-resistant population denoted as LTVR. This population combines the heat tolerance and early bulking ability of *S. tuberosum* germplasm bred under the summer conditions of the Northern Hemisphere on the one hand, with virus resistance from native *S. tuberosum* group Andigena and Neotuberosum on the other. Recurrent selection with evaluations in a range of environments on the Peruvian coast led to the development of multiplex progenitors of extreme resistance to PVY and PVX, and the selection of virus-resistant clones better adapted to the warm conditions of the lowland tropics in each selection cycle. More recently, advanced germplasm from European and other long-day breeding programs was incorporated to improve resistance to *Potato leafroll virus* (PLRV), while enhancing earliness and market traits.

In the Central Potato Research Institute of India there has been interest in Tuberosum (female) × short-day Andigena hybrids in breeding for the sub-tropical plains where the potato crop is grown under short days (Gopal et al., 2000; Kumar

and Kang, 2006). A number of Indian potato cultivars have already been developed from Tuberosum × Andigena crosses, including 'Kufri Pukhraj,' 'Kufri Giriraj,' 'Kufri Chipsona II,' and 'Kufri Shailja' (Kumar et al., 2008).

5.6 TPS

Most potato cultivars are clonally (vegetatively) propagated through seed (daughter) tubers and are genetically uniform. There are, however, circumstances where cultivars based on current methods of true potato seed (TPS) propagation are an attractive proposition despite being genetically variable. Breeding cultivars for TPS was started at CIP in 1972 with the aim of high yields and acceptable uniformity. Methods and progress have been reviewed by Golmirzaie et al. (1994) and Ortiz (1997). TPS propagation appeared most attractive in the torrid zones of the lowland tropics and subtropics. Here the difficulty of establishing a TPS crop, later maturation, and less uniformity could be outweighed by three advantages (Golmirzaie et al., 1994). Seed costs are reduced due to the much smaller amounts of planting material required. Planting time is flexible because the farmer does not have to consider the physiological age and condition of seed tubers. Finally, tubers are free from tuber-borne diseases with the possible exception of the few caused by true seed-borne viruses. In practice TPS potatoes have been established in Bangladesh, China, Egypt, India, Indonesia, Nicaragua, Peru, Philippines, southern Italy, and Vietnam (Almekinders et al., 1996; Ortiz, 1997; Simmonds, 1997). They have proved extremely useful for food security, particularly for small-scale farmers (Chujoy and Cabello, 2009). Chilver et al. (2005) have recently reviewed on-farm profitability and prospects for TPS and concluded that widespread geographic adoption is unlikely in the immediate future, but that investment in a small but sustained TPS breeding effort can be justified in both China and India.

5.7 Somaclonal Variation and Mutation Breeding

Somaclonal variation can occur when plant regeneration and multiplication involves tissue culture, particularly when there is a callus phase. After such variation was first reported in protoclones of cultivar Russet Burbank (Shepard et al., 1980), it was investigated as a breeding method in its own right. Desirable improvements on parent cultivars were reported and assessed under field conditions, but most somaclonal variations comprised undesirable changes that did not merit further breeding effort (Kumar, 1994; Veilleux, 2005). Hence today somaclonal variation tends to be viewed as a source of undesirable variants that need to be screened out of breeding programs that involve plant regeneration.

Likewise, although natural mutations are the ultimate source of all genetic variations, the potato is not ideal for deliberate mutation breeding because it is a clonally

propagated tetraploid crop. Nevertheless, limited success has been achieved, for example, selecting gamma-ray mutants of the cultivar Lemhi Russet for improved resistance to blackspot bruise and low-temperature sweetening (Love et al., 1993).

5.8 Genetic Transformation

Potato transformation using *Agrobacterium*-mediated systems was developed during the 1980s, first with *A. rhizogenes* (Ooms et al., 1986) and then more successfully with *A. tumefaciens* (Stiekema et al., 1988; Dale and Hampson, 1995). The gene of interest would be incorporated into the bacterial plasmid along with a promoter and selectable marker, such as resistance to an antibiotic or herbicide; the bacterium would be co-cultured with freshly cut tuber discs or leaf or internode explants of the potato; and regeneration of shoots with the selectable marker would take place in plant tissue culture in the presence of the selectable agent. Recently both Chang et al. (2002) and Morris et al. (2006) have been able to simultaneously co-transform potatoes with two genes using a single selectable marker. The transgene constructs were multiplied separately in *A. tumefaciens* clones, and cultures of the *Agrobacterium* were mixed and incubated with the potato internode explants. From 300 explants, Morris et al. (2006) generated 38 independent transformants of which four contained both transgenes. Clearly this increases the speed and efficiency of transformation as a breeding method and makes it a more attractive proposition. The choice of promoter is important for gene expression. The CaMV 35S promoter has frequently been used for constitutive gene expression, but others have been developed for higher constitutive expression and for leaf or tuber expression (Douche and Grafius, 2005).

Monsanto was the first to commercialize GM potatoes in North America from 1995. The GM cultivars were Russet Burbank, Atlantic, Snowden, and Superior with a *Bt* gene for pest resistance from the bacterium *Bacillus thuringiensis.* Subsequently virus resistance was added (Davies, 2002). Trait stability was demonstrated in field trials across a number of years, as was the greatly reduced use of pesticides (Duncan et al., 2002). But leading processing and fast food outlet companies in North America were reluctant to purchase GM potatoes, because of consumer concerns over GM potato products, and Monsanto stopped marketing GM potatoes in 2001. There has also been resistance to GM potatoes in the European Union. The first GM potato for non-food use to be cultivated in Europe is likely to be Amflora, a pure amylopectin starch potato developed by BASF Plant Science by switching off the gene for the granule-bound starch synthase (GBSS), the key enzyme for the synthesis of amylose. However, in 2009 there were still delays in its approval for commercial production because of concerns over the antibiotic resistance marker which it contains. The first GM potatoes for human consumption in Europe and North America are likely to come from recent advances in producing marker-free transformants and from new cisgenic and intragenic approaches (see Section 7.5).

6 Current Goals of Breeding

6.1 Asia, Africa, and Latin America

In Asia, Africa, and Latin America there is a need for increased potato production to meet increasing demands for food from human population growth and thus achieve food security. Both China and India are planning large increases in production by increasing the area under cultivation, with India also projecting increases in yield (Anderson, 2009). Interestingly, however, if China could markedly increase its area of potatoes planted with healthy seed from the current 20%, and effectively control late blight, bacterial wilt, and viruses, the yield per hectare would be expected to double (Anderson, 2009). This brings home the point that new cultivars with inbuilt resistance to these and other diseases and pests are highly desirable. Furthermore, selection for higher and stable yields would also be worthwhile but should take place in environments with appropriate inputs of fertilizers and water, otherwise genotype × environment interactions might prevent yield increases being realized in farmers' fields. Expansion of potato growing into a wider range of environments might require adaptation to changes in the growing season (planting time and length of season) and resistance to abiotic stresses could become important. These include drought, heat, cold, mineral deficiency, and salinity, with water stress being the most important one that affects potato production in most areas of the world. If intercropping increases in warm climates, then shade crops (e.g., sugarcane in India) could help to reduce heat stress in potatoes, but heat-tolerant cultivars are still desirable.

Although the potato is a wholesome food, further improvements in its nutritional and health properties are worth considering. There is current interest in improving the health of poor people by breeding staple foods that are rich in micronutrients (biofortification). CIP has already found accessions in its germplasm collections with higher contents of iron and zinc (Burgos et al., 2007). In vitro assays have also shown variation among potato genotypes in the bioavailability of Fe, and that in particular, the consumption of yellow-fleshed potatoes, which are generally higher in carotenoid contents than white or cream-fleshed potatoes, may enhance the bioavailability of Fe from other sources in the diet. While heritability of carotenoid concentrations in potato has not been determined, the heritabilities of Fe concentration and vitamin C, which also enhances bioavailability, are moderately high, indicating that breeding for increased bioavailable Fe in potato is feasible.

Furthermore, higher beta-carotene (vitamin A) content is now a realistic target for potato given recent success in modifying carotenoid biosynthesis through genetic transformation (Ducreux et al., 2005), and increased protein content and better amino acid balance have been achieved through transformation with a non-allergenic seed albumin gene (*AmA1*) from *Amaranthus hypochondriacus* (Chakraborty et al., 2000). Finally, as mentioned in the Introduction, processors are building factories in countries where the potato is primarily grown as a staple food, and this is a trend that is likely to continue. In these circumstances there is going to be a need for new locally adapted cultivars that meet the stringent requirements

of processors. Appropriate tuber morphology and texture, adequate solids, and low reducing sugar content are important as well as freedom from mechanical damage, bruising, and internal defects.

6.2 Europe, North America, and Oceania

In Europe, North America, and Oceania, food security has been achieved and a number of countries have yields in excess of 40 tonnes/ha. The emphasis is on trying to increase potato usage in an economically and environmentally sustainable way. New cultivars are required which give more yield of saleable product at less cost of production, whether the potatoes are for processing or table use. However, in the European Union, for example, there is political pressure for reduced use of pesticides and fungicides and better use of water and fertilizers, both nitrogen and phosphate, all resources required to achieve high potato yields and quality. Hence, new cultivars are required to meet these objectives together with the quality ones demanded by processors and supermarkets. Finally, there is a need for new cultivars to meet consumer demands for convenience foods and novel products, preferably with improved texture and flavor and health-promoting attributes. Processors are currently under pressure to reduce acrylamide formation in crisps (chips) and French fries because of concerns about its effects on human health (Amrein et al., 2003; Pedreschi, 2009; Pinhero et al., 2009). As acrylamide is formed in processed potato products by Maillard browning reactions between reducing sugars and the amino acid asparagine, lower levels of these compounds are obvious targets for conventional breeding and genetic engineering. Cultivars with a low GI could be of value in lowering the glycemic load of the western diet, thus decreasing the risk of type-2 diabetes, cardiovascular disease, and obesity (Storey, 2007). For example, following cooking, a portion of high amylose starch recrystallizes to form so-called resistant starch, which acts as a form of dietary fiber (Karlsson et al., 2007). The introduction of inulin to potato is another way of improving its nutritional value by reducing its energy density through increased dietary fiber. Hellwege et al. (2000) have developed transgenic potato tubers that synthesize the full range of inulin molecules naturally occurring in globe artichoke (*Cynara scolymus*).

In countries with a starch industry there are considerable opportunities to breed or genetically engineer potatoes with novel starches for use in food and non-food products. Potato starch has numerous useful functional properties such as thickening, coating, gelling, adhesion, and encapsulation. Some of these functionalities are unique to the polymer as a result of the structure and organization of its linear amylose (17–25% of starch) and highly branched amylopectin (Li et al., 2006). Genetic engineering has already generated the two extreme types of potato starch. High amylopectin starch was produced by the down-regulation of the granule-bound starch synthase gene that controls amylose synthesis (Visser et al., 1991) and high amylose starch by concurrently down-regulating two starch branching enzymes, A and B (Schwall et al., 2000).

6.3 Climate Change

Haverkort and Verhagen (2008) reviewed the likely consequences of climate change on potato production based on the International Panel on Climate Change report (IPCC, 2007). Climate change will bring a rise in temperature, an increase in carbon dioxide concentration in the air, and an altered precipitation pattern. In parts of the world such as the Mediterranean and Sahelian Africa, potato yields will go down as the suitable heat-free period of the year for production becomes shorter. In these areas breeding for heat tolerance will be important because of the adverse effect of high temperatures on tuberization. Furthermore, with a higher evaporative demand, water will be used less efficiently and breeding for drought tolerance will also be important. In contrast, potato yields in temperate climates may increase due to higher carbon dioxide concentrations in the air and a longer growing season. In northern Europe, there will be a decreasing number of days with frost, more rain in winter and less in summer, with more erratic but heavier rain storms. Water availability for irrigation will be important to maintain yields and quality.

Throughout the world then, there is a drive for cultivars that make better use of water, and either avoid drought (faster tuber bulking) or are tolerant of drought. Not surprisingly, there is increased research on root architecture (Iwama, 2008), looking at the effects of variation on water use and the correlation between field and glasshouse screens. Furthermore, effects of root architecture on fertilizer use cannot be ignored. Potatoes need large inputs of phosphate fertilizer, compared with other crops, and in Europe, for example, most potato growing is in nitrate vulnerable zones.

Climate change is also likely to bring new challenges in reducing losses from pests and diseases. Higher temperatures and longer growing seasons are likely to alter the geographic ranges of pests and diseases and the pressure they exert on potato production (Haverkort and Verhagen (2008)). As a consequence, breeders may need to alter some of their priorities.

6.4 TPS

As mentioned earlier, in the torrid zones of the lowland tropics and subtropics, cultivars based on current methods of true potato seed (TPS) propagation are an attractive proposition. The aim is still high yield and acceptable uniformity. If they could be produced, apomictic potatoes would be a solution to the uniformity problem. Furthermore, synthetic seed is an alternative to TPS that would avoid the need to develop parents that generate uniform hybrids. Hence there is currently renewed interest in somatic embryogenesis in potato, and sufficient progress has been made for serious consideration to be given to exploiting synthetic potato seed, as seen in the review by Veilleux (2005).

6.5 Breeding Objectives and Selection Criteria

Breeding objectives will vary from country to country, but all programs are likely to involve selection for higher yield, appropriate maturity and dormancy, tuber characteristics that affect quality and suitability for particular end uses, and resistance to abiotic and biotic stresses. To be of practical benefit, however, objectives need to be translated into the improvements required over existing cultivars and into selection criteria that can be used by breeders. The objectives and criteria that follow are all relevant to current breeding goals, but priorities will need to be assigned in any particular program as progress is most noticeable where the focus is on few rather than many traits. More details on each trait and its inheritance can be found in the review by Bradshaw (2007b). Not included are physiological and morphological parameters of crop growth, root architecture, and water and fertilizer use efficiency, but these could well become integrated into future breeding programs.

6.5.1 Yield, Dry Matter Content, Maturity, and Dormancy

- High fresh-weight yield
- Stability of yield across target environments and years
- Tuber number and size appropriate for end use
- Dry matter (specific gravity) and starch content appropriate for end use
- Maturity appropriate for growing season
- Dormancy appropriate for length of storage

6.5.2 Tuber Shape and Defects

- Regular shape and shallow eyes to reduce wastage
- Round shape for crisps (chips) and long oval for French fries
- Lack of external defects (growth cracks, mechanical damage and bruising, and greening)
- Lack of internal defects (hollow heart, brown center, and internal rust spot)

6.5.3 Nutritional and Health Value, Pigmentation, and Glycoalkaloids

- Higher contents of antioxidant pigments: carotenoids (yellow flesh) and anthocyanins (red and purple flesh)
- Increased vitamin C content
- Increased micronutrient content (biofortification)
- Glycoalkaloid content below 20 mg/100 g fresh weight
- Reduced potential for acrylamide production in roasted and fried products (lower reducing sugars and asparagine)

6.5.4 Cooking and Processing Quality

Improved flavor (aroma and taste, volatile and non-volatile compounds)
Appropriate texture (ranges from waxy to floury (or mealy), dry matter and starch content, and even distribution for processing)
Freedom from after-cooking blackening and enzymic browning
Light-colored fry products post-harvest and after storage (lower reducing sugars)
Novel starches for countries with a starch industry (altered ratio of amylopectin to amylose)

6.5.5 Resistance to Abiotic Stresses

Resistance to water stress (drought avoidance, drought tolerance, and water-use efficiency)
Heat tolerance
Cold tolerance
Salinity tolerance
Mineral deficiency tolerance

6.5.6 Resistance to Major Pests

Potato tuber moth (PTM, *Phthorimaea operculella* Zeller): larvae mine foliage, stems and tubers in field and storage, most serious insect pest worldwide, particularly in warm tropical and sub-tropical climates.
Colorado potato beetle (CPB, *Leptinotarsa decemlineata* Say): can defoliate crop in field, major pest in northern latitudes, particularly in North America, Europe, Russia, and Central Asia.
Andean potato weevil (APW, *Premnotrypes* spp.), green peach aphid (GPA, *Myzus persicae*, primarily as virus vector), and leafminor flies (LMF, *Liriomyza huidobrensis*): major pests in many developing countries.
Cyst nematodes (*G. rostochiensis* Woll. and *G. pallida* Stone): penetrate and feed on roots, most damaging nematodes worldwide.
Root-knot nematodes (*Meloidogyne* spp.): the southern root-knot nematode (SRN, *M. incognita*) is the main problem in warm climates, whereas the Columbia root-knot nematode (CRN, *M. chitwoodi*) and the northern root-knot nematode (NRN, *M. hapla*) are the main ones in temperate regions, with *M. chitwoodi* and *M. fallax* emerging problems in Europe.

6.5.7 Resistance to Major Diseases

Oomycete Disease of Foliage, Stem, and Tubers

Late blight (*P. infestans* (Mont.) de Bary): the most serious disease of potato worldwide.

Fungal Diseases of Foliage, Stem, and Tubers in Warm Climates

Verticillium wilt (*Verticillium dahliae* Kelb.): most common soil-borne disease
Early blight (*Alternaria solani* (Ellis and Martin) Jones and Grout): most common air-borne disease
Fusarium wilt (*Fusarium solani* (Mart) App. and Wr. var. *eumartii* (Carp.) Wr. and *F. oxysporum* Schlecht.): soil-borne disease

Fungal Diseases of Tubers

Wart (*Synchytrium endobioticum* (Schilb.) Perc): persistent soil-borne, disfiguring, blemish-forming disease of tubers
Powdery scab (*Spongospora subterranea* (Wallr.) Lagerh): persistent soil-borne, disfiguring, blemish-forming disease of tubers
Dry rot (*Fusarium coeruleum* (Lib.) ex Sacc.), *F. sulphureum* (Schlecht.), *F. sambucinum* (Fuckel), and *F. avenaceum* Sacc. (Corda ex. Fr.): tuber-borne disease, important cause of spoilage of tubers in storage in warmer climates
Gangrene (*Phoma foveata* Foister): tuber-borne disease, was important cause of spoilage of tubers in storage in northern Europe
Black scurf (*Rhizoctonia solani* (Kühn.) and stem canker): tuber-borne blemish disease
Skinspot (*Polyscytalum pustulans* (Owen and Wakef.) M.B. Ellis): tuber-borne blemish disease
Silver scurf (*Helminthosporium solani* Dur. and Mont.): tuber-borne blemish disease
Black dot (*Colletotrichum coccodes* (Wallr.) Hughes): tuber-borne blemish disease

Actinomycete Disease of Tubers

Common scab (*Streptomyces scabies* (Thaxt.) Waksman and Henrici): persistent soil-borne, disfiguring, blemish-forming disease of tubers

Bacterial Diseases of Foliage, Stem, and Tubers

Bacterial wilt or brown rot (*Ralstonia solanacearum* Smith): the most serious disease of potato after late blight in the developing world.
Blackleg (stem) and soft rot (tuber in storage) (*Pectobacterium* spp.): *P. carotovorum* subsp. *atrosepticum* is common in temperate climates whereas *P. chrysanthemi* predominates in warmer areas and *P. carotovorum* subsp. *carotovorum* is well adapted to both climatic regions.
Bacterial ring rot (*Clavibater michiganensis* subsp. *sepedonicus* (Spieck and Kotth.) Davis et al.): a recurring seed-tuber-transmitted disease problem in temperate regions, despite many countries treating it as a quarantine disease and having a zero-tolerance policy for the import of seed potatoes.

Viral Diseases

PLRV (*Potato leafroll virus*): the most damaging and widespread of the viruses, aphid transmitted in a persistent manner

PVY (*Potato virus Y*): next in importance, aphid transmitted in a nonpersistent manner and hence harder to control with aphicides

PVA (*Potato virus A*): worldwide distribution

PVX (*Potato virus X*): worldwide distribution

PVM (*Potato virus M*): can be a devastating virus in the seed and ware potato production areas of Central and Eastern Europe

PVS (*Potato virus S*): worldwide distribution

TRV (*Tobacco rattle virus*): locally important in cooler climates, transmitted by Trichodorid nematodes, causes spraing symptoms in tubers, a particular problem for processors

PMTV (*Potato mop-top virus*): locally important in light sandy soils, transmitted by *Spongospora subterranea*, causes spraing symptoms in tubers, a particular problem for processors

PSTVd (*Potato tuber-spindle viroid*): a true seed-transmitted disease which is treated as a quarantine disease in countries where it is not endemic

7 Breeding Methods and Techniques

7.1 Breeding Cultivars at the Tetraploid Level for Clonal Propagation

7.1.1 Parents and Crossing

Potato breeding worldwide has traditionally involved making crosses between pairs of parents with complementary phenotypic features and this is still the main route to new cultivars. Increasingly parents will have genes introgressed from wild species and they may also be from complementary groups of germplasm to exploit yield heterosis (Bradshaw, 2009b). The aim is to generate genetic variation on which to practice phenotypic selection across a number of vegetative generations for clones with as many desirable characteristics as possible for release as new cultivars. As genetic knowledge of the potato accumulates, it will become easier to choose parents known to possess desired major genes and large-effect QTLs and to select genotypically for these in their offspring. Major genes have already been mapped for flesh, skin, and flower color, for tuber shape and eye depth, and for resistances to late blight, cyst nematodes, root-knot nematodes, viruses (PVY, PVA, PVX, PVM, PVS, and PLRV), and wart (Bradshaw, 2007b; Jansky, 2009). Large-effect QTLs have also been mapped for total glycoalkaloid content (TGA), maturity and resistances to late blight, Verticillium wilt, cyst nematodes, and PLRV (Bradshaw, 2007b; Bae et al., 2008; Sorensen et al., 2008; Finkers-Tomczak et al., 2009). In contrast, many economically important traits are still best viewed as complex polygenic traits,

despite a number of QTLs being found, and these include dormancy, dry matter and starch content, fry color, resistance to *Pectobacterium* (*Erwinia*), tuberization, and yield. For these traits breeders will still have to rely primarily on phenotypic data and the concepts of quantitative genetics to determine crossing strategy. With a highly heritable trait like fry color the midparent value is a good predictor of the mean performance of the offspring and a few carefully chosen crosses can be made (Bradshaw et al., 2000a). In contrast, with only a moderately heritable trait, such as yield, offspring mean is less predictable and more crosses need to be made to ensure that they include the best possible ones.

Today most cultivars come from deliberate artificial hybridizations. The floral characteristics of potatoes and methods of artificial hybridization and self-pollination have been described by Plaisted (1980). Details can also be found in Caligari (1992), Douches and Jastrzebski (1993), and in the textbook on *Breeding Field Crops* by Sleper and Poehlman (2006). A temperature of 19°C and 16 h of daylength are recommended for crosses involving *S. tuberosum* group Tuberosum so that an extension of natural daylength is required for crossing in the tropics and subtropics. In contrast, group Andigena is in effect day-neutral for flowering, but not of course for tuberization. Not all desired crosses are successful as problems can arise from clones failing to flower, buds and flowers dropping either before or after fertilization, low pollen production, and failure to produce viable pollen (male sterility). Breeders usually encourage flowering by the periodic removal of daughter tubers and sometimes graft young potato shoots onto tomato or other compatible *Solanaceous* plants. Stems with flowers attached can also be cut and placed in jars of water with an anti-bacterial agent to reduce contamination (Peloquin and Hougas, 1959).

7.1.2 Clonal Generations

Potato breeding at SCRI before 1982 was typical of most relatively large programs, both then and now (Mackay, 2005; Bradshaw, 2007a, 2009a). The seeding generation (SG) in the glasshouse comprised 100,000 genetically unique seedlings from some 200–300 crosses. Visual selection reduced this number of potential cultivars to 1,000 clones in replicated yield trials at a ware site in the third clonal generation (TCG). The first clonal generation (FCG) comprised 50,000 spaced plants at a high-grade seed site with a short growing season, rather atypical of normal ware production. The second clonal generation (SCG) of 4,000 unreplicated three- or four-plant plots was also grown at the seed site. Decreasing numbers (1,000, 500, and 200) of potential cultivars were then assessed for 3 years in replicated yield trials at a local ware site before the most promising ones (60, 10, and 5) underwent 3 years of more extensive testing at a number of sites, including overseas ones. One or a few clones would then be entered into 2 years of official statutory trials (National List Trials) and registered for Plant Breeders' Rights. Multiplication from disease-free stock would start with a view to commercialization. During these intermediate and final stages of selection the production of seed tubers was separated from the trials that were grown under ware conditions, designed as far as possible to resemble

Fig. 1.4 Early generations (spaced plants and unreplicated small plots) of potato breeding program at seed site in Scotland (*top*) and replicated yield trials at ware site in Scotland (*bottom*) (Source: SCRI)

those of good commercial practice (Fig. 1.4). Clones undergoing selection were assessed for their yield and agronomic performance, external and internal defects, and cooking and processing characteristics, as described by Bradshaw et al. (1998, 2003). They were also assessed for their disease and pest resistance in special tests as described by Mackay (1987) and Bradshaw et al. (2000b). More recently the time from seedlings to cultivar has been reduced by 3 years by using progeny tests to discard whole progenies, by starting replicated trials earlier at more than one site and by using micropropagation to multiply promising clones for more extensive testing (Mackay, 2005). More detailed information on handling the intermediate and later generations, including the conduct of yield trials and selection for disease and pest resistance, can be found in the review by Bradshaw (2007b).

Research in the 1980s found that intense early-generation visual selection for most quantitative traits was very ineffective, particularly between seedlings in a glasshouse and spaced plants at a seed site (Bradshaw and Mackay, 1994; Bradshaw et al., 1998). Selection for tuber skin and flesh color and shape can, however, be done if these are important for particular consumers. The solution to ineffective

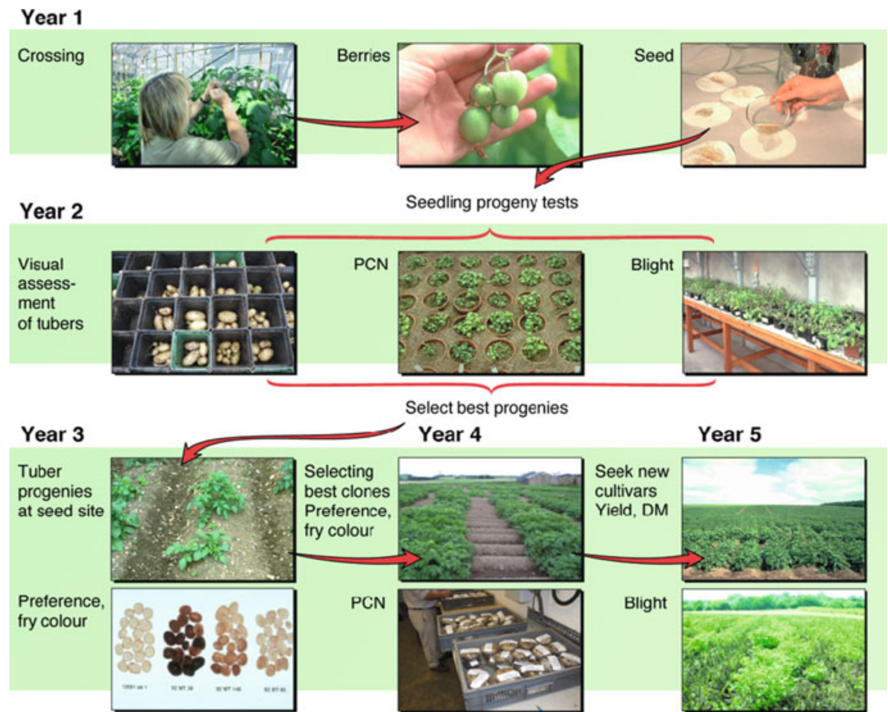

Fig. 1.5 Potato breeding at SCRI: progeny tests used to discard whole progenies (= full-sib families) in years 2 and 3, followed by clonal selection within best families in year 4 (unreplicated small plots at seed site) and in year 5 (yield trials at ware site), followed by further clonal selection and next round of crossing and selecting (Source: SCRI)

early-generation visual selection developed and implemented at SCRI was the use of progeny tests to discard whole progenies (= full-sib families) before starting conventional within-progeny selection at the unreplicated small-plot stage (Bradshaw and Mackay, 1994; Bradshaw, 2007a, 2009a). Today at SCRI, once promising clones have been identified after both the first and the second year of ware (yield) trials, they are used as parents in the next round of crossing and selecting to keep the momentum of the program going (Fig. 1.5).

7.2 *Introgression*

Introgression of genes from wild and cultivated species was introduced in Section 5.3. Introgression is essentially backcrossing, and in the past it took from three to seven backcrosses to transfer a major dominant resistance gene into a successful cultivar (Ross, 1986). Molecular marker-assisted introgression offers the possibility of faster progress than can be achieved by traditional backcrossing (Hermsen,

1994; Barone, 2004; Iovene et al., 2004). This is because one can select genotypically against the unadapted species genome as well as genotypically for the desired gene(s). With adequate molecular marker coverage of all 12 potato chromosomes, it is possible to estimate the optimal combination of population sizes and number of backcross generations and to select in a very precise way for the desired products of meiosis in each backcross generation (Hospital, 2003). But one is still dependent on the occurrence of favorable intra-chromosomal recombination and linkage drag (retaining undesirable genes through linkage to selected gene(s)) can be a problem. The recurrent parent(s) will be from tetraploid group Tuberosum when the starting material is one of the following: somatic hybrids between tetraploid *S. tuberosum* and primitive diploid (1EBN) species such as *S. bulbocastanum*; autotetraploids produced by colchicine treatment of diploid (2EBN) species such as *S. vernei*; and hexaploid (4EBN) species such as *S. demissum.* In contrast, the recurrent parent(s) will be from diploid (dihaploid) group Tuberosum, diploid group Phureja, or diploid group Stenotomum when introgression is from a 2EBN wild species. A comparison of tetraploid and diploid introgression was made by Bradshaw and Ramsay (2005).

Where introgression is performed at the tetraploid level, the result may not be a genotype with 48 Tuberosum chromosomes, including one (or more) of which has the introgressed gene(s). This need not affect the performance of the genotype and its vegetative propagation but could affect its fertility and use as a parent for further breeding. The result of an introgression from *S. brevidens* was a high-yielding clone, C75–5+297, with resistances to both tuber soft rot and early blight (Tek et al., 2004). Molecular and cytogenetic analyses revealed that C75–5+297 had 47 chromosomes, including four copies of chromosome 8, three from potato and one from *S. brevidens* that was the only part of the wild species genome present. In contrast Barone et al. (2001) did obtain 48 chromosomes and evidence of recombination between *S. commersonii* (a 1EBN diploid) and *S. tuberosum* chromosomes in their molecular marker-assisted introgression of tuber soft-rot resistance.

As potatoes are heterozygous outbreeders, use of the same recurrent parent during introgression results in a self of the recurrent parent and hence inbreeding depression. This can be avoided by using different Tuberosum parents for each backcross, but results in an entirely new cultivar, which may or may not be the desired outcome. The only way to introduce a gene into a known cultivar is by genetic transformation, which is discussed in a later section. It also has the advantage of no linkage drag and hence should be cleaner and faster.

7.3 Base Broadening and Population Improvement

Broadening the genetic base of modern breeding programs was considered in Section 5.5. All of the schemes considered were in essence population improvement for a number of generations (recurrent selection) to provide tetraploid or diploid parents suitable for crossing with adapted tetraploid parents (often cultivars) in the breeding of finished cultivars. Some of the schemes involved selection for

tuberization in long days. These were usually simple phenotypic mass selection schemes, but sometimes involved within family selection (Haynes and Lu, 2005). The rate of progress depends to a large extent on the length of each cycle. The Neotuberosum program of Simmonds (1969) operated on a 2-year cycle. In the first year large populations of seedlings were grown in the field and selected for high yields of tubers of acceptable sizes, shapes, and colors. In the second year the selected tubers were planted in an isolation site and open-pollinated berries harvested to provide the next generation of seedlings. In the USA Plaisted (1987) introduced the deliberate pollination of selected clones with bulk pollen to avoid inbreeding depression from selfing in his Neotuberosum program. He also achieved more vigorous plants for selection by raising his seedlings in pots to produce seed tubers for planting field trials. This enabled him to screen the seedlings in the glasshouse for resistance to late blight and viruses, but it also increased the length of each cycle to 3 years.

The diploid breeding program of Carroll (1982) relied on natural insect pollination and seed set and included both seedling and tuber populations with a minimum generation cycle of 2 years. In practice the generation time was 3 years once seedlings were raised in a polythene tunnel rather than the field. The self-incompatibility of the diploids was presumed to ensure cross-pollination. The diploid program of Haynes (Haynes and Lu, 2005) involved selection within 72 half-sib families and operated on a 2-year cycle for 10 generations, the first five to six for general adaptation followed by four to five for high specific gravity. The program was then continued on a 4-year cycle in which a seedling generation in the glasshouse was followed by 3 years of field evaluation in which the number of clones per family was reduced from 100 through 4 to 1. Open-pollinated seed was collected from the 288 clones evaluated in the second year in the field, but only used from the 72 clones selected after the third year in the field.

At SCRI, Bradshaw et al. (2009) have shown that full-sib family selection in a tetraploid breeding program can operate on a 3-year cycle with limited within family selection and on a 5- or 6-year cycle with more extensive within family selection. They recommended the 5- or 6-year cycle once genes have been combined from sufficient parents to achieve the program's objectives, in their case combining resistances to late blight and cyst nematodes with increased yield and acceptable fry color.

7.4 Breeding Cultivars for True Potato Seed

Breeding cultivars for TPS was started at CIP in 1972 with the aim of high yields and acceptable uniformity. None of the current breeding methods can deliver genetic uniformity and hence all of them involve selection for acceptable uniformity. Current TPS breeding aims to produce tetraploid cultivars, either from $4x \times 4x$ crosses in which heterosis is exploited between group Tuberosum and group Andigena (Simmonds, 1997) or from $4x \times 2x$ crosses in which the $2x$ parent produces a high frequency of $2n$ gametes by FDR so that 83% of its heterozygosity is

transmitted to the offspring (Golmirzaie et al., 1994; Clulow et al., 1995) or from $2x \times 2x$ crosses in which both parents produce $2n$ gametes, again with a very high frequency of $2n$ pollen produced by FDR (otherwise the offspring will contain more diploids than tetraploids) (Ortiz and Peloquin, 1991). Simmonds (1997) argued, however, that diploid TPS cultivars should not be ruled out for the future, and De Maine (1996) produced TPS families of long-day-adapted Phureja that appeared as uniform for tuber size and shape as selected clones. Open pollination of diploids will result in almost 100% outcrossing because of self-incompatibility. Cross-pollination between field plots of *S. phureja* has been estimated to decline from 5.1% at 10 m to 0.2% at 80 m (Schittenhelm and Hoekstra, 1995), figures which can be used to decide isolation distances for natural true-seed multiplication. Open pollination of tetraploids will normally result in varying amounts of self-pollination and inbreeding depression, which may not be outweighed by their apparent intrinsic yield advantage over diploids. Furthermore the seed fertility (seed yield) of diploids is greater than that of tetraploids. Whether or not diploid inbred lines and true F1 hybrids can be produced in the future for maximum heterosis and uniformity is a matter for further research. The inbreeding depression from selfing meant that hand-pollinated tetraploids were superior to open-pollinated ones, but their production was more expensive because of the cost of emasculation of the flowers of the female parent (Simmonds, 1997).

Today, the technology to produce hybrid TPS uses male sterility to minimize hybridization costs and mother plant management to increase seed production (Chujoy and Cabello, 2009). One hundred to 150 kg TPS can be produced from 1 ha. In large TPS seed stocks, dormancy is released by high temperature treatment for 4–6 months. TPS is sown in seedbeds to produce minitubers or to raise seedlings for transplanting to the field. The seedling transplants, or more successfully the minitubers, are then used to produce either seed tubers or potatoes for consumption. The seed tubers derived from TPS cultivars should ideally be selected for tuber traits each generation to ensure that the produce is within the desired yield and quality range.

7.5 Genetically Modified Potatoes

Genetic modification is the act of inserting one or more agriculturally important genes into the genome of a potato plant by in vitro techniques and by using modified *A. tumefaciens* as a natural gene transfer tool (Jacobsen, 2007). It thus allows targeted improvements of successful and widely grown potato cultivars. Today marker-free transformants can be produced, most easily by using transformation vectors without selection markers. PCR analysis of regenerated plants has shown relatively high percentages (5%) of transformation (Jacobsen, 2007). The products of transformation do require screening to select the best transformants for commercialization, including demonstration of substantial equivalence to the parent cultivar (Davies, 2002). Commercialization also involves the demonstration that it is safe both to grow and eat the genetically modified (GM) potatoes. While no GM potatoes are currently being grown commercially, prospects are good for the future provided skeptical consumers can be convinced of their value.

Today a distinction needs to be made between transgenic, intragenic, and cisgenic potatoes. Cisgenes are genes from cultivated potatoes and their cross-compatible wild relatives. A cisgene includes its native introns and is flanked by its native promoter and terminator in their normal sense orientation. These restrictions do not apply to intragenes. They are produced by isolating specific genetic elements from cultivated potatoes and their cross-compatible wild relatives, rearranging (recombining) them in vitro to create 'intragenic' DNA, and then inserting the resulting expression cassettes into a potato using plant-derived transfer DNAs (P-DNAs) and marker-free transformation. P-DNAs resemble *Agrobacterium* T-DNA borders that can be used to support DNA transfer from *Agrobacterium* to plant cells creating 'intragenic' plants (Rommens et al., 2007). Finally, transgenes are ones derived from other organisms (e.g., bacteria and fungi) or other plant species which are not cross-compatible with cultivated potatoes.

7.5.1 Genes from Cultivated Potatoes and Their Cross-Compatible Wild Relatives

Desirable (dominant) genes can be found in cultivated potatoes and their cross-compatible wild relatives, cloned, and introduced by genetic transformation. Gene isolation in potato can be done via map-based (i.e., position-based) cloning using BAC technology and dense AFLP genetic maps, but it is time consuming (De Jong, 2005). Sometimes shortcuts can be taken by using a candidate gene approach or allele-mining, but their success is not guaranteed. However, gene isolation should be much faster now that the potato genome has been sequenced (see Section 8.1). Past examples are the molecular cloning of natural resistance genes and their transfer into well-adapted but susceptible cultivars (see book chapter by Simko et al., 2007). Currently there is a major research effort in the Netherlands (DuRPh Program) using cisgenic modification of potatoes in a quest for durable resistance to late blight (Haverkort et al., 2009). In 2009, for the first time, they trialled potatoes in which they had stacked two resistance genes (R-genes) by marker-free cisgenesis.

7.5.2 Gene Silencing

Where the control of biochemical pathways of interest is understood, down-regulation of gene expression using gene silencing has proved useful for achieving some desired modifications, in particular to starch composition, processing traits, and other quality traits (see review by Bradshaw, 2007b). The gene silencing has been achieved by RNA interference in the broadest sense (i.e., post-transcriptional gene silencing) and mimics recessive loss of function mutations (Jacobsen, 2007). For example, in antisense technology the potato plant is induced to produce large amounts of antisense RNA capable of trapping the messenger RNA from the gene being transcribed in an untranslatable RNA duplex. However, of particular current interest are the intragenic potatoes which are being trialled from the Simplot Program in the USA in which three genes are silenced. They combine reduced black spot bruise (polyphenol oxidase (*Ppo*) gene) with lower reducing sugars out

of cold storage, reduced amounts of processing-induced acrylamide, and increased starch levels (starch degradation-associated *R1* and phosphorylase-L (*PhL*) genes) (Rommens et al., 2006). The Simplot Program has also been able to achieve low-acrylamide French fries and potato chips (crisps) by tuber-specific silencing of two asparagine synthase genes (Rommens et al., 2008). Glasshouse-grown tubers of the transformed intragenic plants contained up to 20-fold reduced levels of free asparagine and both their French fries and potato chips accumulated as little as 5% of the acrylamide present in the controls. Tuber yield, shape, and sensory characteristics were not affected.

7.5.3 Novel Traits

Finally, and more controversially, the use of exotic sources of genes in transformation has the clear value of permitting the introduction of traits not found in cross-compatible relatives and hence of novel function. The genes used to date have mainly been ones that code for proteins that are toxic to pests and pathogens, ones whose expression interferes with virus multiplication in host cells, and ones that code for key enzymes in biochemical pathways in other organisms, often, but not always other plant species. Examples can be found in the book chapter by Bradshaw (2007b), but typical ones can be briefly summarized as follows.

Resistance to the Colorado potato beetle (Duncan et al., 2002) and the potato tuber moth (Mohammed et al., 2000; Davidson et al., 2004; Douche and Grafius, 2005) has been achieved with genes that encode proteins from the bacterium *B. thuringiensis*; to the potato cyst nematodes with genes that encode cysteine proteinase inhibitors (cystatins) (Urwin et al., 2003); to viruses PLRV and PVY with replicase and coat protein genes, respectively, from these viruses (Duncan et al., 2002); and to blackleg and soft rot with a gene encoding a chicken lysozyme enzyme (Serrano et al., 2000). Expression of a gene for a derivative of the antimicrobial peptide dermaseptin B1, from the arboreal frog *Phyllomedusa bicolor*, has been shown to increase resistance to diseases, such as late blight, dry rot, and pink rot, and to markedly extend the shelf life of tubers (Osusky et al., 2005). Protein content has been increased and amino acid balance improved by expression of a non-allergenic seed albumin gene (*AmA1*) from *A. hypochondriacus* (Chakraborty et al., 2000), and carbohydrate composition has been improved by inulin production from the expression of fructosyltransferases from globe artichoke (*C. scolymus*) (Hellwege et al., 2000). The primary flavor compound methional has been enhanced by increasing the level of soluble methionine (Di et al., 2003). Carotenoid content has been improved, including the production of beta-carotene from expressing an *Erwinia uredovora crtB* gene encoding phytoene synthase (Ducreux et al., 2005) and the production of astaxanthin from expressing an algal (*Haematococcus pluvialis*) *bkt1* gene encoding beta-carotene ketolase (Morris et al., 2006). The conversion of sucrose to glucose and fructose, and hence cold sweetening, has been minimized by expressing a putative vacuolar invertase inhibitor protein from tobacco (Greiner et al., 1999).

8 Integration of New Biotechnologies in Breeding Programs

We have just considered progress in the production of genetically modified potatoes. Earlier we saw how potato breeders can use somatic (protoplast) fusion to achieve difficult or impossible sexual hybridizations, androgenesis (anther culture) for haploidization, and embryo rescue to secure a hybrid where embryo abortion is due to a defective endosperm. We also saw that today somaclonal variation tends to be viewed as a source of undesirable variants that need to be screened out of breeding programs that involve plant regeneration and that the potato is not ideal for deliberate mutation breeding because it is a clonally propagated tetraploid crop (it is easier to achieve loss of function by gene silencing). In contrast, somatic embryogenesis holds promise as an alternative to TPS. In the next section we shall see how micropropagation has become a key aspect of the production of clean seed tubers for planting material. In this section we need to consider the impact of advances in genetics for gene discovery and marker-assisted selection.

8.1 Gene Discovery (Linkage Maps, Sequencing, and Microarrays)

During the last 20 years, at least a dozen linkage maps have been constructed with DNA-based markers, mostly in experimental populations of diploid potato (Celebi-Toprak et al., 2005; Gebhardt, 2007a, b). Many qualitative and quantitative traits have been mapped onto potato chromosomes. Knowing their map positions was instrumental for cloning the major genes mentioned earlier, identifying candidate genes underlying quantitative traits, and developing diagnostic markers for use in marker-assisted selection, which is considered in the next section. Diagnostic markers are also being developed through association mapping and population genetics in tetraploid varieties and breeding lines (Gebhardt, 2007a).

The first draft DNA sequence of the potato genome (840 Mb) was published on September 23, 2009 (http://www.potatogenome.net). In fact two potato ‘varieties’ were sequenced, RH, a diploid, heterozygous potato variety and DM, a doubled monoploid. The initial choice of the Dutch-led sequencing consortium was RH. A potato genomic bacterial artificial chromosome (BAC) library of 78,000 clones was fingerprinted and aligned into about 7,000 physical map contigs. The BAC ends were sequenced and approximately 30,000 BACs were anchored to the ultra-high density genetic map of potato, composed of 10,000 unique AFLP™ markers. A BAC by BAC sequencing strategy was then adopted but overall progress was slow. The heterozygosity of RH limited the progress of physical mapping and was expected to complicate assembly of genome sequence. Hence whole genome shotgun sequencing of DM was undertaken as it was expected to eliminate the complexity in assembly. The consortium is now working on high-quality drafts of both genomes, the results of which should aid gene discovery and the development of molecular breeding methods.

Progress is also being made in understanding gene expression and how gene function at the biochemical level relates to observed phenotypes. Where genetic

and biochemical information is available on metabolic pathways, such as those for carbohydrates, anthocyanins, and carotenoids in potatoes, candidate genes are being postulated and sought for improving both qualitative and quantitative traits. For example, Zhang et al. (2009) have confirmed that the potato *R* locus codes for dihydroflavonol 4-reductase and that a specific allele of the *dfr* gene is required for the production of red pelargonidin-based anthocyanin pigments. Furthermore, advances in metabolite profiling are allowing a systems approach to understanding biochemical pathways and hence the key genes to target for desired phenotypic (trait) changes. By 2006 there were about 220,000 EST (expressed sequence tags) sequences for potatoes in the public database GenBank (http://www.ncbi.nlm.nih.gov) and these can be regarded as a catalogue of partially sequenced genes that are expressed in target potato cells and organs of interest for crop improvement. The value of ESTs for gene discovery, identifying candidate genes and developing markers, will increase as they are located on the genetic and physical maps of potato, and as the extent of colinearity of the different Solanaceae genomes is established. Colinearity can be exploited in comparative genomics where known gene position and function in one genome are used to make inferences to other genomes (e.g., from tomato to potato, pepper, and eggplant). The availability of considerable quantities of EST data facilitated the development of microarrays for global gene expression studies in which different genotypes (e.g., cultivars and breeding clones) can be compared in different environmental situations, including the presence of pests and diseases. The Potato Oligo Chip Initiative (POCI) of Wageningen University in the Netherlands uses the Agilent '44 K feature platform' system. Their microarrays contain at least 75% of the potato transcriptome (messenger RNAs = expressed genes) and they should allow a more complete analysis of gene expression than has previously been possible.

8.2 Marker-Assisted Selection

As knowledge increases about the number and chromosomal locations of genes affecting important traits, breeders should be able to design better breeding programs. The advantages of molecular marker-assisted introgression were considered in the previous section. We now need to consider a tetraploid program where breeders will be able to choose parents that complement one another for desirable genes and then select for these genes in the offspring. Breeders will be able to determine the seedling population size required for certainty of finding the desired combination of genes or, more realistically, the number of cycles of crossing and selection required before this is achievable in practice in the size of population that they can handle. A big impact on the efficiency and rate of progress would be the identification of superior clones with the desired combination of genes as seedlings in the glasshouse and the use of modern methods of rapid multiplication to progress them to commercialization. This will require molecular marker-assisted selection (MAS) or preferably direct recognition of the desired genes, as has recently been

achieved for the *RB* gene for late blight resistance from *S. bulbocastanum* (Colton et al., 2006). Progress to date has been slow but is expected to increase.

Diagnostic markers are ones that have a very high probability of detecting the desired gene and hence are of value to breeders. Examples of ones for major genes for disease resistance are as follows. Kasai et al. (2000) developed sequence characterized amplified region (SCAR) markers to the *Ry* gene (from group Andigena, on chromosome 11) for extreme resistance to PVY. Bakker et al. (2004) identified an AFLP marker (EM1) that co-segregates with the *H1* gene for resistance to *G. rostochiensis* pathotype Ro1 and recommended its conversion to a cleaved amplified polymorphic sequence (CAPS) marker for use in marker-assisted selection because such markers are cheaper and easier to handle. Colton et al. (2006) developed a polymerase chain reaction (PCR)-based DNA marker for tracking the *RB* gene for resistance to late blight. Likewise, Sliwka et al. (2006) developed a PCR-based DNA marker for the *Rpi-phu1* gene (from group Phureja) for resistance to late blight. Finally, Gebhardt et al. (2006) demonstrated the use of diagnostic markers for *Rx1* (extreme resistance to PVX), *Sen1* (resistance to *S. endobioticum* pathotype 1, the cause of potato wart), and *Gro1* (resistance to all known pathotypes of *G. rostochiensis*), as well as *Ry*, for pyramiding these major genes for pathogen resistance in potato. The pyramiding of such major genes, together with QTL alleles of large effect for disease resistance, is likely to be the first main use of marker-assisted selection because of the removal of the need for costly, complex, and sometimes inaccurate disease testing. One of the sub-projects in the current EU Framework 6 Integrated Project BIOEXPLOIT is developing marker-assisted breeding for disease resistance in potatoes. Two examples of such MAS are the APPACALE program in Burgos, Spain and the Zamarte Breeding Company program in Kamien Kraj, Poland (Carrasco et al., 2009). Furthermore, the breeding programs at the Crops Research Centre, Oak Park, Ireland and the SCRI, Dundee, Scotland plan to use a diagnostic marker for a QTL of large effect ($GpaIV^{s}{}_{adg}$) for resistance to *G. pallida* Pa2/3 (Moloney et al., 2010).

9 Seed Tuber Production

TPS seed production was dealt with in Section 7.4. Here we are going to consider asexual reproduction which allows a genetically unique seedling to be maintained, multiplied, and grown as a new cultivar. A useful account of the various multiplication procedures can be found in a special issue of Potato Research, The Canon of Potato Science (Struik et al., 2007). Potato yields and quality are certainly best when crops are planted with disease-free seed tubers in the correct physiological state. Experience around the world during the twentieth century showed that this is most likely to be achieved through statutory seed certification schemes operating in areas where potatoes are grown only for seed. Such areas will usually be geographically and climatically less favorable to the aphid vectors of viruses which cause systemic infection (Jeffries et al., 2006). A typical seed production scheme with certification is now described.

Seed production starts from pathogen-free microplants which are produced by the certifying authority (or under license) in sterile laboratory conditions. In Scotland, for example, SASA (http://www.sasa.gov.uk) is the certifying authority for the Seed Potato Classification Scheme (SPCS). Single-node cuttings from tuber sprouts, from tubers supplied by the breeder, can be taken to provide rooted plantlets for pathogen testing. The breeder's stock should have been grown to a high health status and officially inspected and approved. If pathogen (mainly virus) elimination is required, larger plants can be grown from the plantlets and exposed to a heat treatment or to chemotherapy (e.g., Virozole) prior to meristem-tip culture. Dissected portions of the meristematic region of a shoot tip are placed on a liquid nutrient medium for plant regeneration. Once the resulting, or original, plantlets have been confirmed to be pathogen free, they can be used for rapid multiplication by in vitro nodal cuttings under artificial, aseptic conditions in the laboratory. The healthy plant material is cut into individual stem portions, each with one axial bud and an attached leaf (single-node cutting), which are placed on agar media in jars. The axial bud grows into stems with numerous buds from which more single-node cuttings can be taken. Once sufficient have been produced, they are allowed (with the help of growth regulators) to develop into fully rooted in vitro plantlets (transplants). These are then used by licensed commercial growers to produce minitubers (pre-basic TC (tissue culture)) in a greenhouse or screenhouse with high health status. The transplants can be grown in soil where they are likely to produce from two to five minitubers per plant. However, many more (up to 40) uniform tubers can be achieved from frequent harvests when the transplants are grown in aeroponic, hydroponic, or nutrient film cultures. In aeroponic culture the 'below-ground' plant parts are suspended in air and intermittently misted with nutrient solution. In hydroponic culture the transplants are grown in static nutrient solution whereas the nutrient flows along the lower roots in nutrient film culture. The minitubers are then grown by officially approved commercial growers in the field to produce pre-basic (field grown) seed tubers for further field multiplication. Subsequent generations result in grades of basic seed and finally certified seed in the amount required by ware growers who produce potatoes for consumption. Most European countries plant whole seed tubers whereas cut tuber pieces are commonly used in African, American, and Asian countries. There are usually from four to six field generations.

In Scotland seed crops are officially inspected twice during the growing season and tubers must also be inspected for diseases and disorders and meet the required standards before they can receive the official label required for marketing. The land used for seed crops must be free from wart disease and also tested and confirmed free from cyst nematodes. The interval between potato crops in the rotation must be 7 years for pre-basic seed crops and 5 years for basic seed crops. Certified seed can only be sold for ware, it cannot be replanted in Scotland. It is common practice for the certifying authority to hold in vitro pathogen-free nuclear stocks of cultivars under multiplication and also to fingerprint cultivars with molecular markers for unique identification (http://www.sasa.gov.uk).

Two other types of asexual reproduction are worthy of mention. First, it is easy to take and root stem cuttings from potato plants and this can be a useful way to

rapidly multiply potential cultivars for more extensive trialling in a breeding program. Second, in vitro plantlets can be induced to produce microtubers in the axils of leaves of cuttings. These can be of value for germplasm conservation and for storage and exchange of germplasm, but perhaps are of most value in potato research.

Finally it is important to point out that strict quarantine procedures are required when potatoes are transferred from one country to another to prevent the introduction of diseases, particularly non-indigenous ones. Again advances with in vitro techniques are proving useful. Lang (2001) has described how CIP supplies its new cultivars to farmers in East Africa through a seed multiplication scheme that starts in Kenya with nodal cuttings being taken from virus-free sprouts. These are supplied in vitro from CIP headquarters in Lima, Peru to the Quarantine Station in Kenya.

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Chapter 2
Cassava

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1 Introduction

Cassava (*Manihot esculenta* Crantz) is the most important crop among the tropical root and tuber crops (Pujol et al., 2002; Meireles da Silva et al., 2003). Along with maize (*Zea mays* L.), sugarcane (*Saccharum* spp.), and rice (*Oryza sativa* L.), cassava is among the most important sources of energy in the diet of most tropical countries of the world.

Until four decades ago, with few exceptions, little scientific effort had been made to improve the crop in relation to the relevance of cassava for tropical and subtropical agriculture. However, with the creation of the International Institute of Tropical Agriculture (IITA) in Nigeria and the International Center of Tropical Agriculture (CIAT) in Colombia in the early 1970s, and rapid consolidation of several National Agriculture Research programs, a new era began for cassava with the implementation of successful breeding projects, modernization of cultural practices, and development of new processing methods (Cock, 1985; Jennings and Iglesias, 2002). National research centers in Brazil, Colombia, China, Cuba, India, Indonesia, Nigeria, Thailand, and Vietnam, among many other countries, have conducted successful research on cassava as well.

Currently cassava is a fundamental component in the diet of millions of people. Scott et al. (2000) estimated that for the year 1993, annual production of cassava was about 172.4 million tonnes, with a value of approximately US $ 9.31 billion. Between 1961–1963 and 1995–1997, cassava production increased at a rate of 2.35% per year (Scott et al., 2000), a trend comparable to that found in other crops, such as wheat (4.32%), potato (4.00%), maize (3.94%), yams (3.90%), rice (2.85%), and sweet potato (1.07%). Between 1994 and 2005, cassava productivity was expected to increase at 1.1% per year. In fact, worldwide productivity increased by about 18.4% in the last 10 years. Progress in increasing productivity,

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however, is not uniform. Vietnam doubled its productivity in a 10-year period from 8.5 tonnes/ha in 1997 and 1998 to 16.2 tonnes/ha in 2006 and 2007 (FAOSTAT). Thailand achieved remarkable progress in the last decade as well with average yields in 1997 and 1998 of 14.8 tonnes/ha up to 22.0 tonnes/ha in 2006 and 2007.

Cassava is a very rustic crop that grows well under marginal conditions where few other crops could survive. A large proportion of cassava varieties is drought tolerant, can produce in degraded soils, and is resistant to the most important diseases and pests. The crop is naturally tolerant to acidic soils and offers the convenient flexibility that it can be harvested when the farmers need it. Cassava is a perennial plant handled as annual. It does not have a pre-established development such as that of the cereals where the plants germinate, grow, flower, fill the grain, mature, and die. Cassava grows when conditions are favorable, and when they are not the plant drops its leaves and assumes dormancy until favorable conditions return. Farmers often abuse the advantages that the crop offers negating the minimum requirements for a sustainable and competitive production.

The most important commercial product of cassava is the storage root, full of starch. Roots are not tubers and, therefore, cannot be used for reproductive purpose. Cassava roots have a very short shelf life due to a process known as post-harvest physiological deterioration (PPD). PPD rapidly renders the roots unpalatable and unmarketable (Han et al., 2001; Reilly et al., 2003, 2007). Consequently, cassava roots need to be consumed or processed soon after harvesting (van Oirschot et al., 2000). The short shelf life of the roots severely limits the marketing options by increasing the likelihood of losses and the overall marketing costs. In addition, the access to urban markets and processing facilities is restricted to production sites that are relatively close to them. PPD begins with vascular streaking, which is a blue-black discoloration of the xylem parenchyma, followed by general discoloration of the storage parenchyma. Five to seven days later, microbial activity may cause further deterioration. The processes involved in PPD resemble typical changes associated with the plant's response to wounding and trigger a cascade of biochemical reactions, in which reactive oxygen species are central. Specific genes involved in PPD have been identified and characterized and their expressions evaluated (Reilly et al., 2001).

Stem cuttings (stakes) are the most common source of planting material and are used for the commercial propagation of the crop. Cassava foliage is not widely exploited in spite of its high nutritive value, although consumption of leaves by human populations is relatively common in certain countries of Africa and Asia. Foliage is also used for animal feeding. Crude protein content in leaves typically ranges from 20 to 25% of dry weight (Gomez et al., 1983; Buitrago, 1990; Babu and Chatterjee, 1999), but levels as high as 30% have been identified (Buitrago, 1990). Exploitation of foliage in cassava is expected to increase because of the recent developments and testing of mechanical harvesters and alternative cultural practices to exploit it (Cadavid Lopez and Gil Llanos, 2003). Relevant traits for most cassava breeding projects include high and stable production of fresh roots and adequate levels of dry matter content. These are characteristics typically valued by the industry and farmers as well.

The inherent potential of cassava, its capacity to grow in marginal environments, the recent success in identifying high-value traits, and the incorporation of new tools for genetic enhancement, as described in several of the references provided in this review, offer bright prospects for the crop and the people that depend on it.

2 Origins and Domestication

All 98 species of the genus *Manihot* are native in the Neotropics from where it was introduced to other regions of the world (Rogers and Appan, 1973). The origin of cultivated cassava is still unclear. Three relevant questions were raised by Allem (2002) regarding the botanical origin (parental wild species that eventually lead to the emergence of *M. esculenta*); the geographic area where this emergence took place and the region where it was domesticated (agricultural origin). The prevailing hypothesis is that cultivated cassava originated in South America (Allem, 1990; 2002; Olsen and Schaal, 2001), but many of these questions remain open.

Although it is frequently considered a polyploid species, the analyses conducted during diakinesis and metaphase I indicate the presence of 18 small and similar bivalents in cassava (Hahn et al., 1990). In some cases occurrence of univalents/trivalents and late bivalent pairing has been observed. Cassava is therefore a functional diploid ($2n = 2x = 36$) (de Carvahlo and Guerra, 2002; Jennings, 1963; Nassar and Ortiz, 2008). It has been suggested that certain portions of the genome may be duplicated and, therefore, cassava may be a segmental allotetraploid (Maggon et al., 1969).

2.1 Species Involved

Rogers (1963) listed *M. carthaginensis*, *M. aesculifolia*, *M. grahami*, *M. flabellifolia*, and *M. saxicola* as the most closely related species to cultivated cassava based on morphological, ecological, and geographical evidence. Allem postulated in 1994 and 1999 that modern cultivated cassava originated directly from wild relatives of *M. esculenta* subsp. *flabellifolia*. This suggestion was further supported by Olsen and Schaal (2001). Nassar and Ortiz (2008), on the other hand, suggested that cultivated cassava arose as a result of hybridization of two species and proposed that *M. pilosa* would be one of them. The crop may have been domesticated more than once (Allem, 2002; Nassar and Ortiz, 2008).

2.2 Reproductive Biology

Commercial propagation of cassava is by stem cuttings (Fig. 2.1). However, sexual reproduction, a key element for conventional breeding, is common and relatively easy to achieve (Alves, 2002; Kawano, 1980). Most breeding programs generate new genetic variation through crossing. Controlled pollinations generate full-sib

Fig. 2.1 Reproduction in cassava. (**a**) Good-quality planting cuttings; (**b**) keeping stems under the shade of a tree; (**c**) cutting stems in the field (Rayong, Thailand); (**d**) male flowers; (**e**) female flowers; (**f**) botanical seeds

progenies. Alternatively, in polycross nurseries insects do the pollinations. In this case the exact origin of the pollen is not known, and half-sib families are produced. A certain proportion of seed from polycross nurseries may be the result of self-pollinations.

Occasionally botanical seed has been used for commercial propagation (Iglesias et al., 1994; Rajendran et al., 2000) but it is not a generalized practice. Propagation from true seed occurs occasionally in farmers' fields and, as such, is also the starting point for the generation of useful genetic diversity (Eke-Okoro et al., 2001; Elias et al., 2001b; Pujol et al., 2002). Shamans or efficient farmers have been found to

be key players in this informal genetic improvement process (Salik et al., 1997; Sambatti et al., 2001).

Cassava is a monoecious species, with female flowers opening 10–14 days before the male flowers on the same branch. Self-pollination can occur because male and female flowers on different branches or on different plants of the same genotypes can open simultaneously (Jennings and Iglesias, 2002). Flowering depends on the genotype and environmental conditions. Branching occurs when an inflorescence is formed (Fig. 2.2). Because erect, non-branching types are frequently preferred by farmers, the crossing of elite clones in certain regions may become more difficult because of the scarcity of their flowers. Synchronization of flowering remains a difficult issue in cassava breeding. Some clones flower relatively early at 4 or 5 months after planting (MAP), whereas others flower only at 8–10 MAP. Because of this and the time required for the seed to mature, it takes generally more than a year to obtain seeds of a planned cross. On average, between one and two seeds (out of the three possible formed in the trilocular fruit) per pollination are obtained. Several publications illustrate the procedures for controlled pollinations in cassava (Kawano 1980; Jennings and Iglesias 2002). Seeds often have a dormancy period of a few months after maturity, and they require relatively high temperatures (30–35°C) for optimum germination (Ellis et al., 1982).

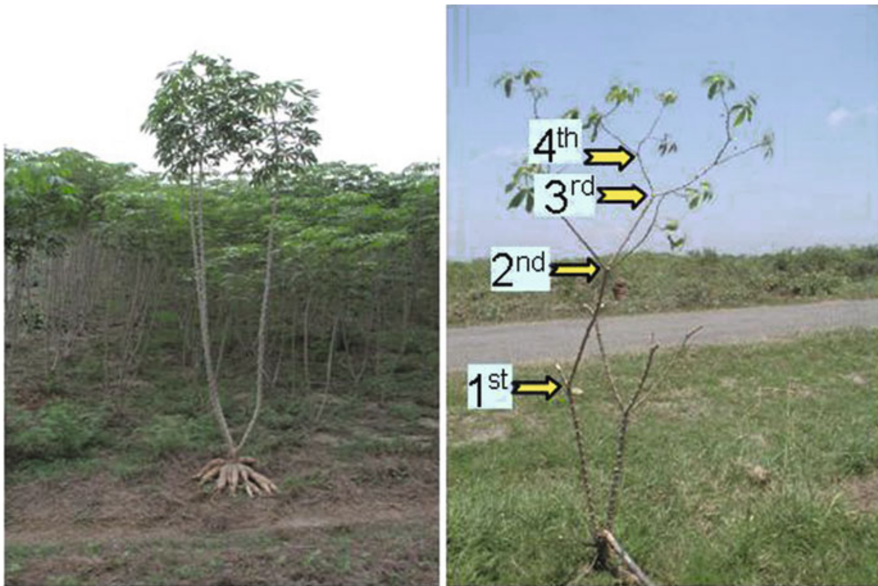

Fig. 2.2 Illustration of a cassava plant. The photograph on the *left* shows a plant close to harvesting time. The storage roots have swelled considerably and plant architecture is erect. The photograph on the *right* illustrates a plant that has been dissected slightly to expose how branching occurs in cassava. Four different flowering events along the life of this plant resulted in the respective four levels of branching

There is no evidence of incompatibility, so crosses can be done easily (except for the scarcity or absence of flowers in certain genotypes). There is no evidence of self-incompatibility either, so it is technically possible to make self-pollinations and obtain viable botanical seed. Male sterility is a frequent phenomenon and is currently being used to measure pollen flow.

2.3 History of Crop

Cassava originated in South America and was domesticated less than 10,000 years ago, with evidence of ancient cultivation in Brazil, Peru, Colombia, and Venezuela (Elias et al., 2001a; Allem, 2002; Nassar and Ortiz, 2008). Early European sailors and explorers soon recognized the advantages of the crop and carried it from Brazil to West Africa by the end of the sixteenth century. Cassava started to spread in East Africa later and one century on had reached the islands of Réunion, Madagascar, and Zanzibar (Janssens, 2001). From there, traders later introduced it to Asia. Cassava does not grow in temperate regions and therefore, until recently, its products were not well known outside the tropical and subtropical regions where it is grown and consumed. Recognizing the occurrence of many exceptions, cassava has become a major industrial crop in several countries of Asia, has a dual role in food security and as an industrial feedstock in Latin America and the Caribbean (LAC) and plays a key role in food security for sub-Saharan Africa.

3 Genetic Resources

Allem and co-workers suggested in 2001 that there are three *M. esculenta* subspecies: *esculenta* (cultivated cassava), *flabellifolia*, and *peruviana*. These three subspecies along with the closest wild relative (*M. pruinosa*) constitute the primary gene pool. The morphological characteristics of cultivated cassava are highly variable and there are numerous morphological descriptors that can be used for cultivar characterization (Alves 2002). However, within cultivated cassava the concept of varietal group has not been used.

The secondary gene pool includes *M. triphylla*, *M. pilosa*, *M. brachyloba*, *M. anomala*, *M. pruinosa*, *M. gracilis*, *M. tripartita*, *M. leptophylla*, *M. pohlii*, *M. glaziovii*, *M. dichotoma*, *M. aesculifolia*, and *M. chlorosticta.*

3.1 Germplasm

Early efforts were made to explore and assemble a large collection of cassava germplasm. CIAT holds in trust the worldwide cassava germplasm collection with more than 6,000 accessions. Three main types of accessions can be mentioned: (a) wild relatives of cassava within the *Manihot* genus; (b) traditional landraces

grown by farmers in Africa, Asia, or LAC; and (c) improved cassava germplasm produced by breeding projects in the continents mentioned above. The collection is maintained in vitro at CIAT experimental station located in Palmira, Colombia.

It can be said that the domestication of cassava has been completed only recently. Therefore, many landraces can still be directly released as commercial varieties once they have proved to have stable and competitive productivity in a given environment. In this regard cassava differs from other crops such as the cereals where breeding has resulted in a large genetic distance between improved germplasm and landraces. Another feature of the germplasm collection is the limited knowledge that has so far been generated from it. Morphological descriptions of the accessions are now available. A large screening of starch quality traits from more than 4,000 genotypes was recently published (Sánchez et al., 2009). Still, a more aggressive approach to analyze self-pollinated progenies (in search of useful recessive characteristics) and further analysis of the collection in search of new sources of tolerance or resistance to abiotic and biotic stresses is required. Three pre-breeding activities conducted at CIAT in collaboration with IITA and research institutions in Thailand and Brazil will be described below.

Extensive exploration to increase the germplasm collections and to develop approaches that will allow for an efficient evaluation of such germplasm is also urgently needed. The lack of genetic variability in many breeding programs can be overcome through an enhanced exchange of germplasm. The availability of partially inbred genetic stocks that allows the exchange of source material using botanical seeds will facilitate germplasm exchange.

3.2 Pre-breeding

3.2.1 Inter-specific Crosses

Wild *Manihot* germplasm offers a wealth of useful genes for the cultivated *M. esculenta* species (Hahn et al., 1980b; Chavarriaga et al., 2004). Several accessions of *M. esculenta* subsp. *flabellifolia*, *M. peruviana*, and *M. tristis* have high levels of proteins (Asiedu et al., 1992). This trait was observed also in crosses with *M. oligantha* (Nassar and Ortiz, 2008). A source of tolerance to PPD has been identified in *M. walkerae* (Bertram, 1993) and introgressed into cassava (Cuambe, 2007). The only source of resistance to the cassava hornworm and a widely deployed source of resistance to cassava mosaic disease (CMD) were identified in fourth backcross derivatives of *M. glaziovii* (Jennings, 1976; Chavarriaga et al., 2004). Moderate to high levels of resistance to white flies have been found in inter-specific hybrids of *M. esculenta* subsp. *flabellifolia*. Resistance was recovered easily in F_1 inter-specific hybrids, suggesting a simple inheritance of the trait. Accessions of *M. crassisepala* and *M. chlorosticta* are the only genotypes from the primary and secondary gene pool of the crop discovered to possess the waxy starch phenotype (Ceballos et al., 2006a). Apomixis has been observed in crosses with *M. neusana* (Nassar and Ortiz, 2008).

CIAT and EMBRAPA-Brazil are actively searching for desirable traits in *Manihot* species other than *M. esculenta* with the support of the Generation Challenge Program.

3.2.2 Inbreeding to Unmask Useful Recessive Traits

Early attempts to identify useful starch quality traits in cassava germplasm (Sánchez et al., 2009) failed to achieve the objective because in most instances these traits are recessive in nature. Therefore, the cassava breeding project initiated a systematic effort to self-pollinate accessions from the germplasm collection and then patiently analyze the segregating progenies. Some important root quality discoveries have been made and are described in a separate section below. In addition, other traits such as male sterile genotypes or characters affecting plant morphology have gradually emerged.

A new generation of self-pollinated germplasm is evaluated in the field every year. S_1 progenies from MVEN 331 segregated for a distinctive feature: leaves without petioles and a very erect plant type (Fig. 2.3) with absence of branching (at least for the first 6–8 months of age). This plant type offers interesting commercial applications. The most immediate one would be for the production of dried cassava foliage. One of the bottlenecks for this new market for cassava is the costs involved in the harvesting of the foliage. The only practical approach is a mechanical harvest that would also drag a considerable amount of young stems and petiole tissue. It should be easy to envisage a system where the kind of plant shown in Fig. 2.3 is harvested and, since the leaves have no petiole, and there is no branching (or few), leaves could be easily peeled off the stem. The result would be a reduced cost of harvest and, because of the reduced proportion of petiole and young stems, a better quality of foliage with reduced fiber content. This last characteristic would be fundamental for the use of dried foliage in the composition of diets for the poultry industry.

A second important potential application of this mutation would be the possibility of drastically increasing plant densities in commercial planting of cassava. It should also be easy to accept the idea that this new plant type could allow higher plant densities in cassava fields. Perhaps as many as 30,000 plants/ha could be used. This concept is important because most of the genetic gains achieved in different crops through the last century relates to modifications in plant architecture. The use of semi-dwarf wheat and rice varieties led to the highly successful green revolution. In the case of maize, if there is a single characteristic that can explain the consistent gains observed after the first introduction of commercial hybrids, it is reduced plant height with increased tolerance to higher plant densities (Duvick, 1999; Fehr, 1987; Troyer 2006). It should be mentioned that the petioles mutation was already known. What this research provides is evidence that it is simply inherited and has now allowed the segregation of several different phenotypes, a few of them also having the erect plant architecture.

There are many examples reported in the literature where tolerance to different herbicides has been found in different crops (canola, cotton, lentil, lettuce,

Fig. 2.3 Illustration of a plant type mutation resulting in a petioleless leaf and a very erect architecture. This plant type offers interesting possibilities for the future of cassava

maize, rice, sugar beet, sunflower, tobacco, tomato, and wheat), which led to the release of trade marks such as Clearfield, RoundUp Ready, and Liberty Link (Sherman et al., 1996; Tan et al., 2005; 2006; Tan and Bowe, 2008). In most of these cases, tolerance to herbicides was based on recessive or partially dominant genes and self-pollinations have facilitated their identification. During 2009 the cassava breeding project initiated activities to screen for natural tolerance to herbicides. A group of about 800 S1 genotypes were planted in six different blocks. Each genotype was represented by two plants in each block. Plants in each block will be treated with a different herbicide in search of sources of resistance. CIAT has had for many years transgenic cassava with resistance to BASTA, but restricted for research purposes only (Calderón-Urrea, 1988). This technology offers great potential once the intellectual property rights and biosafety issues are overcome.

3.2.3 Developing Partially Inbred Genetic Stocks

One of the most important uses of accessions from the germplasm collection is their role as source of useful traits. Currently the exchange of sources of specific traits is made through the shipment of in vitro accessions carrying the desired trait. Shipment of in vitro germplasm is expensive and troublesome. Therefore, exchange of germplasm is limited.

If a given genotype is to be used as source of a desirable trait, its value is in the trait itself, not in the whole genotype. CIAT and IITA have initiated a new approach by developing partially inbred genetic stocks. The source germplasm is self-pollinated to increase the degree of homozygosity (for the desirable trait). S_1 genotypes (homozygous for simply inherited traits) could then be obtained. There are three different scenarios for identifying homozygous genotypes among the S_1 (segregating) progeny: (a) if the trait is recessive (e.g., amylose-free starch) only homozygous recessive genotypes will express it and selection can be done using the phenotype of the S_1 genotypes; (b) if the trait is dominant and molecular markers are available, co-dominant markers such as SSR can be used to identify homozygous S_1 genotypes; and (c) if the trait is dominant and no molecular marker is available a second self-pollination would be necessary to identify S_2 progenies that do not segregate for the trait (indicating that the progenitor S_1 genotype was homozygous).

These partially inbred genotypes would become the backbone of these genetic stocks. A menu of options for tolerance/resistance to abiotic/biotic stresses, plant architecture, starch quality, nutritional quality, or other desirable traits will be gradually developed. The breeding value (for the trait) of these homozygous S_1 genotypes doubles if the assumption of heterozygosity for the trait in the elite S_0 genotype holds true. The selected S_1 genotypes could then be registered as a source of the desirable trait. Also, the S_1 genotype could be self-pollinated to produce S_2 seed, which would also be homozygous for the desirable gene(s). The storage and exchange of these S_2 botanical seeds would be considerably less expensive and faster than maintaining germplasm in vitro or in the field. Phytosanitary restrictions for the exchange of botanical seed are less limiting compared with the shipment of in vitro or vegetative cuttings. Finally, crosses of S_1 genotypes homozygous for different desirable traits can be made to produce new S_1 genotypes combining more than one desirable trait in a homozygous condition. Genetic stocks combining germplasm developed by IITA, CIAT, EMBRAPA, and other national programs in Africa, Asia, and Latin America could then contribute to a more dynamic exchange of germplasm and a more efficient exploitation of cassava genetic resources.

In the case of germplasm collections, 30–50 S_1 genotypes could be used to represent accessions and kept as botanical seed as a backup (the original genotype would be lost but its genes would be maintained).

4 Major Breeding Achievements

Conventional plant breeding has one of the highest rates of return among the investments in agricultural research. The remarkable increase in the productivity of many

crops during the twentieth century was reportedly due to genetic gains achieved through crop breeding (Fehr 1987). Cassava has also benefited from technological inputs in the area of breeding (Kawano, 2003). New varieties in Africa, Asia, and LAC have satisfied the needs of farmers, processors, and consumers, bringing millions of dollars in additional income to small farmers. The applications of tissue culture technologies have also made positive contributions (DeVries and Toenniessen, 2001) as well as the definition of adequate cultural practices, particularly in relation to fertilization protocols (Howeler, 2007). Genetic transformation and molecular biology (Blair et al., 2007; Calderón-Urrea, 1988; Fregene et al., 1997, 2000; Puonti-Kaerlas et al., 1997) offer great potential but have not had any measurable commercial impact yet.

During the past 30–40 years, significant progress has been achieved in the initial phase of the scientific genetic improvement of cassava. It can be said that with the turn of the millennium the adaptation of the crop to more intensive cultivation systems was completed. This process involved assembling major traits such as improved yield (mainly through a higher harvest index), low cyanogenic content (when desirable), improved plant architecture, and resistance/tolerance to the major diseases and pests. All these activities contributed to the general aim of increasing productivity and improving stability of production.

Two major factors influence the impact of plant breeding: the quality of the germplasm developed (i.e., its yield potential and stability) and the degree of adoption by farmers. Adoption of new varieties tends to be faster and more dynamic where there are markets for whatever the farmer produces. On the other hand, in subsistence farming where different crops play mostly a food security role, the adoption of new varieties is frequently slower and/or limited. Participatory plant breeding has a clear advantage for subsistence farming where many subtle criteria define the success of a given variety and its chances of adoption by farmers. The impact of conventional breeding in cassava tends to reach a maximum when and where the crop is largely used for processing and, therefore, there are strong markets for it. Figure 2.4 illustrates the increases in productivity of cassava in Thailand and Vietnam (strong markets for processed cassava) compared with the performance in LAC and sub-Saharan Africa. Yield productivity in Thailand and Vietnam increased an average of about 0.5 t/ha/year since 1990, whereas in South America and Africa the increase was much slower with 0.09 t/ha/year (FAOSTAT).

In addition to increases in productivity, Kawano (2003) reported a major improvement in dry matter content of cassava varieties released in SE Asia and also demonstrated the importance of selection for adequate levels of harvest index, particularly in early stages of the selection process. Jennings and Iglesias (2002) provided an assessment of the significant progress achieved to develop cassava cultivars tolerant to the main viral diseases (CMD and CBSD), bacterial blight, and super-elongation diseases. Resistance to CMD has been deployed and analyzed from the molecular point of view (Fregene et al., 2000, 2004; Egesi et al., 2007). Important progress in identifying and deploying tolerance/resistance to CBSD has also been achieved in recent years (McSween et al., 2006).

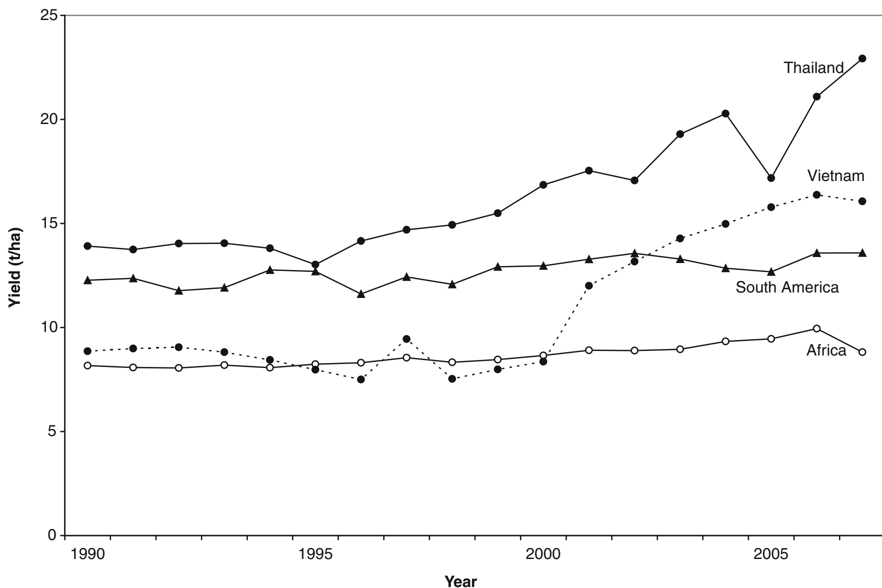

Fig. 2.4 Average yields in countries with strong markets for cassava in Southeast Asia (Thailand and Vietnam) compared with the average productivity in Latin America and sub-Saharan Africa (FAOSTAT)

5 Current Goals of Breeding

High and stable productivity will also be key traits for the future but there will also be increasing opportunities and needs for developing cassava cultivars particularly suited to specific end uses. It is therefore envisaged that multipurpose varieties will gradually give way to specific varieties with special quality traits developed for different processing pathways. This situation will hopefully lead to a closer interaction between the processing and production sectors of the different value-added chains.

Climate change will most likely have an impact in regions where cassava is a key commodity. Currently cassava can be grown in very contrasting set of conditions from the Amazon basin to arid conditions in sub-Saharan Africa or the northeast of Brazil. The crop, therefore, has the capacity to adapt to the predicted changes in climate. Climate change will negatively affect cassava through changes in the occurrence of pests and diseases. There is likely to be a shift in these biotic problems. The other negative impact that climate change will have is around unpredictability of rains both in amount and timing. The problem with these changes is not the lack of adaptation of cassava to the new environmental conditions but the time required for farmers to change from one variety (adapted to the old conditions) to a new one (adapted to the new conditions).

5.1 Cassava Utilization

Cassava is a unique crop because every part of the cassava plant can be utilized. There are many different uses of cassava (Balagopalan, 2002; Ceballos et al., 2006a) which are described below. These alternative uses, in turn, create an array of requirements that can be satisfied by conventional breeding. The roots are by far the most important product of cassava. Table 2.1 provides a summary of the most important characteristics of cassava roots. There is variation in starch quality in relation to its amylose percentage with a mean around 21% (Sánchez et al., 2009; Wheatley et al., 1993). Cassava roots are low in protein and fat contents with an average below 2% (dry weight basis). There have been, however, some preliminary results suggesting that protein content in the roots can be considerably higher (6–8%) in some landraces, particularly from Central America (Ceballos et al., 2006b). Yellow cassava roots have considerable amounts of carotenoids (Chávez et al., 2000, 2005; Iglesias et al., 1997) and through conventional breeding the original levels have been increased threefold in a period of 4–6 years (CIAT, 2009).

Table 2.1 Qualitative characteristics of cassava roots (Chávez et al., 2005; Ceballos et al., 2008; Morante et al., 2009; Sánchez et al., 2009)

Trait	Average	Min.	Max.
Dry matter content (%)	33.5	14.3	48.1
Cyanogenic glucosides (ppm)	325	14	3,274
Starch (% of dry weight)	84.5	65	91
Amylose content (% total starch)	20.7	15.2	26.5
Starch granule size (μm)	16.29	13.97	18.73
Total sugars (% of dry weight)	3.75	0.20	18.8
Reducing sugars (% of dry weight)	1.31	0.0	15.7
Total carotenoids (μg/g fresh root)	8.84	3.39	18.87

Cyanogenic glucosides (CG) are found in every tissue of cassava, except in the seed. The most abundant CG is linamarin (about 85%), with lesser amounts of lotaustralin. The CG, synthesized in the leaves and transported to the roots, is broken down by the enzyme linamarase to produce hydrogen cyanide (HCN), a volatile poison (Andersen et al., 2000; Du et al., 1995; McMahon et al., 1995; Wheatley and Chuzel, 1995). Linamarin and linamarase accumulate in different parts of the cell, thus preventing the formation of free cyanide. However, most processing methods disrupt the tissues, allowing the enzyme to act on the substrate for a rapid release of cyanide. CG accumulation varies with genotypes, environments, agronomic practices, age of the plant and plant tissue, being highest in the leaves and peel of roots (Cock, 1985). Concentration of CG in the roots frequently defines the specific uses a given variety can have.

5.1.1 Starch

Cassava is one of the four most important sources of starch worldwide, together with maize, potato (*Solanum tuberosum*), and wheat (*Triticum aestivum* and *T. durum*) (Davis et al., 2003; Ellis et al., 1998). Because of the low levels of protein and fat in the roots, the starch from cassava roots has excellent characteristics and is relatively inexpensive to extract. Key traits for this industry include white parenchyma, high dry matter content, and variation in the starch quality properties and composition.

5.1.2 Animal Feeding

Cassava is a competitive source of calories for animal diets because of its efficiency converting light into chemical energy. However, cassava roots have reduced levels of fat and protein, and lack vitamins and minerals compared with maize. Roots can be processed into dried chips, meals, or pellets for animal feed. The price of dried cassava roots, when used for animal feeding, is lower than that of maize (typically around 70% the price of maize) because of its reduced nutritional value. Key traits for this industry are high dry matter content and if possible at all, increased nutritional value (particularly in relation to protein and vitamins content). This industry may also consume dried foliage which is an excellent source of proteins, minerals, and vitamins.

5.1.3 Bio-ethanol

This is a relatively new end use for cassava and is the result of increased prices of oil and technological developments for the hydrolysis of starch prior to the fermentation process. For this industry a key breeding objective would be to maximize the productivity of energy per hectare. In certain circumstances, therefore, when fresh roots are ground and used for fermentation without being first dried, it may be acceptable to release clones with high fresh root productivity per hectare, even if they do not possess a minimum of dry matter content. This kind of clone would not be acceptable for the starch or dried chip industries because of unacceptably higher prices of processing. Another characteristic that would be desirable for this industry is roots whose starch is easier to degrade into simple sugar syrups. Carvalho et al. (2004) described a "sugary" mutation in cassava collectively known in Brazil as "*mandiocabas*."

5.1.4 Cassava for Processed Food

There are many different ethnic uses of cassava for processed food (gari, fufu, kokonte, farinha, casabe, gaplek, etc.) (Cock, 1985). Each of these products requires specific organoleptic and physico-chemical properties, which in turn imply that there is a large variation of requirements for these end uses.

In some cases, high CGs are preferred because they confer a particular taste to the product, or they prevent theft, or they protect from monkeys and other mammals,

or simply because of cultural preferences. In others cases (e.g., pre-cooked frozen croquettes for the export markets) very low levels of CG are a critical requirement. Cultivars with less than 100 mg CG/kg fresh weight in the roots are considered "sweet." Above this level cassava roots are considered "bitter." In an extensive evaluation of a large sample of genotypes, Sánchez et al. (2009) found that breeding has tended to reduce the cyanogenic potential of cassava roots.

5.2 *Breeding Objectives*

Breeding objectives depend on the ultimate use of the crop. The processing industry has relatively few requirements which can be summarized as high and stable productivity and a minimum dry matter content of about 35%. However, the globalization of economies during the 1990s has opened up opportunities never available to cassava before because both governments and private sectors realized that the crop was a key but underutilized commodity (Ceballos et al., 2004). These changes made clear that, in addition to high and stable productivity, cassava breeding projects had the opportunity of expanding and exploiting genetic variability that would generate clones with increased value for the different industrial processes where cassava can be a strategic raw material. Examples of key traits for the different industries have been described in the section about cassava utilization.

When cassava is used as a food security crop, additional requirements need to be addressed for a variety to be adopted. Human consumption frequently emphasizes cooking quality or starch characteristics over productivity as a determining trait. Good cooking quality is often associated with other morphological traits, such as the color of the peel of the roots, the leaf petiole, or the shoot. Farmers frequently reject any change in such morphological traits, although they may have little or no correlation with actual cooking quality. Because of those types of farmers and consumers' preferences, participatory research and breeding approaches have been incorporated in cassava breeding (DeVries and Toenniessen, 2001; Gonçalvez Fukuda et al., 2000; Gonçalvez Fukuda and Saad, 2001).

Other root quality traits relevant to different cassava breeding programs around the world are the cyanogenic potential in the roots (Dixon et al., 1994) and early bulking capacity. Unfortunately it is very difficult to monitor clones that have the early bulking trait because breeding programs need to standardize their harvesting time (typically at 11–12 MAP), particularly when genotypes are represented by only a few plants. Early bulking is typically assessed after several selections have been made and potentially useful genotypes may have unknowingly been discarded.

5.2.1 Abiotic Stresses

Stable production relies on tolerance to biotic and abiotic stresses, which vary with the environment. There are a variety of abiotic factors limiting cassava productivity which would probably accentuate as a result of changes in the climate generating wider fluctuations in relevant weather parameters. The crop is frequently grown in

drought-prone regions and/or on low-fertility soils. It can also be found in alkaline or acidic soils, most frequently the latter. Some traits associated with adaptation to these conditions have been suggested (Jennings and Iglesias, 2002), such as leaf longevity (CIAT 2001; Fregene and Puonti-Kaerlas, 2002; Lenis et al., 2006), optimum leaf area index, and ideal plant architecture (Hanh et al., 1979; Kawano et al., 1998; Kawano 2003). The capacity of the stems to withstand long storage periods (sometimes up to 2 months) from harvest to planting is an important trait (Fig. 2.5). This characteristic affects final density of established plants and is fundamental for areas with relatively long dry spells or erratic rainfall, because the storage period may extend to the point that it compromises their viability. While there is known genetic variation for stem storability, it has not been a major breeding objective of any program so far.

Fig. 2.5 Illustration of missing plants in cassava trials. The stems of some genotypes can lose their capacity to sprout after a short period of storage from the time they were harvested to the time they were cut and used for planting a new crop

Although it is a self-inflicted reaction, PPD and the resulting short shelf life of cassava roots after harvest is frequently grouped as an abiotic stress. Consequently, cassava roots need to be consumed or processed soon after harvesting (van Oirschot et al., 2000). The short shelf life of the roots severely limits the marketing options by increasing the likelihood of losses, the overall marketing costs, and by limiting the access to urban markets or processing centers to production sites close to them. Extending the shelf life by only 2–3 weeks would offer huge advantages to the cassava community.

5.2.2 Herbicide Tolerance

Herbicide tolerance in crops offers several advantages. The handling of herbicides can be made in a much more efficient way, applying them at the optimal timing when weeds are most vulnerable. This implies that there is a reduction in the amount of herbicides used, reducing costs of production on the one hand and having a positive impact on the environment on the other. Perhaps more important is the possibility that herbicide tolerance allows direct planting, which without proper technologies

to handle the problem of weeds, is often unviable. Direct planting also offers several advantages: it allows the maintenance of a mulch of crop residues on the soil, thereby reducing soil erosion and maximizing the capture and conservation of water and soil nutrients. Direct planting reduces the operations of soil preparation at planting time, which offer the dual advantages of reducing costs and the negative impact on the environment. There are a few alternative approaches to develop cassava tolerant to herbicides, and one of them is inbreeding accessions from the germplasm collection.

5.2.3 Disease and Pest Resistance

The distribution of diseases is not uniform worldwide. In Africa, Cassava Mosaic Disease (CMD) and Cassava Brown Streak Disease (CBSD) are important constraints. A disease similar to CMD is also present in southern India, but they are fortunately neither present in the rest of Asia nor in the LAC. On the other hand frogskin disease causes roots to become "corky" and commercially unusable but it is only present in LAC. The causal agent has not yet been identified, although it has been suspected for many years that it may be a virus or a phytoplasm. Bacterial blight, induced by *Xanthomonas axonopodis* pv. *manihotis* (also known as *X. campestris* pv. *manihotis*), is found worldwide and can have devastating effects on yield and the availability of planting material, particularly in Africa and LAC (Hillocks and Wydra, 2002). Several fungal diseases may also affect cassava productivity. Super-elongation disease, induced by *Sphaceloma manihoticola* (Teleomorph: *Elsinoe brasiliensis*), is widespread in the Americas, from Mexico to Southern Brazil. In tropical lowlands with high rainfall, *Cercospora, Cercosporidium, Phaeoramularia*, or *Colletotrichum* species can affect cassava productivity (Jennings and Iglesias, 2002). *Phoma* species cause leaf and stem lesions in the tropical highlands. Several species of *Phytophthora* induce root rot. Root rots are also induced by different species of the genera *Sclerotium, Armillaea*, and *Fusarium*. Fortunately, there are sources of genetic resistance to most of these diseases (CIAT, 2001; Hilloocks and Wydra, 2002).

Pests that feed on cassava can reduce productivity through direct feeding damage or as vectors for diseases. The green mite (*Mononychellus tanajoa*) devastated cassava fields upon its introduction in Africa in the 1970s (Nyiira, 1975). Other mites important for cassava are *Tetranychus urticae*, *T. cinnabarinus*, *Mononychellus caribbeanae*, and *Oligonychus peruvianus* (Bellotti, 2002). The mealybugs *Phenacoccus manihotis* and *P. herreri* feed on cassava fields of Africa and LAC, respectively. IITA and CIAT collaborated in the introduction into Africa of agents for the biological control of mealybugs found in Brazil and Colombia. This is one of the most successful interventions in the deployment of biological control agents with huge economic and environmental benefits (Neuenschwander, 1994). Unfortunately *P. manihotis* has recently been introduced into Asia. Thrips (particularly *Frankliniella williamsi* and *Scyrtotrips manihoti*) considerably reduce yields of susceptible genotypes. Clones with pubescent leaves in their early stages of

development offer excellent levels of resistance to these insects (Belloti, 2002), and this trait has been broadly incorporated into improved varieties.

Whiteflies are among the most widespread pests in cassava. *Aleurotrachelus socialis* is the predominant species in northern South America, where it causes considerable crop damage through direct feeding. *Bemisia tabaci* is widely distributed in tropical Africa and several Asian countries. Until 1990, *B. tabaci* biotypes found in the Americas did not feed on cassava. The major effect of *B. tabaci* is through its role as a vector of the devastating CMD disease in Africa. Several other species of whiteflies affect cassava in different regions. Genetic resistance to whiteflies in cassava has been found particularly for *A. socialis* in several germplasm accessions from the CIAT collection (Bellotti, 2002). Based on breeding work at CIAT, Colombia released the first whitefly-resistant variety of any crop in 2002, targeted toward the Tolima Valley, where whiteflies typically devastate plantations. There are several other arthropod pests affecting cassava roots, foliage, and/or stems, particularly Lepidoptera, Diptera, and Hemiptera. There is little or no genetic resistance to those pests and their management is commonly achieved through biological control measures. Attempts to produce transgenic resistance to the horn worm in cassava have succeeded with the introduction of *cry* genes encoding insect-specific endotoxins (Bt toxins) from *Bacillus thuringiensis* (Fregene and Puonti-Kaerlas, 2002; Ladino et al., 2001; Taylor et al., 2004).

6 Breeding Methods and Techniques

6.1 Evaluation and Selection Scheme Used in Cassava Breeding

Cassava genetic improvement starts with the assembly and evaluation of a broad germplasm base, followed by production of new recombinant genotypes derived from selected elite clones and careful evaluation in a set of representative environments. Directed and systematic cassava breeding began only a few decades ago and, therefore, the divergence between landraces and improved germplasm is not as wide as in crops with a more extensive breeding history. As a result, landrace accessions, as explained above, probably play a more relevant role in cassava than in other crops. Parental lines are selected based mainly on their per se performance and little progress has been made in using general combining ability (Hallauer and Miranda, 1988) as a criterion of parental selection.

The botanical seed obtained by the different crossing schemes (Kawano, 1980) may then be planted directly in the field or first germinated in greenhouse conditions and then transplanted to the field when they are about 20–25 cm tall (Jennings and Iglesias, 2002). Root systems in plants derived from botanical seed or vegetative cuttings may differ considerably. The taproots from seedlings tend to store fewer starches than roots from cuttings (Alves, 2002; Rajendran et al., 2000). Because of this, it is difficult to correlate the root yield of clones at later stages in the evaluation/selection process, with early results from the plants obtained from botanical

seeds (Morante et al., 2005). However, when seeds are germinated in containers and later transplanted, the taproot often does not develop, and the seedling-derived plant may be more similar to subsequent stake-derived plants regarding the shape of their starchy roots.

For general purpose breeding, once a set of crosses is planned, it takes 2 years for the crosses to be ready. Certain crosses can be made (and the seed harvested) within a year, but crossing blocks are generally maintained for up to 2 years in the field thus allowing several flowering events per plant so numerous seeds per cross and many crosses per parental line can be obtained. Most of the seed is obtained from the second flowering event (6–8 MAP) through the third or fourth event (about 14–18 MAP). The last pollinations are then made early enough for the seed to mature and be harvested and processed for germination. Table 2.2 describes a typical recurrent selection cycle. The first stage of that cycle includes the 2 years for the recombinant seed to be produced, harvested, and processed.

Table 2.2 Typical recurrent selection scheme for an individual target environment. As planting material becomes available larger plots, replications and larger number of locations become feasible. A consistent reduction of genotypes is also achieved through selection

Year	Type of trial	No. of genotypes	No. of plants/plot	No. of replications	No. of locations
1–2	Crosses among elite clones to produce recombinant botanical seed				
3	F1	2,500–4,000	1	1	1
4	Clonal evaluation trial	2,000–3,000	7–8	1	1
5	Preliminary yield trial	200–350	10	3	1
6	Advanced yield trial	50–100	20–25	3	1–3
7–8	Regional trials	10–30	25	3	6–12

The vegetative multiplication rate of cassava is low. From one plant, 5–10 cuttings typically can be obtained, although this figure varies widely by genotype. This situation implies a lengthy process to reach the point where replicated evaluations across several locations can be conducted, just because of the time required to produce enough planting material. It takes about 5–6 years from the time the botanical seed is germinated until the evaluation/selection cycle reaches the regional trial stage when several locations can be included (Table 2.2). One further complication in a cassava program is the number of factors that can affect quality of planting material. For example, the original positioning of the vegetative cutting along the stem affects considerably the performance of the plant it originates. Cuttings from the mid-section of the stems usually produce better performing plants than those at the top or the bottom. This variation in the performance of the plant, depending on the physiological status of the vegetative cutting, results in larger experimental errors and undesirable variation in the evaluation process.

There is some variation among different cassava breeding programs regarding the numbers of genotypes and plants representing them through the different stages of selection. However, the numbers presented in Table 2.2 are fairly common and illustrate the different stages required to complete a selection cycle and the kind of

selection pressures that are generally applied (Ceballos et al., 2004, 2007a; CIAT, 2009). The first selection can be conducted in the third year on the nurseries with plants derived from botanical seed (F1 in Table 2.2). Because of the low correlations between the performance at this early stage of selection and when the genotypes reach replicated trials, the early selections are based on high heritability traits, such as plant type, branching habits, and, particularly, reaction to diseases (Hahn et al., 1980a, b; Hershey, 1984; Iglesias and Hershey, 1994; Morante et al., 2005). At IITA, combined selection for resistance to CMD and bacterial blight (*X. axonopodis* pv. *manihotis*) begins with about 100,000 seedlings and only about 3,000 genotypes survive this first stage of selection, which is based on single plant performance.

The second stage of selection is called the clonal evaluation trial (CET). The surviving genotypes from the single plant selection conducted during the F_1 stage produce the 6–10 vegetative cuttings required for this second step. The capacity to produce this number of cuttings is in fact another selection criterion used at the F_1 stage. CETs usually range from 2,000 to 3,000 clones. Within a given trial, however, the same number of plants is used to avoid the confounding effects between number of plants and genotypic differences. Because the competition between neighboring genotypes in the CET may favor more vigorous plant architectures, selection at this stage still relies heavily on high heritability traits, such as harvest index (Kawano et al., 1998; Kawano, 2003). Plant type is an important selection criterion at early stages of selection; plants whose main stem does not branch until it reaches atleast 1 m are preferred (Kawano et al., 1978; Hahn et al., 1979). Other selection criteria at this stage include high dry matter and cyanogenic potential (Iglesias and Hershey, 1994). Between 100 and 300 clones survives the CET. A common feature in the first two stages of selection for most programs is that selection is frequently visual with no data recording to manage a larger number of materials at lower costs. One important trait that makes the harvest of large trials, such as the CET, expensive and time demanding is the measurement of dry matter content (DMC) in the roots. The productivity of cassava depends ultimately on the amount of fresh roots produced and the DMC of those roots. Heritability for DMC is considered to be intermediate.

The following stage of selection is the preliminary yield trial (PYT). At CIAT, PYTs are currently based on the evaluation of 10 plants in three replications. The 10 plants in each replication are planted in two 5-plant rows. If possible, rows are spaced only 0.8 m apart (instead of the standard 1.0 m), and one empty row is left between plots to increase within-clone competition and reduce between-clone competition. Alternatively, row spacing can be maintained at 1.0 m but then the plant-to-plant distance within the row is reduced to 0.8 m. In this case also an empty row is left separating plots with different genotypes. Large genetic variability occurs among clones, even within the same family. Although poorly performing clones are mostly eliminated at the CET stage, there is still a considerable variation in the PYT trials. This highlights the need for a gradual process of selection and the need to avoid strong selection pressures in early stages.

With the initiation of replicated trials, the emphasis of selection shifts from high heritability traits to those of low heritability, such as yield. Starting with PYT and

increasingly during the advanced yield trials (AYT) and the regional trials (RT), a greater weight is given to yield and its stability across locations. Cooking quality trials (relevant for the different ethnic ways cassava may be consumed) also begin at these stages, when the number of genotypes evaluated is more manageable. The AYTs are typically grown in 1–2 locations for 1 or 2 consecutive years. They have three replications per trial and plots are four (or five) rows with five plants per row. Yield data are taken from the six (nine) central plants of the plot and the remaining 14 (16) plants are used as source of planting material for the next season. The RTs are conducted for at least 2 years in 3–6 locations each year. Plots have five rows with five plants per row. Yield data are taken from the nine plants from the center. Clones that show an outstanding performance in the RT are released as new varieties after a few years of informal evaluation in semi-commercial evaluations with key farmers. They are also sooner rather than later incorporated into the crossing blocks as progenitors to initiate a new recurrent selection cycle.

The breeding scheme described above can be classified as mass phenotypic recurrent selection. No family data are used in the selection process and individual clones are evaluated and selected or discarded. It has been suggested that data are recorded in all entries at the CET level (Ceballos et al., 2004, 2007a), and estimates of general combining ability of the progenitors that generated the CET are obtained.

6.2 Strategies for Improving the Efficiency of Cassava Breeding

6.2.1 Rapid Cycling Recurrent Selection for High Heritability Traits

The HarvestPlus initiative aims at improving the nutritional quality of several crops (Pfeiffer and McClafferty, 2007). In the case of cassava the main focus is to increase the levels of pro-vitamin A carotenoids in the root. As explained above, the normal recurrent selection cycle in cassava requires about 8 years for completion (Morante et al., 2005, Table 2.2). Carotenoids content is a highly heritable characteristic and taking advantage of this feature, and in response to the need for rapid progress, a rapid cycling recurrent selection system was implemented.

Basically root samples of each F1 plant are harvested 10–12 MAP, but leaving the plant still growing in the field. As results from the laboratory become available, the same plants could be used to generate new recombinant seed. Within 2–3 years new recombinant seed is produced from the genotypes with higher carotenoid content values. This system, unusual for cassava, produced outstanding results. Figure 2.6 illustrates the gains from selection for high carotenoids content. This figure presents the results of the seedling (F1 trials) nurseries during the harvest of nurseries in the last 4 years. Every year an average of almost 3 μg carotenoids/g of fresh root was added to the best genotypes. These are unprecedented gains for a crop like cassava. These results also highlight the importance of adapting the breeding schemes to the specific needs and characteristics of the trait to be improved.

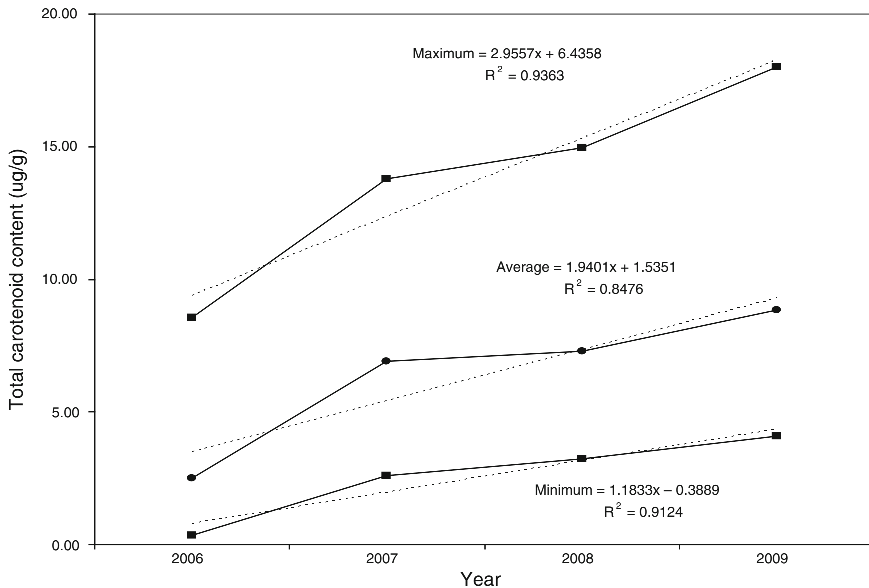

Fig. 2.6 Gains from selection for high carotenoids content using the rapid cycling recurrent selection approach. Data present the results of the seedling (F1 trials) nurseries during the last 3 years of work

6.2.2 Correcting Data for Missing Plants

A major problem in cassava breeding is the impact that the storage of stems has on their sprouting capacity. Evaluations with a uniform plant stand are fundamental for reliable results. However, it is very difficult to correct data for missing plants within an experimental plot. Linear covariance analysis of crop yield data using plot stand as the covariate is not a satisfactory approach especially when the plants are missed soon after sprouting or early in the growing season. The following formula to adjust for missing plants in AYT or RT has been developed (Pérez et al., 2010):

$$y_a = y_o \left[1 + \left(1 - \frac{N_0}{N_a} \right) 0.727 \left(\frac{N_0}{N_a} \right)^{-0.805} \right],$$

where y_a is the adjusted plot yield, y_o the observed plot yield, N_a is the ideal plot stand (in the case of AYT and RT, nine plants), and N_0 is the number of plants actually harvested. This correction should be extremely useful in overcoming the problems of missing plants and lack of uniform plant densities illustrated in Fig. 2.5.

6.2.3 Inbreeding to Exploit Heterosis

Cassava, as a typical cross-pollinated species, shows good levels of heterosis (Cach et al., 2005a, b; Calle et al., 2005; Jaramillo et al., 2005; Pérez et al., 2005a, b)

as well as severe inbreeding depression (Contreras Rojas et al., 2009). Given the length of each cycle of selection and the complexities derived from improving non-additive genetic effects such as dominance and epistasis, the availability of fully inbred progenitors would allow a gradual but consistent building up of heterosis and, eventually, the identification (or creation) of heterotic populations where reciprocal recurrent selection could be used. However, to produce fully inbred genotypes through successive self-pollinations is not practical in cassava for two major bottlenecks: (a) it would require 12–15 years to reach the S_6 stage of inbreeding (six successive self-pollinations) and (b) based on CIAT experiences in attempting to produce self-pollinated generations beyond the S_2 or S_3 level, favored plants with profuse flowering result in the generally disliked early branching types. There is an ongoing effort to develop a protocol for the production of doubled haploids through microspore culture. This could be an ideal approach for producing highly homozygous clones in less than 2 years.

6.2.4 Inbreeding to Reduce Genetic Load

Inbreeding has been intentionally omitted in the breeding scheme. Therefore large genetic loads are likely to remain hidden in cassava populations and useful recessive traits are difficult to detect. Contreras Rojas et al. (2009) reported that on average S_1 progenies produce only 30% of the root yield harvested in their respective progenitors. This degree of inbreeding depression highlights the size of the genetic load (undesirable alleles in breeding populations) still present in cassava. The introduction of inbreeding would allow (actually require) the selection of tolerance to inbreeding depression, which basically means breeding to eliminate the undesirable alleles collectively known as genetic load.

6.2.5 Inbreeding to Identify Useful Recessive Traits

An amylose-free mutation has been reported for many other crops (maize, potato, rice, wheat, barley, etc.). Amylose-free starch offers special functional properties that are beneficial for the starch industry (Davis et al., 2003; Ellis et al., 1998). In addition, it has been shown to reduce processing costs in the production of ethanol from starchy crops (Sharma et al., 2007). In 2006 a spontaneous amylose-free starch mutation was discovered in cassava (Ceballos et al., 2007b). ECOTILLING (see Section 7.3) can be used in the future for the identification of specific spontaneous mutations in different populations and will also benefit from the availability of a sequenced genome of cassava.

6.2.6 Inbreeding to Allow for the Backcross Scheme

In the sections on breeding objectives and development of high-value clones, several examples of useful traits (putatively) controlled by single genes have been provided. Their discovery will have undoubtedly a huge impact on cassava production and processing. However, since progenitors in cassava breeding are not homozygous it

is impossible to introgress these single genes through sexual crosses and recover the original progenitor. The current strategy relies on making crosses with the source of the desirable trait and basically, breeding a new variety again. This is a very expensive and inefficient process. If progenitors were homozygous the backcross scheme could be easily implemented. Backcrossing has been one of the most successful breeding approaches used consistently in many different crops (Allard, 1960).

6.2.7 Improving Testing Methodologies

Several characteristics rely on expensive or time-consuming tests. This is particularly the case of breeding for improved nutritional quality. The standard methodology for estimating protein content, for example, has been through the indirect method of quantifying N by the Kjeldahl method and then multiplying it by a 6.25 constant. There is growing evidence that the N-to-protein conversion factor, in the case of cassava roots, may be considerably lower because of the presence of non-protein sources of N. A direct method for the quantification of total soluble proteins contents has recently been tested using the Bradford colorimetric method (BioRad dye reagent). Preliminary results confirmed a large variation in N as well as protein content where high levels are threefold higher than low levels of protein (Chávez et al., 2009). This system is considerably less expensive and faster than the traditional approach based on N quantification.

Another example is in breeding for increased levels of carotenoids content. The current system relies on a tandem selection based first on visual assessment of the intensity of root pigmentation, followed by total carotenoids content determination by spectrophotometry (on selected genotypes) and then by HPLC analysis to determine β-carotene content. Depending upon the equipment used, between 8 and 24 samples per day can be processed by HPLC. This means only about genotypes per harvesting season. An alternative approach would be the use of near-infrared reflectance spectroscopy (NIRS) which could allow (once the standard curves are developed) screening as many as 400–600 genotypes per day. An additional advantage is that predictions for several traits can be simultaneously obtained from a single NIRS reading (total carotenoids, β-carotene, protein, dry matter and protein content, cyanogenic potential, total and reducing sugars, amylose/amylopectin ratio, etc.).

6.2.8 Stratification of Large Trials

A major problem with the CET is its large size (easily 2 ha in size) and the unavoidable environmental effect in the selection. This problem is particularly relevant in the case of cassava, because the target environments for cassava are typically in "marginal" agriculture conditions and prone to large variation. Since CETs are frequently the first stage of evaluation, only a few stakes (<10) are available for trials. So the introduction of replications that could help overcome this problem is not practical.

The same simple principles as suggested by Gardner in 1961 were introduced for the evaluations of CETs several years ago. The field where the CET is going to be planted is divided into three “blocks” of about equal size trying to maximize differences among blocks and minimize variation within each block. Clones could actually be replicated (i.e., two replications of four plants rather than one replication with eight plants) but the logistical complexity and additional efforts to generate twice as much data proved to be too much compared with the advantages (CIAT, unpublished data). However, clones are grouped in either full- or half-sib families. Generally, since many clones are available from each family, they can be randomly allocated to one of these three “blocks.” In other words instead of planting all the clones from a given family together, one after the other, they are split into three groups. This approach provides two interesting advantages: (a) there is a replication effect for the families because all the clones from a given family are distributed in three “repetitions” in the field. The averages from all the clones are less affected by the environmental variation in such a large experiment and (b) selection is made within each block therefore reducing the environmental component affecting the selection. This is similar to the stratified mass selection suggested by Gardner in 1961. This approach effectively overcomes the environmental variation that can be measured by comparing the means of each block. In general, variation in the order of 10–20% has been observed among average performances of the three blocks. These are, in other words, the gains in the precision attained by introducing the stratification of the CETs.

6.2.9 Estimation of General Combining Ability of Progenitors

One of the major decisions taken by any breeder is the selection of parents used to produce a new generation of segregating progenies. In cassava, this decision has been mainly based on the per se performance of each clone. Nonetheless, some empirical knowledge of the quality of progenies produced by different parents could be developed. This lack of organized information on the breeding values of parental lines used in the breeding projects was partially due to the fact that no data were taken and recorded during the first stages of selection or else, they were incomplete. Therefore, it was not possible to generate a balanced set of data that would allow the breeder to have an idea of the relative performance of the progeny of each elite parental line. In other words, no formal process to assess the breeding values of the progenitors used in the cassava breeding projects was available (Allard, 1960; Simmonds and Smartt, 1999).

To overcome this problem, the decision was taken to record data and to introduce the use of selection indexes (Baker, 1986). Selection is made within each stratum as explained in the previous section. Data from each family are then pooled across the three blocks in which it was planted. The stratification means that, in a way, there is a replication effect at the family level. Since a given progenitor may be used more than once, data from all the families in which each progenitor participated are pooled to obtain an idea of the general performance of all the progenies from a

given parental clone. The advantages of this approach have already been reported (Ceballos et al., 2004, 2007a).

7 Integration of New Biotechnologies in Breeding Programs

Biotechnology tools have been adapted to cassava and are currently incorporated in different projects for its genetic improvement. A molecular map has been developed (Fregene et al., 1997, 2000; Mba et al., 2001) and marker-assisted selection is currently used for key traits, such as resistance to CMD. The recent sequencing of the cassava genome will certainly contribute to a more efficient implementation of molecular markers, which will also benefit from the availability of homozygous germplasm (doubled haploids) developed through microspore or anther culture (CIAT, 2009). The efficiency of TILLING (induced mutations) and ECOTILLING (a similar tool applied to spontaneous mutations) will also be greatly facilitated by the sequencing of the cassava genome. Finally genetic improvement of cassava by genetic transformation is also now possible.

7.1 Use of Molecular Markers

Molecular markers represent a limitless source of neutral markers for the quantitative assessment of genetic diversity and "signposts" in gene and genetic diversity. They rely on differences in the nucleotide sequences (either nuclear or in organelles) that can be uncovered using diverse methods based upon PCR and DNA–DNA hybridization or both. Molecular markers are abundant and many of them show high level of polymorphism and the assays can be done at any stage in the development of the plant. They are not influenced by the environment but require that phenotypic data associated with them have properly taken into consideration genotype-by-environment interactions.

Markers have been used to generate several molecular genetic maps for cassava (Fregene et al., 1997; Mba et al., 2001; Okogbenin et al; 2006). In an attempt to make marker technology more widely applicable in breeding programs, highly polymorphic SSR markers were mainly used in the construction of subsequent genetic maps for cassava. Presently over 525 SSR markers have been used in the development of the new SSR-based maps which have yet to be published. An initiative toward completing the saturation of the cassava genetic map has also resulted in the generation of expressed sequence tags (ESTs) and SNPs. Several ESTs have been developed for cassava (Lopez et al., 2004; Lokko et al., 2007) with over 80,000 ESTs for cassava available.

The generation challenge program (GCP) is currently supporting an initiative to develop SNPs for drought tolerance. A physical map of the cassava genome was constructed by fingerprinting 70,000 BAC clones and sequencing the ends of 9,000 clones distributed throughout the genome. The availability of a physical

map is an important tool for map-based cloning of agronomically relevant genes in cassava. The selected low-copy sequences spread throughout the genome will be re-sequenced in a panel of 10 cassava genotypes to identify SNPs that can be used for genetic mapping. Recently, a general genotyping array of 1,536 cassava SNPs has already been designed from cassava EST sequences. SNPs are ideal markers as they allow the use of genotyping platforms that can assay many individuals for thousands of SNP markers in parallel. The strategy for utilizing markers is primarily driven by their availability and cost of genotyping platforms.

One of the primary objectives of genetic mapping and gene tagging efforts in cassava is to provide tools that can increase the cost effectiveness and efficiency of cassava breeding. It includes pests and diseases, traits expressed only at the end of the crop's growing cycle, and those for which phenotype is difficult to measure. Various markers have been used to tag several traits in cassava. Molecular markers have been used to tag three different sources of CMD resistance originally found in *M. glaziovii*, TME3, and TMS97/2205 (Fregene et al., 2000; Akano et al., 2002). Two SSR markers have been found associated with CGM (NS1009 and NS346). About six markers were found associated with CBB explaining 9–27% of the phenotypic variance of response to five Xam strains (Jorge et al., 2000). Early bulking is another trait evaluated in cassava and results from the analysis of this trait showed that it was mostly affected by harvest index and dry foliage. Three QTLs explaining 25–33% of phenotypic variance were found for dry foliage while five other QTLs associated with harvest index were identified with phenotypic variance in the range of 18–27% (Okogbenin and Fregene, 2002). Several other gene tagging projects have since been conducted or are ongoing in cassava at CIAT for other traits such as PPD, whiteflies, and β-carotene. Except for CMD and CGM, markers identified for several traits have yet to be validated and there is the need to test these markers and to further conduct fine mapping of the genomic regions for the markers with a view to developing better markers to enhance their application in marker-assisted selection (MAS). Generally molecular markers have been applied in cassava genetic improvement in the following areas.

7.1.1 Genetic Diversity

Phylogenetic relationships of *Manihot* species were revealed through the use of RFLP markers, indicating little variation within species and a close relationship between *M. chlorosticta* and *M. esculenta* (Hayson et al., 1994). Olsen and Schaal (2001) used microsatellites to postulate possible ancestors of cultivated cassava. AFLP markers have been used for quantitative assessment of genetic relationships in a representative sample of the crop's diversity and six wild taxa (Roa et al., 1997). In this study, *Manihot* species *M. esculenta* subsp. *flabellifolia*, *M. trisitis*, and *M. esculenta* were found to be more similar to cassava than its Mexican relative *M. aesculifolia* indicating that cassava might have its origin in these close relatives (Roa et al., 1997). Evidence of introgression into cassava from *M. glaziovii* was also observed in an AFLP evaluation of genetic diversity in a large collection of cassava from the South American center of diversity (Second et al., 1997). In other

studies, markers have also been used to obtain a quantitative assessment of genetic similarity in cassava (Beeching et al., 1993; Second et al., 1997; Elias et al., 2000) and to study the genetic structure of germplasm resistant to disease (Sanchez et al., 1999; Fregene et al., 2000), including the genetic structure and the basis of genetic differentiation of cassava landraces in Africa (Mkumbira et al., 2003).

Germplasm studies with markers have also revealed intravarietal polymorphism, indicating that a variety could also be made up of more than one genotype (Elias et al., 2000). Markers have also been used to study the effect of disease on genetic diversity in cassava. Kizito et al. (2005) reported the loss of rare alleles in areas with high CMD incidence in Uganda. From Genetic diversity studies conducted at CIAT, an SSR diversity kit of 36 SSR markers has been developed (Fregene et al., 2004) and is presently being used in breeding programs for genetic diversity analysis. Other applications include the use of markers for the identification of duplicates in germplasm (Chavarriaga-Aguirre et al., 1999) and the analysis of germplasm from the littoral and Amazonian regions of Brazil.

7.1.2 Marker-Assisted Selection

MAS is best used to investigate traits that are difficult or expensive to evaluate. Markers can be particularly useful where pathogen or pest pressure is low, variable, erratic, or just absent. To be useful for MAS, markers must adequately account for a large proportion of the genetic variance. MAS can increase the efficiency of breeding schemes by identifying plants with the desired trait at a very large stage, enabling much smaller populations to be grown in the field. This in turn reduces the cost of breeding, but more importantly, enables a larger number of families to be produced and analyzed.

Cassava Mosaic Disease

Several variants of the disease exist in Africa and South Asia (Swanson and Harrison, 1994). CMD is the most important disease of Cassava in Africa considering the high evolutionary capacity of the virus and largely accounts for yield losses of over a billion US $ in Africa alone. The discovery of markers linked to a dominant resistance gene (CMD2) has allowed the selection of resistant CMD cassava genotypes in the absence of the pathogen (Akano et al., 2002; Okogbenin et al., 2007). Five markers were initially identified for this gene with the closest being RME1 (a SCAR marker) and NS 158 (a SSR marker) at distances of four and seven cM, respectively. MAS has been carried out using multiple flanking markers involving these two markers with 68% efficiency (Okogbenin et al., 2007). Recent fine mapping of the CMD2 region to identify closely linked markers and positional cloning of the gene have been done with the construction of BAC libraries and characterization and screening of the BAC clones. This resulted in the identification of two SSCP–SNP markers which have been identified between RME1 and CMD2. Current efforts are being directed toward screening for new sources of CMD resistance in African

germplasm. Where different sources of CMD resistance exist, this is indistinguishable phenotypically, and markers are being used to screen identified genotypes in the breeding populations in Africa. Under the Genotyping Support Services activities of the GCP, crosses were carried out using six parents resulting in the development of nine F_1 segregating populations. Four of the populations were selected and phenotypically evaluated and analyzed with 530 SSR markers to identify new sources of CMD resistance different from CMD2. IITA is currently conducting further research to improve the molecular markers currently available for resistance to CMD.

Cassava Green Mite

Two markers were found to be associated with the reaction to CGM (NS1009 and NS346) and have been used in MAS as part of validation studies. In preliminary evaluations, progenies selected with the markers showed good resistance to the pest in East Africa but not in Nigeria where moderate tolerance was observed for CGM based on the markers. The phenotypic differences between both African sub-regions might be due to variation in the CGM pressure which is higher in Umudike, Nigeria where a long dry season of 4–5 months in the humid transition ecology often results in high CGM incidence compared with Tanzania and Uganda where the pressure is less severe.

7.1.3 Introgression of Useful Traits from Wild Relatives

As stated above, wild *Manihot* germplasm offers a wide array of useful genes frequently related to tolerance to biotic and abiotic stresses. The use of wild relatives, however, implies the need to reduce or eliminate a large proportion of the undesirable donor genome. Linkage drag can lengthen the process, making it a daunting task for breeders. The desirable traits can frequently be observed in F_1 inter-specific hybrids indicating dominant or additive gene action of the gene(s) involved. Simulation by Stam and Zeven (1981) indicated that markers could reduce linkage drag and the number of generations required in backcross scheme. Hospital et al. (1992) corroborated this in achieving a reduction of two backcross generations with the use of molecular marker selection. Frisch et al. (1999), through a simulation study, found that use of molecular markers for the introgression of a single target allele saved two to four backcross generations. They inferred that marker-assisted selection had the potential to reach the same level of recurrent parent genome in generation BC_3 as is reached in BC_7 without molecular markers. These studies, however, are based on schemes where the recurrent progenitor is homozygous, a condition yet to be satisfied in the case of cassava.

7.2 *Genome Sequencing of Cassava*

The Joint Department of Energy's Joint Genome Institute (JGI-DOE) initiated a pilot cassava genome sequencing project under its Community Sequencing Program

(CSP; http://www.jgi.doe.gov/CSP), using a whole genome shatgum (WGS) strategy. The draft genome sequence has just recently been completed in 2009 with the support of 454 Life Sciences using the Genome Sequencer FLX platform with long-read GS FLX Titanum chemistry to rapidly generate the DNA sequence data. The cassava sequencing project had other participating institutions such as the International Laboratory for Tropical Agricultural Biotechnology at the Danforth Plant Science Center in St. Louis; USDA laboratory in Fargo, ND; Washington University, St. Louis; University of Chicago; The Institute for Genomic Research (TIGR); Missouri Botanical Garden; the Broad Institute; and Ohio State University.

More than 61 million reads were generated and assembled in a draft genome that contains an estimated 95% of cassava genes. The annotated draft genome sequence is available at DOE JGI's phytozome website (www.phytozome.net/cassava). It is one of the first large genome projects to primarily use 454 Life Sciences long-read sequencing platform which enabled both improved quality of the sequence and its rapid generation.

The new development in cassava of a genome sequence database will open a new vista to address intractable knowledge gaps in cassava genetics. Sequencing the cassava genome is expected to result in a better understanding of starch and protein biosynthesis, root storage, and stress controls, and enable crop improvements, while shedding light on such mechanisms shared by other important related plants, including the rubber tree and castor bean. The genome organization of cassava, its evolution and the mechanism involved, will also be properly elucidated. The sequencing information is expected to increase knowledge on the molecular interactions mediating growth and development processes in cassava. Functional genomics research will be further accelerated by the wealth of information to be made available. The first application will be in the development of SNPs for mapping with huge potential for the genetic analysis of complex traits. Current efforts are underway to identify a large collection of high-quality SNPs which will be integrated with genome sequence and annotation in order to make a comprehensive genomic resource available to the cassava community.

7.3 Mutation Breeding, TILLING, and ECOTILLING

In addition to the strategic pre-breeding work described above, there are other alternatives for the incorporation of high-value traits in commercial varieties of cassava. Breeders have used chemical products or irradiation such as gamma rays to induce mutations and generate genetic variability with relative success, particularly in the 1950s and 1960s (Maluszynski et al., 2001; Ahloowalia et al., 2004). Mutation breeding has a few drawbacks. Events are totally random, usually recessive in nature and usually appear as chimeras. Therefore, thousands of genotypes need to be evaluated before a useful mutation in the desired gene can be found. With the advent of molecular biology tools, an interesting system was developed to overcome some of the limitations of mutation breeding. DNA TILLING (for *Targeting Induced Local Lesions in Genome*) has been successfully used in different plant species (McCallum

et al., 2000; Perry et al., 2003; Till et al., 2003). Sexual seeds are mutagenized and, to avoid ambiguities caused by chimeras in the first-generation plants ($\mathbf{M_1}$), they are self-pollinated. The resulting plants ($\mathbf{M_2}$) are then evaluated while DNA is extracted from them. For screening purposes, DNAs are pooled eightfold to maximize the efficiency of mutation detection (description of the TILLING method adapted from Till et al., 2003).

CIAT participated in a project led by Universidad Nacional de Colombia and supported by the IAEA (International Atomic Energy Agency). About 4,000 seeds from six different cassava clones were irradiated with gamma rays (using a Cobalt 60 source with a dosage level of 200 Gy) or with fast neutrons. As many as 5,000 $\mathbf{M_2}$ seeds, from about 140 different $\mathbf{M_1}$ plants have been obtained. Several mutations were identified in the $\mathbf{M_2}$ generation but only the two most interesting will be described, together with a future use of ECOTILLING.

7.3.1 Small Granule – High Amylose Starch Mutation

This mutation has been reported and described already in the literature (Ceballos et al., 2008). The initial discovery was facilitated by the unusual starch granule size which is about one-third the normal size for cassava. Figure 2.7 illustrates how different the starch granules of this mutation are, not only in relation to size but also regarding their surface. Normal cassava starch granules have a very smooth surface. However, the surface of the granules in the mutated genotype is very irregular and rough (Fig. 2.7).

The small size and irregular surface of the starch granule would make this mutation ideal for ethanol production because it facilitates the activity of starch-degrading enzymes (Lehman and Robin, 2007; Thu et al., 2007). The

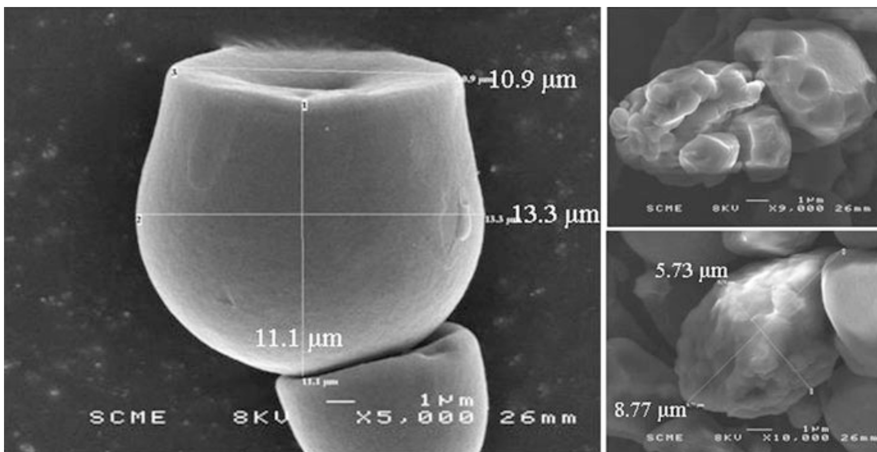

Fig. 2.7 Scanning electron microscope photographs comparing a wild-type starch granule (*left* photograph) and the small granule starch mutant (*right*)

production of bio-ethanol from starch requires its degradation (liquefaction and saccharification) prior to the initiation of fermentation. However, the mutation also has a biochemical abnormality, with almost twice the normal levels of amylose (Ceballos et al., 2008; Sánchez et al., 2009). Amylose is more difficult to degrade (Sharma et al., 2007). It is not possible at this time to say if the morphology of the starch granule will be more prevalent than the biochemical characteristics (high amylose) of the mutation in the process of starch hydrolysis. Only when enough roots can be produced will the analysis be made.

The higher-than-normal level of amylose in these mutations has important commercial implications. Increased amylose levels leads to slowly digestible and resistant starches (Jobling, 2004; Lehman and Robin, 2007), which have distinctive advantage in health, particularly in diabetes management. Slowly digestible starches may influence satiety and help control overweight problems and have also been linked to improved mental performances (Lehman and Robin, 2007). In addition, high amylose starches in different crops offer advantages in the production of sweets, adhesives, corrugated boards and in the paper industry, and reduces the uptake of fat in certain fried products (Jobling, 2004). Very high levels of amylose result in "resistant" starches. Maize starches with more than 50% and up to 90% amylose can be produced commercially. Resistant starches cannot be digested but they are fermented in the large intestine, resulting in the production of butyrate that has been found to be beneficial to colon health (Jobling, 2004).

7.3.2 Tolerance to PPD

A second high-value trait that was identified in the mutagenized population was tolerance to post-harvest physiological deterioration (PPD). Two genotypes were tentatively characterized as PPD tolerant and one of them (2G15-1) proved to have quite good levels of tolerance (Fig. 2.8). As quantification of the reaction to PPD requires many commercial-size roots, these genotypes were first multiplied to produce the number of roots required. In addition, several other genotypes considered as potential sources of tolerance to PPD were evaluated. Relevant results from an evaluation conducted in April–May 2009 are presented in Fig. 2.8. Three genotypes did not show any symptoms of PPD even after 40 days of storage (GM 905-66, AM 206-5, and WAXY 4). The tolerance to PPD in GM 905-66 can be explained by the antioxidant properties of the high carotenoids concentration in the roots of this genotype. AM 206-5 and Waxy-4 are partially inbred genotypes related to each other and have in common the waxy starch mutation. The tolerance to PPD in these two genotypes is not considered to be a pleiotropic effect of the waxy starch mutation but most likely it is due to a gene linked to the waxy starch locus. The mutagenized genotype (2G15-1), and one of the backcrosses from the inter-specific cross with *M. walkerae* (BC289-30), also showed low values of PPD. This is a remarkable finding where many different sources of tolerance to PPD seem to have been discovered and highlights the importance of aggressive and systematic screening of germplasm for different traits (Morante et al., 2010).

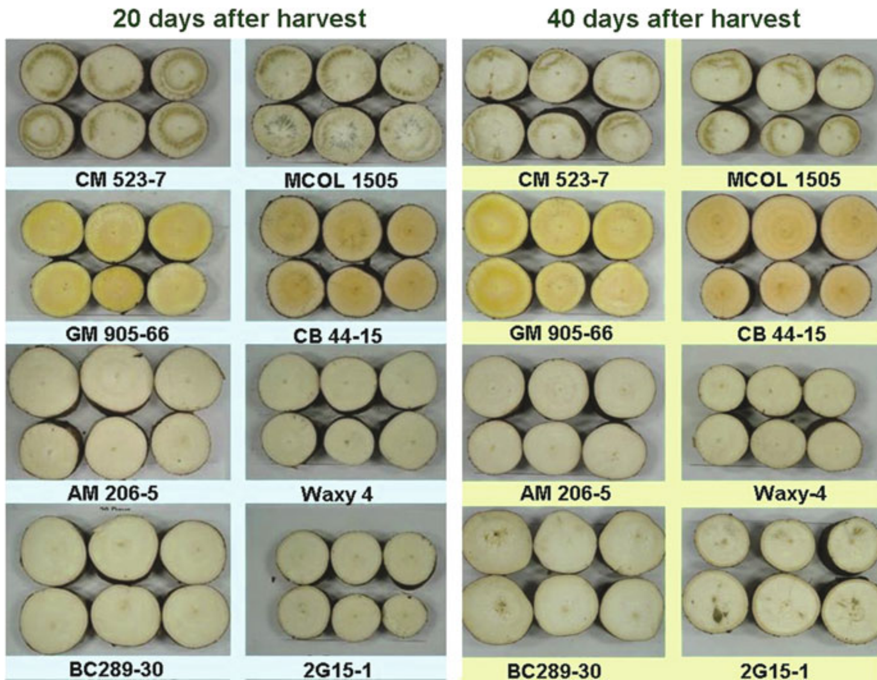

Fig. 2.8 Reaction to PPD 20 and 40 days after harvest. *Top* photographs show reaction in the susceptible checks (CM 523-7 and MCOL 1505). *Second row* shows the reaction in high-carotene clones (GM 909-66 and CB 44-15). The *third row* shows photographs of two waxy starch genotypes (AM 206-5 and Waxy-4). Genotype BC289-30 (*last row*) is a backcross from an inter-specific cross with *M. walerae*. Finally genotype 2G15-1 is from the mutagenized population

7.4 Genetic Transformation

Genetic transformation protocols are available and have been used successfully for the incorporation of different genes but mostly in just one cultivar. Insect and disease resistance, tolerance to herbicides, manipulation of starch content and quality, enhanced nutritional quality, reduction of cyanogenic potential, and enhanced leaf retention are among the traits incorporated (Munyikwa et al., 1997; Taylor et al., 2004). Additional work needs to be done to increase the efficiency of genetic transformation to a wider range of elite germplasm, to overcome the regulatory issues (particularly where it is used for food), and to improve our capacity to regulate the expression of the transgene.

8 Commercial Propagation and Production of Planting Material

Commercial propagation of cassava is by stem cuttings (Fig. 2.1). Different rapid multiplication methods have been developed ranging from the use of microstakes

to tissue culture techniques. One-node microstakes have been used successfully for rapid multiplication schemes. For these conditions, irrigation is highly desirable. The size of the stake to be used depends on the moisture in the soil at planting time (adequate rains or access to irrigation would allow shorter stakes), storage period of the planting material (the shorter the period the shorter the stakes can be), varietal characteristics (some clones have better sprouting capacity than others), and overall physiological and nutritional quality of the planting material. Alternatively, 2-node microstakes can be grown at high density in moist chambers where they sprout. The resulting shoots (15–20 cm long) are harvested about every 3 weeks and their lower section immersed in water for them to produce roots and then transferred to soil. The facilities needed for an efficient hardening of these small plants are not sophisticated. Availability of a cool or fresh environment in shade (screen houses are ideal) where extreme temperature can be avoided and adequate humidity provided is required. When the plantlets are about 2-month old they can be transplanted to the field.

Tissue culture approaches (pre-existing meristems and somatic embryogenesis) have been used for rapid multiplication of cassava (Fregene et al., 2002). The common products of these two protocols are the small plants grown in vitro, which would require a hardening process described by Segovia et al. (2002). The critical period for the hardening process, after the plants are taken from the in vitro condition, lasts for 1 week. Hardening (using facilities described above) starts with the transfer of the small plant from the in vitro condition to a container (plastic bags or seedling trays) with a mixture of soil and sand that has hopefully been previously sterilized with high temperature (100°C). A high moisture condition is required for the first week after transplanting. Different alternatives have been proposed ranging from the use of moist chambers to the use of plastic disposable coffee cups with small holes at the base and placed inverted over the plant so it is completely covered (Fregene et al., 2002; Segovia et al., 2002). After the first week the plants are gradually exposed to conditions with lower humidity and higher temperature and in 2 months can be transplanted to the field.

8.1 Field Management Requirements

In general, there is no production of planting material for cassava independent from the commercial fields for root production. It is recommended that an area in the production field is assigned as source of new planting material for next cycle. This area (about 10% of the total area) is specially managed following the same criteria used for specially designed nurseries. From this point on, the distinction between nurseries or sections of commercial fields will not be made.

When a new variety is identified, or a clean planting material (through meristem culture) of an old variety is produced, specific multiplication nurseries are planted. In this case the primary product is the planting material rather than the roots. This would be particularly relevant for planting material that is certified to be clean of the

viral diseases present in Africa or frogskin in the Americas. When planting material is certified to be disease free, special efforts are made to prevent their re-infection, avoiding the contact of the new, clean crop with the insect vectors of the diseases (e.g., white flies). Although the use of insecticides may be considered, it is not 100% effective. White flies are prevalent in lowland environments and are seldom present beyond 1,800 m above sea level. Crop rotations are important in the case of fields that have been affected by root rots because the inoculum would remain in the soil and infection of a new crop would be likely to occur.

The production of planting material needs to be properly managed to avoid lack or excess of water (irrigation, drainages, etc.), prevent attacks by pests and diseases, and provide adequate soil fertility. The ultimate objective is to have cassava plants (10–18 months of age) that have stems with optimum sanitary and physiological conditions, properly nourished and irrigated. Adequate soil fertility is important because it maximizes quick sprouting in the next generation, with vigorous and healthy plants and a uniform plant stand.

8.2 Monitoring Nurseries

Production of planting material begins with materials that are free from pests and diseases. The producer will first verify, before planting, the absence of contaminants and volunteers from previous seasons, as well as the availability of irrigation and drainages in the field. Other inspections would take place during the development of the crop to monitor crop establishment 1 month after planting (m.a.p.); then at least every other month until harvest (typically 10–12 m.a.p). In certain conditions, planting material is harvested from older plants (i.e., 18 months). This is the case when cassava is grown at high altitude (>1,500 m.a.s.l.), in conditions with short rainy period or cold winters (in latitudes >20°). In these conditions, the visits can be spread.

During the visits the whole nursery should be screened for potential sanitary problems. Plants attacked by diseases or pests should be eliminated. Proper availability of nutrients and water should be guaranteed. Weed control should be carefully done in the first 3 months of the crop. The varietal purity of the nursery can be checked 3–5 m.a.p. The most distinctive descriptors of cassava (color and length of the petiole, shape of leaf lobules, presence of pubescence in the shoot, and color of the stem) allow an easy identification of off-type plants that can then be eliminated. For some diseases such as CBSD or frogskin disease, it is necessary to inspect the roots since they may offer the only source of symptoms that allow the identification of infected plants (Calvert and Thresh, 2002).

It is desirable that 5–7 m.a.p. an official inspection of the plant multiplication nursery is made. Up to 1% of off-type plants can be accepted. For CBB and SED, up to 2% of plants with symptoms is acceptable, provided they are discarded. Depending on disease pressure and the variety being multiplied, CMD and CBSV acceptable levels at that time can range from 0 to 5%.

8.3 Harvest and Storage of Planting Material

Any part of the cassava stem can be used for propagation purposes. However, the thickness of the stem used for cuttings should not be less than one half the diameter of the thickest part of the stem of the particular variety being used. Cuttings from green stems (slightly lignified) will germinate, but they are susceptible to attack by pathogens and insects and tend to dehydrate rapidly. Cuttings from stems older than 18 months are too lignified, contain small amounts of food reserves, and have reduced viability, delayed and slow sprouting, and/or poor vigor. It is recommended that planting material be taken from stems ranging from 8 to 18 months of age. The younger the plant the more lignified should be the part of the stem selected for the cutting. One practical way of knowing whether a stem is sufficiently mature is to determine the relationship between the diameter of the pith and the stem cutting in a transversal cut. If the diameter of the pith is equal to or less than 50% of the diameter of the stem, it is sufficiently mature to be used for planting (Fig. 2.9).

Fig. 2.9 Cross section of a cassava stem showing a relationship between diameter of the pith and total diameter lower than 50% and the latex (symptom of good quality) exudation

It is preferable to maintain the planting materials standing in the nurseries, rather than harvest them too early and then stored for 2–3 months. Cassava can be kept in the field because there is no physiological maturity for the plant. The young branches are cut and discarded and the main stems, offering the quality standards described above, are cut and tied together in bunches of about 50 stems. On average, each stem yields 5–7 stakes. However, depending on age and varietal characteristics

stems can yield from 3 to 12 stakes. There is no dormancy period and stakes can be planted immediately after harvest, when even thin (green) stems could sprout and produce a vigorous plant. Each bunch is identified with a plastic tag with the name of the variety, date, and location of harvest clearly written using permanent ink markers or graphite pencils. At harvest stems are screened for the presence of (damage by) insects, particularly stem borers. If relevant for the region, roots should be checked for FSD or CBSD symptoms.

Stems can be stored as they have been harvested from the field (long stems about 1–2 m long) or else, cut to the proper size for planting (about 20 cm long). To prevent dehydration during storage, however, it is recommended that the stems be cut into planting stakes just prior to their planting. Bunches of long stems are placed vertically on the ground, in shade (usually a tree, Fig. 2.1b) and in an upward position (the apical portion of the stem up). Sometimes farmers cover the stem bunches with remaining foliage of the crop to further reduce dehydration of the stems. The storage area should be shaded and offer high, but not excessive relative humidity (about 80%) and moderate temperatures (20–30°C).

It is recommended that stems are sprayed or submerged in a solution with an insecticide (dimetoathe or malathion) and a fungicide (copper oxychloride). The standard practice is to prepare a solution with 1 l of water, 5 g of Malathion W.P. (4%), and 2 g copper oxychloride (Vitigran 35%). Alternatively the insecticide can be applied as 1.5 cc Malathion (E.C. 57%) or 1 cc dimetoathe (E.C. 40%). This solution has proved to be useful, relatively inexpensive and its use is also suggested for the planting material just prior to planting. In the case of stems that are stored for long periods of time, therefore, they are treated twice with the same solution (immediately after the harvest of the stem and just before planting). Operators should wear protective gloves, aprons, glasses, and breathing equipment.

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Chapter 3
Sweet Potato

Vincent Lebot

1 Introduction

Ipomoea batatas (L.) Lam. (Convolvulaceae, Dicotyledons) produces storage roots rich in carbohydrates and β-carotene, a precursor of vitamin A, and its leaves are rich in proteins. The roots also contain vitamins C, B complex, and E as well as potassium, calcium, and iron. Purple-fleshed ones contain antioxidants such as anthocyanins. In world crop statistics, the sweet potato is ranked seventh, just after cassava, with an annual production around 9 Mt and a cultivated area of 110 Mha (FAO, 2009). In most developing countries, it is a smallholder crop tolerant of a wide range of edaphic and climatic conditions and grown with limited inputs. It is also quite tolerant of cold and being cultivated at altitudes as high as 2,500 m, it has become the staple of communities living in the highlands of Uganda, Rwanda, and Burundi in Eastern Africa and in Papua New Guinea where annual per capita fresh roots consumption is over 150 kg. Asia is the largest producing region and China alone accounts for almost 60% of world production. In the southern provinces of Sichuan and Shandong, sweet potato is a major source of raw material for food processing industries (Fuglie and Hermann, 2004). Nearly half of the Chinese production is for animal feed (roots and leaves), with the remainder primarily used for human consumption, either as fresh (boiled roots) or processed products (noodles and alcohol). In some temperate countries such as the United States, Japan, and New Zealand, the sweet potato is a high-quality luxury vegetable.

The Convolvulaceae family is composed of herbaceous, woody, or climbing species, well distributed throughout the temperate and tropical latitudes in a wide range of habitats, including sand dunes. Convolvulaceae species have alternate and simple leaves. Their flowers are bisexual with five free sepals, five fused petals, and five stamens fused at the base of the corolla tube. The fruit is a dehiscent capsule. The characteristics of the ovary, styles, and stigmas are used to differentiate up to

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10 tribes of genera (Heywood, 1985). The genus *Ipomoea* is composed of approximately 400 species, mostly annual or perennial herbaceous vines with a few erect shrubs found in the tropics. Some African and Australian *Ipomoea* spp. are collected from the wild as emergency foods. In Southeast Asia and Melanesia, *I. aquatica* is cultivated and eaten as spinach for its delicious leaf tops rich in proteins.

I. batatas is a vine and perennial herb, treated as an annual when cultivated (Fig. 3.1). It has trailing or twining stems measuring 1–5 m long and containing latex. These stems are thin (3–10 mm in diameter) with internodes varying from 2 to 20 cm long, and are prostrate or ascending, often twining, glabrous, or pubescent, light green to dark purple in color, with a length from 1 to 6 m depending on genotype. Sweet potato produces 5–10 storage roots per plant by the thickening of adventitious roots. These storage roots present a cellular arrangement identical to a primary root with a radial vascular bundle.

Fig. 3.1 Crossing block

The sweet potato is a versatile plant offering various products and diverse uses ranging from consumption of fresh leaves or roots to processing into starch, flour, noodles, natural colorants, candy, alcohol, and animal feed. Fried chips are homemade in many tropical countries and are available in small snack quantities in local markets. Most roots are usually cooked (boiled or baked) and their quality after cooking is a complex combination of color, flavor, texture, and fiber content.

2 Origin and Domestication

2.1 Species Involved and Place

The oldest remains have been radiocarbon dated to 8,000 years old in the caves of the Chilca Canyon in Peru (Woolfe, 1992). An analysis of their starch granules indicates that, although they were significantly smaller in size compared to the modern cultivars, they are definitely from the species *I. batatas* (Perry, 2002). However,

based on the number of related species and the analysis of their morphological variation, the geographical center of origin of *I. batatas* has been thought to be between the Yucatan Peninsula in Mexico and the Orinoco River in Venezuela. True wild populations of *I. batatas* do exist in Central America. The greatest variation is probably found from about Oaxaca south to Costa Rica. Panama does not seem that variable and may represent a bottleneck in the diversity of the species (Austin, 1977, 1987).

Cultivated forms of sweet potato have $2n = 6x = 90$ chromosomes. The sweet potato and other relatives are grouped in section *Batatas* which includes 16 taxa (McDonald and Austin, 1990). Crossability between various related species of the section *Batatas* has been demonstrated (Diáz et al., 1996). Morphological analysis of the related species indicates that *I trifida* is the closest wild relative to the sweet potato but *I. tabascana* is also morphologically very close (Austin, 1977, 1987). Cross pollinations between wild species, followed by selection and domestication of interesting genotypes, could have produced the hexaploid species *I. batatas.* Austin (1987) suggests that natural hybridization between *I. trifida* and *I triloba* resulted in the generation of the wild ancestors of the present *I. batatas*.

Autopolyploidy of *I. trifida* and the occurrence of $2n$ gametes might also have been involved in the origin of *I. batatas*. It is, therefore, possible that *I. batatas* would have been generated by natural hybridization between several wild species rather than descending from a single ancestor. However, Austin (1987) also thinks that the so-called *I. trifida* hexaploids are in fact *I. batatas* escaped from cultivation and believes that wild *I. trifida* tetraploids are the result of crosses between *I. trifida* diploids and *I. batatas* hexaploids.

Molecular marker (RFLP, RAPD, and SSR) studies confirm the relationship of *I. batatas* with *I. trifida* (Jarret et al., 1992; Jarret and Austin, 1994; Buteler et al., 1999). Restriction analysis of chloroplast DNA indicates that *I. trifida* is probably one of the ancestors of *I. batatas* (Huang and Sun, 2000). The genetic relationship between these two species and *I. tabascana*, studied using the *exon* and *intron* β-amylase gene sequences, indicates that *I. batatas* is closer to *I. trifida* than it is to *I. tabascana* (Rajapakse et al., 2004). Molecular work based on microsatellite markers (SSRs) supports the hypothesis that sweet potato is an allo-auto-hexaploid with two non-homologous genomes (B1B1B2B2B2B2) with tetradisomic inheritance (Zhang et al., 2001).

Fluorescence in situ hybridization (FISH) confirms that among the various diploid species studied, *I. trifida* appears to be the closest relative to *I. batatas* and chromosome organization indicates that *I. batatas* is more closely related to *I. trifida* than to *I. tabascana*. FISH investigations conclude that more than one progenitor is involved in the phylogeny of the hexaploid sweet potato. An allopolyploid hypothesis is retained with a composition made up of at least two different genomes, one from a closely related species and one from a distant relative (Srisuwan et al., 2006).

As the natural distribution of *I. trifida* is between Peru and Mexico, the domestication of the sweet potato occurred within this vast geographical region. The remaining question is: On which side of the Panama isthmus did domestication occur? Central America is a region rich in related species, with great diversity

within *I. trifida* and *I. batatas*, and would appear to be the primary center of origin. It is unknown, however, if two distinct genepools exist on each side of the Panama isthmus and if they correspond to two independent domestication processes. It is also unclear, if southern American genotypes correspond to a second diversification resulting from domesticated sweet potatoes introduced from Central America by migrating populations or if they have been domesticated from the local wild genepool. Peru has more morphological variation within *I. batatas* cultivars and this is not surprising given the zeal with which Peruvian villagers latch onto each minor phenotypic change and apply a new common name (Austin, personal communication, 2009).

2.2 History of Crop

The sweet potato was widely cultivated throughout Central and South America before the first European contact. Columbus introduced the plant to Europe on his return from his first voyage in 1492. In the sixteenth century, with the exploration and colonization of Africa, Portuguese explorers transferred clones collected around Hispaniola directly to Africa, India, and the East Indies. Spanish explorers carried the plant, probably in the form of storage roots, in easterly and westerly directions. By the mid-nineteenth century, the plant was grown from Zanzibar to Egypt. Fukiense sailors took the storage roots not only from the Island of Luzon in the Philippines to Fukien in South China in 1594, but also from Fukien to Taiwan and to the Okinawa Islands and Japan in 1674 (Yen, 1982; Zhang et al., 2009a). The sweet potato was grown by European settlers in Virginia as early as 1648, in Carolina by 1723, and in New England by 1764. In the latter part of the eighteenth century, the sweet potato was cultivated throughout the southern states (Edmond and Ammerman, 1971; Smith et al., 2009).

The origin of the crop in the Pacific has been a long standing enigma. Carbonized remains of sweet potato from Easter Island and the Island of Hawaii predate the first European contacts. It could have been distributed to New Zealand by 1150–1250 AD where it was the staple of the Maori people when Captain Cook arrived. It is therefore assumed that the distribution of the sweet potato throughout the Pacific Islands was done by Polynesians. However, Barrau (1957) was the first to propose three possible routes for its introduction to the Pacific, the so-called *kumara*, *batatas*, and *kamote* lines. The first one corresponds to a Polynesian introduction from Peru during prehistoric times, the second from the Portuguese via the East Indies, and the third thanks to the Spanish galleons voyaging between Acapulco and Manila (Yen, 1974, 1982). Surprisingly, based on palynological and soil depositional evidence, it has been suggested that the sweet potato could have arrived in the New Guinea Highlands as early as 1200 BP, but the radiocarbon dated remains might also have been misidentified for tubers of local yam species (*Dioscorea* spp.) (Ballard et al., 2005). DNA analysis, however, shows that Papua New Guinea cultivars have significantly diverged from their ancestors in South America, indicating their ancient presence on the big Island of New Guinea. There is little relationship between the

Peru-Ecuador germplasm and that of Oceania, suggesting that this region might not be the origin of the Pacific material. Human dispersal from Mesoamerica, the *kamote* line, is the most probable source of introduction (Zhang et al., 1998, 2000; Rossel et al., 2001).

3 Varietal Groups

There are two botanical groups in *I. batatas*. Living populations of wild *I. batatas* var. *apiculata* have been described in the state of Veracruz, Mexico. This botanical variety is distinguished from *I. batatas* by indehiscent capsules. McDonald and Austin (1990) noted that the fruits of all cultivated forms of *I. batatas* var. *batatas* they examined were dehiscent but that they were indehiscent in all *I. batatas* var. *apiculata*. The indehiscent character seems to be important for seed dispersal. Var. *apiculata* capsules have been floated in water for several days without opening and sinking. Their seeds are apparently still viable after being in salt water for a week. It is likely that other wild populations of *I. batatas* exist, in addition to *I. batatas* var. *apiculata*, and that taxonomists would identify new botanical varieties if opportunities occurred.

All attempts made to structure the existing morphological variation found within cultivated *I. batatas* into meaningful varietal groups have failed because there is a continuum of variation. Sweet potato cultivars vary in the color of the skin, and in the color and chemical composition of the flesh of their storage roots. These roots can be fusiform, globular, round, or ovate with smooth, ridged, or rough surface. The root skin color varies from white to yellow, orange, red, purple, or brown and the flesh color may be white, yellow, orange, reddish, or purple. Their leaves are highly variable, sometimes on the same plant, depending on their age. They are simple with laminas mostly ovate but they can be entire to deeply digitately lobed, with their base usually cordate. Their tips can be acute or obtuse and the leaves can be glabrous or with variable pubescence. Their color is also highly variable, from light green to deep purple, sometimes with purple stain at their base, or with green or purple veins beneath.

From a marketing point of view, however, there are three broad groups of cultivars: these are those intended for processing (traditional or industrial) which are often very high in dry matter and starch content and have a white flesh; those for the fresh (table) roots market for daily consumption in developing countries, with high dry matter content and low sugars; those for the US market, the "moist" North American types of cultivars, with low dry matter content, high sugar and high carotene contents. Of course, the quality standards are not the same for the three groups. In the United States, varieties are classified into two types: dry-flesh and moist-flesh and surprisingly this classification is not based on the moisture content of the root but rather on the characteristics of the solids in cooked roots (Smith et al., 2009). Moist-flesh varieties convert starch into sugars more efficiently when cooked and are sweeter than the dry-flesh varieties. Variability in sugars among varieties is very high and has been recorded as high as 38.3% of DM in American

cultivars. Although they have not been thoroughly investigated, the hundreds of cultivars found in Papua New Guinea and Island Melanesia, are low in sugars, allowing an important daily consumption. A local variety of Vanuatu presents total sugar content as low as 1.49% of DM (Lebot et al., 2009). Finally, some cultivars have more specialized uses, either for the human consumption of fresh leaves or for animal feed, or for the production of colorants (anthocyanins) for the food industry (Fig. 3.2).

4 Genetic Resources

The two major approaches to sweet potato germplasm conservation are field collections and in vitro gene banks. The maintenance of ex situ collections is laborious and frequently suffers losses due to biotic and abiotic stresses. One of the problems faced by curators while describing accessions is that these can exhibit very similar or almost identical morphotypes and yet be genetically distant, while they can also present different morphotypes although they are clones of each other. Sweet potato is also particularly prone to somatic mutations and it is not uncommon for curators to observe that some of their accessions may change over the propagation cycles. This situation renders the morphological description process insufficient for accurate classification and for structuring the diversity within the species. Although molecular markers are increasingly used, their costs and practical usefulness for curators are the subject of debate. New approaches are needed, especially those focusing on chemotypes and the characterization of chemical composition (i.e., starch, proteins, sugars, carotenoids, anthocyanins) for specific uses (i.e., processing, feed, colorants, fresh roots, or leaves).

The CIP (*Centro Internacional de la Papa*) germplasm collection contains no less than 4,234 accessions of *I. batatas* originating from 57 different countries, 1,212 accessions from 70 wild and related *Ipomoea* species, 198 advanced cultivars under development, and 1,746 breeding lines totaling 7,477 accessions from 71 species (Schafleitner, 2009; Tay, 2009).

Several wild *Ipomoea* spp. may contribute genes such as those for resistance to pests and diseases. Resistances to the weevils (*Cylas* spp.), scab (*Elsinoe batatas*), and black rot (*Ceratocystis fimbriata*) have been found in *I. trifida.* The wild *Ipomoea* species' adaptation to difficult environments also makes them attractive but their practical use is often constrained by sexual barriers to crossability with *I. batatas* due to different ploidy levels. The crossability between two different species depends on the balance of chromosome numbers (see Table 3.1) and on the homology between the two genomes. It is less effective between hexaploid *I. batatas* and diploid and tetraploid *I. trifida* compared with hexaploid *I. trifida*.

New Guinea Island is the second largest center of genetic diversity in the world. The number of varieties grown has been estimated at some 5,000, of which about 1,600 are maintained in two ex situ collections, one in Aiyura for the highlands and one in Keravat for the lowlands (NARI, 2003). There are several thousands

Fig. 3.2 Types of sweet potato and landraces

Table 3.1 The species and natural hybrids of the section *Batatas* (after Austin, 1977, 1987)

Species	Ploidy	Geographical range
Ipomoea batatas (L.) Lam.	6*x*	American tropics
I. cyanchifolia Meisner	2*x*	Brazil
I. gracilis R. Brown	4*x*	Australia
I. × *grandiflora* (Dammer) O'Donnell	2*x*	Southeast South America
I. lacunosa L.	2*x*	Eastern USA
I. × *leucantha* Jacquin	2*x*	Widespread in the Tropics
I. littoralis Blume	4*x*	Pacific Islands, Mexico
I. peruviana O'Donnell	2*x*	Peru
I. ramosissima (Poir.) Choisy	2*x*	Central and South America
I. tabascana MacDonald & Austin	4*x*	Mexico
I. tenuissima Choisy	2*x*	Caribbean Islands
I. tiliacea (Willd.) Choisy	4*x*	Caribbean
I. Trichocarpa Elliott	2*x*	North and South America
I. trifida (HB & K) G. Don.	2*x*, 4*x*, 6*x*	Central and South America, Mexico
I. triloba L.	2*x*	Caribbean and Central America
I. umbraticola House	2*x*	Costa Rica, Mexico, Nicaragua

of other accessions conserved in collections worldwide and the major ones are located in AVRDC (Taiwan), China, Indonesia, Vietnam, the Philippines, and the United States. Morphological characterization is routinely conducted with internationally standardized morphological descriptors (IPGRI type). Most collections are, however, poorly tapped by breeders because of the lack of useful information on accessions. A major task is to eliminate viruses to facilitate international exchange. The reduction of collection size is another major objective for curators. The identification of duplicates within the Peruvian sweet potato germplasm collection has permitted its reduction from 1,929 accessions to 909 (Mok et al., 1998).

Molecular markers used to study the diversity existing within the CIP germplasm collection indicate that Central America presents the highest total number of alleles, number of region-specific alleles, and greatest heterozygosity, while the Peru–Ecuador region presents the lowest values on all three counts. This information is used to develop core collections in order to assemble maximum allelic diversity into a minimum number of accessions. These results also support the hypothesis of Central America as the primary center of diversity and the most likely center of origin of the sweet potato (Gichuki et al., 2003). The lower molecular diversity in the Peru–Ecuador region suggests human distribution from the primary center of origin and suggests that the Peru–Ecuador region should be considered as a secondary center (Zhang et al., 2001). However, a study conducted with 25 SSR markers to characterize and assess the diversity of a subset of the CIP germplasm (540 accessions) has shown that within *I. batatas* many genepools can be observed and that among them a considerable genetic distance exists (Grüneberg et al., 2007). This result would tend to favor the case for several independent domestications in different geographical locations.

On-farm conservation strategy has been identified as a complementary approach to compensate for the losses inevitable in the ex situ collections. Since smallholders throughout the tropics tend to grow different cultivars together, this might represent a sound basis for developing an in situ strategy (Rao and Campilan, 2002).

The research needs scoring the highest priorities in most developing countries are still related to germplasm conservation. They involve the control of viruses and improvement in the availability and quality of planting material (Fuglie, 2007). CIP is distributing internationally elite cultivars in vitro or as true seeds and an important number of cultivars are available for testing. The distribution of true botanical seeds has practical advantages compared to in vitro clones, as it presents fewer quarantine problems and reduced distribution costs and risks. It also permits the rapid distribution of allelic diversity on a broad scale and selection for adaptation to local conditions.

5 Major Breeding Achievements

Two major difficulties have been encountered in the development of scientific breeding methods. Firstly, because of its polyploidy, the sweet potato is a difficult species for Mendelian genetics and segregation ratios are quite complex unless a single dominant allele is involved. Second, the sweet potato is almost always self-incompatible. Furthermore, sterility problems are common, probably as a result of high polyploidy. The practical consequences of self-incompatibility and sterility have been recognized by different researchers in different countries (Martin, 1988; Wilson et al., 1989; Mihovilovich et al., 2000): it is very difficult to produce seeds by self-pollination and hand pollination cannot produce more than four seeds and often only one or two per capsule. However, despite these practical constraints, one of the first modern breeding programs was implemented by Louisiana State University in the 1920s. Since the 1950s, breeding programs have been implemented, mostly in temperate climates, in the United States (South Carolina), China, Japan, and the subtropical Taiwan (AVRDC).

5.1 Cultivars from Around the World

In the United States, successes in breeding were achieved in the years following the Second World War. The leading variety, *Beauregard* (copper-rose skin and yellow flesh), is a polycross selection released by the Louisiana State University and occupies nowadays approximately 65% of the area planted annually (Smith et al., 2009).

In China, *Xushu 18* (purple skin and white-yellow flesh) was bred by the Institute of Agriculture of Xuzhou in 1972 and is today the leading variety with an annual area of 1.5 Mha. *Nanshu 88* (pale red skin and pale yellow flesh) was released in 1988 by the Nanchong Institute of Agriculture, in Sichuan, and is mainly used for food as fresh tuber and feed processing (Zhang et al., 2009a).

In Japan, the leading table variety, *Beniazuma* (deep red skin and yellow flesh), was released by the National Institute of Crop Science in 1981. Cultivars used for starch production have been developed by the Kyushu National Agricultural Experiment Station but there is still a need to improve starch content and properties to expand uses. New cultivars with high anthocyanin content and high yields are being developed. *Ayamurasaki*, for example, has elongated, fusiform, and uniform good shape roots with dark purple skin and a deep purple flesh. This cultivar is now recommended as a superior source for the production of foods with health benefits (antioxidants) and some foods and beverages already use the anthocyanin pigments (Suda et al., 2003).

In Uruguay, polycross population breeding combined with recurrent selection started in 1988 and allowed substantial progress for most traits and cultivars adapted to diverse regions and uses were released in 2002. Nowadays, *INIA Arapey* (purple skin, orange flesh) is grown over 75% of the area and spreading to Brazil and Argentina.

In Papua New Guinea, the National Agricultural Research Institute released, in 2004, varieties resistant to scab disease (*E. batatas*) with high dry matter*: K9, B11, Nuguria 5*, and *SI 278*.

Since the early 1980s thanks to the leadership of CIP, various programs are now being conducted in the tropics and are releasing improved varieties. Successful breeding has been achieved in Colombia, India, the Philippines, Indonesia, and Vietnam. Many improvement programs in Africa (especially in Burundi, Mozambique, Nigeria, Rwanda, Uganda) have received CIP material in tissue culture and true seed form. When tested in local environmental conditions, some of them outperform local varieties and are released to farmers.

5.2 Industry and Fresh Market

A fairly common difficulty for breeding programs is to satisfy the requirements of both the industry and the fresh market. An industrial variety, for example, must have a high dry matter yield potential (t/ha) with a high dry matter content (% of fresh weight). For human fresh consumption, flesh color and good cooking quality are important traits. High carotene varieties are preferable for areas where fresh yellow-fleshed roots are popular for human consumption (Africa and Melanesia). For the feed industry, high-protein accessions need to be identified. Considering the regional variation in needs for new sweet potato varieties, it is important to decentralize germplasm evaluation and breeding work. CIP has developed a fully decentralized network for sweet potato breeding encouraging strong collaboration with partners in national programs in order to select locally for resistance to diseases, pests, and abiotic stresses and for market needs. In fact, the quarantine problems associated with the movement of germplasm are such that centralized breeding would not achieve much in terms of improved variety dissemination (Mok et al., 1998).

6 Current Goals of Breeding

The sweet potato is used to produce various products: fresh food, processed starch, alcohol, colorants, and foliage for animal feed. Throughout the world breeders are attempting to improve traits sought by various markets and the challenge is to improve different chemical compositions (i.e., starch, cellulose, sugars, proteins, or anthocyanins) for different uses. High and stable yields and virus and weevils resistance are priorities worldwide. All farmers are looking for earliness, either for commercial production or for subsistence. For subsistence cropping systems the varieties should also be able to produce well during an extended harvest season allowing ground storage. Farmers utilizing foliage for animal feed look for vigorous canopy development and high-protein content.

6.1 Yield

The yield of the sweet potato is determined by the length of the growing period. It seems that in Japan, yields of 120 t/ha can be obtained in 8 months in Kyushu and 80 t/ha in 6 months in Okinawa (Agata, 1982). In Vanuatu, yields of 60–80 t/ha can be obtained in 5 months with local cultivars and no fertilizers. The physical components of this yield are the number and the mean size of the storage roots at harvest, which depend on the foliage characteristics, the patterns of storage root growth, their mean weight and shape (Lebot, 1986); and all are important traits for breeders.

6.2 Quality

Qualitative characters are easily distinguished one from the other (for example, storage root skin color) and are generally controlled by only one or two set(s) of genes. Quantitative characters such as the shape and yield of the storage roots are indistinct and continuously grade into each other, involving many sets of genes. White flesh color seems to be dominant over orange flesh. For such characters, progenies often resemble the parents and genetic improvement through recurrent selection is rapid. It has been determined that flesh color, flesh oxidation, percentage dry weight, percentage crude starch, skin color, resistance to root-knot nematode, and vine length all have high heritabilities. Heritability of the storage roots weight is about 61% for the family and 59% for individual genotype response (Jones et al., 1976; Jones, 1986; Martin, 1988).

Among the numerous characteristics that breeders want to improve is the short shelf life of the sweet potato after harvest, between 2 and 4 weeks depending on the climate. In most countries, high dry matter content in the storage roots is an important characteristic as it is often associated with good eating quality and long shelf life. High dry matter also corresponds to processing efficiency. Selection for high dry matter content is very effective because there is tremendous variation for this

trait in the germplasm, ranging from only 14% to more than 44%, and because the heritability of dry matter has been estimated at 75–88%. Chinese breeders, for example, are introducing accessions from various sources and especially from CIP from where more than 100,000 botanical seeds and 55 advanced breeding lines have been introduced. The population mean for dry matter content is constantly increasing for this trait through population improvement (Zhang and Li, 2004).

The broad sense heritability of starch digestibility is very high and its improvement is theoretically feasible but necessitates facilities for routine analysis and screening of numerous genotypes. Apparently, the starch content is determined mainly by the additive effect of polygenes and, therefore, the accumulation of genes controlling high starch content is recommended. The Japanese approach for increasing starch content is via inbreeding by selfing and sib cross. The first step is to develop inbred lines derived from different parents and the second step is the evaluation of their specific combining ability. Apparently, inbreeding depression occurs in root yield but not in dry matter and heterosis is observed through crosses of inbred lines in root yield rather than in DM content. DM content is probably controlled by additive gene effects while root yield is controlled by dominant gene effects (Komaki et al., 1998). A few genes with simple dominance seem to control the inheritance of fiber size, while the total fiber content is controlled by several genes, suggesting that low-fiber improvement is quite feasible.

Unfortunately, carotene content seems to be negatively correlated with dry matter while starch content and dry matter are positively correlated: both are associated with eating quality (Wang, 1982). A study conducted on 183 accessions, corresponding to 27 local and introduced varieties and 156 hybrids, has shown that starch is negatively correlated with protein, sugars, and cellulose content and high selection pressure on starch will inevitably decrease other major compounds (Champagne et al., 2009).

Considerable variation exists for crude protein content in sweet potato. Lebot et al., (2009) have reported a range of 2.6–10.2% of dry weight among 167 different accessions with most of them averaging around 4–5%. A high broad sense heritability of 90% for protein has been reported with significant genotype × location interaction but no genotype × year interaction (Collins et al., 1987). However, Zhang and Li (2004) obtained narrow-sense heritability of family means for crude protein of 57% while narrow-sense heritability among individuals was only 15%. Moreover, they recorded a negative genetic correlation between crude protein content and dry matter and concluded that mass selection may not be effective in increasing crude protein because the heritability based on individuals is too low.

In the United States, breeders are attempting to reduce the sugar levels. The cultivar *GA90-16*, an open-pollinated seedling selection derived from a polycross nursery has been released because of its lower levels of odor-active volatiles and endogenous sugars. In baking trials, it has a bland flavor compared to the intensely flavored cv. *Jewel*. However, when prepared as French fries, *GA90-16* absorbs less fat than *Jewel* (Kays et al., 2001).

Chemical analysis costs are so high that unless a simple screening tool is available, it is difficult to include these chemical traits for routine screening. However,

major compounds can now be quantified using near-infrared reflectance spectroscopy (NIRS) and this technique seems promising for sweet potato breeders (Lebot et al., 2009).

6.3 Resistance to Biotic and Abiotic Stress

Resistances to pests and diseases are also priorities for breeders. Sweet potato weevils (*Cylas formicarius*, *C. puncticollis*, and *C. brunneus*) are the most important pests in the world. Despite intensive efforts to develop resistant cultivars, little has been achieved so far and attention is now turning to deeper formation of storage roots and early maturing varieties, which are less exposed to weevil infestation.

Root-knot nematodes (*Meloidogyne* spp.) and reniform nematodes (*Rotylenchulus* spp.) can cause significant damage. Susceptible cultivars infested with nematodes, wilt, appear stunted, and their infested fleshy roots crack and show growth deformities. Numerous pathotypes of *Meloidogyne* spp. exist and multiple genes could be involved in the resistance to root-knot nematodes. There is a need for standardized evaluation of root-knot nematodes damage in breeding programs and to identify reliable sources of resistance to numerous pathotypes (Cervantes Flores, 2000).

Several leaf fungi can cause significant yield reduction. Scab (*E. batatas*) is important in the humid tropics of Asia and the Pacific. Screening of thousands of seedlings for resistance to scab has been done successfully in Papua New Guinea and Vanuatu lowlands and resistant populations are now available (Fig. 3.3). *Alternaria* leaf spot is a widespread disease. Humid and cool weather (e.g., on the plateaus of Ethiopia or Uganda) is favorable for lesion enlargement. The airborne spores are spread through infected planting material, splashing rain, and water. The death of vines occurs rapidly and stem and leaf debris are sources of inoculum.

Fig. 3.3 Scab and Hill Trial (HT)

Infected material should be removed and burnt. Some local cultivars are tolerant to this pathogen. *Cercospora* leaf spot is also present in the warm humid tropics of Asia, South America, and Africa. Selection of tolerant cultivars is the most practical approach to control the disease. In the United States, *Fusarium oxysporum* sp. *batatas* penetrates healthy plants through open wounds. Yield losses may be up to 50% and are more likely in warm weather and in dry soils. There are resistant or tolerant cultivars, including *Jewel, Redgold, Nemagold*, and *Centennial*. In addition to resistance, other controls include crop rotation, selection of seed roots from disease-free fields, and regular fungicide applications (Clark and Moyer, 1988).

Viruses are transmitted by sap-sucking insects such as aphids and whiteflies. The *sweet potato feathery mottle virus* (SPFMV) and the related *sweet potato virus 2* (SPV2) are mostly transmitted by aphids. The *sweet potato virus G* (SPVG) and the *sweet potato mild virus* (SPMV) are mostly transmitted by whiteflies and especially by *Bemisia tabaci*. These viruses may cause only mild symptoms but it has been observed that symptomless plants may still have a considerable yield reduction. The *sweet potato chlorotic stunt virus* (SPCSV) can cause some dwarfing of the plants and the purpling or yellowing of leaves. When the SPCSV and the SPFMV both infect a plant, they interact synergistically to cause the *sweet potato virus disease* (SPVD). In East Africa, SPVD is a serious constraint to food productivity and security. Affected plants usually produce small storage roots and a severe reduction in yield. Genotypes of a breeding population are grown together with rows of highly susceptible genotypes to allow efficient evaluation of resistance to SPVD and screening.

The most important environmental stresses are drought, poor soil fertility, excess moisture, and cold in high altitudes. Superior genotypes usually have an efficient photosynthetic surface determined by the length of the stem and the number of leaves per unit length of stem. Bushy types can develop with short stems, an ample photosynthetic surface over a small area and are, therefore, attractive. Simple visual tools are developed to screen efficiently thousands of genotypes for such traits. The vine survival and vigor after planting are the key characteristics for successful varieties in drought-prone areas. There is considerable genetic variation for vine survival but drought adaptation is a complex and poorly documented trait. However, fast screening methods using visual tools can be incorporated into breeding programs to screen several thousands of plants in their first clonal generation (Andrade et al., 2009).

6.4 G × E

Sweet potato yield is very sensitive to environmental changes. The lack of association between high yield and stable performance suggests that G × E interactions are very important and there is a need to study the yield performance of selected genotypes in diverse climatic conditions (Manrique and Hermann, 2001; Tekalign,

2007). Selecting new cultivars in an environment with adverse conditions seems to be an efficient and practical way of identifying cultivars with good environmental adaptability (Janssens, 1984, 1988). The G × E interactions are larger than, or nearly equal to, the genetic variation of yield traits and different locations differ in their selection ability for storage root yield. However, low-yielding or marginal environments are not disadvantaged when breeding efforts are conducted in more favored locations (Grüneberg et al., 2005).

In order to satisfy varied regional needs for improved varieties and to take into consideration G × E interactions, CIP has adopted the convergent–divergent scheme which involves maximizing the use of local and diverse genetic resources, promoting collaboration among breeders, and selecting varieties for wide adaptability. Seeds from various sources are used to form the base population. The seedlings are planted and superior genotypes are selected and intercrossed and seeds of the population from cycle 1 are returned to the different collaborating sites. During the intercrossing, each location can introduce elite germplasm for introgression of specific genes (Mok et al., 1998).

An accelerated breeding scheme (ABS) is now suggested by CIP and envisages a selection process in 4 years rather than 8. It is based in the early breeding stages on the simultaneous assessment of traits on thousands of genotypes in 1 m row plots at three to four locations without replications. For later breeding stages the first selection step includes 300 clones × 3 locations × 2 replications and the second selection step 40 clones × 6–12 locations × 2 replications. For sweet potato, the genotype × environment interactions (G × E) are so high that it is better to increase the number of locations as soon as possible in the process, rather than the number of replications. ABS reduces the time needed for breeding as only 2 years are needed for the early stages (1 year of crossing and propagation and 1 year of testing). However, the human and financial resources involved in three environments for yield selection are significant. Also, to work, ABS requires a high heritability of traits assessed simultaneously. Furthermore, it is based on the assumption that genotype × year interaction is not very important and hence more experiments are needed to strengthen this new breeding scheme approach (Grüneberg et al., 2009).

6.5 Participatory Plant Breeding

Participatory plant breeding is attractive and offers potential for rapid progress. In Uganda, it was observed that farmer varieties performed on average better than official breeders varieties (Abidin et al., 2005). Researchers and farmers collaborated to identify selected cultivars among the seedling populations for a wider range of attributes. Important attributes needed by farmers, such as suitability for sequential piece-meal harvesting or tolerance to abiotic or biotic stresses such as drought or pest damage, were found to be difficult to predict by researchers during their on-station work (Gibson et al., 2008).

6.6 Interspecific Hybridization

Interspecific hybridization has been used in Japan since the 1950s to improve sweet potato. Some traits of *I. trifida* are of interest to breeders, especially drought tolerance and disease resistance. After interspecific hybridization and subsequent introgression, a few cultivars have been selected (Shiotani and Kawase, 1987).

6.7 Feeding Pigs

Traditionally, sweet potato leaves can be used for feeding pigs in fresh, dry, and ensiled forms. In China, a very high proportion of the total annual production is used and in some areas, fresh vines and leaves are harvested three to four times during a growing season for pig feed. The breeding of cultivars with high biomass, dry matter, and protein contents may lower the price and raise sweet potato feed energy and protein levels closer to those of cereals but more research is needed.

7 Breeding Methods and Techniques

7.1 Breeding Methods

Recurrent selection appears to be the most suitable breeding method as it permits minor and recessive genes to be selected with a progressive increase in a population. With this type of mass selection, the capture of additive effects is straightforward. It consists in the selection of a number of genotypes for one or more desirable traits and their hybridization in a polycross block by honey bees or by controlled crosses. The numerous seedlings are screened for desirable traits and the best are utilized with or without the best parents in a new polycross block for a second cycle (Figs. 3.1 and 3.3). This simple breeding method results in the rapid accumulation of suitable genes but has to be complemented with efficient screening techniques, and for many traits the accurate measurement of a particular trait is often the weakest operation. Hand pollination is also used but is time consuming and produces on average only two to three seeds per pollinated flower, often less. Two selected accessions can be established in large, isolated, crossing blocks with seeds collected only on mother plants located in the middle of the plot, but there is always a risk of contamination.

7.2 Hybridization Techniques

The flowers are solitary or in clusters of up to 22 buds growing out of the leaf axils. Each flower opens only once, just after sunrise and starts to fade by noon. The sweet potato fruits are glabrous or hirsute, dehiscent capsules measuring 5–10 mm

in diameter. They contain up to four seeds (Fig. 3.4). However, hand pollinated flowers usually produce capsules with only two seeds and open-pollinated ones produce capsules with one to three seeds. Seeds of large size germinate more rapidly than smaller ones. Small seeds often represent up to 50% of the total number of seeds obtained, depending on the genotypes involved. These seeds are brown or black, glabrous, angular and measure approximately 2–3 mm long. If the seeds are subjected to favorable conditions for germination, the embryo grows rapidly. After germination, the radicle appears first and grows downward, developing into the primary root system.

Most sweet potato genotypes flower naturally within the short days of the tropics and it is not necessary to use grafting on to free-flowering *Ipomoea* spp., girdling or day-length control which breeders in temperate countries have to use to induce flowering during long days (Edmond and Ammerman, 1971). In crossing blocks, parent clones isolated from other flowering sweet potatoes are open pollinated by insects. Sweet potato flowers best during the cool season in tropical countries. In the southern hemisphere, for example, the crossing block is planted during the first 2 weeks of April. Flowering begins 6 weeks later and continues for 3–4 months. Seeds are harvested from June to September. In Taiwan, in the northern hemisphere, the best season for pollination is from the beginning of November to the middle of December, when the average daily temperature is between 20 and 25°C with a maximum seed set occurring when the mean daily temperature is about 23.9°C (Wang, 1982).

The vine cuttings of the parent clones are planted at 1 × 1 m with two cuttings per planting position. Usually 10 plants of each genotype are enough although more may be needed for genotypes with poor flowering. Staking facilitates hand pollinations and insect pollination. In practice, the climbing vines of four plants are mixed together on a pyramid-like system with four 2 m-high stakes tied together. Eventually, wires connect the pyramids to allow trellising of the vines (Fig. 3.5)

Fig. 3.4 (**a**) Pollinators

Fig. 3.4 (continued) (**b**) capsules and seed

Fig. 3.5 Trellising of the vines

which promotes flowering. Application of nitrogen fertilizers in crossing blocks is not recommended as it promotes lush and leafy vines without flowering. Various insects and diseases can reduce flowering, especially scab (*E. batatas*) in the wet tropics.

When hand-pollinating, it is necessary to ensure that flowers are well protected from pollination by insects. Buds and flowers that will open the following morning are prevented from opening by clipping the tip of the corolla. The best time to clip flowers is during the afternoon or the evening of the day before hand pollination. The flower from the male parent is carried to the female parent and the clip is gently removed without destroying the corolla. The petals are then spread and the anthers of the male parent are rubbed gently over the stigma of the female parent. In order to prevent pollen contamination, the corolla of the pollinated flower is tied together so that insects cannot reach the stigma. Another technique uses 2 cm long pieces of drinking straws which are pushed on the unopened corollas 1 day before anthesis. After hand pollination, the corolla is again rolled and pushed into the straw. For genetic studies, the female parent flowers are emasculated by hand to eliminate all possibility of pollen contamination.

Success rates depend on the vigor of the parent plants and the weather, but approximately 50% of the pollinated flowers produce two seeds. Seeds mature between 4 and 6 weeks after pollination. Each capsule is harvested by hand when it is fully brown and the pedicel has dried. It is necessary to collect them every morning as mature capsules fall off easily or dehisce and release their seeds. Seeds are then extracted and those that are lightweight or deformed are discarded. Once

properly dried they can be stored and remain viable for up to 20 years if the storage conditions are well controlled (18°C, 50% RH) and for at least 5 years in a simple desiccator with silica gel lodged in a refrigerator.

7.3 Seed Scarification

Since sweet potato seeds have a very hard coat, they germinate slowly and irregularly. The most practical way of scarifying substantial volumes of seeds is to soak them in concentrated sulfuric acid (98% H_2SO_4) in a glass beaker for 40 min. The seeds are then rinsed under running water for 5–10 min. This technique gives about 95% germination success (Wilson et al., 1989). It is also possible to simply soak the seeds overnight in water and this will improve their germination, although it will be irregular, occurring over several weeks. Immediately after scarification or soaking, the seeds are placed individually in *Jiffy*® pots or an equivalent.

7.4 Clonal Generations

Seedlings are planted at 0.5 × 0.5 m and are ready to harvest at 10 weeks. Cuttings are then replanted for “hill trials” and high selection pressure is generally applied in the first clonal generation. Only the best genotypes are propagated for their first “row trial.” Subsequent clonal generations are selected for a combination of different traits while the number of plants per genotype is increased for bulking sufficient planting material to allow multi-location trials (Table 3.2).

8 Integration of New Biotechnologies in Breeding Programs

8.1 Molecular Markers

RFLPs have been used to conduct a phylogenetic study of section *Batatas*. RAPD and AFLP markers have also been used together to identify relationships among some related species in the *Batatas* group (Jarret et al., 1992). Because of the hexaploid nature of the sweet potato and the complexity of its genome makeup, molecular markers research has been limited to germplasm evaluation and characterization and to map making attempts. The total percentage of scorable primers from sweet potato is, however, quite low (Ukoskit and Thompson, 1997; Ukoskit et al., 1997).

Microsatellites have been used to analyze the genetic diversity and genetic relationships among genotypes produced via polycross breeding (Hwang et al., 2002) and to identify paternity using a minimal number of loci (Buteler et al., 2002). Microsatellite markers appear promising because they can disclose multiple alleles at a particular locus although they are more expensive than others. To promote

Table 3.2 Characters evaluated in each clonal generation

No. of genotypes in trial	Planting pattern	Characters evaluated
2,000 seedlings in seedling nursery (SN)	50 × 50 cm, 1 seedling per genotype	Fungal leaf disease (i.e., scab) score at harvest, vine length and thickness, storage root skin color and flesh color
100 genotypes in Hill trial (HT)	1 × 1 m, three plants per genotype (two cuttings per mound)	Fungal leaf disease score, early maturity, little-leaf score, virus score, vine length and thickness, storage root skin and flesh color, yield (low, medium, high), and specific gravity measured in the field
100 genotypes in preliminary trial (PT)	1 × 1 m, six plants per genotype (two cuttings per mound)	Fungal leaf disease score, early maturity, little-leaf score, virus score, vine length and thickness, storage root skin and flesh color, root shape, skin smoothness, skin cracking, number of storage roots per plant and individual size, yield (low, medium, high), and specific gravity measured in the field
25 genotypes in intermediate trials IT-1 and 13 genotypes in IT-2	1 × 1 m, 10 plants per genotype (two cuttings per mound)	Fungal leaf disease score, early maturity, little-leaf score, virus score, vine length and thickness, storage root skin and flesh color, root shape, skin smoothness, skin cracking, number of storage roots per plant and individual size, marketable weight, edible weight per plant, and specific gravity measured in the field
7 genotypes in advanced trials AD-1 and 5 genotypes in AD-2	1 × 1 m, 16 plants per genotype, 4 scored plants (two cuttings per mound)	Same as IT plus tuber dry weight and eating quality
2 genotypes in on-farm trials	Planting patterns determined by farmers Number of replications and number of plants per replication determined by availability of cuttings Trial is best located in the middle of the farmer's field	Average fungal leaf disease score over the season, virus score, marketable weight and tuber numbers, and eating quality as judged by farmers and farmers' choice of which clone will be replanted

After Wilson et al., 1989

the implementation of genetic analyses in the Japanese breeding programs, a set of microsatellite markers have been developed which cover the entire genome. Seventy-five SSR (simple sequence repeats) loci showed length polymorphisms and out of these loci, 71% were found to be associated with some genes (Hu et al., 2004).

AFLPs have been used to detect markers suitable for identification of plants possessing a resistant reaction to root-knot nematode (*Meloidogyne incognita*) (Mcharo et al., 2005). AFLP markers have also been found to be useful to identify genotypes susceptible or resistant to *sweet potato virus disease* (SPVD) in Kenya (Miano et al., 2008).

Although these results appear promising on paper, for the time being, there is limited practical use of molecular markers in sweet potato, for example for marker-assisted selection at the field level. This is quite surprising considering the global importance of the crop and the fact that sweet potato is an important crop in developed countries (the United States, China, Japan). Because of the polyploid nature of sweet potato, the genetic distance-based approach will continue to be the mainstream one in molecular diversity analysis. The complementary use of AFLP and SSR markers might be an attractive solution but, at the moment, none of them can allow linkage with particular phenotypic traits. AFLP genotyping can give a large amount of data in a short period of time and this is useful for reliable assessment of diversity using distance-based approaches. Meanwhile, a small set (e.g., around 10 pairs) of high-quality SSRs can trace the movement of specific alleles, which provide a complementary role to the distance-based multi-variant analysis. Ideally a large number of SNPs would be preferred as these are most efficient in data generation. CIP is at present developing a diploid *I. trifida* map for easy to handle mapping work enabling comparative genomics (Schafleitner, 2009).

Amy beta is a single gene that encodes β-amylase, a starch-hydrolyzing enzyme that confers sweetness to sweet potato. A highly conserved region of *Amy* beta has been sequenced in sweet and non-sweet cultivars. Comparison of the sequences revealed the presence of *In-Del* sequences in null cultivars, which was thought to be a loss-of-function mutation causing a change from sweet to non-sweet. These findings could represent the basis of selection markers for non-sweetness in sweet potato breeding (Anwar et al., 2009).

8.2 Genetic Transformation

Genetic transformation is the focus of much interest. Two different approaches are being used, one mediated by *Agrobacterium tumefaciens* and the other by direct gene transfer using particle bombardment.

The regeneration protocol mediated by *A. tumefaciens* uses two different plasmids which are transferred to *A. tumefaciens* by electroporation. Although regeneration is genotype dependent, this system appears to work efficiently and allow the transformation and regeneration of genotypes widely cultivated in Asia and Africa (Mok et al., 1998).

Various experiments have been conducted in different countries. Because weevils digest their food with the help of certain proteinases, it may be possible to block their

digestion by incorporating proteinase-inhibitor genes into the sweet potato genome using transformation and regeneration protocols. Trypsin inhibitors are therefore potential candidates for the development of transgenic sweet potato with resistance to weevils (*Cylas* spp.) and genes coding for them have been selected (Newell et al., 1995). After propagation and isolated screenhouse trials, transgenic lines have been evaluated in the field in Cuba and China for their resistance to *C. formicarius* (Mok et al., 1998).

In Japan, transgenic plants of cv. *Kokei 14* have been obtained from embryogenic calluses using *A. tumefaciens* mediated transformation. After their evaluation in the greenhouse it was concluded that some agronomically important genes can be introduced easily with this system (Otani et al., 2001). Starch composition can also be altered by genetic transformation: out of 26 transgenic plants obtained, one plant showed the absence of amylose in the storage roots (Kimura et al., 2001).

In South Korea, a successful and reliable *Agrobacterium* transformation of the bar gene conferring herbicide resistance has been achieved and the method seems to have the potential to develop new varieties with enhanced tolerance to the herbicide "Basta" (Choi et al., 2007). Stable transgenic plants are now obtained in 6–10 weeks after infection with *A. tumefaciens* and PCR is used to confirm the stable integration of transgenes into the genome (Luo et al., 2006). Efficient production of transgenic plants using the bar gene for herbicide resistance has also been achieved through the use of embryogenic suspension cultures. Transgenic plants exhibited functional expression of the bar gene in an in vivo assay for herbicide resistance (Zhang et al., 2009b).

The second genetic transformation approach, based on direct gene transfer using electroporation or particle bombardment, is also producing encouraging results. In Japan, the coat protein gene of the sweet potato feathery mottle virus (SPFMV) was introduced into the sweet potato genome. Calluses bombarded with particles and regenerated plants were confirmed as hosting the transgenes using PCR analysis (Murata et al., 1998). Transgenic lines were vegetatively propagated and infected with the SPFMV. Three months after, an ELISA assay showed that virus accumulation was suppressed in the transgenic lines compared with normal ones, confirming the efficiency of the transformation system (Okada et al., 2001, 2002). Transgenic plants expressing the SPFMV coat protein gene were challenged by graft inoculation with field infections and were field evaluated. All of the transgenic plants were protected in the long term against SPFMV compared with control plants. These results suggest that transgenic sweet potato can express resistance to SPFMVs in the field (Okada and Saito, 2009).

The enhancement of tolerance to various environmental stresses is also attractive and has been attempted. Sweet potato was transformed with spermidine synthase genes derived from *Cucurbita ficifolia*. The transgenic plants were tested for their tolerance to salt and drought. One of their most interesting traits was the increase in the number of storage roots produced under both non-stress and stressful environments. The transgenic plants also showed increased tolerance to chilling- and to heat-mediated damage compared to the normal plants. It was concluded that sweet potato could be made more tolerant to environmental stresses through introduction of the spermidine synthase genes (Kasukabe et al., 2006).

However, a study conducted to evaluate the nutritional quality of genetically modified sweet potato on growth, lipid metabolism and protein metabolism of hamsters has shown that they contain less protein to maintain normal animal growth (Shreen and Pace, 2002).

Obviously, more research is needed to assess the potential of these transformation techniques for the sustainable improvement of the crop.

8.3 Somatic Hybridization

Somatic hybridization is also of practical interest. Some wild species present interesting traits but incompatibility with *I. batatas* cultivars limits their use. Somatic hybridization could therefore represent an attractive system to transfer useful genes. A somatic hybrid has been produced by protoplast fusion between sweet potato cv. Kokei No. 14 and *I. triloba*. Protoplasts isolated from embryogenic suspension cultures of Kokei No. 14 were fused with petiole protoplasts *of I. triloba* L. using the polyethylene glycol-mediated protocol. A total of 176 plants were obtained but only one plant, designated KT1, was found to produce storage roots. Drought tolerance, dry matter content, soluble sugar content, and fertility of this somatic hybrid are being evaluated for potential use in breeding (Yang et al., 2009).

9 Commercial Production

True botanical seed of sweet potato is primarily used for breeding and not for planting the crop which is normally done by vegetative means using vine cuttings. The storage roots of *I. batatas* differ from normal roots in their capacity to produce buds and sprouts on the root skin and selected roots are commonly used to establish nurseries.

Tissue culture applications are numerous in the healthy and rapid propagation of selected genotypes. Several protocols have been developed and have shown their efficiency for in vitro propagation. Significant differences in callus characteristics are apparent and some are due to the protocols but genotypes are the most important sources of variation (Templeton-Somers and Collins, 1988).

In most developing countries, farmers themselves manage their planting material stocks and there is no seed industry. To ensure availability of planting materials when the rains start, dry season conservation is necessary in field nurseries established in shaded or wet areas or in plots where irrigation is feasible.

In temperate countries, where planting must be seasonal, the production of healthy propagules has to be done at a certain time of the year, rapidly, and in sufficient volumes. Nurseries are established in the field in raised beds prepared with loose soil mixed with compost or organic manure. Healthy storage roots from selected plants are buried in beds and when the vines have grown long enough (30–50 cm), they are cut at their base and planted directly into the new field. Most field beds are covered with clear plastic where selected storage roots are laid on the

ground for sprouting. Good drainage is necessary to prevent rotting of the bedded roots. Each root can produce up to 15 plants and as many as six sprouts may grow on the same root at the same time. Cuttings are then held in the upright position in the shade for 24 h to promote rooting.

Single-node leafy cuttings are also used to produce low-cost, pathogen-free transplants but necessitate great care and high labor inputs. Transplants produced with artificial light result in a higher yield of storage roots after a complete cycle in the field than those produced with traditional stem cuttings. These transplants have a survival rate close to 100% and a rapid and uniform growth because they have an already developed root system before their field transplantation, unlike traditional stem cuttings (Saifu Islam et al., 2002). Although this technique is clearly promising for rapid propagation of elite material, there are still several practical steps requiring simplification before it could be used by smallholders and commercial growers.

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Chapter 4
Yams

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1 Introduction

Yams, belonging to the genus *Dioscorea*, family Dioscoreaceae, are an important food crop in tropical and subtropical regions (Ayensu and Coursey, 1972). Yam plants are dioecious monocotyledonous vines, cultivated for their starchy tubers. Yam is a multi-species, polyploid, and vegetatively propagated crop, exclusively propagated by means of "setts," small tubers or small pieces of tuber.

The word "yam" is believed to have originated from the tribal African word, "niam" (Coursey, 1967), meaning "to sample" or "taste." It is also known as "igname" (French) and "ñame (Spanish). Tubers are eaten boiled, baked, fried, or pounded into a fufu dough form. They are high in vitamin C, dietary fiber, vitamin B6, potassium, and manganese and low in saturated fat and sodium.

Out of the 10 cultivated yams (Table 4.1, Figs. 4.1, 4.2, and 4.3), *Dioscorea rotundata, D. cayenensis, D. alata,* and *D. trifida* are the major species, while the six others are often referred to as minor ones. *D. alata, D. rotundata,* and *D. cayenensis* belong to the section Enantiophyllum, which includes species with an anticlockwise twining stem and entire leaves. *D. trifida* belongs to the section Macrogynodium and is characterized by a clockwise twining stem and lobed leaves.

The species *D. rotundata* and *D. cayenensis* (also referred to as *D. cayenensis–rotundata* complex) are the most commonly cultivated in the world and represent 95% of yam production worldwide. *D. rotundata* plants have $2n = 40$ chromosomes, whereas *D. cayenensis* includes accessions with $2n = 60$ and 80 chromosomes (Dansi et al., 2000c, 2001). The most serious pests affecting these species are nematodes (Kwoseh et al., 2007) and potyviruses (Thouvenel and Dumont, 1990; Bousalem et al., 2000a, b, 2008).

D. alata, or the greater yam, is the most widely cultivated yam species throughout the tropics. It is a polyploid species that includes accessions with $2n = 40$,

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Table 4.1 Geographic origin of important cultivated *Dioscorea* spp.

Dioscorea spp.	Common names	Botanical section	Geographic origin
Dioscorea alata	Greater, water, winged yam	Enantiophyllum	Southeast Asia, Melanesia
D. rotundata	White Guinea yam	Enantiophyllum	West Africa
D. cayenensis	Yellow Guinea yam	Enantiophyllum	West Africa
D. trifida	Aja, aje, cush–cush, yampi	Macrogynodium	South America
D. esculenta	Lesser yam, Asiatic yam	Combilium	Southeast Asia, Melanesia
D. opposita-japonica	Chinese, Japanese yam	Enantiophyllum	Japan, China
D. bulbifera	Aerial, bulbil, bearing yam	Opsophyton	South America, Africa, Asia, Melanesia
D. nummularia	Spiny yam, wild yam	Enantiophyllum	Melanesia
D. transversa	Marou, wael	Enantiophyllum	Australia, Melanesia
D. pentaphylla	Five-leaved yam	Lasiophyton	Southeast Asia, Melanesia

60, and 80 chromosomes (Abraham and Nair, 1991; Gamiette et al., 1999; Malapa et al., 2005; Arnau et al., 2009). Ploidy level has been found to be correlated with growth vigor, increased tolerance to abiotic and biotic stress, and higher tuber yield (Malapa, 2005; Abraham and Arnau, 2007; Lebot, 2009). Anthracnose, caused by the fungus *Colletotrichum gloeosporioides,* is the most serious disease affecting this species (Winch et al., 1984; Onyeka et al., 2006).

D. trifida is the most important edible *Dioscorea* in tropical America. Its flesh is considered to be the most flavorful of all the yams (Martin and Degras, 1978). This species has $2n = 80$ chromosomes (Essad, 1984; Bousalem et al., 2006). Potyviruses are the main limiting factor for the production of this yam. Potyviruses cause significant economic damage and seriously impede the development of this yam as a viable crop. They are directly involved in the regression of *D. trifida* in the Caribbean and French Guyana (Degras, 1993; Bousalem et al., 2000b, 2003).

Minor cultivated species are important in specific regions or countries. *D. esculenta* (Combilium section) is commonly cultivated in Southeast Asia and Melanesia, *D. opposita-japonica* (Enantiophyllum section) in China and Japan, *D. transversa* (Enantiophyllum section) in Australia and Melanesia, and *D. nummularia* (Enantiophyllum section) in Indonesia and the Pacific. There are also several wild species that are still a major source of food for some communities, such as the endemic species of Madagascar, *D. soso, D. nako, D. bemandry, D. alatipes, D. hombuka* (Brachyandra section), and the species *D. hamiltonii* (Enantiophyllum section) consumed by forest-based communities in India.

Most of the varieties cultivated are accessions selected by farmers from among existing landraces. Breeding in yams as compared to other tuber crops such as potato or cassava had literally not been attempted until recently. It was only in the 1960s that the first breeding work in edible yams was undertaken, beginning with *D. trifida*

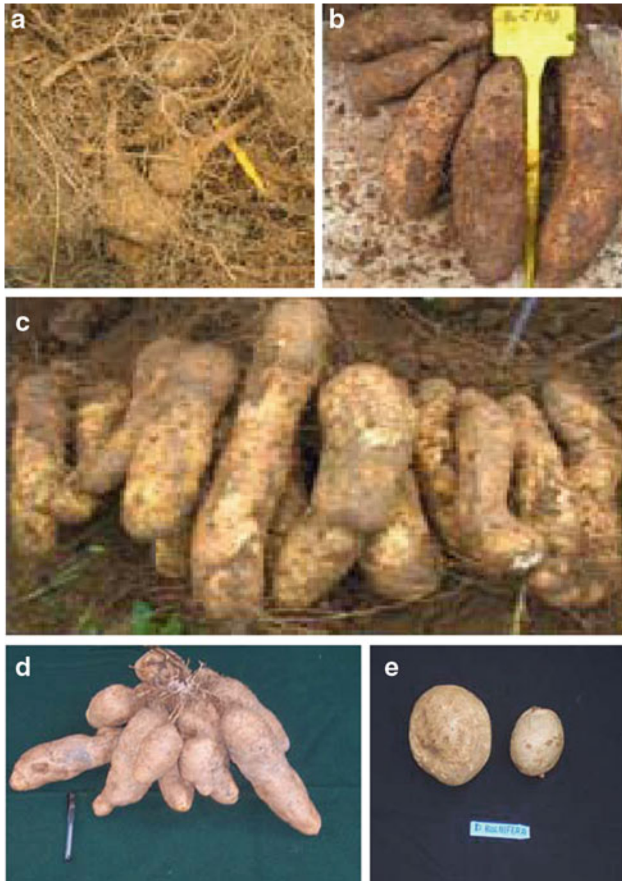

Fig. 4.1 Tubers of different *Dioscorea* spp. **a** *D. nummularia*, **b** *D. alata*, **c** *D. rotundata*, **d** *D. esculenta*, **e** *D. bulbifera*

in the Caribbean (Degras, 1969), followed by *D. rotundata* in the 1970s in Nigeria (Sadik and Okereke, 1975), and only recently in the 1980s with *D. alata* in India (Abraham et al., 1986). The lack of knowledge about the origin, diversity, and genetics of these species has seriously limited the effectiveness of genetic improvement programs.

Progress has recently been made, thanks to the use of different biotechnological tools and, in particular, molecular markers, cytogenetic techniques, and in vitro culture. Essential knowledge has recently been acquired about the ploidy level and the basic chromosome number of the three major species cultivated: *D. rotundata, D. alata,* and *D. trifida*. Based on microsatellite segregation analysis, Scarcelli et al. (2005) and Bousalem et al. (2006) came to the conclusion that *D. rotundata* and *D. trifida* species, previously assumed to be tetraploid and octoploid, respectively, are actually diploid ($2n = 40 = 2x$) and tetraploid ($2n = 80 = 4x$), respectively.

Fig. 4.2 Variability in flesh color of *D. alata* varieties

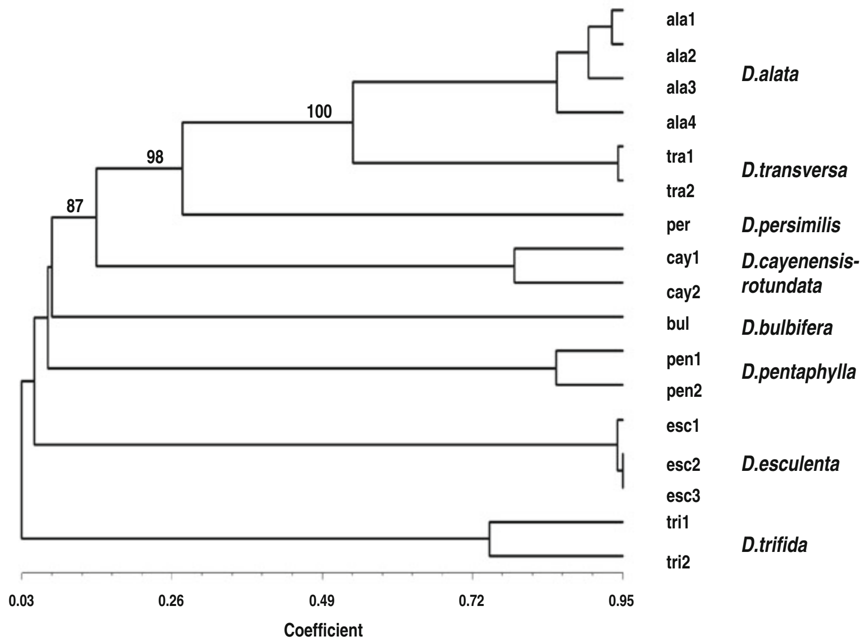

Fig. 4.3 UPGMA dendrogram based on 493 AFLP markers showing relationships among 17 *Dioscorea* clones belonging to eight different species (Malapa et al., 2005)

Another recent study, also based on microsatellite segregation analysis, revealed that *D. alata* accessions with $2n = 40$, 60, and 80 chromosomes are diploid, triploid, and tetraploid, respectively, and not tetraploid, hexaploid, and octoploid, as usually assumed (Arnau et al., 2009). In addition, results were confirmed by isozyme segregation patterns for *D. rotundata* species. These three studies provided the

genetic evidence that the basic chromosome number of these species would be $x = 20$ and not $x = 10$ as previously believed.

The two first genetic maps were published for *D. alata* (Mignouna et al., 2002d) and *D. rotundata* (Mignouna et al., 2002e). Markers linked to anthracnose and potyvirus resistance were identified by IITA, which could be used for marker-assisted selection (Mignouna et al., 2002a, b).

Molecular markers are also useful for determining the genetic diversity of germplasm collections and make it possible to select genetically distant parents to maximize heterozygosity and heterosis in the progenies.

D. alata polyploid hybrids [$2n = 60$ ($3x$), from $2n = 40 \times 2n = 80$, and $2n = 80$ ($4x$), from $2n = 80 \times 2n = 80$] were produced for the first time by conventional hybridization, thanks to the discovery of the fertility of *D. alata* tetraploid varieties and to the development of an in vitro immature embryo rescue method (Arnau et al., 2006; Abraham and Arnau, 2007). This opens new perspectives in greater yam breeding since the higher ploid hybrids are more vigorous and higher yielding. In addition, flow cytometry analysis revealed the natural production of diploid gametes in *D. alata* species by detection of hybrids with non expected ploidy levels in progenies produced. Studies are in progress to understand this phenomenon and to explore its ability to transfer heterozygosity and agronomic values of diploids to the tetraploid level.

Recent efforts devoted to the collection and characterization of the genetic diversity of *D. trifida* in French Guyana, as well as the recent discovery of the existence of wild *D. trifida* (Bousalem, 2004; Bousalem et al., 2006; Hochu et al., 2006; Bousalem et al., 2010), lead to new possibilities for the genetic improvement of this species, which has suffered significant genetic erosion as a result of potyviruses.

2 Origin and Domestication

The four major cultivated yams are believed to have originated in tropical areas on three separate continents: West Africa [*D. rotundata* (white Guinea yam) and *D. cayenensis* (yellow Guinea yam)], Southeast Asia and the South Pacific (*D. alata*), and South America (*D. trifida*).

It is thought that the Guinea yams originated in the so-called yam belt or civilization of the yam (Coursey, 1967; Lagemann, 1977; Orkwor and Asadu, 1998), which extends from central Cote d'Ivoire to the mountains of Cameroon and includes the following countries: Cameroon, Benin, Togo, Ghana, and Cote d'Ivoire. Their taxonomy and phylogenetic origin are still obscure. Guinea yams can be broken down into two phenotypically distinguishable types: white yams (white-fleshed tubers with a 6–8 month growth period) and yellow yams (yellow-fleshed tubers with an 8–12 month growth period). However, there are many intermediate forms. Several authors have treated the two types as two different species: *D. rotundata* and *D. cayenensis* (Burkill, 1960; Ayensu, 1972; Akoroda and Chheda, 1983; Chair et al., 2005; Scarcelli et al., 2006a; Tostain et al., 2007), but other authors consider them to be a species complex: *D. cayenensis-rotundata* (Hamon

and Touré, 1990; Dansi et al., 1999). Two species, which still exist in savannah areas (*D. abyssinica* Hochst ex Kunth) and in humid forests (*D. praehensilis* Benth), in West Africa, are considered to be wild relatives of *D. rotundata* (Hamon and Touré, 1990; Terauchi et al., 1992; Mignouna and Dansi, 2003b; Chair et al., 2005; Scarcelli et al., 2006b). *D. cayenensis* is assumed to have a complex origin with the possibility of having been domesticated from various wild species such as the perennials *D. burkilliana* or *D. mangenotiana* (Dansi et al., 1999; Mignouna and Dansi, 2003b) or from interspecific hybrids. Terauchi et al. (1992) and Ramser et al. (1997) hypothesized that these yams had an interspecific origin with the male plant belonging to one of the following wild rainforest species – *D. burkilliana, D. minutiflora,* or *D. smilacifolia* – and the female plant belonging either to *D. abyssinica, D. praehensilis, D. liebrechtsiana,* or *D. rotundata*.

The origin of *D. alata* is still a matter of debate because it has not yet been found in its wild state in nature. De Candolle (1886) first suggested an Indo-Malayan origin of the species. Prain and Burkill (1939) subsequently placed it further northward in Indochina where two closely related wild Southeast Asian species (*D. hamiltonii* and *D. persimilis*) are found. According to these authors, *D. alata* is a true cultigen that has been selected from these species or from their natural hybrids. Both species are characterized by long, deeply buried tubers that superficially resemble some cultivated but inferior varieties of *D. alata*. Although there is no further evidence to support this second hypothesis, it has been widely accepted (Alexander and Coursey, 1969; Martin, 1974; Degras, 1993; Mignouna et al., 2002d). Based on segregation analysis in sexual progenies using molecular markers, Mignouna et al. (2002d) emphasized an allotetraploid (amphidiploid) genome structure of $2n = 40$ types involving two different genomes: PPHH (P = *D. persimilis*, H = *D. hamiltonii*). However, the recent demonstration (Arnau et al., 2009) of diploidy of $2n = 40$ plants (previously considered to be tetraploid) casts doubt on its allopolyploid origin.

Recently, Malapa et al. (2005, 2006) used AFLP markers to show that *D. alata* is closer to the species *D. nummularia* and *D. transversa* than to *D. persimilis*, two species that are restricted to Southeast Asian islands and Oceania (Bourret, 1973; Lebot, 1997). They proposed that *D. alata,* together with these two species, may belong to a Southeast Asian–Oceanic gene pool that is confined in large part to the former Sahulian and Wallacean regions.

According to Alexander and Coursey (1969), yam-based agriculture appears to have developed about the same time in West Africa and in Southeast Asia, around 5000 BP. It has been suggested that this process could have started ca. 7000 BP for the West African yams (Dumont et al., 2005) and 6000 BP for the greater yam (Lebot, 2009), although there is no accurate dating to support this hypothesis. The process of domestication of a vegetatively propagated crop such as the yam cannot be thought of as a sudden cultural change, comparable to the "Neolithic Revolution," but rather as a gradual evolution taking place over centuries or perhaps millennia (Ayensu and Coursey, 1972). An original practice referred to as domestication, ennoblement, or paraculture still exists among farmers in West Africa, especially in Benin and to some extent in Nigeria (Dumont and Vernier, 2000;

Vernier et al., 2003; Mignouna and Dansi, 2003b; Zannou et al., 2006), as well as in the Solomon and Vanuatu Islands in Melanesia (Lebot, 2009). Farmers select edible wild forms that they subject to intense vegetative multiplication and selection over several years, leading to morphological and biochemical changes in the plants, mainly at the tuber level. This practice has been associated with *D. praehensilis*, *D. abyssinica,* and *D. burkilliana* in West Africa and *D. nummularia* in Melanesia. Studies on the genetic nature of germplasm ennobled in Benin have also revealed spontaneous interspecific hybrids of these wild species with cultivated yams (Scarcelli et al., 2006a, b). This process therefore contributes to the enhancement of the genetic diversity of cultivated yams. Mignouna and Dansi (2003b) called for a revision of the taxonomy of *Dioscorea* species because it is not conceivable that individuals identified in the wild such as *D. praehensilis* or *D. abyssinica* can directly become *D. rotundata* or *D. cayenensis* following "domestication" without any genetic change.

An Amazonian origin has long been recognized for the American species, *D. trifida* (Degras, 1993), despite the fact that its wild ancestor has not been identified and that its domestication is poorly documented. Moreover, no signs of a yam-based American society like the ones found in Africa and in the South Pacific have ever been found (Coursey, 1967). Recently, the existence of *D. trifida*'s wild yam relative was reported by Bousalem et al. (2010) in French Guyana.

3 Varietal Groups

Cultivars of Guinea yams cultivated in Nigeria (Akoroda, 1983; Onyilagha, 1986), Côte d'Ivoire (Hamon et al., 1986; Hamon, 1987), Benin (Dansi et al., 1999), and Cameroon (Mignouna et al., 2002c) have been characterized on the basis of morpho-agronomical characteristics and classified into varietal groups. Hamon (1987) characterized 806 accessions from Cote d'Ivoire and grouped these into 20 varietal groups. Similarly, 560 accessions collected throughout Benin were classified into 26 cultivars groups based on their morphological similarities (Dansi et al., 1999). Certain varietal groups are restricted to specific regions while others are present in several countries. This is the case of the varietal group "Kponan," which includes early-maturing varieties that are highly valued for the preparation of fufu. This is also the case of two varietal groups, "Alakissa" and "Baridjo," of Benin that both include late-maturing varieties of the *D. cayenensis* type that correspond, respectively, to the "Yaobadou" and "Baniakpa" varietal groups of Côte d'Ivoire.

Cultivars of *D. rotundata* are classified into early- and late-maturing cultivars. The early-maturing cultivars are harvested twice. The first harvest occurs 3–5 months after emergence and the tubers are used for culinary preparations. The second harvest occurs after the end of the vegetative cycle and produces many small tubers that are used as seeds. Early-maturing varieties require more fertile soil than late-maturing varieties. Late-maturing cultivars are harvested once a year at the end of the vegetative cycle and each plant produces many tubers of different weights that

vary depending on the cultivars. The late-maturing cultivars are the best adapted for the production of chips (Dumont et al., 2005).

Cultivars of *D. alata* cultivated in New Caledonia (Bourret, 1973), India (Velayudhan et al., 1989), and Vanuatu (Malapa, 2005) have been characterized on the basis of morpho-agronomical characteristics. Bourret (1973) studied 100 cultivars from New Caledonia and classified them into four major morphotype groups. Velayudhan et al. (1989) conducted a study on 140 local cultivars from India and identified 15 groups.

Malapa (2005) studied 331 accessions collected throughout the Vanuatu islands where a remarkable morphological variation exists. He identified 35 groups of morphotypes including some resistant or tolerant to anthracnose. Groups "M1" and "M7" consist of diploid varieties, which are late maturing (7–9 months) and tolerant to anthracnose. Groups "M8" and "M17" include tetraploid varieties, which have thick leaves and are late maturing (7–9 months) and tolerant to anthracnose.

4 Genetic Resources

The concept of genetic resources concerns all materials potentially of use for the improvement of yield, adaptability, quality, and resistance to diseases. It includes landraces, wild ancestors, related species, and breeding stocks.

The greatest phenotypic variation of *D. alata* is observed in the south of the Southeast Asian Peninsula, in Indonesia, Malaysia, and Melanesia (Bourret, 1973; Coursey, 1976; Martin and Rhodes, 1977; Lebot et al., 1998). The Pacific islands harbor one of the richest in situ collections of *D. alata*, consisting of more than 1,000 cultivars (Lebot, 2009). Several national collections were assembled and characterized in Fiji, New Caledonia, Papua New Guinea, the Solomon Islands, and Vanuatu as part of a regional root crop program in the 1980s (Jackson, 1994) and later as part of an INCO project in 2000 (SPYN, South Pacific Yam Network; Lebot, 2003). A core sample was assembled and is maintained in vitro at the Regional Germplasm Centre managed by the Secretariat of the Pacific Community (SPC) in Fiji. A wide diversity also exists in India where many national ex situ collections have been established by the CTCRI (363 acc.), NBPGR (195 acc.), and AICRPTC (211 acc.).

In addition, an international *D. alata* germplasm collection of 235 accessions was assembled and evaluated in Puerto Rico by the US Department of Agriculture in Mayaguez in the 1970s (Martin and Rhodes, 1977). However, this collection was not maintained.

The greatest phenotypic variation of Guinea yams is observed in West Africa, which corresponds to their area of origin. Many national ex situ collections have been established in Côte d'Ivoire (Hamon et al., 1986; Hamon, 1987), Benin (Dansi et al., 1999), and Cameroon (Mignouna et al., 2002c) as well as other countries, to assess the genetic diversity at the country level. However, their maintenance has

been difficult and numerous accessions have been lost. This is primarily due to the high cost of maintaining these collections.

The IITA has the largest collection of yams in the world in its gene bank, including eight species and about 3,200 accessions. Most of the accessions were collected from West and Central Africa, and *D. rotundata* makes up about 67% of the collection. All of the accessions are grown annually in the field, but 1,544 of these are also conserved in tissue culture as in vitro plantlets. A core collection of 391 accessions from six species has been defined on the basis of morphological characteristics (Mahalakshmi et al., 2007). The CTCRI in Trivandrum (India) and INRA and CIRAD in Guadeloupe (French West Indies) each maintain several hundred *Dioscorea* accessions in the field and in vitro.

A major constraint for breeding is that a large number of varieties do not flower or produce only a reduced number of flowers. This is particularly important in the species *D. alata*. This reduction in flowering is due to exclusive vegetative propagation over a long period of time. The use of seeds leads to renewed fertility. Moreover, it has been observed that most hybrids obtained by controlled pollination, as well as those obtained from lowly fertile varieties, are nevertheless highly fertile. It was therefore possible to establish collections for breeding purposes that contain a large number of profusely flowering fertile clones. Doubled diploids were also developed from elite *D. alata* varieties in order to extend the range of 4*x* parents that could be used in the polyploid variety production program (Arnau et al., 2006).

Cytogenetic techniques and different markers (isozymes, RFLP, RAPD, AFLP, SSR) have been used to characterize the diversity of germplasm collections of *D. rotundata*/*D. cayenensis* (Hamon and Touré, 1990; Asemota et al., 1996; Dansi et al., 2000a; b; Mignouna et al., 1998, 2002b; Mignouna and Dansi, 2003a; Mignouna et al., 2003; Tostain et al., 2007) and *D. alata* (Lebot et al., 1998; Malapa et al., 2005; Sheela et al., 2006; Abraham and Arnau, 2007; Arnau et al., 2007b, 2009). Few studies have dealt with *D. trifida* (Essad, 1984; Bousalem et al., 2006), which is also the case for wild species and minor cultivated species. *D. nummularia* and *D. transversa* are both particularly interesting because they are resistant to anthracnose and closely related to *D. alata* (Malapa, 2005; Malapa et al., 2005).

The acquisition of knowledge about the genetic diversity of these species at both the agronomic and cytogenetic levels is essential to be able to use them in genetic improvement programs. *Dioscorea* is one of the most problematic genera for cytogenetic studies (Essad, 1984). Chromosome counting is difficult because of the small size of chromosomes, their tendency to stick together, and their satellites that are sometimes as large as the chromosomes themselves (Essad, 1984; Gamiette et al., 1999; Bousalem et al., 2006). On the basis of published cytological studies on 72 *Dioscorea* species, it was concluded that the basic chromosome number of *Dioscorea* species is $x = 10$ or 9 (Essad, 1984). However, recent studies have shown that the basic chromosome number of the three major cultivated species, *D. rotundata, D. alata,* and *D. trifida,* is $x = 20$ and not $x = 10$ as previously believed. More research is needed to re-examine the basic chromosome numbers of other *Dioscorea* species.

5 Breeding Achievements and Goals

In all the major edible yam species, the main breeding objective is to combine the traits of high and stable tuber yield, good tuber quality [oxidation rate (browning of the cut tuber), taste, texture, dry matter content], tuber characteristics that facilitate harvesting and are valued by consumers (size, shape), and resistance to abiotic (drought and low soil nutrients) and biotic stresses (virus, fungi and nematodes). Potyviruses are the main limiting factor for the production of *D. rotundata, D. cayenensis,* and *D. trifida* species, and anthracnose for *D. alata*. In white yam, nematodes pose a more severe problem than in other species. Yam cultivation is also limited by the high cost of labor and the need for fertile land. Better yield stability under different cultivation conditions can be acquired by maximizing heterozygosity and by the production of highly heterozygous polyploid hybrids. Labor inputs can be reduced by selecting genotypes that do not require costly staking operations and that produce tubers that are easy to harvest.

Host plant resistance to yam nematodes (Kwoseh et al., 2007; Mohandas et al., 1996) and viruses (Mignouna et al., 2001b; Odu et al., 2004a, b, 2006a) has been identified. The genetic basis of the resistance to a Nigerian isolate of yam mosaic virus (YMV), genus Potyvirus, was investigated in *D. rotundata* (Mignouna et al., 2001b). Results showed that it can be expressed by the action of a single dominant gene, *Ymv-1*, in simplex condition or a major recessive gene in duplex condition. High levels of resistance to viruses in white yam have been introduced into new varieties that combine this resistance with high yield and good quality (Egesi and Asiedu, 2002). Collaborative evaluation of IITA-derived breeding lines with national yam programs in Africa has led to the official release of a number of varieties of *D. rotundata* in Nigeria and Ghana (Agbaje et al., 2002, 2003; Agbaje and Adegbite, 2006; Otoo and Asiedu, 2005). In India, several varieties of white yam have also been developed by the CTCRI and released to growers, including a dwarf variety (Nair et al., 1987; Abraham et al., 1989).

Because of the scarcity or absence of flowering in cultivars of the greater yam (*D. alata*), this species was long considered to be sexually sterile (Kay, 1973; Rao et al., 1973; Coursey, 1967, 1976; Martin and Rhodes, 1977; Martin and Delphin, 1978; Wilson, 1982). The possibility of sexual reproduction was demonstrated by the CTCRI during the 1980s. Pollination studies were integrated into pollen studies and cytological investigations. These studies revealed the general prevalence of high pollen fertility of the male clones that were primarily $2n = 40$ types. The $2n = 40$ types showed normal meiosis with 20 bivalents. The somatic chromosome numbers of the female clones were primarily $2n = 60$, followed by $2n = 40$ and 80 types. In artificial pollinations, only $2n = 40$ females formed viable seeds. Thus, a relationship between polyploidy level ($2n = 40$) and sexual fertility was established in *D. alata* (Abraham and Nair, 1991). It was also observed that the male clones were largely fertile, $2n = 40$ types, whereas the female clones were largely sterile, $2n = 60$ types. It was this differential distribution of fertile $2n = 40$ types among male and female clones that eluded the researchers and branded the species as being sexually sterile.

The breeding work carried out since then has all been entirely devoted to the creation of $2n = 40$ varieties through artificial pollination between $2n = 40$ types. Different sources of resistance to anthracnose have been identified in germplasm collections (Mignouna et al., 2001a; Lebot, 2003; Abang et al., 2003; Malapa, 2005; Odu et al., 2006b; Onyeka et al., 2006; Egesi et al., 2007; Abraham and Arnau, 2007; Arnau et al., 2008). Crosses using these cultivars have led to the creation of resistant hybrids (Egesi et al., 2005; Ano et al., 2005; Abraham and Arnau, 2007; Arnau et al., 2008). The genetic basis for the resistance to a moderately virulent strain in Nigeria has been investigated. This strain is the predominant virulent phenotype in Nigeria and represents a genetically heterogeneous population (Abang, 1997). Resistance to this strain appears to be controlled by a major dominant locus (Mignouna et al., 2001a). More knowledge about the genetic basis of other resistances is necessary to develop varieties that carry several resistance genes and to provide more sustainable resistance.

The first breakthrough in greater yam breeding was accomplished by the development of the first hybrid selection by the CTCRI (a variety known as ‘Sree Shilpa’) and its official release in the state of Kerala in India in 1998. This variety is a medium-to-high yielder (28 tonnes/ha), with two to three tubers, free of anthracnose, viral diseases, and pests such as nematodes.

Very recently, the collaborative research work between the CTCRI (India) and CIRAD (France), sponsored by the Indo-French Centre for the Promotion of Advanced Research (IFCPAR), has made another breakthrough in greater yam breeding possible. Joint studies have led to the discovery of fertile $2n = 80$ types that were hybridized to produce $2n = 80$ progenies. Crosses between $2n = 40 \times 2n = 80$ were also successful, producing $2n = 60$ progenies. Thus, for the first time, higher ploid hybrids ($2n = 60$ and $80 = 3x$ and $4x$) were produced in the greater yam by conventional hybridization. This has opened up new perspectives in greater yam breeding since the higher ploid hybrids are more vigorous and higher yielding (Arnau et al., 2006; Abraham and Arnau, 2007; Arnau et al., 2007a).

6 Breeding Methods and Techniques

The breeding scheme begins with the selection of parents for hybridization based on data from the characterization and evaluation of germplasm at different levels: agronomic attributes, tuber quality, ploidy status, flowering ability, trait-combination ability, etc. Separate hybridization blocks of male and female parents are established for varieties with similar flowering dates. Male and female plants are planted next to each other and the vines are staked to common supports, allowing the floral branches to mix and pollination to be carried out by thrips. This practice is easy and useful and the purity of crosses can be ensured if biparental combinations are planted in isolated plots. The required separation distance between blocks has not been systematically established for yams. This distance usually exceeds 500 m. Moreover, many desirable parents can be planted together to generate polycross seeds. Multiple

planting dates are necessary to enhance synchronization of the flowering of male and female parents.

Hybridization by manual pollination is the best way to ensure biparental crossing when the parental flowering dates do not coincide or if the male parent does not flower during a particular year. Pollination by the "pencil method" (Abraham and Nair, 1990) usually results in a very high degree of fruit set and seed set. However, it is laborious and time consuming since the male flowers are tiny (1–2 mm in diameter) and the anther transfer to the stigma requires some amount of skill and experience. When jointly conducted by two people, this method can result in about 240 successful pollinations per hour. Since the male flowers are ephemeral and open at noon, the best time for pollination is during the noon hours. Since the female flowers remain open for 6–7 days with a fully receptive stigma, the limiting factor in continued pollination during the day is the short pollen viability period that diminishes after 2 or 3 p.m., depending on the prevailing weather conditions. However, pollination time can be enhanced by forcibly opening the male flowers 1 h before their natural opening to take out the anthers for deposit on the stigmatic surface.

Pollen storage is of prime importance for the production of progeny from desired parents when the parental flowering dates do not coincide or if the male parent does not flower during a particular year. Daniel et al. (2002) have described conditions for preservation of yam pollen. The cold storage of pollen without pre-drying at –20 or –80°C for periods as long as 2 years or more is an efficient means of conservation.

The maturation of fruits and seeds takes about 12 weeks and continues long after leaves have senesced. The fruits (capsules) eventually turn from green to yellow and then brown. At that stage, they are collected in large paper envelopes before they split open to naturally disperse their seeds. The paper envelopes are hung in a dry area at ambient temperature for a few weeks to allow the capsules to dry further. The seeds are then extracted from the capsules if they have not already dropped into the envelope.

The seeds are either drilled in nursery beds or sown individually in Jiffy 7 peat pellets. Pre-sowing seed disinfectants are known to affect the percentage of seeds that germinate. Most yam seeds will germinate without treatment but the most effective way to disinfect them without significantly affecting germination is to soak them for 20 min in 10% w/v calcium hypochlorite. Yam seedlings are transplanted to nursery beds or to the field for first year evaluation.

Only certain traits can be evaluated during the first generation (F1) and the first clonal generation (C1). These include flesh color, the browning of the cut tuber, and disease symptoms following natural infection by anthracnose and viruses. Yield stabilizes in the second clonal generation (C2), with positive correlations with that of the subsequent generations and, hence, selection may take place from this stage (Abraham, 2002). The evolution of tuber shape during the different generations has been studied in *D. alata* progenies (Abraham et al., 2006). The oval-shaped tubers often undergo shape changes from the seedling stage to clonal generations, whereas the cylindrical tuber shape seems to be fairly stable across generations. The tuber shapes remain stable as a clonal trait after the second clonal generation and direct

selection can take place from this stage. The variables that can be evaluated from the second clonal generation include shoot vigor, tuber shape, disease severity, tuber yield, and yield components.

Early clonal trials are limited by the low multiplication ratio of the tubers, requiring three to four annual cycles before multilocational yield trials can be established. The yield trials are conducted in collaboration with farmers at multiple sites to allow assessment of the influence of potential genotype × environment interactions on the traits of interest.

In crosses where embryo development is not normal, in vitro culture of embryos has proved itself to be successful (Arnau et al., 2006). This is especially the case in the greater yam when a $2n = 40$ female parent is crossed with a $2n = 80$ male parent to produce $2n = 60$ triploid progenies.

Flow cytometry is used to check the ploidy level of *D. alata* hybrids obtained by intra-cytotype crosses. It is also used to determine the ploidy status of new clones introduced into germplasm collections before they can be used in the breeding program.

7 Integration of New Biotechnologies into Breeding Programs

7.1 Molecular Markers

Molecular markers are increasingly used to examine the genetic diversity of cultivated and wild yam species (see Section 4). Microsatellite markers have been developed and used to characterize the genetic diversity of CTCRI (India) and CIRAD (France) *D. alata* germplasm collections, within the framework of a collaborative project sponsored by the Indo-French Centre for the Promotion of Advanced Research (IFCPAR) (Abraham and Arnau, 2007). These markers are now routinely used to select genetically distant parents to maximize heterozygosity and heterosis in the progenies. The characterization of the IITA's Core Collection with SSR is also in progress.

The development of genetic maps allows the use of marker-assisted selection (MAS). Genetic linkage maps based on AFLP markers have been constructed for *Dioscorea tokoro*, a wild yam (Terauchi and Kahl, 1999) and for the cultivated species, *D. rotundata* (Mignouna et al., 2002e), and *D. alata* (Mignouna et al., 2002d). The *D. rotundata* map is based on 341 markers segregating in an intraspecific F1 cross. Separate maternal and paternal linkage maps were constructed, comprising 12 and 13 linkage groups, respectively. Several QTLs with an effect on resistance to YMV were identified, three on the maternal linkage map and one on the paternal linkage map, showing that both parents contributed to the phenotypic resistance of the progeny. The *D. alata* map is based on 469 markers segregating in an intraspecific F1 cross. These markers were mapped on 20 linkage groups. One QTL located on linkage group 2 was found to be associated with anthracnose resistance, explaining 10% of the total phenotypic variance. The genome coverage of the

D. rotundata and *D. alata* maps is 56 and 65%, respectively. The saturation of these maps with co-dominant markers such as microsatellites or simple sequence repeats (SSRs) and expressed sequence tags (ESTs) is necessary for full utilization of their potentials and greater ease of application in yam breeding. Recently, a USAID Linkage Project coordinated by the IITA and Virginia State University (VSU) (USA) generated several thousand ($\geq$ 80,000) expressed sequence tags (ESTs). In addition, a joint CIRAD-INRA project is developing new microsatellites for linkage purposes. To date, only about 60 SSR markers developed from seven different yam species, *D. tokoro* (Terauchi and Konuma, 1994), *D. rotundata* (Mignouna et al., 2003)*, D. alata, D. abyssinica, D. praehensilis* (Tostain et al., 2006), *D. japonica* (Misuki et al., 2005), and *D. trifida* (Hochu et al., 2006), are available in yams.

The bulked segregant analysis approach was successfully used for the identification of RAPD markers linked to YMV and anthracnose resistance genes. Two RAPD markers, $OPW18_{850}$ and $OPX15_{850}$, closely linked in coupling phase with the dominant YMV-resistance locus Ymv-1 were identified (Mignouna et al., 2002b). These markers successfully identified the resistance gene in resistant genotypes among a sample of 12 *D. rotundata* varieties. Similarly, two RAPD markers, $OPI17_{1700}$ and $OPE6_{950}$, closely linked in coupling phase with the anthracnose resistance locus, Dcg-1, were identified (Mignouna et al., 2002a). These RAPD markers will be easier to use for indirect selection once converted into co-dominant PCR-based sequence characterized amplified regions (SCARs).

Conventional breeding of yam is time consuming due to various factors including the long growth cycle. The identification of DNA markers linked to key traits that affect yam yield and quality will make it possible to accelerate the gene transfer process. In addition, the identification of markers linked to other identified anthracnose resistance sources will facilitate the pyramiding of different genes.

Molecular markers can also be jointly used with isozymes and cytogenetic analysis to clarify the ploidy status of other cultivated and wild *Dioscorea* species. A study is in progress using microsatellite segregation analysis to determine if the tetraploid *D. alata* clones are autopolyploid or allopolyploid, making it possible to optimize the breeding methods for the production of polyploid varieties.

In addition, flow cytometry analysis revealed the natural production of diploid gametes in *D. alata* species. Screening progenies produced by crosses between diploid and tetraploid varieties ($2x \times 4x$) showed that instead of obtaining 100% triploid hybrids, some crosses produce 30% tetraploid hybrids and some females are capable of forming unreduced diploid gametes. Moreover, crosses between two diploid varieties ($2x \times 2x$) resulted in progenies with a majority of diploid hybrids, but with some triploid and tetraploid hybrids as well, suggesting that some males can also form unreduced diploid gametes. Studies using microsatellite markers are in progress to determine what type of cytological event leads to the formation of $2n$ gametes: SDR (second division restitution) or FDR (first division restitution). This will make it possible to determine if the $2n$ gamete formation phenomenon can be used to transfer heterozygosity and agronomic values of diploids to the tetraploid level.

7.2 *In Vitro* Culture

In vitro culture allows the conservation of germplasm and the multiplication of disease-free plants. A significant amount of research has been undertaken in this area since the 1980s (Mantell et al., 1978, 1980; Ng and Hahn, 1985; Ng, 1988; Ng and Ng, 1990; Saleil et al., 1990; Malaurie et al., 1993, 1995; Filloux and Girard, 2006). It also makes it possible to obtain tetraploid lines by chromosome doubling of diploids (Arnau et al., 2006) and immature embryo rescue. In vitro embryo rescue should facilitate wider crosses with related species and wild yams to give access to a much wider range of genes.

7.3 Somatic Hybridization and Transgenesis

Given the scarcity or absence of flowering in some yam cultivars, somatic hybridization could be useful for producing hybrids from elite, non-fertile varieties. Genetic transformation could be useful for more efficiently transferring virus and anthracnose resistance to commercial varieties. Plant protoplasts are the starting material for the production of somatic hybrids through cell fusion and transgenic plants through protoplast transformation. These two technologies require the development of reliable systems of regeneration. Much research was devoted to this subject in the 1990s, but with limited results (Mantell, 1994; Tor et al., 1998; Mantell and Boccon-Gibod, 1998).

8 Seed Production

8.1 True Seed in Breeding

Seeds can be produced by artificial or natural pollination. Artificial pollination requires the skilled handling and timely use of the ephemeral male flowers for pollen/anther transfer to stigma. To ensure the purity of the offspring in biparental crosses, female flowers are bagged before opening and the pollinated female flowers are then kept bagged for 7–8 days since the stigma remains fertile for 6–7 days. Natural pollination can be directed (for specific crosses) or non-directed (polycross) for producing bulk seeds. The fruits mature in 10–12 weeks and the seeds are collected before they are dispersed from the dehiscent capsules. Yam seeds are winged and adapted to wind dispersal. White yam seeds have a dormancy period of about 3 months, whereas greater yam seeds have no dormancy period (Abraham, 1992). In the latter, the seeds start germinating 10–12 days after sowing and are ready for transplantation in 6 weeks.

8.2 Seed Tubers for Commercial Propagation

Farmers usually save from 10 to 30% of their harvest to use as planting material for the next crop. This can either be small tubers or large ones which before planting

are cut into 20–40 small pieces called mini-setts (Lebot, 2009). When small tubers are kept year after year, tuber quality can deteriorate and tuber-borne diseases can accumulate. The mini-sett technique is now used increasingly by commercial growers for multiplying stocks of new cultivars and for providing seed tubers for farmers to plant. Rooted stem cuttings can also be used for the production of planting setts of disease-free minitubers. Ideally the starting material should be virus-free plants, resulting from tissue culture and virus indexing. In vitro nodal segments can then be used for rapid clonal multiplication. In vitro plantlets are also the most practical way of distributing germplasm internationally. Finally it is worth mentioning that some species and cultivars produce bulbils, and these are a good source of seed setts (Lebot, 2009).

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Chapter 5
Taro and Cocoyam

José Quero-Garcia, Anton Ivancic, and Vincent Lebot

1 Introduction

Aroids are an economically important source of food for numerous tropical countries. They are mostly consumed for their corms and cormels but leaves and petioles can also be part of the diet. According to FAO databases (www.fao.org 2007) taro (*Colocasia esculenta* (L.) Schott) and cocoyam (*Xanthosoma sagittifolium* (L.) Schott) produce the lowest average yields (6.5 tons/ha) of all root crops. World production in 2006 was approximately 11.9 million fresh tons from 1.8 million hectares but significant taro producers such as India, Bangladesh, Burma, Indonesia, Papua New Guinea, and Cuba do not supply production figures. Since taro production was around 4 million tons in 1961, its cultivation is stable or even growing and follows the global trend of demographic growth. Aroids are considered minor crops but they are a staple food for numerous poor populations from tropical countries. Taro is cultivated as a backyard or a home garden crop, or within a shifting agro-forestry system with very limited inputs. But even in countries where crop management techniques are quite elaborate (i.e., Hawaii and Egypt for taro and Florida for cocoyam), it is clearly observed that the yields per unit of area and time are too low (Lebot, 2009).

The Araceae family includes close to 110 genera and more than 2,500 species (Bown, 2000). It is subdivided into nine sub-families and both taro and cocoyam belong to the Colocasioideae sub-family. One of the major characteristics of aroids is the spadix and spathe type of inflorescence (Figs. 5.1 and 5.2). A spadix is a club-like racemose inflorescence bearing flowers on a fleshy axis. The spathe is a large bract subtending and unsheathing the inflorescence. Flowers are generally small and can be unisexual or bisexual. According to Hay (1990), plants from the

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Fig. 5.1 Taro inflorescences (Source: Vincent Lebot)

Colocasioideae sub-family have unisexual flowers. A second major trait of aroids is protogyny. Female flowers become receptive before pollen is shed, which enhances cross-pollination. Pollination is carried out by insects and the beginning of flowering is characterized by the release of attracting odors. Flowering in the Araceae family is characterized as well by thermogenesis, which has recently been documented for taro, giant taro (*Alocasia macrorrhizos* (L.) G. Don), and *Colocasia Gigantea* (Ivancic et al., 2004a, 2005, 2008). Taro and cocoyam are the most important root crops of the Araceae family. Both are very ancient aroids and are included in about half a billion people's diet. There are three other relatively important aroids: giant taro, swamp taro (*Cyrtosperma merkusii* (Hassk.) Schott), and elephant foot yam (*Amorphophallus campanulatus* (Roxb.) Blume). Cocoyam has overtaken taro as the main edible aroid in many tropical areas (Onokpise et al., 1999; Matthews, 2002). Despite this increasing importance, breeding efforts have been by far more intense for taro than for cocoyam. The genus *Xanthosoma* has about 40 species, grown as ornamentals and as food crops. The taxonomic position of the cultivated *Xanthosoma* species is unclear and there is a tendency to call them all *X. sagittifolium*.

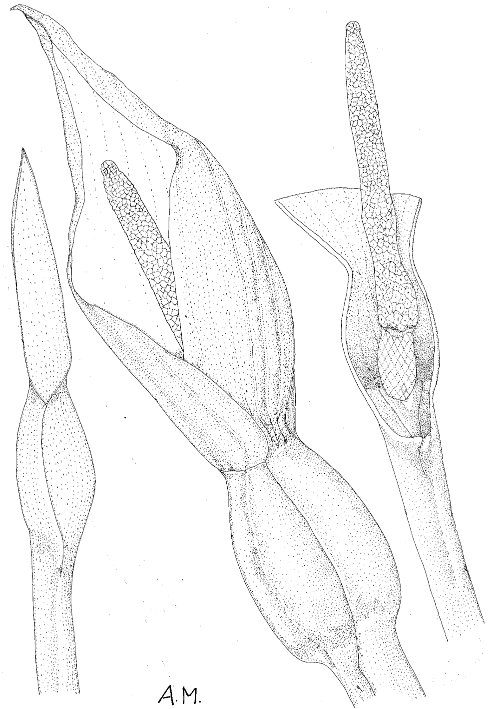

Fig. 5.2 Cocoyam inflorescences (Source: Vincent Lebot)

2 Origin and Domestication

2.1 Taro

Taro is probably one of the oldest crops on earth and its domestication would have occurred more than 10,000 years ago. Although some authors (Yen and Wheeler, 1968; Matthews, 1990) consider that taro originated in the Indo-Malayan region, between Myanmar and Bangladesh, there is not enough evidence to prove it. Furthermore, there has not been a comprehensive and detailed genetic study including cultivars and wild materials from the whole area of distribution of taro (India, Southeast Asia, South China, Melanesia, and Australia).

The first studies on taro domestication claimed that taro was first domesticated in Southeast Asia before being transported to the south toward the Pacific Islands, the north toward China and Japan, and the west toward the Mediterranean area (Cyprus and Egypt) (Yen and Wheeler, 1968). However, several authors have recently argued that at least an independent process of domestication has occurred in the Pacific (Matthews, 1990; Lebot and Aradhya, 1991; Lebot, 1999). Archaeological studies have documented an independent emergence of agriculture, during the early and mid-Holocene, at Kuk Swamp in the highlands of Papua New Guinea (Fullagar et al., 2006). Moreover, two issues concerning a unique domestication event in

Southeast Asia have been raised. The first migrants to reach Australia and Papua New Guinea, 40,000 years ago, had to cross 100 km of open ocean to reach the Sahul plate (Belwood, 1979; Yen, 1982). It is unlikely that they carried on their fragile rafts planting materials of banana, taro, or yams. Second, the presence of typical wild taros has been reported in areas such as Papua New Guinea, Solomon Islands, Northern Australia, and New Caledonia (Coates et al., 1988; Ivancic and Lebot, 1999). It is dubious that migrants from Southeast Asia would carry wild taros in their maritime expeditions. Wild taros could have been dispersed from Sunda to Sahul by birds. Caillon et al. (2006) observed that fruits and seeds from taro in Vanuatu could be eaten by a purple swamphen, *Porphyrio porphyrio* (fam. Rallidae).

Studies conducted with biochemical and molecular markers have given support to the existence of two independent domestication processes in Sunda and Sahul (Lebot and Aradhya, 1991; Kreike et al., 2004; Noyer et al., 2004). An isozyme study conducted within a set of 1,417 cultivars from Asia and Oceania allowed the differentiation of two genepools, one from Asia and one from the Pacific (Lebot and Aradhya, 1991). This first study included solely cultivars from Indonesia as Asian representatives but a more recent isozyme survey covering four Asian and three Pacific countries confirmed this result (Lebot et al., 2002). Taro (*C. esculenta*) might be a species of Gondwanean origin (i.e., present in the southern precursor-supercontinent which included Antarctica, Africa, Madagascar, Australia-New Guinea, and New Zealand, as well as Arabia and the Indian subcontinent) and its geographic distribution might be larger than initially envisaged. Different waves of domestication might have independently occurred in distant places. Taro is a species cultivated from 0 to 45° of latitude and from 0 to 3,000 m of altitude; such phenotypic plasticity might reflect a very ancient natural distribution (Lebot, 2009).

At the time of the great discoveries by Western explorers, taro was already largely cultivated throughout the tropics, but also in temperate latitudes, such as China, Nepal, Japan, Korea, the Mediterranean, and New Zealand (Lebot, 2009). Taro reached Egypt about 2000 BP (Plucknett et al., 1970) and was introduced in sub-Saharan Africa very early, via the Nile or most likely via Madagascar (Lebot, 2009). The slave trade allowed the introduction of taro in the West Indies and Cuba during the eighteenth century. More recently, taro was likely re-introduced into Cuba, after the Spaniards, by Chinese immigrants during the nineteenth century and by the Japanese just before the Second World War.

2.2 Cocoyam

Concerning cocoyam, the situation is by far less documented than for taro. It is likely to have been domesticated on the northern side of the Amazon basin. It was introduced to Central and West Africa, between the sixteenth and seventeenth centuries when it was brought by Portuguese slavers into Sao Tomé and Principe (Bown, 2000). The introduction of cocoyam in Asia and Oceania occurred in the nineteenth

century and was the result of missionaries' intervention (Coursey, 1968; Wilson, 1984).

3 Varietal Groups

3.1 Taro

Taro is considered a very polymorphic species with at least two botanical varieties (Purseglove, 1979):

C. esculenta var. *esculenta* named as dasheen and
C. esculenta var. *antiquorum* named as eddoe.

These two varieties are mainly differentiated by the shape and size of the main corm and cormels. Var. *esculenta* genotypes have a larger main corm and smaller side cormels whereas var. *antiquorum* genotypes usually have a relatively smaller central corm and well-developed side cormels (Photos 5.1 and 5.2).

Concerning taro ploidy levels, only diploids and triploids, with, respectively, $2n = 28$ and $3n = 42$ chromosomes, have been observed in nature (Yen and Wheeler, 1968; Coates et al., 1988; Sreekumari, 1997). Basic chromosome number is x=14 (Kuruvilla and Singh, 1981; Matthews, 1990; Kokubugata and Konishi, 1999). In the Pacific and certain regions of Southeast Asia, cultivars and wild taros are mainly diploids. Kreike et al. (2004) identified 34 triploids within a sample of 255 genotypes from seven different countries (Indonesia, Malaysia, Papua New

Photo 5.1 Dasheen taros sold in a market in the island of Tanna, Vanuatu (Source: José Quero-García)

Photo 5.2 Eddoe taros (*left bottom corner*) sold in a market in Bangkok, Thailand (Source: Vincent Lebot)

Guinea, Philippines, Thailand, Vanuatu, and Vietnam). In Asian countries such as India or China, distribution is much more even (Sreekumari and Matthews, 1992; Matsuda, 2002). Triploids are more frequent at high latitudes and altitudes, both in India (Sreekumari and Matthews, 1992) and in China (Zhang and Zhang, 1990). They are not more vigorous than diploids but the presence of a third chromosome set would entail better adaptive capacities (Sreekumari, 1997). Most authors agree on a formation of triploid genotypes through non-reduced gametes.

Diploids and triploids have traditionally been associated with dasheens and eddoes, respectively (Ivancic and Lebot, 2000), but recent studies proved that, although the majority of diploid genotypes from the Pacific area were of dasheen type, eddoes could also be diploids and conversely, dasheens have been found to be triploids (Lebot et al., 2002; Kreike et al., 2004).

Taros can be cultivated in two different systems: rain fed or irrigated (Photos 5.3 and 5.4). Dasheens are found in both cultivation systems whereas eddoes are only cultivated in rain fed conditions. Although some genotypes may be preferentially adapted to either rain fed or irrigated conditions, numerous intermediate types have been identified (Ivancic and Lebot, 2000).

Apart from these major distinctions (dasheen vs. eddoe, diploid vs. triploid, and rainfed vs. irrigated), numerous morpho-agronomic traits are used by farmers for varietal differentiation. In China, farmers concentrate on petiole color, corm color, shape, and taste. In some Pacific countries, such as Vanuatu, farmers share a more sophisticated system of varietal identification using four main groups of descriptors: petiole colors, leaf characteristics, plant shape, and corm traits (Caillon et al., 2004).

Finally, taro varieties can also be classified according to culinary uses. Caillon and Lanouguère-Bruneau (2004) studied the taro cultivars of the west coast of Vanua

Photo 5.3 Rain fed taro, island of Ambae, Vanuatu (Source: José Quero-García)

Photo 5.4 Irrigated taro, island of Vanua Lava, Vanuatu (Source: Sophie Caillon)

Lava, an island from Vanuatu, and found different cooking techniques, some adapted to specific cultivars.

3.2 Cocoyam

Concerning cocoyam, the main corm is usually acrid and it is only eaten when no other food is available (i.e., during and after cyclones in some Pacific islands). Young leaves of some cultivars can be used as a vegetable and can be an important source of proteins and vitamins. Cultivars are differentiated mainly by leaf pigmentation, plant size, cormel shape and number, cormel tip shape and pigmentation, spatial arrangement of cormels, and cormel flesh pigmentation (Lebot, 2009).

4 Genetic Resources

4.1 Taro

Since the first taro breeding programs were launched in the 1970s, numerous ex situ collections have been created in different countries, in particular within the Asia-Pacific region, although collections were also formed in Africa and the Caribbean. However, despite these efforts, most collections were lost due to financial, political, or climatic factors (Ivancic and Lebot, 2000). To date, close to 6,000 taro accessions have been collected and described in different countries.

TANSAO (Taro Network for Southeast Asia and Oceania), an EU-funded project, composed of four Asian and three Pacific countries, aimed at exchanging a core sample composed of elite cultivars of each country member. The objective was to exchange cultivars representative of the genetic diversity (evaluated in two stages, first with isozymes and second with AFLPs) of this vast region (Lebot et al., 2004b). In the Pacific, the project TaroGen (Taro Genetic Resources: Conservation and Utilization) aimed at creating a regional in vitro center of genetic resources conservation at Suva, Fiji. The objective was to conserve close to 20% of the accessions collected in each country, by maximizing the morpho-agronomic variation and SSR genetic diversity (Godwin et al., 2004).

When using morpho-agronomic descriptors to characterize genetic resource collections, many authors have attempted to apply analytical methods in order to classify the different taro cultivars or morphotypes. Lebot et al. (2002) used factorial analysis of correspondence (FAC) and clustering hierarchical techniques such as UPGMA when analyzing the morpho-agronomic variation of 2,289 accessions from the TANSAO countries. No clear structure was produced and a vast continuum of groups (or clusters) was obtained. A stratification of large collections based on the use of six important traits and a dichotomous key was proposed (Quero-García, 2000; Lebot et al., 2002; 2004a). Quero-García et al. (2004), while working with the Vanuatu taro collection (452 cultivars), validated this methodology and compared the genetic diversity of three different types of sub-sets with AFLP markers. Singh et al. (2008) proposed another methodology for the constitution of core collections by studying the Papua New Guinea taro collection (859 accessions). Cluster analysis based on morpho-agronomic traits allowed the selection of 20% of the accessions. Subsequently, by using seven SSR markers, this first sample was reduced and rationalized to 10%.

Studies aimed at characterizing the genetic diversity of taro genetic resources were initially conducted with isozyme markers (Table 5.1):

The most striking result in the study of Lebot and Aradhya (1991) was the fact that the isozyme polymorphism within Polynesian and Micronesian cultivars was almost nil. This could be explained by the absence of sexual reproduction in this area, where cultivars rarely flower and active pollinators have not been observed. The strong phenotypic variation found in Polynesian taros would correspond to the accumulation of somatic mutations through a very intense rate of

Table 5.1 Main diversity studies conducted with isozyme markers

Region	Acc Nb.	Isozyme Nb.	Authors	Year
Oceania–Indonesia	1,417	7	Lebot and Aradhya	1991
Oceania–Southeast Asia	2,081	6	Lebot et al.	2002
Asia–Southeast Asia	113	13	Ochiai et al.	2001
China–Japan–Taiwan	102	4	Matsuda	2002

clonal propagation. Within the TANSAO project, Lebot et al. (2002) found 319 different zymotypes. However, six zymotypes represented 51% of the total number of accessions and the genetic variation within these zymotypes was rather limited. The genetic base of analyzed cultivars was very narrow. Conversely, wild accessions had much higher isozyme diversity. In terms of isozyme diversity, Asian countries were richer than countries from the Pacific.

Asian studies consistently demonstrated that few bands were specific to triploids, confirming their auto-polyploid origin. Within each geographic region, diploids and triploids tended to form different genetic groups, suggesting an agro-ecological and sexual differentiation between both ploidy levels.

Isozyme markers were able to produce large-scale studies and to confirm hypotheses about the origin, domestication, and dispersal of taro. However, they did not allow the differentiation of major agronomic groups, in particular within the Pacific region, characterized by a great morphological variability (Photo 5.5).

More recently, DNA markers have been used to characterize taro genetic resources (Table 5.2). In most studies, these markers confirmed previous isozyme results in terms of geographical differentiation, between large regions (Asia vs. Oceania) or between countries. However, differences between regions in terms of genetic diversity are not as strong as revealed with isozymes; thus, genetic diversity of taros from Pacific countries is not as reduced as compared to that from Southeast Asian countries. Furthermore, there is no concordance between genetic diversity and morpho-agronomic variation. Thus, molecular markers do not allow the differentiation between the main varietal groups, such as eddoe and dasheen taros, rain fed or

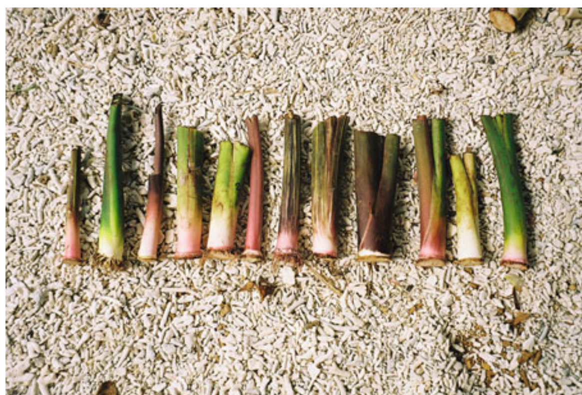

Photo 5.5 Phenotypic variability for taro petiole colours (Source: José Quero-García)

Table 5.2 Main diversity studies conducted with molecular markers. The number of SSR primers pairs is indicated into parenthesis

Marker type	Region	Acc Nb.	Authors	Year
RAPD	Asia–Southeast Asia	83	Ochiai et al.	2001
AFLP	Oceania–Southeast Asia	256	Kreike et al.	2004
AFLP	Vanuatu	120	Caillon et al.	2006
SSR (7)	Oceania	515	Godwin et al.	2004
SSR (22)	Oceania–Southeast Asia	144	Quero-García et al.	2006a
SSR (9)	Vanuatu–Southeast Asia	385	Sardos	2009

irrigated taros, taros with or without stolons. Nevertheless, compared with isozyme markers, DNA markers produce reliable fingerprints for taro cultivars, even in areas characterized by a narrow genetic base, such as most of the Pacific countries.

Ex situ conservation of taro has faced numerous difficulties in the past and alternative methods have been tested. Thus, in vitro conservation was acknowledged as a safer and cheaper technique. Several teams have optimized in vitro protocols and have verified genetic stability by using morpho-agronomic and molecular markers (Taylor et al., 2004; Hussain and Tyagi, 2006). Another attractive solution is the droplet vitrification cryopreservation technique for which Sant et al. (2006, 2008) have optimized the protocol.

The cheapest way of preserving the genetic variation is represented by true botanical seeds. However, there are problems associated with seed viability, which does not exceed 5 years (Ivancic and Lebot, 2000; Price et al., 2007).

Finally, in situ conservation approaches have been proposed for taro genetic resources conservation. In Vanuatu, Caillon et al. (2006) found that the genetic diversity, assessed with AFLP markers, contained in a typically taro-producing village, was not significantly less than the diversity contained in a core sample issued from the taro national collection. They proposed a dynamic in situ conservation (DISC) strategy, favoring the broadening of the genetic base. This approach has recently been adopted in Vanuatu, where 41 elite cultivars from TANSAO have been distributed to farmers from 10 different villages. From a theoretical point of view, Lebot et al. (2005) called this approach 'geographical distribution of allelic diversity' (DAD). In order to compare the allelic diversity already present in farmers' villages and the one incorporated via TANSAO elite cultivars, Sardos (2009) conducted an extensive SSR study. She found 86 alleles within the 344 taro cultivars sampled in the farmers' villages and 52 new alleles within the TANSAO subset of elite cultivars. In order to validate this in situ dynamic conservation approach, further studies will aim at evaluating the extent of incorporation of the TANSAO genes into the local genepool.

4.2 Cocoyam

Concerning cocoyam, available data are from the mid-1990s. The total number of *X. sagittifolium*, *X. violaceum*, *X. nigrum*, *X. brasiliense*, and *X. yuca* accessions

was close to 400 (FAO, 1995) and the largest collection was established in Cameroon. In Cuba, Milián et al. (2001) characterized *X. violaceum*, *X. atrovirens*, *X. caracu*, and *X. sagittifolium* accessions. They concluded that the taxonomy of the genus *Xanthosoma* is not clearly defined at a specific level. Major collections of *X. sagittifolium* from Cameroon, Equatorial Guinea, Gabon, Ghana, and Togo were also created during the 1980s (Tambong et al., 1997; Onokpise et al., 1993, 1999).

DNA markers have only recently been used for cocoyam genetic diversity studies. Schnell et al. (1999) studied 18 cultivars of *Xanthosoma* spp. from the USDA germplasm collection based at Puerto Rico with RAPDs. The genetic dissimilarity between these accessions was rather low and the authors stated that the existing collection was of limited value as a genetic resource. They found some unexpected groupings, including different species (*X. caracu* and *X. violaceum*) and different morphotypes (with white and purple cormels). Their results confirmed those of Milián et al. (2001) and the authors considered that all these traditionally differentiated species could be in fact variants of the same species. In Ghana, Offei et al. (2004) analyzed 70 accessions, again with RAPD markers. They found significantly higher polymorphisms compared with the results of Schnell et al. (1999) and considered that this genetic diversity might result from sexual reproduction events through open pollination.

5 Major Breeding Achievements

5.1 Taro

Breeding programs of taro were initiated from the late 1970s. Their main characteristics are summarized in Table 5.3.

Table 5.3 Main characteristics of the most important taro breeding programs

Country	Year	Breeding goals	Breeding scheme	Varieties released
Fiji	1978	Quality, yield	Bi-parental crosses	3
Samoa	1982	Quality, yield	Bi-parental crosses	1
Samoa	1996	TLB resistance	Recurrent selection	6
Solomon Islands	1978	TLB resistance	Bi-parental crosses, backcross	1
Solomon Islands	1992	TLB resistance	Recurrent selection	0
Papua New Guinea	1993	Quality, yield, TLB resistance	Recurrent selection	5
India	1995	Quality, yield, TLB resistance	Bi-parental crosses	3

TLB, Taro Leaf Blight
Source: Quero-García (2004).

The Fijian breeding program proved that it was relatively easy to find good hybrids when crossing good parents (Sivan and Tavalqia, 1984; Wilson et al., 1991). Despite the narrow genetic base utilized, strong phenotypic variation was observed for important traits such as yield, petiole and corm colors, and number of stolons. A popular hybrid, *Samoa Hybrid*, was released and was largely accepted by farmers.

In Samoa, the first breeding program had a regional perspective, with the distribution of clones to six other Pacific countries. The result was the release of hybrid *Alafua Sunrise* in 1988. It was superior to the local cultivar, *Niue*, with a yield gain of 50–130%, although it was not largely accepted by farmers. Overall, *Samoa Hybrid* and *Alafua Sunrise* produced good yields in all countries free of TLB (caused by *Phytophthora colocasiae*), suggesting a good adaptability to different environments (Rao et al., 1998).

When TLB entered Samoa in 1993, taro cultivation was literally wiped out (Chan et al., 1998). Because of strict quarantine rules, only local varieties had been used for breeding and no resistance source was present in the country. This disaster raised new interest for taro breeding in the whole Pacific area but also in Asian countries such as India. In Samoa, a new program was initiated in 1996. Introduced sources of resistance to TLB came from Palau, Pohnpei, and the Philippines. After the first breeding cycle, 10 clones were identified with good resistance to TLB, good vigor, and taste. After multiplication and multi-site evaluation, six clones have been officially released to farmers, of which four yielded higher than the reference variety at the majority of evaluated sites (Fonoti et al., 2008).

Breeding programs in the Solomon Islands were the first to search for TLB resistance. The recurrent selection program was stopped in 1992 after the first cycle and although several lines are maintained, they have not been further evaluated. Only one line from the initial backcross program, *LA 16*, has been distributed to farmers. It is resistant, produces big corms, and has an acceptable taste (Rao et al., 1998).

The Papua New Guinea breeding program has managed to conduct the largest number of breeding cycles. Numerous expectations with regards to TLB resistance were raised by this program within the other Pacific countries, although, because of ABVC, Alomae-Bobone virus complex, distribution of genetic materials from Papua New Guinea remains delicate. Cycle 1 was based on a population composed of several agronomically superior cultivars and four wild genotypes used as TLB resistance sources. A poor eating quality was observed within hybrids, along with other wild undesired traits, such as the production of numerous stolons and flowers. Only eight clones appeared to be superior in repeated field trials. At the end, only one was found suitable for the farmers (Okpul, 1997). During cycle 2, a half-diallel design was adopted with the 18 best lines selected from cycle 1. Three lines from this second cycle showed large adaptability, after having been tested in seven different agro-ecological sites and have been released to farmers (Okpul, 2005). From the third breeding cycle, six elite lines were selected for multi-location trials. Line *C3-E10* was superior to the other five lines on the basis of high yield, TLB resistance, yield stability over a range of environments and good eating quality. It has been released to farmers and for regional multi-location trials in other Pacific countries (Singh et al., 2006).

In India, at the Central Tuber Crops Research Institute (CTCRI), in Trivandrum, Kerala, taro breeding consisted initially in clonal selection. Two high-yielding varieties, *Sree Reshmi* and *Sree Pallavi* were identified. Both are triploid, which indicates that in this country triploidy confers a higher yield potential. Contrary to Pacific countries, breeding in India concentrates solely on eddoe genotypes. Several sources of TLB resistance, both within the cultivated and wild genepools, were identified (Santha Pillai et al., 1993). In 2004, the first genetically improved taro hybrid, named *Sree Kiran*, was released in India. Today, five new hybrid lines are being evaluated in on-farm trials at different agro-climatic regions in the Kerala State (Sreekumari and Abraham, 2006). One original feature of the Indian breeding program is the utilization of the induction of polyploidy. Since triploids were found to be the most productive and vigorous, direct selections can be made from triploid cultivars but the latter cannot be used for breeding due to sterility. Hence, the aim is to produce artificial triploids by crossing diploids with induced tetraploids. Colchicine treatments on cormel buds from selected diploid individuals produce tetraploids which are taller and have comparable cormel yield with their diploid genitors. These individuals are presently being multiplied to promote flowering and to cross them with diploid cultivars (Sreekumari et al., 2004).

5.2 Cocoyam

Cocoyam improvement has been initiated in Cameroon where few viable seeds were obtained, perhaps due to ploidy differences (Tambong et al., 1997; Onokpise et al., 1999). Attempts were also made to produce new forms through in vitro culture (Tambong et al., 1998). Experiments conducted with local cultivars in Vanuatu have, however, shown that there are no major difficulties to producing large progenies (several hundreds of full-sibs).

6 Current Goals of Breeding

6.1 Taro

Yield is the most important goal in all taro programs. It is a complex, quantitative trait which depends on genotype, environmental factors, and $G \times E$ interactions (Ivancic and Lebot, 2000). Further complexity comes from the influence over yield of the size, the nature (mother plant vs. sucker), and the sanitary state of planting materials. Calibration of propagules is therefore a necessary preliminary step before any evaluation process. Plant height and leaf area are important factors correlated with taro yield (Simin et al., 1995; Lebot et al., 2006). Yield can be improved by increasing the individual potential of each taro plant or by increasing the yield per surface unit (through high plantation densities). Optimal densities can be better achieved with plants having an erect leaf orientation.

The second goal of taro breeders is eating quality. A good taro cultivar is determined by the chemical composition of the corms (i.e., by its chemotype) and by a regular and attractive corm shape. It must be non-acrid (low amount of calcium oxalate crystals) and with a relatively high dry matter content. Corm flesh color is sometimes associated with a good quality; for instance, in Vanuatu, yellow corms are particularly appreciated. Corm quality and corm yield appear to be negatively correlated.

Apart from yield and eating quality, a taro ideotype is determined by its maturity period (early varieties are preferred), corm shape, the number of suckers, the absence of stolons, the number of leaves, and the verticality of petioles. Corm shape is, however, determined by the presence of inflorescences which are a major cause of deformation. It has been observed that hybrids produce more flowers than traditional cultivars, which may be problematic when flowering is too profuse.

Concerning taro pests and diseases, much is known about a small group of them but for the majority, little or nothing is known about their economic or biological impact (Carmichael et al., 2008). The main threat for taro cultivation is TLB caused by *P. colocasiae*. Lebot et al. (2003) studied the intra-specific variability of *P. colocasiae* isolates from all TANSAO countries through isozyme and RAPD markers. A very high diversity was revealed, suggesting a high capacity of the pathogen to evolve rapidly in isolated insular regions. These results showed that once a cultivar is found resistant in one country, it will be exposed to genetically different isolates in other countries. Thus, breeding for TLB resistance should be conducted against local isolates in each country affected by TLB. Concerning the genetic mechanisms involved, Ivancic et al. (1996a) showed that resistance reactions could be highly variable. In addition to strong and distinct reactions, there were weaker ones, probably involving minor genes.

Taro viruses are not considered as dangerous as TLB, but Harding (2008) believes that they are one of the reasons for the decline in taro cultivation in the Pacific region over the past 30 years. Recent research efforts have allowed the identification of approximately five viruses infecting taro, from four taxonomic groups, namely *Dasheen mosaic virus* (DsMV), *Colocasia bobone disease virus* (CBDV), *Taro bacilliform virus* (TaBV), *Taro vein chlorosis virus* (TaVCV), and *Taro reovirus*. When TaBV combines with CBDV it causes Alomae-Bobone disease, which is the most damaging taro viral disease, largely spread in the Solomon Islands and Papua New Guinea. Breeding for resistance to this viral complex was conducted in the Solomon Islands and, although hybrids from the first generations were heavily affected, differences appeared in their recovery. Fully recovered plants were considered to have at least some tolerance (Ivancic et al., 1993).

Apart from viral vectors, such as taro leafhopper (*Tarophagus proserpina*) and aphids, the most important taro pests are the taro beetles (*Papuana* spp. and *Eucopidocaulus* spp.), particularly in Melanesian countries. Resistance to taro beetle is one of the most difficult breeding objectives. In Papua New Guinea, breeders are favoring corms which develop above ground since taro beetle damage is underground. This character can be easily transferred from wild populations. However,

plants are physically unstable, eating quality can be seriously affected by the wild genetic load, and it is likely that beetles would end up damaging corms above ground if no other corms were available.

6.2 Cocoyam

Concerning cocoyam, breeding in Cameroon has been oriented toward cocoyam root rot disease (CRRD) caused by *Pythium myriotylum*, which is the most serious constraint to production (Nzietchueng, 1988). A test has been developed to assess genotypes. It showed that root rot disease might be associated with an increased peroxidase activity in the roots. Breeders might apply this test to screen large progenies (Nyochembeng et al., 2007).

7 Breeding Methods and Techniques

Breeding of taro has been previously thoroughly documented by Wilson (1989) and Ivancic and Lebot (2000). Basically, the taro breeding process can be divided into three main steps:

– creating genetically variable populations
– selection of superior individuals
– release of new cultivars

Genetically variable populations in taro have been mainly created through hybridization, controlled or natural. Open pollination, within an artificial polycross population with a well-managed flowering induction system, is a simple and cheap way of producing high genetic variation. Self-fertilization of self-compatible genotypes is also possible but has not been exploited much because of inbreeding depression risks. In all cases, it is crucial to select good parental material to enable continuous and synchronized flowering. As many taro varieties do not flower naturally, flowering induction techniques may be used (e.g., treatment with gibberellic acid, removal of leaves or stress induced by drought, excess or low temperatures).

Since taro has a spadix with female and male flowers, the procedure for controlled hybridization has six steps: preventing insect pollination before hand pollination, emasculation, pollination, preventing insect pollination after hand pollination, labeling, and harvesting of seeds. The taro fruit is a berry which can contain over 50 seeds and a fruit cluster or a ‘fruit head’ is composed of numerous berries (Photo 5.6). Thus, a very high number of seeds can be potentially produced from a single cross. The highest number of seeds per fruit cluster recorded so far in Papua New Guinea was estimated to be 22,133 seeds on a wild taro used as a genetic donor of resistance (Ivancic et al., 1996b). Taro seeds are small, about 1.4 mm long (Ivancic and Simin, 1996). They have to be planted into special pots filled with

Photo 5.6 Taro 'fruit head' containing numerous berries (Source: Vincent Lebot)

adequate soil and placed into 'water beds.' Seed germination and raising of young seedlings require a warm and humid environment and special care. Low temperatures are a serious constraint to seed germination and this issue should be considered if taro breeding programs are initiated in cool non-tropical regions.

Concerning the choice of parental materials, it is now accepted that different taro genepools should be combined. The first taro breeding programs used narrow genetic bases. The occurrence of heterosis was far from evident and most of the hybrids produced were susceptible to the main taro diseases. An interesting approach would be to combine genotypes from the two main genepools, Asia and the Pacific. Pacific cultivars are the result of intense local selection; they produce corms of good quality, but are susceptible to pests and diseases. Conversely, in Asia, co-evolution with numerous and diverse strains of *P. colocasiae* has produced resistant genotypes but because taro is not as important as in the Pacific, most cultivars produce numerous suckers and stolons and have irregular corm shapes. For this breeding approach to be successful there needs to be international exchange of germplasm (Lebot, 2005).

The most common and simplest selection method in taro breeding is selection from a population derived from a crossing between two elite cultivars. This type of selection is useful when it is directed at one or a few genetically controlled characters and when the parental material is highly cultivated. Backcrossing has been used when one characteristic had to be changed without influencing the others. The most typical use of efficient backcrossing in taro breeding is breeding for resistance against diseases, e.g., vertical resistance to TLB. This method was first used in the Solomon Islands (Patel et al., 1984). In order to avoid inbreeding depression effects, the recurrent parent is replaced in each new backcross by a different (phenotypically similar) genotype. Finally, recurrent selection has been successfully used in the Solomon Islands and Papua New Guinea with the aim of introgressing horizontal or durable TLB resistances (Ivancic and Okpul, 1995). In taro recurrent selection, when wild materials are used, it is important that all

wild genetic sources are included at the beginning because it takes a lot of time to remove the undesired 'wild alleles' from the population. Another important condition is to establish a good and simultaneous flowering in each cycle. However, the outstanding growth vigor and high flowering ability of the genotypes with predominant 'wild' characteristics can impair a balanced distribution of pollen among genitors. In more advanced cycles, breeders should avoid crosses with genotypes carrying too many 'wild' genes. Finally, the evaluation of individuals in a population cannot always take place before flowering. Improving breeding efficiency could be achieved by conducting the selection process at an early stage of development without waiting for the plants to be uprooted. This approach has been developed for determining taro corm flesh and corm fiber colors, which were correlated to the color of different petiole zones (Ivancic et al., 2003). Also, a vegetative growth index (VGI) that takes into account four vegetative traits proved to be useful for the rapid assessment of genotypes with good yield potential (Lebot et al., 2006). One of the lessons of the Papua New Guinea breeding program was the difficulty of getting rid of wild deleterious traits. Today, whenever possible, taro breeders are favoring resistance sources within the cultivated genepool (Lebot, 2009).

Knowledge on the quantitative genetics of important agronomic traits for taro breeders is scarce. Most investigations have been looking at the broad-sense heritability of major traits by comparing the performance of genotypes in randomized complete blocks followed by variance analysis (Dwivedi and Sen, 1997). These authors found relatively high broad-sense heritabilities for the traits number of suckers and yield. More recently, Quero-García et al. (2006b) conducted experiments in Vanuatu during two clonal generations involving progenies from controlled crosses (FS families). Family and narrow-sense heritabilities from parent–offspring regression were calculated. Heritabilities were higher for number of suckers and dry matter content than for yield (expressed as corm weight). Family heritability values were higher than narrow-sense heritabilities values, suggesting the possibility of using family selection as opposed to individual plant selection, in the first cycles of a recurrent breeding program. Finally, these authors aimed at answering an important question for taro breeders: Should breeding be based on few very large families or on numerous small families? A high percentage of valuable hybrids were only observed among a small number of families. When working with a narrow genetic base, such as the one used in Vanuatu, it could be best to create a few large full-sib families by selecting the best parents, provided they are not too closely related. However, when using a collection with high genetic diversity, breeders should conduct a high number of crosses in order to find the best heterotic combinations. Further trials involving crosses from distant origins will be needed to test these hypotheses. On the other hand, although taro flowering and synchronization are not always easy to optimize, future factorial or diallel designs should be implemented in order to provide precise estimates of additive and dominance variances for taro agronomic traits.

Before any new hybrids are released, extensive multi-location trials have to be conducted in order to test their adaptability and stability. Trials aimed at quantifying G $\times$ E interactions have only been implemented in Papua New Guinea. Seven

elite taro lines resistant to TLB and a susceptible, but highly preferred control cultivar, were tested in seven diverse agro-ecological environments. Pooled analysis of variance detected significant G $\times$ E interactions with a low broad-sense heritability for yield. Concerning resistance to TLB, heritability was much higher and all tested resistant lines showed wide adaptability (Okpul, 2005).

Other conventional taro breeding techniques such as mutational breeding through irradiation or inter-specific and inter-generic hybridizations (with *C. gigantea* and *Alocasia macrorrhizos*) have been attempted without much success (Ivancic and Lebot, 2000).

8 Integration of New Biotechnologies in Breeding Programs

As mentioned above, molecular markers have recently been developed for the characterization of taro genetic resources. Apart from rationalizing and managing germplasm collections, molecular markers may also be useful for providing precise estimations of genetic distances between taro varieties. Quero-García et al. (2009) conducted a study in order to assess the relationship between genetic distance of parents, assessed with AFLP and SSR markers, and offspring hybrid vigor. Only progenies from crosses between cultivars from Vanuatu were studied. The correlations between genetic distances and hybrid vigor were low and statistically not significant with the exception of the trait number of suckers. Low heterosis was observed and further trials involving a larger genetic base should be conducted.

The use of molecular markers for mapping and QTL detection studies is also at an early stage in taro. Only two genetic maps have been published; they involved two different F_1 crosses (with 123 and 100 full-sibs, respectively) from the Vanuatu breeding program and contained mostly AFLPs and a small number of SSRs (Quero-García et al., 2006c). QTL detection was performed on data from one clonal generation for traits number of suckers, corm dimensions (length and width) and weight, and dry matter content. Although heritabilities were higher for number of suckers and dry matter content, only six putative QTLs were found for yield and related components. In order to optimize future taro QTL detection trials, larger progenies should be created, and more replications used, especially when dealing with low heritability traits. Also, crosses between genetically distant varieties should be performed. Progenies from genetically close cultivars might be in a poor polymorphic state for the studied traits, which could explain the non-detection of QTLs for heritable traits such as dry matter content. The genetic maps produced by Quero-García et al. (2006c) were not completely saturated and future maps should include a larger number of co-dominant and informative markers such as SSR or SNP markers. The new generation ultrahigh-throughput sequencing techniques and the rapid decrease of sequencing costs appear promising for taro mapping activities. QTL detection studies might be implemented in marker-assisted selection (MAS) programs.

Taro breeding is based, as for all root crops, on the evaluation of very large numbers of hybrid clones. Moreover, taro multiplication rate is relatively slow in

comparison to other root crops (Pardales, 1993). For all these reasons, MAS strategies could be rapidly beneficial for genetically simple or highly heritable traits. A first and simple way of using MAS strategies could be to eliminate potential parents carrying deleterious alleles from germplasm collections. This could be attractive for the branching corm trait, which is relatively frequent in the Vanuatu progenies of the breeding program and which is controlled by at least two different loci (Ivancic et al., 2004b).

Transgenic technologies could provide interesting solutions for complex traits for which sources of genetic variation have not yet been identified. These could concern tolerance to the Alomae-Bobone virus complex, resistance to taro beetle, or resistance to abiotic stresses, such as salinity or drought, which are likely to become a threat for taro cultivation, in the context of global climate warming. The University of Hawaii has developed an efficient *Agrobacterium tumefaciens*-mediated transformation method, with a 43-fold higher transformation efficiency than the particle bombardment method (He et al., 2008).

9 Seed Production

The 'true' taro seed is systematically produced and used only in genetic breeding and scientific investigations. The main reasons for not using 'true' seed in agricultural production are high level of heterozygosity (causing genetic segregation), difficult germination, and slow growth of seedlings. In agricultural production, taro is multiplied vegetatively (i.e., by side shoots, stolons, and corm heads). There is no 'seed' industry per se and no organized supply of planting materials. Smallholders are managing their own planting material and regularly destroying plants exhibiting disease symptoms. In a few countries (e.g., Thailand and Vietnam), farmers manage nurseries nearby their houses during the off-season period of the year in order to keep their planting material alive and in good health. The economic value of the crop is not sufficient to encourage in vitro propagation of healthy plants, although reliable protocols exist.

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Chapter 6
Sugar Beet

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1 Introduction

World sugar production is around 160 Mt yearly with a per capita consumption of about 23 kg. Total utilization is increasing approximately 1.4% annually thanks to the improved standard of living in densely populated countries like China and India. About one-quarter of world production is extracted from beets (*Beta vulgaris* L. ssp. *vulgaris*), and the remainder from cane (*Saccharum officinarum* L.). The chemical composition of both commercial sugars is sucrose (more than 99.5% in white crystalline sugar) despite the crops being very different in their climatic requirements and photosynthetic pathways. Beets yield better in temperate climates, especially in areas such as France, Germany, northern USA, whereas cane requires a tropical to subtropical environment (India, Australia, Cuba, Brazil, etc.). Sugar from beet and cane has completed in the market place since the earliest sugar beet factories produced sugar in the early 1800s. One advantage cane processing enjoys, among other things, is that cane factories can be energy sufficient due to the burning of bagasse (fibrous matter remaining after crushing the cane stalks), whereas the power for processing beets generally relies on fossil fuels. The cost of cane sugar is currently lower and the price differential for sugar extracted from beets and from cane follows the price of crude oil.

2 Origin and Domestication

Sugar beet is classified *Beta vulgaris* L. ssp. *vulgaris* sugar beet group (Lange et al., 1999). The second ssp. is *Beta maritima* (L.) Arcang., classified by Linnaeus (1797) as a separate species. The genus *Beta* L., of the family *Amaranthaceae* (formerly *Chenopodiaceae*), is subdivided into four sections (Table 6.1). All cultivated beets are included in the sub-species *vulgaris* that belongs to the species *vulgaris* and to

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Table 6.1 Taxonomy of the genus *Beta* (Letschert, 1993; Ford-Lloyd, 2005)

	Genus *beta*		
	Section *Beta* (syn. *Vulgares* Ulbrich)		
$2x$, $3x$, $4x$[a]	*Beta vulgaris* L.	ssp. *vulgaris*	Leaf beet group[b] Garden beet group[c] Fodder beet group[d] Sugar beet group
		ssp. *maritima* (L.) Arcang.	
		ssp. *adanensis* (Pamuk.) Ford -Ll. et Will.	
$2x$, $4x$	*Beta macrocarpa* Guss.		
$2x$	*Beta patula* Ait		
	Section *Corollinae* Ulbrich		
$2x$, $4x$	*Beta lomatogona* Fisc. et May.		
$2x$	*Beta macrorhiza* Stev.		
$4x$	*Beta corolliflora* Zos. ex Buttler		
$4x$	*Beta intermedia* Bunge		
$4x$, $6x$	*Beta trigyna* Waldst. et Kit.		
	Section *Nanae* Ulbrich		
$2x$	*Beta nana* Boiss. et Heldr.		
	Section *Procumbentes* Ulbrich (syn. *Patellares* Tranzschel)		
$2x$	*Beta procumbens* Sm.		
$2x$	*Beta webbiana* Moq.		
$4x$	*Beta patellaris* Moq.		

[a]Number of chromosomes ($2x = 18$; $3x = 27$; $4x = 36$; $6x = 72$).
[b]Also named Mangold, Spinach beet, Chard, Swiss chard etc.
[c]Also named red beet.
[d]Also named forage beet.

the section *Beta* (Letschert, 1993; Letschert et al., 1993; Lange et al., 1999; Ford-Lloyd, 2005). Wild beets (i.e., the species and sub-species (ssp.) of the genus *Beta* outside of *B. vulgaris* ssp. *vulgaris*) have only been used as potential sources of useful traits for cultivated beets, particularly disease resistance characters (Coons, 1975; Lewellen and Whitney, 1993; Asher et al., 2001). Artificial hybridizations between section *Beta* species and sections *Corollinae*, *Nanae*, and *Procumbentes* have proved very difficult (McGrath et al., 2007). Sea beet [*Beta vulgaris* L. ssp. *maritima* (L.) Arcang.] was domesticated pre-historically somewhere in the Middle East (Coons, 1949; 1954; Campbell, 1984). Because the wild species normally flowers 2–3 months after emergence, the first growers would likely have selected beets with delayed bolting and flowering. In this way, as for several vegetables, the growing season was extended under cultivation, with the leaves being used as food (Campbell, 1984; McGrath et al., 2007). Following a long period of mass selection, cultivated beets became predominantly biennial and entered their reproductive

phase after overwintered vernalization (Biancardi, 1984). About 1000 BC, leaf beet was grown in Greek Mediterranean countries and later spread through the Roman Empire where the crop was named *Beta* (von Lippmann, 1925). Here, a second cultural variant with expanded hypocotyl and root became an important vegetable. The precise origin of table beet (also named garden or red beet) is obscure. During the Middle Ages another cultural variant of beet, characterized by larger roots suitable for livestock fodder, was developed in northern Europe (Campbell, 1984).

After the discovery that fodder beets contained the same kind of sugar as cane, the fourth crop variant was selectively bred in Germany toward the end of the 1700s (Achard, 1803; Knapp, 1958). This selection led to the first sugar beet variety (Fischer, 1989), the "Weisse schlesische Rübe" (White Silesian Beet). Achard built the first beet sugar factory at Cunern (Silesia), which began operation in the spring of 1802 (Winner, 1984). After a few years of expansion, the crop acreage decreased quickly in favor of cane, due to changes in international trade. Beet cultivation and the construction of factories began again in Germany around 1830, partially because sugar beet culture improved greatly the yield of rotation crops (Coons, 1949).

During the early breeding efforts, sugar yield increased rapidly as the result of new analytical and breeding methods developed in France (McFarlane, 1971). Cultivation methods were improved with the employment of chemical fertilizers and steam tractors, which allowed deeper plowing and better soil management. In the twentieth century, improvement was characterized by continuous progress in breeding and agronomy leading to a reduction in growing costs and an increase of sugar yield (Robertson-Scott, 1911; Winner, 1993). The singling of seedlings was needed because the multigerm "seed" (fruit) sown was composed of two to five fused true seeds. Approximately 100 man-h/ha that had been required to thin and single stands to the desired population density was eliminated after the discovery of genetic monogermity (Savitsky, 1950). The adoption of monogerm seed greatly reduced hand labor and stimulated a rapid evolution of cultural practices. Pelleted seed with incorporated chemicals improved sowing precision and provided better protection against seedling diseases (Leach and Bainer, 1942; Winner, 1993). Sugar beet was one of the first crops protected with chemicals (arsenic, nicotine, sodium fluoride, sulfur, copper salts, etc.) and herbicides (Winner, 1993). The discovery of some genetic resistances to diseases increased sugar yield while reducing dependence upon pesticides. Approximately half of the improvement in sugar yield can be attributed to breeding (Sneep et al., 1979). The most important improvements over the last 50 years have been the introduction of hybrid varieties, the pest and disease resistances, including that to rhizomania and sugar beet cyst nematode, the meristem multiplication techniques, and breeding assisted by molecular biology (Biancardi et al., 2005). Thanks to integrated research efforts, the increase of sugar yield per hectare in advanced European countries is about 1.4% annually (Bosemark, 2006).

Sugar beet in the northern hemisphere is usually sown in late winter or early spring. Depending upon climatic and soil conditions, the crop is harvested after 5–9 months of growth. In Mediterranean climates, sowing may be in autumn (see Section 4.5) with spring/summer/fall harvest. Mechanically topped and lifted roots are either transported to the factory quickly or placed in storage piles, depending on the temperature and weather conditions and the throughput of the factory. The tops

(e.g. crowns, petioles, and leaves) are removed from the beet because of the low sugar content and the high concentration of processing impurities (see Section 5.1 and Fig. 6.5). After washing, sugar is extracted with hot water diffusion from thinly sliced roots. The "raw juice" is purified with repeated treatments of lime and carbon dioxide. After filtration, the "thin juice" is concentrated by evaporation. When sucrose concentration becomes greater than 60%, crystallization of sugar is initiated in "thick juice" under partial vacuum and high temperature conditions. Molasses, a brown and heavy syrup containing about 45% sugar, are separated from crystalline sucrose by centrifugation. Crystallized raw sugar undergoes further processing to obtain nearly pure, commercial, white sucrose (McGinnis, 1982). Molasses are used for animal feed and for production of alcohol, glutamate, yeasts, etc., or may be returned through the factory for further sugar removal and separation of sucrose by molecular sieving and ion exchange. The pulp, i.e., the non-soluble part of the sliced roots after sugar extraction, is used mainly for animal and pet food.

3 Genetic Resources

Although sugar beet is a relatively new agricultural crop and was not cultivated until the early 1800s, beet was domesticated as a leafy pot herb in pre-historical times (Ford-Lloyd et al., 1975; De Bock, 1986). It is thought that the gene pool of white fodder beet provided the genetic base for early sugar beet varieties. It has been suggested that this narrow germplasm base has left sugar beet with a narrower genetic pool than that of other open-pollinated crops (Bosemark, 1979; 1989; Lewellen, 1992). Because early sugar beet development and production was in the temperate climate of Northern Europe, which was relatively disease free, there was little pressure to select or maintain high levels of host-plant resistance (Lewellen, 1992). As sugar beet production moved out of Northern Europe into warmer zones, endemic diseases were encountered that severely limited yield and for which there were no known resistances (Lewellen, 1992). The first attempts to screen exotic and wild-beet germplasm at the beginning of the 1900s, primarily for disease resistance, were undertaken in response to this increasing pest and disease pressure.

One of the first successful attempts to use exotic germplasm was in the Po Valley of Italy in the early 1900s, where the high humidity and warm night temperatures provide optimal conditions for cercospora leaf spot (CLS) caused by the fungus *Cercospora beticola* Sacc. Here we find the first documented instance of collecting sea beet germplasm (*B. vulgaris* ssp. *maritima*) to use in a sugar beet breeding effort. Munerati et al. (1913) recognized the potential of the sea beet growing in the Po Delta as a source of host-plant resistance to CLS. The germplasm produced in this breeding program, the Rovigo series (R148, R581, etc.) and the varieties "Cesena" and "Mezzano," has been adopted worldwide and is the source of most CLS-resistant germplasm in use today (Munerati, 1932; Biancardi and De Biaggi, 1979).

In other countries of Europe, researchers studied sea beet and crossed it to sugar beet (Rasmussen, 1933; Tjebbes, 1933). There were other efforts to develop

CLS-resistant varieties as Munerati had done (Stehlik, 1949; Schlösser, 1957), and varieties with resistance to other diseases (Margara and Touvin, 1955, reviewed by Asher et al., 2001). However, it is difficult to estimate the extent to which sea beet germplasm was used in commercial breeding programs, especially because of undesirable traits that could potentially be introduced with its use, e.g., fangy roots, annualism, high fiber content in the root, elongated crowns, red pigment (in root, leaf or petiole), and lower sugar production [reviewed by Coons (1975) and by Panella and Lewellen (2005)].

Commercial sugar beet seed production was initiated in France around 1810 by the firm Vilmorin. About 10 years later breeding activities including mass selection (mother root selection) and progeny test selection (Oltmann, 1984) were begun. Vilmorin is credited with being the first to use progeny test methods for improvement of any crop. In Germany, the first firms active in sugar beet seed production were Ziemann (around 1830), Rimpau (around 1841), and Knauer (1849). Because of the strategic importance of seed supply for the sugar factories, numerous breeding and seed production centers were developed in nearly every country where sugar beet was cultivated. Due to the proprietary nature of this activity, the circulation and distribution of sugar beet germplasm in Europe became tightly controlled, as it remains today (Oltmann, 1984). For this reason, sugar beet breeding and germplasm conservation evolved differently to that in the United States and have been largely proprietary.

Until World War I, most sugar beet seed used in the United States came from Europe. The disruption of seed importation from Germany caused by the war led to the establishment of domestic seed production, and by the end of the 1930s, domestic production provided about one-third of the needs of the United States (Coons, 1936). USDA researcher, G.H. Coons, who was familiar with Munerati's work, made collection trips to Europe and Asia to look for sources of CLS, and curly top, resistances in sea beet (Coons et al., 1931) as well as in the other species in the genus *Beta* (Coons, 1975). USDA researchers in the United States made some effort to evaluate this material, and material collected by Stewart in 1969, for resistance to CLS (Bilgen et al., 1969), rhizoctonia root rot caused by *Rhizoctonia solani*, and black root caused by *Aphanomyces cochlioides* (Schneider and Gaskill, 1962). The germplasm was stored in Beltsville, MD, where storage conditions were poor, and what survived was taken by McFarlane to Salinas, CA, for regeneration. The part of the collection that was rescued (in the United States, 93 wild-beet accessions within the range WB1–WB319) was extensively evaluated and has provided genes for many useful traits (Whitney, 1989a, b; Lewellen and Whitney, 1993; Yu et al., 1999; Lewellen and Schrandt, 2001).

A number of changes in sugar beet breeding came together in the 1960s. This confluence caused a genetic bottleneck in this time period, which exacerbated growing disease pressure due to an increase in cultivated area and shortening of the rotation between sugar beet crops. These were the cytoplasmic male sterility (CMS) and genetic fertility restoration system developed by Owen (1954b) and the introduction of new monogerm, CMS and O-type maintainer lines to produce commercial monogerm, CMS hybrid varieties (Savitsky, 1950: McFarlane, 1971). Until

the 1980s, there seemed to be a reluctance to use wild-beet germplasm, perhaps because of earlier experiences that resulted in the introgression of many undesirable traits from the exotic germplasm (Frese et al., 2001). The need for increased resistance to disease and insect pests and a greater productivity rekindled interest in sea beet and other exotic sources of germplasm (Lewellen, 1992).

The Sugar Beet Crop Advisory Committee (now Crop Germplasm Committee-CGC) formed in 1983 represents the sugar beet germplasm user community in the United States. The sugar beet CGC is still an integral part of the USDA-ARS's National Plant Germplasm System (NPGS) (reviewed by Janick, 1989), as well as an official committee of the American Society of Sugar Beet Technologists (ASSBT). Since its inception, this committee has consisted of sugar beet seed industry members, plant breeders, university researchers, and USDA-ARS scientists. The sugar beet CGC has aggressively supported evaluation of the *Beta* germplasm within the USDA-ARS NPGS (Panella and Lewellen, 2007).

The increasing interest in wild germplasm as a genetic resource for improving sugar beet varieties heightened the realization that wild *Beta* germplasm was being lost in the 1980s and 1990s (Pignone, 1989; Doney et al., 1995). The value of the wild relatives in the improvement of the sugar beet crop was well demonstrated (De Bock, 1986; Doney and Whitney, 1990; van Geyt et al., 1990; Lewellen and Skoyen, 1991; Doney, 1993), and using evaluation data from the sugar beet CGC evaluations, the USDA/ARS public sugar beet breeders began introgressing wild germplasm into the sugar beet gene pool (Doney, 1998; Panella, 1998; Panella and Lewellen, 2007). This germplasm was released in the United States to sugar beet seed companies, as well as released internationally (Lewellen, 1991, 1997; 2000a, b; Yu, 2002). The Genetic Resources Information Network (GRIN) Database of NPGS *Beta* collection includes everything from wild relatives (Hannan et al., 2000; Panella et al., 2003) to heritage open-pollinated varieties (McGrath et al., 1999) and germplasm registered in Crop Science (Doney, 1995). Of the 2,550 *Beta* accessions in the NPGS, the 572 sea beet accessions are among the best characterized and evaluated as well as being among the most useful in breeding programs (Panella et al., 2003). As of 2003, about 25,000 evaluation records (descriptors multiplied by accessions evaluated) are in the database (Panella and Frese, 2003). These and other data in the GRIN database can be accessed through the URL: www.ars-grin.gov/npgs.

During the 1980s in Europe, sugar beet breeders were developing a theoretical framework for effectively introgressing new germplasm into elite breeding programs, which has been expanded into a strategy to broaden the germplasm base of the sugar beet gene pool (Bosemark, 1989; Frese, 1990). This prebreeding strategy has been implemented through the World *Beta* Network (WBN), founded in 1989 with the goal of improving international collaboration among users and curators of germplasm collections throughout the world (Frese, 1990). A central database of all *Beta* accessions contained in genebanks throughout the world, the International Data Base for *Beta* (IDBB), maintained at the Federal Centre for Breeding Research on Cultivated Plants (BAZ) Gene Bank (Quedlinburg, Germany), has been developed and supported by the WBN members.

Building on the WBN strategy, public and private plant breeders within the International Institute for Sugar Beet Research (IIRB, Brussels), Genetics and Breeding Group, started developing Doggett buffer populations improved through recurrent selection (Doggett and Eberhart, 1968; Bosemark, 1971). Additionally, Frese (2000) developed an international core collection comprising 805 accessions of the IDBB in various genebanks in Europe and around the world. The GENRES CT95 42 Project, funded through the European Union, evaluated between 300 and 700 accessions of the synthetic core collection for resistance to seedling diseases (caused by *A. cochlioides* and *Phoma betae*), leaf diseases (caused by *C. beticola, Erysiphe betae*, beet yellows virus, and beet mild yellowing virus), the root diseases rhizomania (caused by *beet necrotic yellow vein virus*), and rhizoctonia root and crown rot (caused by *R. solani*), as well as drought tolerance (Panella and Frese, 2003). Data from this project can be accessed and downloaded at the URL: http://ice.zadi.de/idbbonline/beta.php and users can query passport, characterization, and evaluation data (including statistical parameters) (Panella and Frese, 2003). Private and public plant breeders in Europe and throughout the world have taken the results of these evaluations and are beginning to introgress these newly discovered sources of disease resistance into sugar beet (Asher et al., 2001; Biancardi et al., 2002; Luterbacher et al., 2000; Panella and Lewellen, 2007).

4 Major Breeding Achievements

Breeding has obtained significant results in enhancing the yield traits and the genetic resistances against several diseases, in some cases allowing sugar beet to survive even where serious infections are otherwise uncontrollable. Here we look in more detail at a number of achievements which have affected breeding methods and the types of cultivar produced.

4.1 Polyploidy

Efforts to modify the number of chromosomes in sugar beet became successful after the discovery of the mutagenic properties of colchicine (Schwanitz, 1938). The first tetraploid families, having twice ($2n = 4x = 36$) the normal number of chromosomes ($2n = 2x = 18$), were characterized by better root shape and fewer but larger leaves with shorter and stronger petioles than diploid ($2x$) beets (Lasa and Romagosa, 1992). Flowers, seed clusters, and pollen grains were also larger. Seed germination and root development of tetraploid ($4x$) families (genotypes reproduced with open pollination) were, on average, slower compared to their $2x$ counterparts, and bolting resistance was slightly improved. The main disadvantages in selecting genotypes at $4x$ level were due to the slower breeding response and increased difficulties to introduce new traits (Bosemark, 2006).

The $2x$ and $4x$ families are easily crossed, producing triploid ($2n = 3x = 27$) hybrids, manifesting intermediate morphological characteristics. Triploid ($3x$)

Table 6.2 Production system of commercial varieties in chronological order of cultivation

Production systems			Year of introduction[a]	Varieties
Multigerm varieties				
2*x* F *MM*			1802	2*x*, *MM* open pollinated
2*x* F *MM* × 4*x* F *MM*			1951	2*x* + 3*x* + 4*x*, *MM* anisopliod open pollinated
4*x* F *MM*			1966	4*x*, *MM* open pollinated
Monogerm hybrid varieties				
Seed bearers		Pollinators		
2*x* CMS *MM*	×	2*x* F *MM*	1954	2*x*, *MM* top cross
2*x* CMS *MM*	×	4*x* F *MM*	1954	2*x*, *MM* top cross
2*x* CMS *mm* (line)	×	2*x* F *MM* (line)	1955	2*x*, *Mm*[b] single cross
2*x* CMS *mm* (line)	×	2*x* F *MM* (family)	1955	2*x*, *Mm* top cross
2*x* CMS *mm* (F1)	×	2*x* F *MM* (family/line)	1955	2*x*, *Mm* three-way cross
2*x* CMS *mm* (F1)	×	2*x* F *MM* (F1)	1957	2*x*, *Mm* double cross
2*x* CMS *mm* (line)	×	4*x* F *MM* (line)	1959	3*x*, *Mm* single cross
2*x* CMS *mm* (line)	×	4*x* F *MM* (family)	1959	3*x*, *Mm* top cross
2*x* CMS *mm* (F1)	×	4*x* F *MM* (family/line)	1965	3*x*, *Mm* three-way cross
2*x* CMS *mm* (F1)	×	4x F *MM* (F1)	1965	3*x*, *Mm* double cross
4*x* CMS *mm* (line)	×	2*x* F *MM* (family/line)	1974	3*x*, "reverse" *Mm* top cross or single cross
4*x* CMS *mm* (line)	×	4*x* F *MM* (family/line)	[c]	4*x*, *Mm* top cross or single cross

F, male fertile; CMS, male sterile; *mm*, monogerm; *Mm* and *MM*, multigerm.
[a]According to Sneep et al. (1979).
[b]Phenotypichally monogerm because harvested on monogerm plants.
[c]Not released.

hybrids display better sugar yield than their parental averages, indicating heterosis. This important advantage was used for the production of anisoploid varieties. The seed was obtained by crossing 2*x* and 4*x* families transplanted in a 1:3 ratio. The higher proportion of 4*x* plants compensated for the lower competitiveness of their pollen. The bulk harvested seed had a percentage of 3*x* hybrid plants as high as 50%, thus ensuring a superior sugar yield (McFarlane, 1971; Sneep et al., 1979). The remaining seed comprised various proportions of 2*x* and 4*x*. Anisoploid varieties were widely used after 1951 (Table 6.2).

4.2 Monogerm Seed

The flowers in the section *Beta* are joined in clusters of two or more, which develop multigerm "seed," botanically classified as utricle and formed by the aggregation of as many fruits each containing the true seed (Klotz, 2005). After emergence, manual thinning was necessary not only to avoid competition among the plantlets emanating from the multigerm seed, but also to achieve a regular stand of about

80,000–100,000 equally spaced plants per hectare. Since hand thinning was very expensive, mechanically processing multigerm glomerules into single seeds was used (Fig. 6.1) (Knapp, 1958). Sowing with precision machines the "monogerm" seed obtained in this way, the requirement for hand thinning was strongly reduced, but not eliminated. In fact, the complete removal of bigerm seeds was difficult when using the gravity separators widely used during seed processing.

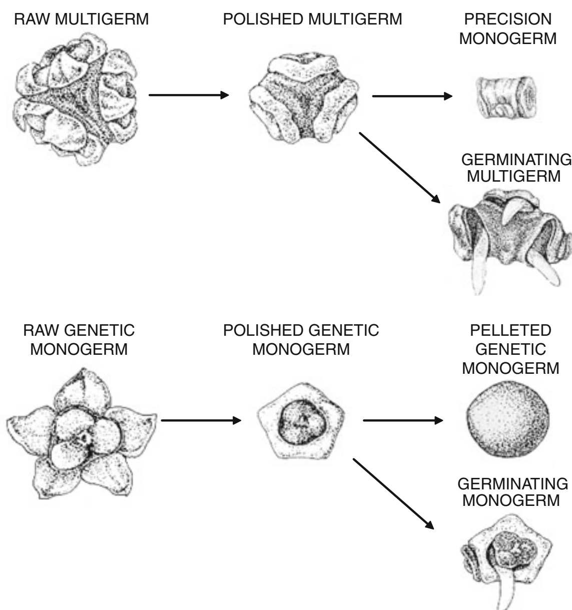

Fig. 6.1 Processing steps of multigerm/precision seed (*above*) and genetic monogerm seed (*below*) (from Biancardi, 1984, modified)

In 1948, plants with single flowers developing monogerm seeds were discovered and deployed (Savitsky, 1950). The first genetic monogerm germplasm, SLC 101, was available in 1951, and the commercialization of monogerm varieties was initiated some years later (McFarlane, 1971). Currently, only genetic monogerm varieties are in use, except in countries where the field emergence is difficult and/or labor costs are still low, such as in Northern Africa and China. The monogerm character depends on a pair of alleles designated *Mm* and it is in homozygous recessive condition. Other forms of monogermy have not been used commercially to date (Brewbaker et al., 1946; Shavrukov, 2000).

4.3 Male Sterility

Commercialization of hybrids became possible in 1955 (Sneep et al., 1979), after the discovery of genetic-cytoplasmic male sterility (CMS) by Owen (1945). The existence of a sterile cytoplasm (S) was demonstrated, resulting in sterility only in presence of two pair of alleles, designated *Xx* and *Zz*, in a homozygous recessive condition. Therefore, the CMS lines must possess the S *xxzz* genotype, whereas

all other combinations produce fertile or partially fertile offspring. The normal (N) cytoplasm always produces fertile progeny. The reproduction of CMS lines required the employment of maintainers bearing the N cytoplasm and the genes *x* and *z* in homozygous recessive condition. The maintainers, to which the monogerm character was soon transferred, were called O-types (McFarlane, 1971). At the beginning, both CMS and monogerm inbred lines (genotypes reproduced with more or less strict self-pollination) were very weak, but after crossing vigor and the seed production improved slightly (McFarlane, 1971). For reproduction, each CMS line needs a corresponding O-type. At least six to eight backcrosses are needed to create similar genotypes differing predominantly by their N- and S-cytoplasms (Fig. 6.2) (Sneep et al., 1979; Skaracis and De Biaggi, 2005). Nuclear (also named genetic or Mendelian) male sterility (NMS) depends on alleles at the locus *Aa* and is expressed in homozygous recessive condition (Owen, 1952). In contrast to CMS, NMS is not suited for commercial hybrid seed production, and consequently its use is limited to some specialized breeding schemes (Bosemark, 1971).

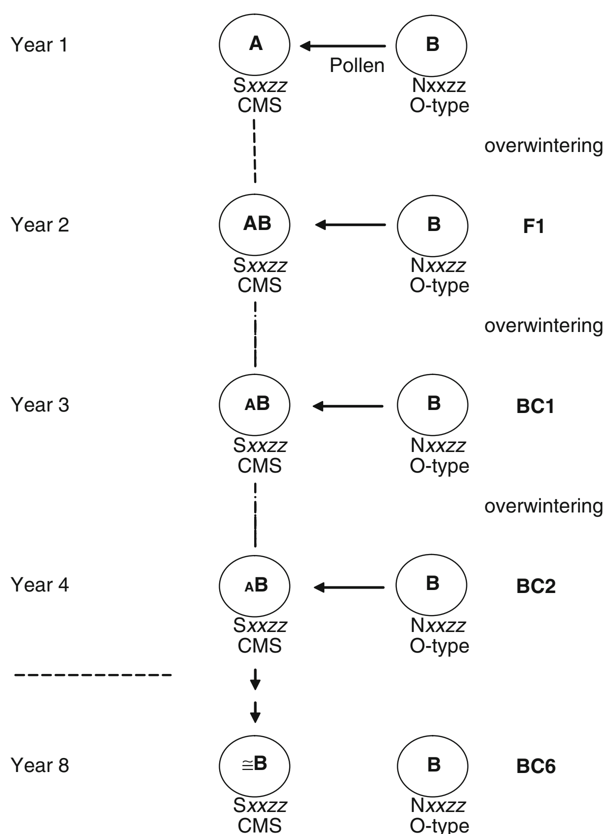

Fig. 6.2 Backcrossing for conversion of O-types to CMS maintainers

4.4 Growth Habit

The cultivated beets are biennial, that is, they require a vernalization period (overwintering) to begin the reproductive phase (Letschert et al., 1993). Under certain weather conditions (cold and increasing day length), biennial beets may vernalize in the field, giving rise to bolting plants (Fig. 6.3), and releasing fertile pollen and producing viable seed (Smit, 1983). Since seed production in Europe takes place in regions where annual beets are quite common, conditioned by alleles at the *Bb* locus where the recessive state confers biennial habit, pollen from annual plants transmits the bolting tendency and can be particularly damaging in seed production areas. The seeds shed from bolting sugar beets in the field pollinated with pollen from annual beet develop as annual beets, also named weed beets (Letschert et al., 1993), causing weedy infestations often difficult to control in subsequent beet crops. Weed beets flower like the wild ones a few months after emergence. The annuality trait depends on the dominant gene *B* (Munerati, 1931; Owen, 1954a). Bolting and flowering in annual genotypes occurs without influence of temperature or day length (Abegg, 1936; Abe et al., 1997).

4.5 Bolting Resistance

Usually a small proportion (around 0.1%) of beets in commercial fields bolts and flowers. High temperatures after the bolting induction may reverse its effects

Fig. 6.3 Bolted beet in field condition

(devernalization) (Smit, 1983). Notwithstanding the complexity of flowering physiology in biennial beets and genotype × environment interactions, selection has improved bolting resistance. Early sowing is effective in inducing bolting as a breeding tool for mass selection. Since early sowing in field conditions is not always possible, different greenhouse systems with combined photo-thermal treatments were developed. Bolting resistance is perhaps best accomplished using progeny testing (McFarlane, 1971). Due to the strong genotype × environment interactions, achieving significant progress in bolting resistance is only possible by selecting in the local climate where the improved variety will be grown (Smit, 1983).

The use of bolting resistant spring varieties enabled earlier drilling, resulting in a longer growth period and in a slightly improved sugar yield. Varieties endowed with a high degree of bolting resistance are also used for autumnal sowing in areas where a mild climate allows the overwintering of the crop (California, southern Spain, southern Italy, North Africa, etc.). Extending autumn sowing northward has good potential to increase sugar yields, but seems quite difficult due to the limited possibilities to significantly improve cold and bolting resistance. The former trait is needed by plantlets to survive winter; the latter is necessary for reducing the effects of intense bolting induction. Among other things, such enhanced bolting resistance would hinder the flowering when seed production is necessary (Smit, 1983). Because bolting of winter beets in cold areas can be as much as 100%, beginning in April, the large biomass yield (roots, leaves, and seed stalks) could be employed for fermentation and biogas production (Kluge-Severin et al., 2009).

Bolting resistance is likely controlled by several genes acting through different mechanisms, but the precise genetics are yet undetermined (McFarlane et al., 1948; Le Cochec, 1989; Jolliffe, 1990; Sadeghian and Johansson, 1993).

4.6 Self-Sterility and Self-Fertility

Sugar beet is primarily self-sterile (or self-incompatible). Self-pollination is quite rare in wild beets. Self-sterility was employed to enhance and maintain the heterosis in multigerm varieties before the discovery of CMS (Owen, 1942). The self-sterility trait generally acts through hindering the growth of the pollen tubes inside the pistils (Savitsky, 1950). According to Owen (1942), self-sterility is explained by multiple alleles S^1–S^{n} and Z^1–Z^n. The hypothesis assumed that a single *S* or *Z* factor carried by the pollen, if not present in the tissue of the stigma, causes fertility. A second model considers gametophytic self-incompatibility conditioned by four *S* loci with complementary interactions. The *S* genes in the pollen encountering the same allele(s) in the pistil result in incompatibility (Larsen, 1977, 1978).

The release of the first monogerm lines, which were also self-fertile, lead to the introduction of the trait into commercial germplasm (Savitsky, 1950; Smith, 1987). Plants carrying the gene S^{F} in a homozygous or heterozygous condition are highly, but not completely, protected against cross-pollination even without any isolation

measure. The trait is useful for the development of inbred lines, and it is employed in breeding programs in combination with NMS.

5 Current Goals of Breeding

The main objective of plant breeding is the development of varieties with the maximum commercial yield at the lowest economic and environmental cost. The yield potential for sugar beets depends also on their suitability for processing, which includes several traits that enhance sugar extraction by the factory (Campbell, 2002; 2005). Varieties must also possess good yield stability across localities and years, which depend on a broad genetic base and on resistances against multiple biotic and abiotic stresses. Apart from these general objectives, several secondary breeding aims are taken into account according to local needs (Barocka, 1985). More than 40 qualitative traits were recognized as follows: annuality, monogermy, Mendelian male sterility, self-fertility, some forms of resistance to rhizomania, etc. (Smith, 1987; van Geyt et al., 1990). The improvement of composite traits, such as yield, processing quality, germination ability, bolting, and several disease resistances, is more difficult due to their quantitative inheritance and genotype × environment interactions.

In Fig. 6.4 an outlook of the selection targets in the different phases of sugar beet development and factory processing is presented. Results are still unsatisfactory for several resistances, not only for incomplete reduction of damage but also for a yield penalty that lowers sugar yield and processing quality. A complete review of the resistances against biotic and abiotic stresses in sugar beet was made by Biancardi et al. (2005).

5.1 Yield and Quality Traits

Gross sugar yield is the most important trait for growers and it depends on the weight of the roots produced per hectare and on the sugar content, i.e., the percentage w/w of sucrose present in the roots. In addition to the gross sugar yield, the extractable sugar must be considered, indicating how much white sugar can be extracted in the factory. This is directly related to processing quality (see below). With increasing quality, the white sugar yield approaches the gross sugar yield. The inheritance of the character "sugar yield" is quantitative and strongly affected by the environment (Powers et al., 1963). A non-additive variance is prevalent in controlling the trait "root production" (Campbell, 2002), while for the "sugar content" the variance is additive without expression of heterosis or dominance (Smith et al., 1973). There is a high correlation between sugar yield and root yield. However, if the root weight is increased by selection, the sugar content tends to be lower and vice versa.

Processing quality includes a number of chemical and physical traits of the harvested beets affecting the quantity of extractable sugar (Oltmann et al., 1984). Many

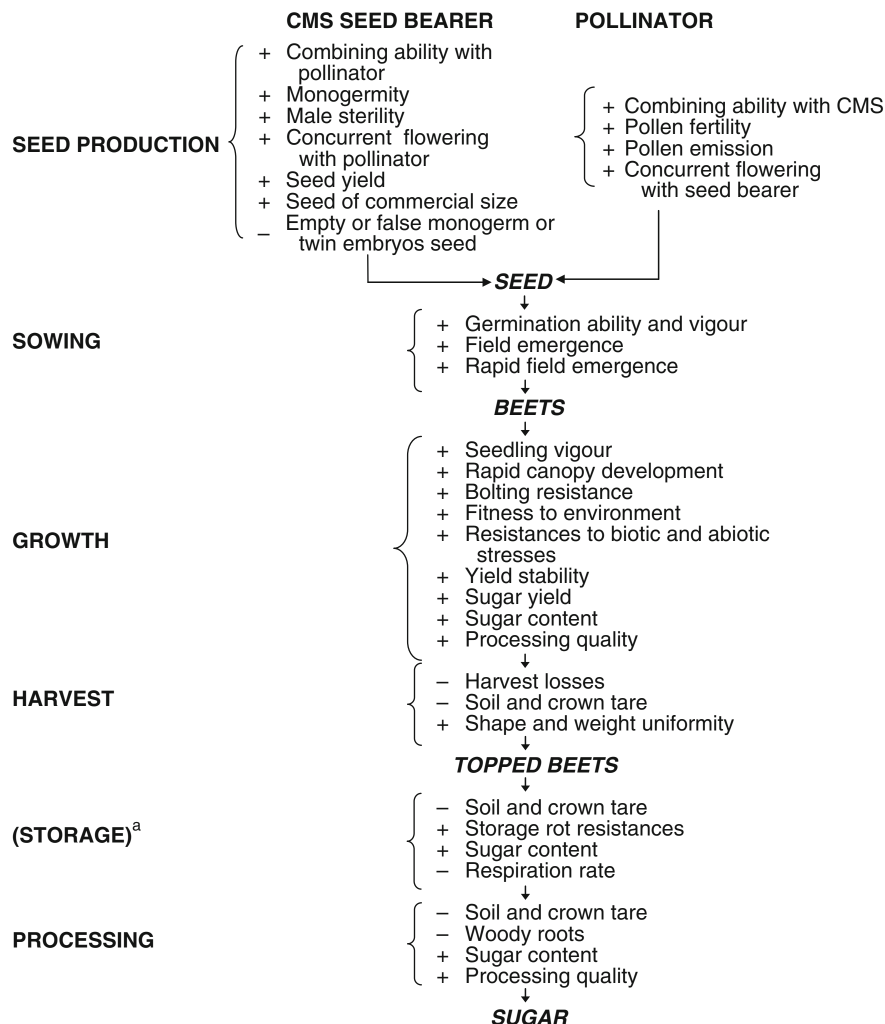

Fig. 6.4 Breeding targets (– less; + more) of some sugar beet traits in different steps of development and processing

of such characteristics are under genetic control, but the effect of cultural practices, harvest, storage methods, environment, etc., normally exerts a greater influence than the genetic control (Harvey and Dutton, 1993). Among the soluble impurities (non-sugars), sodium, potassium, alpha-amino nitrogen, reducing sugars, etc., have received most attention in breeding programs due to their negative effects on sugar extraction (Last and Draycott, 1977; Smith et al., 1977). The concentration of these non-sugars can be easily reduced with mass selection, suggesting an additive

genetic variance (Powers et al., 1963; Smith et al., 1973; Coe, 1987; Smith and Martin, 1989). Breeding for further improvements is complicated by interactions among non-sugars, sucrose concentration, and root weight (Campbell, 2005).

Some anatomical characteristics of the roots are associated with processing quality. Selection of smooth root hybrids (with reduced or without the two vertical grooves) lowers the amount of adhering soil carried to the factory (Fig. 6.5). This is desirable as the soil remaining on the roots after washing causes damage, especially during the slicing and diffusion phases (Theurer, 1993). Smooth root varieties with improved root shape and reduced crown dimension were developed through repeated cycles of mass selection (Mesken and Dieleman, 1988; Saunders et al., 1999). Similar results in improving processing quality are also possible with an appropriate fertilizer management.

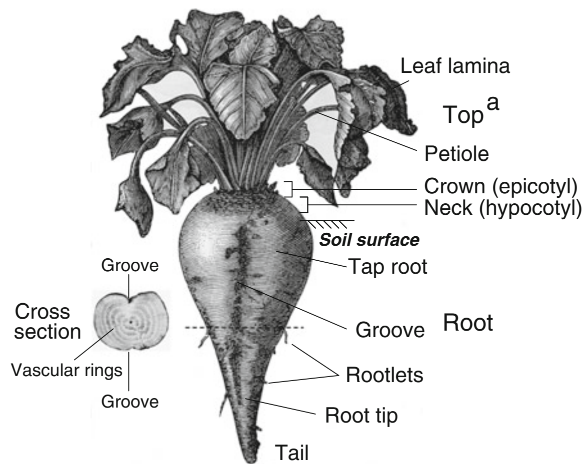

Fig. 6.5 Sugar beet drawing with the common names of the main parts

5.2 *Resistance to Diseases*

5.2.1 Curly Top

The beet curly top virus (BCTV) is transmitted by the beet leafhopper *Circulifer tenellus* Baker that attacks sugar beet throughout the arid areas in Western USA, Southwestern Canada, Mexico, Turkey, Iran, etc. (Duffus and Ruppel, 1993). The BCTV is a mixture of strains, which vary their virulence according to the host conditions, thus changing continuously the reactions required by the resistant varieties (McFarlane, 1971; Stenger and McMahon, 1997; Strausbaugh et al., 2008; Lam et al., 2009). Infected plants show a typical leaf curling, discoloration, and stunting, followed by the death of the young beets under severe infections. Breeding programs were initiated around 1925 by Carsner (1933). Mass selection of roots showing resistance in heavily infested fields proved effective (Coons et al., 1931), and the first resistant open-pollinated variety US1 was released (Coons, 1949). Although mass selection was successful in producing resistant open-pollinated populations,

inbreeding and progeny testing were necessary to continue to improve the varieties and to transfer the monogermy in the multigerm-resistant families (McFarlane, 1969). Much of the breeding for BCTV resistance was done through selfed genotypes endowed with the S^F gene and NMS (Owen, 1952). Improvements in creating uniform BCTV infection in selection fields have been instrumental for breeding progress (Murphy and Savitsky, 1952; Mumford, 1974).

Studies carried out by Abegg and Owen (1936) described a partially dominant genetic factor *C*, linked to the gene for crown color *R*. Murphy and Savitsky (1952) indicated a more intermediate (additive) resistance in F1 hybrids under moderate BCTV infection. In case of severe BCTV attacks on susceptible genotypes, the genetic nature of resistance appeared more composite. Savitsky and Murphy (1954) estimated that two or more genes were involved in the BCTV resistance. According to Hecker and Helmerich (1985), the multigenic traits of resistance should be present in both parents of the hybrid varieties. The genetic control of the disease was successfully integrated, and in some cases replaced, by insecticide treatments against the vector (Strausbaugh et al., 2006). Due to the need to reduce pesticides and other chemicals, further and rapid improvement of the BCTV resistance is necessary.

5.2.2 Rhizomania

This disease is caused by beet necrotic yellow vein virus (BNYVV) carried and inoculated into sugar beet roots by the soil-borne fungus *Polymyxa betae* Keskin. The symptoms are evident especially on the roots as (i) excessive proliferation of the rootlets assuming a beard-like appearance around the tap root; (ii) constrictions of the root tip leading to a wineglass shape; (iii) necrotic rings in the root tip section (Fig. 6.6). Diseased beets, if analyzed, show low sugar content, processing quality, etc. Immunoenzymatic tests (ELISA) performed on the roots can easily quantify the infection.

The virus causes losses of up to 80% in sugar yield (McGrann et al., 2009). Firstly detected in Italy around 1950, the disease is today more or less widespread in all growing areas (McGrann et al., 2009). By means of RNA analyses, three pathotypes of BNYVV were identified (A, B, and P) with different geographical distribution and pathogenic effects on the crop (Koenig et al., 1995; Lennefors, 2006a). The first source of resistance was found in cercospora leaf spot (CLS)-resistant germplasm derived from the multigerm variety “Alba P” (Biancardi et al., 2002). The superior performance of “Alba P” was observed in trials grown in 1957, i.e., before the discovery of the disease agents (Bongiovanni and Lanzoni, 1964). The resistance was classified as quantitative (Lewellen and Biancardi, 1990). A more resistant variety “Rizor” was released in 1985 by SES Italy (De Biaggi, 1987). The “Rizor”-type resistance was recognized as monogenic and dominant, being the hybrid variety produced with susceptible CMS seed bearer. In 1983, Erichsen observed some experimental hybrids yielding five times more than the mean of a diseased field trial (Lewellen et al., 1987). The hybrids, produced by the same CMS line owned by Holly Sugar Company, segregated in a pattern typical for a single

Fig. 6.6 Beets severely diseased by rhizomania

dominant gene, now named *Rz1* (Lewellen et al., 1987; Lewellen, 1988). Later, screening trials carried out in California confirmed that WB42, an accession of sea beet collected in Denmark, was resistant in diseased field condition (Lewellen, 1995a). Lewellen (1995a) identified other sources of resistance with unknown traits in the genotypes C28, R04, R05, C50, WB151, and WB169. Scholten (1997) and Scholten et al. (1999) reported that WB42 resistance was conditioned by a dominant gene, closely linked to the *Rz1* gene. This gene was coded *Rz2*. More recently, Gidner et al. (2005), Grimmer et al. (2008a), and Grimmer et al. (2008b) found similar traits of resistance in the sea beet accessions WB41 and WB258 (Panella and Lewellen, 2007).

The commercially employed types of resistance, Alba, Rizor, and Holly, appear to be derived from sea beet (Biancardi et al., 2002). The monogenic resistances in Rizor and Holly have been mapped to the same chromosomal region (Scholten et al., 1999; Biancardi et al., 2002). Genotypes carrying the monogenic sources of resistance frequently exhibit different levels of expression, probably due to the presence of minor genes interacting with the major allele in heterozygous individuals (Scholten et al., 1996; De Biaggi et al., 2003).

The resistant varieties used today, when tested in severe disease conditions applied in greenhouses, display no more than 80% resistant plants. Improvement of this percentage should allow better sugar yield even in severely diseased fields. Since the resistance in commercial varieties is usually transmitted by the pollinators, this goal should be possible using varieties in which all plants carry the genes of resistance at least in heterozygous conditions. This result is becoming possible by (i) using resistant pollinators and seed bearers; (ii) analyzing with molecular markers for rhizomania-resistance genes all pollinating and/or seed-bearing beets employed in seed production; and (iii) discarding the recessive and, when possible, the heterozygous plants. In addition, further sugar yield improvements should

be possible by combining in the same variety the different sources of resistance (De Biaggi, 2005). This would be essential where the known sources of resistance appear to be overcome by suspected mutations of BNYVV or in presence of the more pathogenic strains of the virus (Liu and Lewellen, 2007; Panella and Lewellen, 2007). Additional advantages may be obtained utilizing some forms of resistance against the vector *P. betae* found in wild species of the sections *Beta*, *Corollinae,* and *Procumbentes* (Paul, 1993; Paul et al., 1994; Barr et al., 1995; McGrann et al., 2009).

5.2.3 Cercospora Leaf Spot

Cercospora leaf spot (CLS), caused by the fungus *C. beticola* Sacc., is a very damaging disease in humid temperate zones (Greece, northern Italy, northern Spain, Austria, southern France, Japan, China, Michigan, etc.). The infection develops as necrotic lesions that enlarge and cause the more or less rapid destruction of the leaves. During the juvenile stage (up until 80–90 days from emergence), sugar beet appears immune to CLS attack, suggesting an inhibitory mechanism for the establishment of the pathogen inside the leaves. Several explanations have been proposed, such as lack of synchronization between hyphae elongation and stomata opening and the narrow passage through the stomata excluding the hyphae (Canova, 1959; Solel and Minz, 1971). None of these hypotheses were confirmed (Ruppel, 1972).

Only one source of quantitative genetic resistance to CLS is employed today (Skaracis and Biancardi, 2000). A second qualitative type of resistance has been reported when plants are infected with pathogen strains present in a limited area of California (Lewellen and Whitney, 1976). The latter resistance was not commercially employed. Species of the section *Procumbentes* exhibit high levels of resistance with unknown genetic characteristics (Biancardi, unpublished data). CLS-resistant genotypes have been derived from crosses initiated around 1915 using sea beets collected along the coasts of Adriatic Sea (Munerati, 1931). After repeated backcrossing in order to reduce the negative traits of sea beet, some resistant lines were released (Coons et al., 1955; Coons, 1975). Selections continued in Italy and in the United States, giving rise to numerous commercial varieties (Coons, 1975; Lewellen, 1992).

The CLS resistance discovered by Munerati is controlled by at least four or five alleles with variable effects depending on the severity of infection (Smith and Gaskill, 1970). Based on QTL analysis, Koch (1997) agrees with these results, attributing part of the difficulties encountered in selection to recessive genes controlling the expression of the trait. Several fungicides proved quite effective in limiting the disease. When the effects of fungicides and resistance complement each other, a satisfactory control of the disease is achieved (Skaracis and Biancardi, 2000).

5.2.4 Beet Cyst Nematode

Cyst nematode (*Heterodera schachtii* Schm.) is one of the most destructive pests of sugar beet. It damages the root system and severely limits root yield and sugar

content. Typical symptoms are the weak development of the beets and the wilted leaves under high temperature and/or intense light conditions. The cysts of the nematode can be quite easily seen on the rootlets with the naked eye. Management of nematodes in sugar beet is becoming harder due to the increasing restriction on fumigations and to the wide number of host crops and weeds. Intervals of at least 4 years between beet crops reduce the nematode initial populations below economic levels.

Interspecific hybridization with embryo rescue and grafting techniques with *Beta procumbens* was employed successfully for transferring resistance to sugar beet (Savitsky, 1960, 1975; Yu, 2005). Nineteen nematode-resistant monosomic addition lines in diploid *B. vulgaris* were identified, each carrying one chromosome from *B. procumbens.* Subsequently, 18 chromosome nematode-resistant genotypes were developed, each with a translocated fragment attached to chromosome 9 that carried the gene $Hs1^{pro-1}$ (Sandal et al., 1997). Homozygous-resistant diploid sugar beet lines have been developed but continue to possess deleterious traits from *B. procumbens* and inefficient pairing in meiosis (Yu, 1983; Heijbroek et al., 1988; Lewellen, 1995b). The positional cloning of the gene $Hs1^{pro-1}$ enhanced the possibility of transferring the resistance to high-yielding varieties (Cai et al., 1997).

Resistance to cyst nematode conditioned by dominant or partially dominant genes was recently found in sea beet (Panella and Lewellen, 2007). Varieties carrying the resistance derived from *B. procumbens* and *B. vulgaris* ssp. *maritima* were released in the United States (Lewellen, 2006, 2007) and Europe. According to Niere (2009), the former source is higher yielding than the latter, which he classified less susceptible or tolerant. Under compared infested and non-infested field conditions, Lewellen and Pakish (2005) showed that the resistance from *B. vulgaris* ssp. *maritima* greatly reduced sugar yield losses and had reduced nematode populations (Lewellen and Pakish, 2005). In both cases, crop rotations in order to reduce the nematode population density and resistance breaking biotypes are advisable.

Root knot nematode (*Meloidogyne* spp.) is not as widely distributed in sugar beet production as cyst nematode, but where it occurs can be very serious. Resistance was identified in *B. vulgaris* ssp. *maritima* and transferred to sugar beet (Yu, 1995; Yu et al., 1999; Yu and Lewellen, 2004).

5.3 Resistance to Abiotic Stresses

Several breeders with different approaches have examined resistance (tolerance) to drought, cold, heat, etc. Appreciable levels of genetic variability were observed despite the masking effects of environmental interactions (Wood et al., 1950; Wood, 1952; Srivastava, 1996; Ober and Luterbacher, 2002; Stevanato, 2005). Traits conferring such resistances were identified also in wild beets (Luterbacher et al., 1998). The potential breeding value for improving stress resistance is still unknown due to the difficulties in transferring and introgressing useful traits from the wild species to high-yielding germplasm. Pidgeon et al. (2006) found positive interactions among the yield of varieties and water availability. Drought-tolerant varieties were

characterized by their specific leaf weight and their succulence index (Ober et al., 2005), both conditioned by unknown genetic factors. For cold resistance, some degree of variance was detected in sugar beet varieties (Dix et al., 1994). According to Wood (1952), the resistances to cold and to cercospora leaf spot appeared correlated. Until now, no real improvement in cold resistance has been reported in literature for sugar beet. In the southern cultivation areas, temperature and light intensity are frequently excessive for the crop. Selection to reduce heat stress was tested by analyzing leaf chlorophyll fluorescence (Clarke et al., 1995; Srivastava, 1996). In this case as well, there was no real progress obtained.

6 Breeding Methods and Techniques

Increases realized in sugar beet production through breeding have been impressive and firstly occurred at a rapid rate (McFarlane, 1971). Mass selection was applied initially, followed by several schemes based on progeny evaluation and combining ability assessment (Smith, 1987). Further advances over the last 40 years were possible using recurrent selection methods and through various biotechnology approaches.

6.1 Mass Selection

Successful application of mass selection in sugar beet requires an adequate level of heritability for improvement (Hecker, 1967). In other words, mass selection is quite efficient for qualitative characters and gives satisfactory progresses when dealing with traits controlled by genes having significant additive effects, as in the case of sugar content (Smith et al., 1973). Root yield, being controlled by genes with non-additive action, shows poor response to mass selection, although the method is quite effective if used with non-selected materials (Bosemark, 1993).

In a typical mass selection scheme, the fields are established earlier than those of the commercial crop. The beets to be selected, also called mother beets, must grow exactly in the same condition (soil, spacing, nutrients, water, treatments, etc.). Mother beets are biennial as the cultivated ones and require overwintering to enter the reproduction phase. Normally they are selected in the first year. Stecklings, i.e., beets drilled normally in August and transplanted in the late winter, are used only for seed production. At harvest, mother beets with undesired phenotypic traits are discarded. In this stage, approximately 10% of the beets closer to the desirable ideotype are selected, i.e., those with a regular shape and without defects. After individual sampling and analysis, the selected beets are treated with fungicides and kept under appropriate temperature and light conditions to induce vernalization. The following spring, transplanted roots are allowed to intercross by open pollination in isolated fields, where the seed of the improved population is harvested for a second selection cycle.

6.2 Family Selection and Line Breeding

The evaluation of genotypes based on the characteristics of their offspring provides a more efficient means for improvement than simple mass selection. Since the middle of the 1900s, family (or progeny) selection, together with its various versions, came into common use. This method allowed the accumulation of favorable genes with additive and dominant effects (Helmerick et al., 1965; Smith et al., 1973) and was successfully used for the development of improved multigerm populations. When quantitative traits are involved, the response in advanced genotypes is quite small. Efficiency of progeny testing requires the populations under improvement to possess sufficient genetic variability for the traits to be improved. Two main methods are still employed: half-sib and full-sib progeny selection (Bosemark, 1993).

6.2.1 Half-Sib Selection

Plants selected as for mass selection (year 1) are vernalized and intercrossed by open pollination (Fig. 6.7) and the seed is collected separately (year 2). The seed of each plant, a half-sib (HS) family, is a mixture of F1 hybrids produced by the seed bearer and by a random sample of pollen released by the plants present in the crossing plot. Due to the possible presence of a variable degree of self-fertility, part of the seed of each HS family could be derived by self-pollination. The seed of the HS families is drilled in field trials to assess the yield performances (year 3). According to the results, the best HS families are selected in the nursery established in the meantime. Usually, the seed quantities of the single HS families are small and consequently the field tests are limited to few replications. The best HS families can be used to repeat years 2 and 3 (HS family selection) or individual HS families can be multiplied under pollen isolation (year 4). Higher seed quantities and reduced heterozygosity in these S1 multiplications allow a more reliable evaluation of the HS families (year 5), but will be accompanied by some inbreeding depression for yield. The stecklings of the superior S1's are joined for seed production (year 6). Field evaluation of HS families provides an indication of their general combining ability (GCA), whereas trials of their respective S1's allow the elimination of other inferior lines. In this way, a quite efficient selection is possible, but the S1 evaluation lengthens the cycle time for recurrent selection. The seed obtained at the end of the selection process can be used directly (or after appropriate test cross evaluations) as pollinators for commercial varieties. Several modifications of the method are possible.

6.2.2 Full-Sib Selection

This method allows a more effective selection because both parents of the full-sib (FS) families are fully determined (Hecker and Helmerich, 1985; Smith, 1987). As with the HS scheme, the FS selection method is mainly used for improving pollinators.

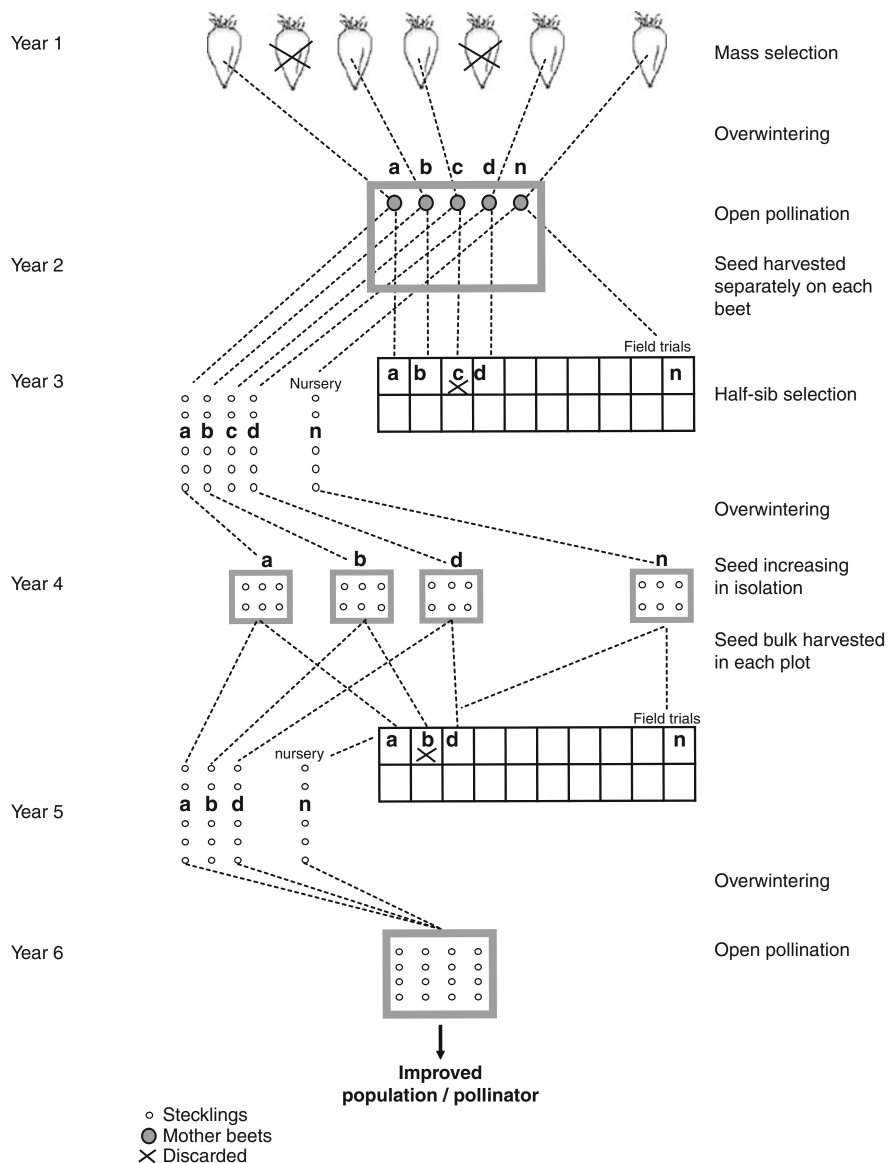

Fig. 6.7 Half-sib selection method

Through normally mass selected mother beets, seed is produced in isolated pair crosses. The new FS families are sown in the nursery and in field trials. The following year, only the better families are intercrossed to obtain an improved population with a superior range of favorable genetic combinations. As with the HS families, the seed multiplication in isolated plots (S1) of the FS families would allow

a more accurate assessment of their yield potential. However, the performance of FS families will be affected by inbreeding depression.

6.3 Recurrent Selection for Combining Ability

Recurrent selection (RS) for combining ability refers to a group of methods suited to improvements through an increased frequency of superior alleles and allelic combinations. The method allows the selection of lines with superior combining ability for use as male or female parents of hybrid varieties. The RS method presents the following common features: (i) plants of a heterozygous family are either selfed (S1) or selfed and crossed to a tester; (ii) after field trials of the S1s or test crosses, the inferior progenies are eliminated; (iii) all possible crosses between the remaining S1 progenies are performed; and (iv) the population resulting from these crosses is used to begin a new selection cycle.

Four main models of RS methods are suitable for sugar beet (Bosemark, 2006): (i) Simple recurrent selection (SRS), based solely on the phenotype or on the evaluation of S1 progenies; (ii) Recurrent selection for general combining ability (RSGCA), where the selection is made according to the evaluation of test crosses with a heterozygous common tester; (iii) Recurrent selection for specific combining ability (RSSCA), where the tester line, usually an inbred line, provides information on the specific (and general) combining ability of the selected families; and (iv) Reciprocal recurrent selection (RRS), in which two populations are simultaneously improved, in the same way as in RSGCA, but one is used as tester to the other, and vice versa. A number of other variations are possible depending on the genotypes, the traits to improve, and the selection targets.

6.4 Hybrid Varieties

With the employment of CMS monogerm lines, several new combinations of varieties became possible (Table 6.2) using as pollinators the same genotypes employed for the multigerm varieties. Seed harvested on monogerm seed bearers is genetically multigerm but phenotypically monogerm, thus only the female monogerm parent was necessary for the synthesis of the first monogerm hybrids. Crossing multigerm $2x$ line or family to $2x$ monogerm CMS line, $2x$ monogerm single cross or top cross hybrids are produced, respectively. If the CMS seed bearer is an F1 between CMS and different O-type lines, the cross with a $2x$ pollinator gives a three-way hybrid. If both parents are F1, a double cross hybrid is obtained. Using $4x$ pollinators, similar combinations at $3x$ ploidy level are possible. The use of $4x$ CMS lines is difficult due to problems of pollen contamination during seed production. Notwithstanding, crossing $4x$ CMS lines with $2x$ or $4x$ pollinators, $3x$ “reverse” and $4x$ hybrids were obtained, respectively. The former varieties were released by some European seed companies, but were not widely grown commercially (Bosemark, 1977).

Commercial varieties are produced crossing inbred CMS lines with pollinators, which can be inbred lines or hybrids between inbred lines. In these cases, single crosses (A × B) and three-way crosses A × (B × C) are obtained, respectively. For improving seed yield, usually the monogerm seed bearer is not an inbred line but a hybrid between a CMS line and a different O-type line from the maintainer. Such CMS F1 produces three-way crosses (A × B) × C or double cross (A × B) × (C × D) hybrids after crossing with an inbred line or a hybrid between inbred lines. Hybrids made with pollinators reproduced by free intercrossing (families) are designated top crosses.

For some decades, the 3*x* hybrids obtained with 4*x* multigerm families and 2*x* CMS F1 seed bearers displayed a superior sugar yield to the 2*x* equivalents, and, at least in Europe, had large commercial success. In the last 25 years, the development of 2*x* pollinators with a broad genetic base (family) enabled the synthesis of 2*x* hybrids with improved performance. Therefore, the use of 2*x* hybrid varieties is becoming prevalent in Europe, as elsewhere, due to a simpler and less expensive breeding process, easier introgression of the resistance traits, better germination quality of the seed, and higher processing quality. Today, at least in more advanced countries, most varieties can be classified as 2*x* three-way hybrids or as 2*x* single cross hybrids. The latter combination is less frequent owing to the lower seed production of CMS lines.

Methods for the synthesis of hybrid varieties are becoming quite similar among the few seed companies currently active. In Fig. 6.8 is represented the method for the synthesis of three-way hybrids employing a monogerm CMS F1 crossed with a multigerm 2*x* pollinator. As previously mentioned, the CMS inbred line is usually crossed with a different O-type. The selection of the best combination CMS × O-type is made testing their general combining ability (GCA). The traits to consider in the F1 progeny are also seed production, a high degree of male sterility, monogermy, the traits of the seed stalk, etc. The selected CMS F1 is crossed with different pollinators each in an isolated field. The seed of test crosses is harvested from the CMS and is accurately tested for the germination traits. The year later, test crosses are drilled in multi-year field trials organized in localities where the future variety should be cultivated. The crosses with superior yield and quality performances are mixed in different ways and go on with testing for at least 3 years. According to the results, the seed of the new variety is reproduced in large amounts for registration procedures and commercialization.

7 Integration of New Biotechnologies in Breeding Programs

7.1 Genetic Maps

Many sugar beet genetic maps have been constructed with molecular markers using (i) anonymous genomic restriction fragment length polymorphisms (RFLP); (ii) randomly amplified polymorphic DNA (RAPD); (iii) amplified fragment length

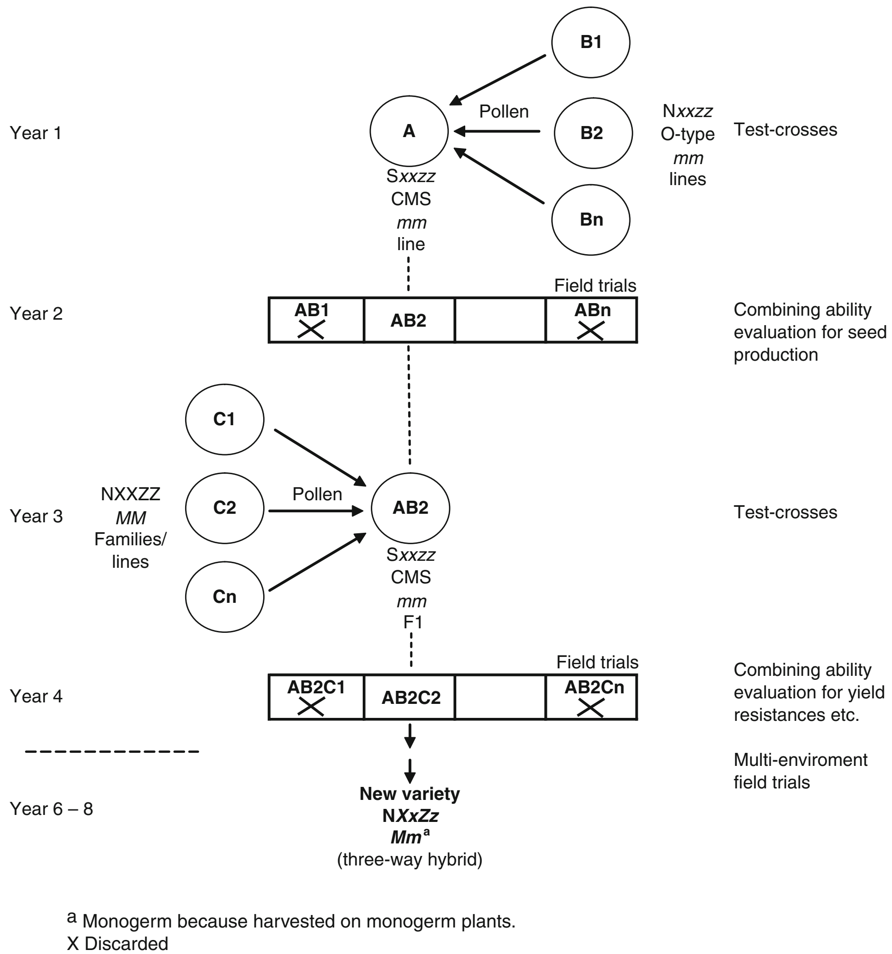

Fig. 6.8 Synthesis of three-way hybrid variety including tests of combining ability for O-types and pollinators

polymorphisms (AFLP); (iv) simple sequence repeats (SSR); (v) single nucleotide polymorphisms (SNP), as well as morphological and isozyme markers (Barzen et al., 1992; Pillen et al., 1992, 1993; Boudry et al., 1994; Barzen et al., 1995; Uphoff and Wricke, 1995; Halldén et al., 1996; Schondelmaier et al., 1996; Nilsson et al., 1997; Schumacher et al., 1997; Hansen et al., 1999; Weber et al., 1999; Rae et al., 2000; Schneider et al., 2001; Möhring et al., 2004; Grimmer et al., 2007a; Schneider et al., 2007). The specific numbering of individual chromosomes, defined in genetic linkage maps, has been standardized. However, most maps have not been updated to reflect a common nomenclature. A series of markers are now publically available that allow standardization of chromosome nomenclature in most, if not all, mapping populations (McGrath et al., 2007 and unpublished). Work by Schondelmaier and

Jung (1997) is now considered the reference since their work has integrated previous cytogenetic information. Schneider et al. (2001) sequenced 37 genes developed from ESTs in two inbred sugar beet lines, and found one SNP per 283 bp within coding regions, and one SNP per 130 bp if introns and 5′ and 3′ flanking sequences were also considered. In 400 specific regions defined by ESTs, Schmidt et al. (2003) showed 75% of sequences derived from 16 divergent *B. vulgaris* germplasm sources are sufficient to detect SNPs, with an average of 4.6 SNPs per 200–600 bp.

Most maps show strong clustering of markers on each linkage group, suggesting restricted genetic recombination, but this observation may be an artifact of the type of marker used (Nilsson et al., 1997). However, this trend is less pronounced using markers derived from expressed genes. Segregation distortion is common. Interestingly, the extreme segregation distortion for linkage group 5 in the sugar × red table beet maps of McGrath et al. (2007) and Laurent et al. (2007) was opposite in their transmission despite both using sugar beet as the maternal parent, with sugar beet alleles favored in the former and table beet alleles in the latter. There appears to be little or no regularity in the organization of duplicated chromosome regions in beets (Halldén et al., 1998), indicating the true diploid nature of the beet genome.

Molecular markers suggest a large amount of genetic diversity is present in wild *B. vulgaris* ssp. *maritima* that is not captured in the cultivated crops. Molecular markers have been used extensively to characterize sugar beet and related *Beta* species (Jung and Herrmann, 1991; Mita et al., 1991; Jung et al., 1993; Senda et al., 1995; Kraft et al., 1997; Shen et al., 1998; McGrath et al., 1999; Wang and Goldman, 1999; Kraft et al., 2000; Cureton et al., 2002; Richards et al., 2004; Poulsen et al., 2007; Fénart et al., 2008). Genetic diversity in cultivated beets is low compared with other beet types (Jung et al., 1993), and cultivated beets may contain only a quarter to a third of the genetic diversity present in sea beets (Fénart et al., 2008; Saccomani et al., 2009).

Markers have been used to discover the location of genes involved in the expression of quantitative traits. Candidate genes involved in the accumulation of sucrose in sugar beets were mapped to the nine linkage groups of beet, and QTL analyses for a number of agronomic traits (e.g., sugar yield, beet yield, sucrose content, and impurity levels) uncovered many potentially useful associations (Schneider et al., 1999; 2002). Loci involved in restoration of male fertility in a sterile cytoplasm, *X* and *Z*, have been located on chromosomes 3 and 4, respectively (Schondelmaier and Jung, 1997), with locus *X* located terminally on chromosome 3 (Pillen et al., 1993; Uphoff and Wricke, 1995; Hagihara et al., 2005a). A third putative locus was found ca. 15 cM from Z on chromosome 4 by QTL analyses (Hjerdin-Panagopoulos et al., 2002). Disease resistance gene analogues have been mapped in beets (Hunger et al., 2003), and these have allowed co-segregation analyses with disease resistance QTLs (Lein et al., 2007, 2008). Interestingly, a complete class of disease resistance genes, the TIR-type, is completely lacking in *B. vulgaris* (Tian et al., 2004). QTL approaches have identified chromosome regions associated with resistance to powdery mildew (Grimmer et al., 2007b), rhizoctonia crown and root rot (Lein et al., 2008), rhizomania (Gidner et al., 2005; Lein et al., 2007), *Aphanomyces* (Taguchi et al., 2009), and cercospora leaf spot (Nilsson et al., 1999; Schäfer-Pregl et al.,

1999; Setiawan et al., 2000). Generally, the genetic component of these measured traits can be portioned into 2–10 chromosome regions, and many of these could be considered oligogenic in their inheritance patterns. Association mapping approaches appear to have good potential for uncovering loci involved in agronomic and disease traits (Stich et al., 2008a, b). However, molecular marker density and phenotypic precision in open-pollinated populations and hybrids are still sub-optimal for fine mapping.

7.2 Sugar Beet Genome

DNA content (C-value) of *B. vulgaris* has been reported as 714–758 million base pairs per haploid genome, with variation reported among sub-species (Bennett and Smith, 1976; Arumuganathan and Earle, 1991). The nine chromosomes of sugar beet are morphologically similar at mitotic metaphase, and centromeres are either metacentric or sub-metacentric. A terminal constriction is on chromosome 1 and carries the major cluster of 18S–5.8S–25S ribosomal RNA genes. Highly repetitive DNA sequences comprise >60% of the beet genome (Flavell et al., 1974) and consist of numerous families of short (140–160 nt) repeating units present at high copy numbers (10^5–10^6 copies/genome) (Schmidt and Heslop-Harrison, 1996), and various classes of transposable elements (Schmidt and Heslop-Harrison, 1998; Staginnus et al., 2001; Jacobs et al., 2004; Dechyeva and Schmidt, 2006; Kuykendall et al., 2008; Menzel et al., 2008; Kuykendall et al., 2009). Organization of centromeric regions has been of interest to understand the molecular processes of chromosome segregation, to understand the process of non-disjunction, and to create plant artificial chromosomes (Gindullis et al., 2001a; Menzel et al., 2008; Jacobs et al., 2009). A generalized picture of beet chromosome structure and organization indicates that *Beta* chromosomes are substantially similar to most other dicot chromosomes, at a gross level.

In most cases, agronomic traits in sugar beet can be assumed to be controlled by genes whose product is a catalytic or structural RNA or protein. In sequenced crop plants, the number of genes is roughly assumed to be between 25,000 and 75,000, although much remains to be discovered about plant genomes. *B. vulgaris* would thus be expected to fall within this range for total gene number (Herwig et al., 2002), although significant differences in gene regulation, gene copy number, and presence or absence of specific gene classes (Tian et al., 2004) could be expected from differences in beet's form and function relative to other species in other plant families. Most *B. vulgaris* ESTs (Expressed Sequence Tags) are from sugar beet. These represent a reasonable cross section of important tissue types (root, leaf, seed, flower), including, for instance, genes induced upon nematode infection (Samuelian et al., 2004). The majority of ESTs were generated after oligo-fingerprinting of cDNA libraries (Bellin et al., 2002; Herwig et al., 2002), and there is good breadth of coverage (>18,000 contigs) but little depth for assessing the level of gene expression changes. In addition, 31,138 genome survey sequences have been deposited,

primarily derived from paired-end BAC and fosmid clones (McGrath et al., 2004; Lange et al., 2008). A number of large-insert libraries (e.g., Bacterial Artificial Chromosome; BAC) and other DNA libraries of beet have been made for various purposes, including cloning flowering genes and the bolting gene, nematode resistance genes, apomixis genes, CMS restorer genes, and centromeres (Jung et al., 1990; Kleine et al., 1995; Gindullis et al., 2001b; Hohmann et al., 2003; Hagihara et al., 2005b; Reeves et al., 2007; Jacobs et al., 2009). An oligo-fingerprinting approach to physical map construction is underway, and a draft *B. vulgaris* genome sequence should be available by 2011. It is anticipated that a genome sequence of beets will suggest means to achieve alternative uses of sugar beet beyond sucrose, molasses, and fodder.

7.3 Applications in Breeding

Using new technologies such as parallel nucleotide sequencing and gene expression profiling, breeders now have direct access to testing specific gene functions, such as those genes differentially expressed in root tissues (Bellin et al., 2002), and not just a correlation of phenotype with genotype. Basically, the internal workings of the beet plant can be made transparent, and thus allow more efficient and rational breeding targets, with results precisely measured and predictable. However, few target agronomic traits in beets have been characterized, and the level of understanding is still rudimentary. Still, some promise has realized. One of the first successful applications of such an approach in beets was to examine seedling vigor and resulted in identification of at least two biochemical pathways leading to enhancement of seedling vigor where little or no heritability was previously surmised (Sadeghian and Khodaii, 1998; De los Reyes and McGrath, 2003; De los Reyes et al., 2003). Differential gene expression analyses of mRNA profiles revealed a number of transcripts differentially regulated between extremes of high and low seedling vigor germplasm, and some were specifically expressed in the high vigor germplasm but not the low, identifying genetic targets for vigor enhancement. It should be noted that development of suitable test environments, such as the in vitro germination assays, could find use as surrogate selection criteria providing a strong association with agronomic performance.

In many cases, a tentative assessment of the biochemical pathways and overall metabolic status of a trait in a particular germplasm in a particular environment can be readily assessed, and this information can provide context and clarity as to the complexity of the phenotype. While specific genes and alleles and their contribution to phenotype are desired for breeding, gene cataloging and discovery are the current state of the art. Genes and proteins expressed during germination, early seedling development, mature beets, post-harvest processes, and disease and pest interactions have been surveyed (Samuelian et al., 2004; Bellin et al., 2007; Larson et al., 2007; Leubner-Metzger, 2007; Hermann et al., 2007; Puthoff and Smigocki, 2007; McGrath et al., 2008; Pestsova et al., 2008; Rotthues et al., 2008; Schmidlin

et al., 2008; Smigocki et al., 2008; Trebbi and McGrath, 2009), resulting in some broad insight into the patterns and processes of genes involved in development and responses to environment. However, at these levels of analyses, few genes can be unambiguously determined, and then only by association with other gene products present in databases, and thus their specific function and role in beets remain to be ascertained.

Specific genes identified by their demonstrated roles in processes important for sugar beet breeding have been sought. Map-based cloning approaches have been attempted, but this approach has been difficult in beets (Gaafar et al., 2005). More useful have been candidate gene approaches, particularly where model systems have uncovered biochemical pathways that have direct relevance for beet improvement. Specifically, the analysis of bolting and vernalization has been facilitated by flowering in *Arabidopsis* (Turck et al., 2008), with many of the genes in this pathway shared with beets (Reeves et al., 2007; Chia et al., 2008; Mutasa-Gottgens et al., 2009; Schulze-Buxloh et al., 2009). Marker-assisted selection is being practiced for at least one trait in sugar beet, that of rhizomania resistance. Commercial markers have been developed for the *Rz*1 gene, and likely *Rz*2, however, the specific primer sequences being used are proprietary and are likely different among the various breeding companies. Markers for rhizomania resistance are available in the public sector (Scholten and Lange, 2000; Amiri et al., 2009), but new ones are desired for other specific genes or alleles conferring resistance to rhizomania or other diseases (Friesen et al., 2006; Grimmer et al., 2007b).

The lack of fundamental knowledge about the number, identity, and diversity of genes and alleles present in beets is a serious hindrance to utilizing directed biotechnologies to introduce and develop novel traits in beets. Technology has matured to the point where transformation, while not easy, is possible (e.g., Liu et al., 2008) and novel and potentially easier methods are being investigated (e.g., Lennefors et al., 2006b, 2008). Tissue-specific expression and production of specialty compounds have been demonstrated using native beet promoters and native secondary compounds (Oltmanns et al., 2006; Thimmaraju et al., 2008), and these proofs of concept will allow rapid deployment of other modifications to the beet genome, either for breeding or as products in their own right. Risks and benefits associated with growing transgenic beets were recently summarized (Gurel et al., 2008; OCED, 2008).

7.4 Micropropagation and Haploidy

Beets are amenable to tissue culture, including clonal propagation through meristem culture, regeneration from callus tissues derived from virtually all plant organs, and somatic embryogenesis (Skaracis, 2005; Gurel et al., 2008). Success is somewhat dependent on the plant genotype, but can be generally achieved by manipulating the media and culture conditions, and in some cases the source explant tissue (Mishutkina and Gaponenko, 2006; Zhang et al., 2008; Xu et al., 2009). Tissue culture is primarily used in preparation for transformation, which is now

relatively routine in the major breeding companies; however, somaclonal variation has been exploited for herbicide resistance and salt tolerance (Gurel et al., 2008). Although culture of meristems for larger scale propagation generally avoids triggering somaclonal variation and preserves the source genotype, micropropagation is not a widely used technology for sugar beet variety development.

Haploid production in sugar beet (reviewed in Skaracis, 2005) has received considerable interest because of its potential for rapid inbreeding and fixation to genetic homozygosity in a single event. Unlike many other crops, anther culture has not proved useful for sugar beets for reasons that are not entirely apparent. Ovule culture has proved more successful, and gynogenetic embryos were shown to originate only from the egg cell (Ferrant and Bouharmont, 1994). The technique is laborious, lengthy, and the relatively low yield of doubled haploid plants (ca. 10%, obtained through chemically induced chromosome doubling of haploid ovules in culture) is currently insufficient for application in breeding programs (Mackay et al., 1999), particularly considering the genetic load in heterozygous breeding lines and fixation of lethal and sub-lethal alleles in doubled haploids. For genetic studies, doubled haploids can be important, and the most famous of them to date, KWS2320, derived from a monogerm breeding line, has been used as the DNA donor of most nucleotide sequence data in beets (Herwig et al., 2002).

8 Seed Production

8.1 *Methods of Seed Production*

Seedling vigor is a complex combination of traits that results in rapid germination, good field emergence, and the uniformity of stands (Stibbe and Märländer, 2002). With an adequate number of beets distributed uniformly, it is possible to optimize light interception by the canopy, and to reduce both the development of weeds and losses occurring at harvest due to irregular size and varied height of beets as they protrude above the soil surface (Snyder, 1963). Quality seed ensures better levels of sugar production. The change from breeding of multigerm to genetic monogerm varieties has made germination traits far more important, because fewer propagules are planted and each planted seed must produce a beet. Overplanting and thinning can sometimes be used to regulate the density of stand, but thinning is laborious and expensive.

Some geographical areas have been identified where the seed yield is better in terms of quantity and, particularly, quality. The most noteworthy of these are the lower Po Valley (Italy), southern France, Turkey, and Oregon (USA). Two systems of seed production are employed for sugar beet. Using the direct system, the genotypes to be reproduced are sown in place where seed will be harvested. Direct sowing is used mainly in Oregon and southeast France. An advantage of this method is that roots develop undisturbed in the same place, they are deeper and broader than the alternative transplanting. Consequently, lodging is less problematic, the crop requires less irrigation, and better vegetative development occurs.

The disadvantages include major losses caused by frost and the risk of weed beet contamination. Beets are spaced at greater distances than in sugar producer's fields and are thus less protected from the frost, having to survive the winter. Temperatures less than –12°C cause severe loss, particularly in monogerm materials (Campbell, 1968). Seed is planted at 6–14 cm intervals within rows that are 60–75 cm apart. A row of pollinators is sown every three or four rows of the CMS line. However, this proportion varies according to environment and to the pollen producing capacities of the pollinator (Smith, 1987). A second sowing method is to plant a mixture of monogerm and multigerm seeds in a ratio of about 10:1. All stalks are harvested, and the new monogerm and multigerm seeds are separated by grading (Hecker and Helmerich, 1985). Planting the parents in distinct rows is preferable since it allows inspection before flowering to eliminate any fertile, anomalous, or off-type plants. Furthermore, it allows trimming the stalks in order to obtain simultaneous flowering of pollinators and seed bearers.

In the indirect system, beets are first planted in a nursery. At the appropriate time, usually after vernalization, the small roots (stecklings) are transplanted into seed production fields located elsewhere (Bornscheuer et al., 1993). The system is more laborious but allows higher levels of seed quality. It is used especially in Italy and southeast France. As in the former system, there is the risk of nursery contamination caused by seed left in the soil by previous beet crops. In order to avoid such situations, it is necessary to know the past rotations of the field, and to leave at least 10 years after the last beet crop (Bornscheuer et al., 1993). Before sowing the nursery, it is necessary to know the germination ability of the genotypes, since the stand affects the dimension of the stecklings. A regular stand reduces plants wasted by a smaller or larger shape than optimal. The ideal stand is between 1,000,000 and 1,200,000 plants per hectare. The distance between the monogerm seeds in the row generally ranges between 2 and 3 cm. For multigerm seed, the distance between depends on the mean number of embryos per seed cluster. The rows are drilled from 20 to 25 cm apart depending on zone, soil, harvesting system, and climate. The nursery is normally planted in August.

The stand of stecklings at transplanting time also depends on sensitivity to cold. It is rare to find damage to multigerm pollinators, but the CMS's and especially the O-types are more sensitive. In order to avoid frost damage, special plastic covers are used to ensure effective thermal insulation. Nursery fertilization roughly follows that recommended for the sugar crop, with attention to the amount of nitrogen, which can cause excessive vegetative development. Great care is taken against diseases, such as cercospora leaf spot, *Phoma*, *Alternaria*, powdery mildew, *Botrytis*, *Pseudomonas,* and *Peronospora*. Insects (flea beetles, aphids, cutworms, etc.) also require adequate chemical control. Due to the required long rotations, control of the cyst nematode is usually not an issue. For weed control, the same herbicides employed for sugar crops are used.

Stecklings are normally harvested in February or March. In colder environments, it is better to harvest before winter and to store the plants in piles with leaves oriented toward the outside of the piles. The dimensions of the roots at transplanting depend on the stand and on weather conditions, but the most important characteristic is uniformity. Generally, roots measuring 3–4 cm across survive transplanting better.

Smaller roots are more suited to mechanical operations and require lower transportation costs, but they are more susceptible to drought. Leaves are trimmed mechanically before uprooting to leave petioles measuring 4–5 cm in length, and the tap root is trimmed at its end to stimulate development of lateral roots. Finally, the stecklings are cleaned of adhered soil and submersed in a fungicide solution to control fungal disease, such as *Phoma*.

Parents of hybrid varieties are usually transplanted into distinct rows. Stecklings are transplanted every 40–50 cm in rows 70–80 cm apart, for a target population density of about 36,000 plants per hectare. Once transplanted, only the petioles must protrude completely above the soil. It is important that the soil surrounding the stecklings is carefully compressed. Weeds are controlled with hoeing between the rows and with herbicides. Attention should be paid to *Phoma*, *Alternaria*, *Uromyces*, *Ramularia*, *Cercospora*, *Erysiphe*, *Botrytis*, *Peronospora*, *Verticillium*, and *Pseudomonas*, which all reduce yield and seed quality. Black and green aphids must be controlled, before and after bolting, due to the risk of virus infection. Any type of chemical treatment is not advisable during flowering. Irrigation after transplanting is often necessary and, if so, it should be repeated during seed ripening. An improvement of yield and quality is possible with drip irrigation, which does not moisten the plants and reduces the risk of pathogens on seeds. Topping (about 10–20 cm) of the seed-bearing stalks favors the development of lateral branches and improves the uniformity of the seed size. It is also useful to synchronize flowering of pollinators and seed bearers.

The growth of the seed stalk and development of flowers continues through harvest, in July–August. Therefore, all plants have a range of flowers under development, from fully closed, forming, and fully functional flowers, together with seeds at different stages of ripening. Pollinators are eliminated at the end of June, since flowers pollinated after this date are unlikely to be ripe by the time seed is harvested from the field. The harvest of the seed bearers begins when most of the seed has turned a light tobacco color and starts to come away easily. Earlier harvests will not lead to great losses, but the seed is partially unripe and there is the risk of poor germination. The loss of seeds increases with later harvests.

Swathing machines are adapted to avoid shaking the plants and the resulting seed shatter and loss of seed. The stalks are laid out in windrows for 1–2 weeks until seed moisture is 10–15%. Rain during this period is very damaging because it promotes the development of fungal parasites on the seeds, and always results in lowered germination. Threshing machines are also equipped for reducing the seed losses. Where the climate does not allow the drying in the field, stalks are transported to the factory to be processed as soon as possible.

Regular stands in sugar beet fields depend not only on germination ability, speed of emergence, etc., but also on other qualities, such as the percentage of empty, shrunken, or false monogerm (twin) seeds. Empty seeds are normal shaped, but they do not contain an embryo (TeKrony and Hardin, 1969; Shavrukov et al., 2000). A quite large percentage of empty seeds were observed, especially in 3*x* monogerm hybrids (Jassem, 1976). Due to their weight difference compared with normal seeds, empty seeds are partially eliminated by gravity tables. Monogerm seeds containing

shrunken embryos are impossible to discard by seed processing. The only method for reducing their percentage is careful selection of the parents, as can be judged by X-ray analysis of the hybrid seed. Another negative trait is the presence of multiple embryos in the same apparently monogerm seed (false monogermy). In this case, two, or rarely, more embryos develop. The "twin embryos" character is heritable and is quite well distributed among 4*x* genotypes and in 3*x* hybrids (Fischer, 1956). Their percentage can be reduced by separation on a gravity table, simultaneously with empty seeds.

Improvement in seed characteristics and emergence of commercial varieties is a slow but continuous process (Longden, 1990). Selection plays a significant role, but much of this progress has been due to seed crop growth and seed processing techniques and also in protecting germinating seedlings with chemicals delivered via pellet seed. Further improvement of germination traits and in speed of emergence was obtained using seed priming, which is a process of pre-germination (Mukasa et al., 2003). The use of primed seed in Western Europe and in the United States is increasing rapidly and is approaching 100% in areas such as France.

8.2 Pollen Isolation

Sugar beet is normally allogamous and self-sterile. Over medium and long distances, wind pollination is prevalent (Artschwager, 1927; Stewart and Tingey, 1927). Pollen granules are spherical with a diameter varying around 16–20 μm (Artschwager and Starrett, 1933). On 4*x* plants, the diameter is 5–10 μm greater (Knapp, 1958). The traits of sugar beet pollen are suited to be carried easily by the wind and for covering long distances.

Except for some self-fertile genotypes, control of pollination is necessary during breeding and the reproduction of basic and commercial seed. Isolation systems include (i) paper or cloth bags for one or more branches of the seed stalk; (ii) cloth or plastic coverings for one or two plants; (iii) glass and metal structures for up to about ten plants; and (iv) space isolation for more numerous groups and for commercial seed crops (Knapp, 1958). Using bags or isolators of small dimensions, the isolation can be completely controlled, but often the yield and quality of the seed are lower due to higher temperature and humidity inside the enclosure (Raleigh, 1936). Space isolation uses distance to lower effectively the pollen concentration in the air (Archimowitsch, 1949). Stewart and Campbell (1952) state that pollen levels reduce as the squared distance from the source, even if air movement and meteorological conditions create large variations.

In commercial seed production, fields must be appropriately separated to prevent or minimize possible contamination. The "home" pollen, with which fertilization is planned, could be mixed with "foreign" (contaminating or background) pollen released by other *Beta* sources (Chamberlain, 1967). Damage caused by pollen contamination on commercial seed multiplications depends not only on the percentage of undesired crosses, but also on the origin of the pollen itself. Although rare, crosses

with wild beets, like sea beet and *B. macrocarpa*, are very damaging due to the transmission of the annual trait. More commonly, contamination is due to weed or ruderal beets, i.e., from plants originating from the seeds of bolted sugar beet growing inside or outside of cultivated fields, respectively. Since weed beets can receive pollen released up to 9.6 km apart (Marco De Biaggi, personal communication), intercrossing is almost unavoidable in some areas (Fenart et al. 2007). If cultivation of transgenic varieties is allowed, the risk of transmission of the modified traits from the cultivated to the weed beets needs to be taken into account (Bartsch et al., 2003).

As the annual trait is dominant, the pollen of wild, ruderal, and weed beets transmits this bolting habit to the progeny. In seed production areas, damage caused by the pollen emitted by bolted sugar beets is quite frequent. Therefore, bolting sugar beets should be eliminated before the flower's opening. Crosses between other types of cultivated beets (leaf, garden, fodder) are also dangerous because they are immediately recognizable in the subsequent sugar beet crop, even if the contamination is very slight.

Crosses can also occur between fields where the seed of different varieties is produced. Morphological and agronomic differences between the commercial pollinators are generally so small that the contamination is difficult to detect, unless there are differences in chromosome number between the pollinators. In such case, if the field is to produce $3x$ hybrids, the presence of foreign pollen released by $2x$ beets causes an increased percentage of $2x$ hybrids. Risks of contamination by pollinators are much lower in fields for producing $2x$ hybrids, as this foreign pollen is less competitive (Scott and Longden, 1970). The minimum distance between seed crops required by law generally leads to low and acceptable levels of contamination if the annual and bolting beets are eliminated in a timely fashion around the fields.

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Chapter 7
Fodder Beet

Karine Henry

1 Introduction

The fodder beet belongs to the family Amaranthaceae (formerly Chenopodiaceae), which includes no less than 105 genera divided into 1,400 species, and whose members are herbaceous dicotyledonous plants (Watson and Dallwitz, 1992). In the literature, it has often been called *mangel*, *mangold*, or *wurzel* instead of fodder beet. Other economically important cultivated groups are the sugar beets, table beets, and leaf beets (e.g., Swiss chards); these too all belong to the species *Beta vulgaris*. The table beets and Swiss chards are mainly used as vegetables, the sugar beets as a source of sucrose, and fodder beets as cattle feed.

Fodder beet is a crop which experienced a remarkable development in many countries of Europe at the beginning of the nineteenth century. Known and appreciated for a very long time, the old varieties of fodder beet constituted one of the basic winter feeds for cattle (Fig. 7.1). After the Second World War, fodder beet growing showed a marked decline mainly due to its intensive labor requirement (thinning), reinforced by the rise in popularity of maize.

Today, fodder beet is no longer a major crop for animal feed in terms of production or area cultivated, even though it has a number of advantages. It has very high productivity, the best of all the fodder crops (both root and top may be utilized), and is very adaptable. Full mechanization of its culture is now possible. Moreover, it has a raised energy concentration, about 1–1.15 UF/kg[1] of dry matter. It is a fresh appetizing food which has a positive effect on both product quality and animal health. However, rationing is sometimes necessary because, as a close relative of sugar beet, its sugar content can be too high. The fodder beet market is still relatively small. Production areas are as follows (Internal data and respective Ministries of Agriculture, 2008): France (13,000 ha), the United Kingdom

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[1] UF/kg is an arbitrary unit referring to energetic equivalent of a kilo of barley.

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Fig. 7.1 Cows consuming fodder beet roots

(10,000 ha), Belarus (8,000–10,000 ha), Denmark (8,000 ha), Ireland (7,600 ha), New Zealand (7,000 ha), Belgium (4,000 ha), Germany (4,000 ha), and Switzerland (1,000–1,500 ha).

The recent changes to European farm subsidies in the Common Agricultural Policy (CAP) are likely to lead to alterations in crop rotations. As the differences between the subsidies for different crops become negligible, the reintroduction of fodder beet or an increase in area may become more attractive. However, activity in the improvement of fodder beet is much lower today than for sugar beet and there are relatively few breeders (Florimond-Desprez, Momont, INRA through Agri-Obtentions, LIMAGRAIN LG, DLF-Trifolium), essentially because of the crop's minor importance. Nevertheless, innovative breeding strategies are in place, greatly aided by the proximity of the sugar beet crop. Furthermore, if the modifications of the CAP lead to increased interest in fodder beet as a crop, renewed interest in its breeding may follow.

1.1 Biology of Species

The fodder beet is in general a diploid plant with 18 chromosomes ($2n = 2x = 18$) although the majority of European fodder varieties are triploid hybrids (by use of a tetraploid pollinator as male parent and a diploid male sterile plant as female parent). Like sugar beet, it is a biennial plant, which produces a fleshy root in the first year and a flowering stem in the second. As a general rule, fodder beet is sown in spring and harvested in the autumn of the same year before the first frosts. During its first (vegetative) year, the plant is generally characterized by leaves of a dark green color and oval form. Their sizes and forms vary according to the genotype, the age of the plant, the climatic conditions, and the appearance of foliar diseases (Artschwager, 1926; Klotz, 2005; McGrath et al., 2007). The first pair of leaves lies

horizontally so as to maximize light interception. During the growing period the root is formed quickly and develops until harvest (Hayward, 1938; Doney et al., 1981; Elliott and Weston, 1993). During the second year, following a period of exposure to cold temperatures (4–7°C), the rosette is transformed into a flower stalk with indeterminate flowering (the height can vary from 50 to 180 cm). The flowers are generally green and petal-less, with both male and female organs (Smith, 1987). The flowers can be monogerms (only one flower per bract axil) or multigerms, gathered by cluster. Each flower is made up of five sepals, which protect the pistil and the ovary (tricarpelate ovary), and five stamens, fixed at their base (Knapp, 1958). The very short style carries two to three stigmas, protecting a single ovary surrounded by a structure called a receptacle (Duke, 1983). The pollen grains are round with a diameter of about 15–22 μm; each flower can produce up to 85,000 pollen grains (Knapp, 1958). Pollination is done by the wind and at a low frequency by insects (Artschwager, 1927). There are two systems of fertilization: self-fertilizing beets, in the presence of a dominant gene *Sf* (Owen, 1945), and self-incompatible beets due to a complex system of self-incompatibility and the existence of protandry. The duration of flowering is spread out over approximately 40 days. After pollination, seed maturation lasts about 6–10 weeks, depending on genotype. The flowers of the same cluster group form a seed which is monogerm if the flowers were single or multigerm if there were several flowers (Cooke and Scott, 1993). The seed is a cork-like structure between 4 and 8 mm in diameter, containing one or more embryos.

2 Origin and Domestication

2.1 Origin

The genus *Beta* comprises four sections: *Beta* (genepool I), *Corollinae* and *Nanae* (genepool II), and *Procumbentes* (genepool III) (Buttler, 1977a, b; Lange et al., 1999), mainly native of Europe and adjacent zones (Fig. 7.2). The sections *Nanae* and *Procumbentes* have limited geographical distributions whereas the wild species of the *Beta* section have been found on the shores of Europe, North Africa, the Middle East, and Asia Minor since prehistoric times. They constitute the general pool from which the domesticated beet could have been derived, with subsp. *maritima* (Sea beet) being the most probable parent. The center of diversity is located in Eastern Turkey, in the zone of overlap between the sections *Corollinae* and *Beta*. The domestication of beets probably started in the areas of the Tigris and Euphrates and continued into Turkey and Greece, areas from which cultivated beets were introduced into Northern Europe (Boughey, 1981). The *Beta* section of the genus *Beta* contains three species: *Beta macrocarpa*, *B. patula*, and *B. vulgaris*, which itself includes three subspecies, *B. vulgaris* subsp. *adanensis*, *B. vulgaris* subsp. *maritime*, and *B. vulgaris* subsp. *vulgaris* (Table 7.1). All plants from the *Beta* section can intermate easily, providing the breeder with new sources of variability. On the other hand, intermatings are rare between plants of various sections of the genus

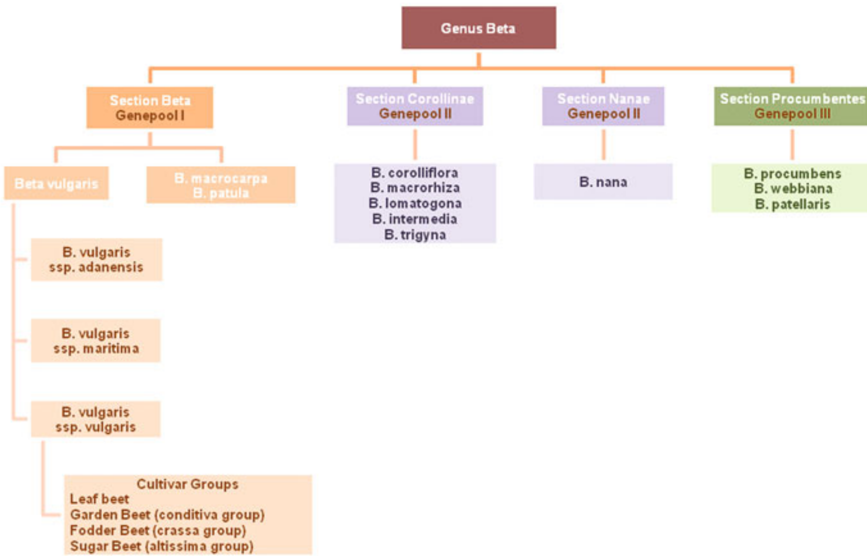

Fig. 7.2 Representation of the various sections of the genus *Beta*

Table 7.1 Main features of the species of the section *Beta* (Letschert et al., 1994; Villain, 2007)

Species	Type	Leaves	No. of flowers/cluster	Localization	Chromosome number
Macrocarpa	Annual	Long, green, and glabrous	3	Europe	$2x = 18$
Patula	Perennial	Small and glabrous	7	Madeira	$2x = 18$
Vulgaris subsp. *adanensis*	Annual or perennial	Long	3	Greece, Turkey, Syria, and Cyprus	$2x = 18$
Vulgaris subsp. *maritima*	Annual, biennial, or perennial	Oval, glabrous, or hairy	2–3	Europe, littoral	$2x = 18$
Vulgaris subsp. *vulgaris*	Biennial	Oval, green clear with dark green	1–3	Cultivated zones	$2x = 18$ or $4x = 36$

Beta, mainly because of the different chromosome numbers. The breeders often use plants from the subspecies *maritima*, for example, whose many genes contributed to current fodder beet cultivars, mainly via sugar beet (Zohary and Hopf, 2000). However, major sources of resistance to pests or diseases have been identified in the sections *Procumbentes* and *Corollinae*.

2.2 History

According to de Vilmorin (1923), the Greeks Hippocrates and Theophraste mention the existence of varieties with fleshy roots which were used especially as medicinal plants, at that time already distinguishing the dark types from the pale types. The beet was then used mainly for its leaves and petioles (more or less marked according to the subspecies). Later, one finds beets (garden, table) in the gardens of Charlemagne (724–814). In a text "Regulation concerning Landed Property," going back to 812 (Capitulare of Villis), *B. vulgaris* is recorded as a plant having to be specifically cultivated in the grounds of the Imperial State. In the Middle Ages, *B. vulgaris* was cultivated for its roots, described in the gardens of the French, Italian, and Spanish monasteries as Roman beet (Nottingham, 2004). At that time, the root was long and fine; there were intermediate types between fodder beet and Swiss chards but we do not know how fodder beets started differing from these types. It is almost certain that since the first use of beet by the Greeks and the Romans, all the types of *B. vulgaris* have been given to cattle. In fact, no distinction was made between fodder and sugar beet before the eighteenth century. The first varieties existed in the Palatinate around 1700 (Knapp, 1958) but it was only in 1778 that Philippe de Vilmorin announced for the first time the cultivation of "pastoral" beet in France. The variety was "Dick Wurzel," a type of beet grown in Germany for feeding cows through the winter. Selections made in varieties such as "Blanche de Silésie" were the origin of sugar beets (Bougy, 1936; Fischer, 1989) and also led to the creation of the fodder sugar beet "Géante Blanche." In the middle of the nineteenth century, there were three or four fodder beet shapes derived from the German "Disette blanche," two red varieties and the "Yellow of Germany," from which "Jaune de Vauriac" was selected in France, as was the ovoid "Yellow of the Bars" which then spread in Germany and Scandinavia (de Vilmorin, 1923; Le Cochec, 1969). If these roots were regarded as too fibrous for human consumption, they certainly provided a relatively abundant and inexpensive food for cattle, with fodder beet being generally preferred to carrots and cabbages.

Various types of fodder beet are distinguished by their color and growth habit. In England, the name "fodder beet" is restricted to the types of beets with smaller roots and high dry matter content; those with broader roots, a stronger proportion of hypocotyl and which sit high in the soil are called "mangels" or "mangolds." This is distinct from Germany, where the term "mangold" refers to the white beet (leaf chard) (Winner, 1993).

3 Varietal Groups

The leaves and roots of fodder beet are given to the cattle or other animals either fresh or in the form of silage. In general they have a very productive output since both root and tops can be used. They have broad roots which are indexed according to their shape and dry matter content. Since the introduction of genetic monogerms

and the use of cytoplasmic male sterility derived from sugar beet (which allows the production of hybrids), fodder beets can be grouped into three categories:

– The fodder beets whose dry matter content is lower than 11%
– The fodder sugar beets with a dry matter content between 11 and 16%
– The sugar fodder beets whose dry matter content is above 16%.

The total dry matter content of the fodder beet roots is measured by oven drying a 100 g sample at 70–80°C for 36 h. However, it can be estimated from the soluble dry matter, quickly measured by refractometry. A table makes it possible to carry out conversions. According to the dry matter content and the methods used, it is necessary to add 1.5–3 points to the soluble dry matter to obtain the total dry matter.

Fodder beets can be categorized further by their root morphology and color (Fig. 7.3, Table 7.2).

3.1 Root Morphology

Observations carried out after marking various zones of the plant at a young stage showed that tuberization affects not only the root but also the hypocotyl (source Comité Technique Permanent de la Sélection,[2] CTPS 1957). Depending on the proportion of tuberous hypocotyl, the fodder root will be more or less buried. The shape of the root is strongly dependent on the mode of tuberization and can be defined using the degree of lengthening and the gradient of tuberization. Various types of fodder beets can be defined according to their shape (source CTPS, 1957; Frandsen, 1958):

– globe type (sphere): tuberization is maximum in the middle and largely decreasing downward; the roots are short and broad, e.g., Yellow of Obendorf
– ovoid type (spindle): tuberization is maximum in the middle; the roots are fairly elongated, e.g., ovoid Yellow of the Bars
– cylindrical type (cylinder): tuberization is essentially the same at different levels; the roots are moderately long, e.g., Yellow or Red of Eckendorf
– cylindro-conical type: e.g., White or half-sugar Giantess
– long type: the roots are very long with a decreasing tuberization from the top downward, e.g., Red Giantess or long Rose of North
– conical type: tuberization is maximum in the upper part then quickly decreasing downward, e.g., very rich sugar-type fodder beets.

[2]The CTPS is in charge of the management of the French catalog of the species and the varieties. It examines the requests for inscription to the official catalog of the new varieties.

Mangel-wurzels of cylindro-conical types

Géante Blanche 4x *Géante rouge 4x* *Jaune de Vauriac 2x* *Jaune de Vauriac 4x* *Q of Mitra jaune pâle 4x*

Mangel-wurzels of long types

Rosver De Kluis 4x Q of Markenta 4x

Mangel-wurzels of cylindrical types ***Beets of the globule types***

Yellow of Eckendorf Red of Eckendorf Eckendorf green 2x Jaune sphere monogerme

Fig. 7.3 Representation of the different kinds of fodder beets ($2x$ = diploid, $4x$ = tetraploid)

In addition, the end of the root can be flattened, broad, blunt, or narrow. The sacchariferous furrow is generally absent or slight, except for the sugar-type fodder varieties with a higher dry matter content (source CTPS, 1957).

3.2 Root Color

It is important to distinguish the color of the skin from that of the flesh. In the buried part, the skin can be white-greyish, yellow, yellow-orange, orange, pink, or

Table 7.2 Characteristics of the principal types of mangel-wurzels

Type	Skin	Shape	Outground (%)	Hypocotyl	Foliage	Flesh	Harvesting	DM% class
White Giantess half-sugar	White, green in the outground part	Cylindro-conical	50	Green or pink	Abundant	White	Easy	6–9
Yellow of Eckendorf	Yellow, greenish (HT)	Cylindrical	70	Yellow	Fairly abundant	White	Very easy	5–8
Yellow of Obendorf	Yellow-orange	Spherical	70	Yellow	Abundant	White and yellow	Very easy	9–11.5
Yellow giant of Vauriac	Orange	Ovoid cylindro-conical	50	Yellow	Abundant	White and yellow	Easy	7–10
Red Giantess	Red	Cylindro-conical	40	Red	Fairly abundant	Reddish	Easy	7.5–10
Red of Eckendorf	Red	Cylindrical	60	Red	Not very abundant	White	Very easy	7–9
Red of Obendorf	Red	Spherical	65	Red	Fairly abundant	White zonée of red	Very easy	10–12

Source: CTPS (1957) and Frandsen (1958)

red. In the above-ground part, the addition of chlorophyll to the other pigments produces a green coloring or intermediate colors: greenish, yellowish, or reddish. The yellow varies from a very pale yellow (straw) to dark orange. The color is mainly due to betaxanthin pigments. The red varies from pale to very dark, characteristic of table beets (Nottingham, 2004). This dark color is also accompanied by a coloring of the petioles and leaf ribs, due to anthocyanins and betaxanthin pigments. For flesh color, distinct light and dark rings are usually visible when the root is cut transversely, especially in the red varieties. The parenchymatous zones and the storage tissue are normally different: the flesh itself is seldom colored but the pigments sometimes accumulate in the parenchymatous zones giving a color to the concentric rings, especially in the red varieties (Nottingham, 2004). Independently, the coloring of the hypocotyl before the four-leaf stage can be green, yellow, pink, red, or orange.

The color of the root, like the color of the hypocotyl, is determined by genes at two loci, G (sometimes quoted Y), with the allelic series *G, gr*, and *g*, and R (on chromosome 2) with the allelic series R, Rt, R^E, R_1, and r (Keller, 1936; Owen and Ryser, 1942; Pedersen, 1944). More recent studies (Linde-Laursen, 1972) show the presence of a third gene, P, which could be involved in the formation of the basic chromogene betaxanthine. The relationship between phenotype and genotype is shown in Table 7.3.

Table 7.3 Relationship between phenotype and genotype for root and hypocotyl color

Phenotype	Genotype
White roots, green hypocotyls	rrgg
White roots, colored hypocotyls	Rrgg, RRgg[a]
Yellow roots and hypocotyls	rrGg, rrGG
Red roots and hypocotyls	RrGg, RRGg, RrGG, RRGG[1]

[a]The existence of different allelic series both in R and G genes leads to different color variations either in hypocotyls or roots, e.g., the gene Rt causes red striping of petioles. The gene R_1 results in rose color instead of red.

4 Genetic Resources

Breeders can use three primary genetic pools to improve fodder beets:

– wild species of the *Beta* genus [particularly *B. vulgaris* subsp. *maritima* (sea beet)]
– sugar beet populations and cultivars
– table beet populations, Swiss chards, and others

Of these, the last two pools are those which have attracted most breeders due to their ease of use and the faster return toward a crop type. However, the wild species of *Beta* including *B. procumbens* and *B. webbiana* have often been used to improve the cultivated species of *B. vulgaris* (Goldman and Navazio, 2003) because these

wild species contain many interesting genes, in particular with regard to disease and pest resistance. More details can be found in the chapter on sugar beet.

5 Major Breeding Achievements

The current varieties of fodder beet mainly originate from old fodder beet varieties initially selected in France and Germany. It is difficult today to appreciate the characteristics of these first fodder varieties because they disappeared with the development of the varieties which we now know. These varieties were either selected in France ("Red Giant," "Pink Giant," etc.) or introduced from Germany ("Yellow and Red of Eckendorf," "Oberndorf") or from England ("Earth," "Mammoth"). The very different resulting forms are an important source of variability for the breeder. During the period prior to 1950, the old varieties that composed the traditional group of fodder beets were characterized by a weak buried part and a dry matter content of about 11%, and they occupied approximately 66% of the cultivated areas (Le Cochec, 1969). From 1956, further breeding progress was made following the introduction of Danish varieties with an intermediate dry matter content that was from 10 to 25% higher than the old varieties, and with a more extreme buried part. The best new varieties were suitable for the feeding of pigs. The next stage was the creation of polyploid (tetraploid and triploid) varieties using the same process as in sugar beets. The discovery of beet monogerms was made by Savitsky and Bordonos in 1930, and the transfer of this characteristic into varieties was finally achieved in 1968. The introduction of the first two genetic monogerm varieties of fodder beet opened the way to a new market since they combined an equivalent yield potential with a greater ease of cultivation with no need for tiresome thinning. The use of these varieties was accompanied by a considerable increase in the sowing density, leading to a large increase in the dry matter yield per hectare. De Vliegher et al. (1994) reported that the dry matter yield of the root improved 0.4% a year with selection in the period 1950–1993. Other improvements accompanied the change in varietal types, such as the development of chemical weed control and modification in manure composition, contributing effectively to a rise in output and profitability of cultivation.

During the period 1950–1980, the primary objective of selection was the use of the system of cytoplasmic male sterility, discovered by Owen in 1945, for the production of hybrids, and the general improvement of combining ability. The breeders were also able to use genes of interest found in sugar beet to improve fodder beets. Among the features transferred to the crop were the gene for self-fertility *Sf*, cytoplasmic male sterility, and monogermy. Since the year 2000, resistance to rhizomania, which is impossible to circumvent, and to rhizoctonia were also introduced, starting with sugar beet germplasm. Two topics are now covered in more detail.

5.1 Cytoplasmic Male Sterility

Two independent systems of male sterility exist in beet; one is genetic (nuclear) male sterility and the other is often referred to as cytoplasmic male sterility (CMS), but strictly speaking is cytoplasmic-genetic male sterility. The nuclear system is monofactorial (A/a), the sterile genotype being homozygous recessive (Owen, 1945). Difficult to use in the production of seeds, it is used primarily to ensure cross-pollination within populations undergoing recurrent selection. Usually used with sugar beet, it has not been introduced on a large scale to fodder beet programs.

The CMS system has been systematically used since the 1980s for the creation of varieties of fodder beet. It is determined by the presence of both cytoplasm S (N is for normal type) and recessive alleles (x and z) for two nuclear genes X and Z (Owen, 1945). The use of the CMS in selection imposes a very heavy work load on the breeder. To obtain homozygous male sterile lines it is necessary to transfer the male sterile character by several backcrosses and also to maintain each line in two isogenic forms, a male sterile line Sxxzz and a fertile maintainer line Nxxzz. (Brian, 1992). The first fodder beet hybrids were "Trestel" (INRA, France, 1975) and "Kyros" (Dansk Planteforaedling, Danemark, 1975) (Doré and Varoquaux, 2006).

5.2 Self-Fertility

In the past, cross-pollination was normal in beet because of a system of self-incompatibility controlled by four loci (Lundqvist et al., 1973) and because of a more or less pronounced mechanism of protandry. However, a dominant gene *Sf* for self-fertility makes it possible to counter these mechanisms and force self-pollination (Smith, 1987). The increase in consanguinity is of course a disadvantage of this system, but the consequences are of little importance for fodder beets where the degree of homozygosity reached is not as great as for sugar beet. Moreover, many S2 lines have been successfully developed. The introduction of the *Sf* allele into fodder beet germplasm has allowed the development of much more homogeneous populations and the easier maintenance of the various components of varieties by reducing the probability of contamination by foreign pollen during seed production.

Finally, during the last 10 years fodder beet breeding has benefited from the advances in molecular biology which have been applied to sugar beet breeding.

6 Current Goals of Breeding

The main purpose of fodder beet breeding is to produce the maximum income to the farmer. This supposes the production of a maximum of dry matter per production unit. However, certain other criteria must be taken into account.

6.1 Production of Biomass

Like any fodder species, the main objective of selection is the productivity of the plant, as judged by the following features.

6.1.1 The Root Yield per Hectare

This is obtained by measuring the net weight of washed roots per hectare. It is expressed in t/ha. It is improved by seeking the best combining ability between parents for composing different varieties.

6.1.2 Soluble Dry Matter Content

This varies greatly according to the cultivars and the types sought by the farmers. The heritability is high, as can be seen from the very fast progress obtained in a few generations of selection, and this suggests a rather simple genetic determination. However, it is negatively correlated with the root yield. It is estimated from refractometer readings (in Brix units) on the juice from a sample of pulp representing the whole variety.

6.1.3 The Soluble Dry Matter Yield per Hectare

This depends on the root yield per hectare, the dry matter content, and certain technological characteristics related to the constraints of mechanization (dirt tare, buried level, etc.). It is calculated from the product of the root yield and soluble dry matter content. In relation to productivity, variety adaptation to mechanical harvesting is also determined by the uniformity of the buried level, form of the crown, and development of the leaf or foliar bunch.

6.1.4 Regularity of Yield

The regularity of yield, making it possible to ensure the productivity of the crop, depends on other complementary factors such as those considered next.

6.2 Bolting Resistance

For the production of roots, the farmer uses only the vegetative phase (first year of development) of growth of fodder beet. The existence of annual plants in wild populations, from which the cultivated forms certainly resulted, supports the presence of genes giving susceptibility to bolting in the cultivated material. The tendency to practice early sowings (in cold conditions) necessarily led to increasing use of bolting-resistant varieties, and thus these wild genes have disappeared. The earlier that bolting occurs, the greater is the yield reduction. The plants which bolt first are usually those with fangi roots which have a much lower yield than normal plants (Nelson and Deming, 1952).

6.3 Resistance to Diseases

The pests and diseases affecting fodder beet are the same as those affecting sugar beet, though this depends on the cultivation area and practices such as crop rotation. The main aim of current research in this area is to combat rhizomania, crown root rot due to *Rhizoctonia solani*, and foliar diseases (mainly *Cercospora* and powdery mildew).

6.3.1 Rhizomania

Rhizomania is caused by BNYVV (beet necrotic yellow vein virus) and is transmitted by a soil fungus, *Polymyxa betae* (Koch, 1982). Chemical or mechanical controls are relatively limited, and only genetic resistance can limit the disease. Thanks to the use of germplasm from sugar beet, the first genetically rhizomania-resistant fodder beet varieties were commercialized at the end of the 1990s. "Rimon" (from Florimond-Desprez) was the first triploid rhizomania-resistant variety registered in France in 1992. It was a white variety. After that "Jary" (Florimond-Desprez) was registered in 1999 as a yellow rhizomania-resistant variety. In 2008, "Rivage," another yellow-resistant variety, was registered and had a real improvement in yield potential.

6.3.2 *Rhizoctonia* Crown Root Rot

This disease is caused by a basidiomycete soil fungus which causes a dry brown rot on fodder beet roots. As with rhizomania, only genetic resistance can reduce its incidence. The first rhizoctonia-resistant variety Rialto (Limagrain LG) was registered in Belgium in 2008.

6.3.3 Foliar Diseases

The protection of fodder beet from foliar diseases has taken on considerable importance during the last 10 years. The incidence of foliar diseases of cryptogamic origin is such that fungicide treatment results in a considerable increase in dry matter yield per hectare. However, within the framework of environmental sustainability, the marketing of varieties more tolerant to foliar diseases (powdery mildew, *Cercospora* leaf spot, rust, etc.) will allow a reduction in the use of fungicidal treatments (Grenelle de l'environnement, 2009).

6.4 Monogermity, Seed Germination, and Emergence

In order to avoid the necessity of thinning, all the modern varieties of fodder beets are monogerms. This characteristic is monogenic, M/m, the dominant phenotype being multigerm (Savitsky, 1952). As the monogermy characteristic is determined by the phenotype of the plant from which the seeds are harvested, the diploid or tetraploid pollinators can be multigerm. This allows effective exploitation of the

larger genetic variability in this gene pool. Concerning seed characteristics, despite the fact that most of the fodder beet varieties rely on a sugar beet female component, it seems that fodder beet varieties have a much higher seed productivity and quality than sugar beet varieties, due partly to the male parent (Bruno Desprez, personal communication).

To ensure crop establishment and final yield, germination and emergence are key stages. The physiological quality of the seeds must therefore be perfect. Even though there are a great many industrial treatments for improving the various components of seed quality, these remain empirical and their development is difficult. Breeders do not always have sufficient scientific data on the agronomic, physiological, and biochemical factors influencing the potentialities of the seeds. Nevertheless, fodder beets could also benefit from the industrial treatments used in sugar beet like activation of seeds (priming or pre-germination). For the moment, nothing is done due to the minor importance of the species.

7 Breeding Methods and Techniques

A modern variety of fodder beet is generally a population of monogerm seeds of a triploid ($3x = 27$) or diploid ($2x = 18$) genotype. These seeds are produced by crossing a male sterile diploid and monogerm female with a diploid or tetraploid ($4x = 36$) pollinator. The females used may or may not be obtained by hybridization between consanguineous lines: (female A × maintainer A or B). The combining ability between parents to improve productivity or regularity may thus be required on two levels: between the lines forming the females (if the female is a CMS F1) and between these females and the pollinators. The use of lines from sugar beet breeding programs to create part of the variety considerably simplifies fodder beet breeding programs. Thus, there are several ways to create a fodder beet variety of a given color as shown in Table 7.4. In addition, it is technically possible to produce two- or three-way hybrid varieties with a uniform genetic structure by using fixed lines (Lecochec, INRA, cv. "Trestel," 1975). However, at the pollinator level, the fodder components of current varieties generally result from mass selection or half-sib crossings and will thus still have an important level of heterozygosity.

7.1 Mass Selection

This is the oldest empirical breeding method. It consists of selecting from a population the plants which seem most interesting on the basis of simple characteristics such as root shape and color, bolting resistance, resistance to rhizomania, and dry matter content. These plants are all inter-mated (Fig. 7.4).

The resulting seeds are sown to constitute the new population to be selected. Mass selection is the simplest method of improvement and that constitutes a major advantage. However, it is a method which is effective only for characteristics with

Table 7.4 Color of the main components of a fodder variety and consequence for the color of the final hybrid

Females			Males	Variety
Ms	O-type		$2x$ or $4x$	Fodder
Male sterile line from sugar beet A	Sugar beet OT line B	White roots/green hypocotyls	Fodder beet with white roots	Half-sugar fodder beet with white roots
Male sterile line from sugar beet A	Sugar beet OT line B	White roots/green hypocotyls	Fodder beet with yellow roots	Fodder or half-fodder beet with yellow roots
Male sterile line from sugar beet A	Sugar beet OT line B	White roots/green hypocotyls	Fodder beet with red roots	Fodder or half-fodder beet with red roots
Male sterile line from sugar beet A	Sugar beet OT line B	White root/red hypocotyls	Fodder beet with white roots	Half-sugar fodder beet with red roots
Male sterile line from sugar beet A	Sugar beet OT line B	White roots/red hypocotyls	Fodder beet with yellow roots	Fodder or half-fodder beet with red roots
Male sterile line from sugar beet A	Sugar beet OT line B	White roots/red hypocotyls	Fodder beet with red roots	Fodder or half-fodder beet with red roots
Male sterile line from fodder beet A	Fodder beet OT line B	White roots	Fodder beet with white roots	Fodder beet with white roots
Male sterile line from fodder beet A	Fodder beet OT line B	Red roots	Fodder beet with yellow roots	Fodder beet with red roots
Male sterile line from fodder beet A	Fodder beet OT line B	Yellow roots	Fodder beet with red roots	Fodder beet with yellow roots
Male sterile line from fodder beet A	Fodder beet OT line B	Yellow roots	Sugar beet with white roots and green hypocotyls	Half-sugar fodder beet with yellow roots
Male sterile line from fodder beet A	Fodder beet OT line B	Yellow roots	Sugar beet with white roots and red hypocotyls	Half-sugar fodder beet with red roots
Male sterile line from fodder beet A	Fodder beet OT line B	Red roots	Sugar beet with white roots and green hypocotyls	Half-sugar fodder beet with red roots
Male sterile line from fodder beet A	Fodder beet OT line B	Red roots	Sugar beet with white roots and red hypocotyls	Half-sugar fodder beet with red roots

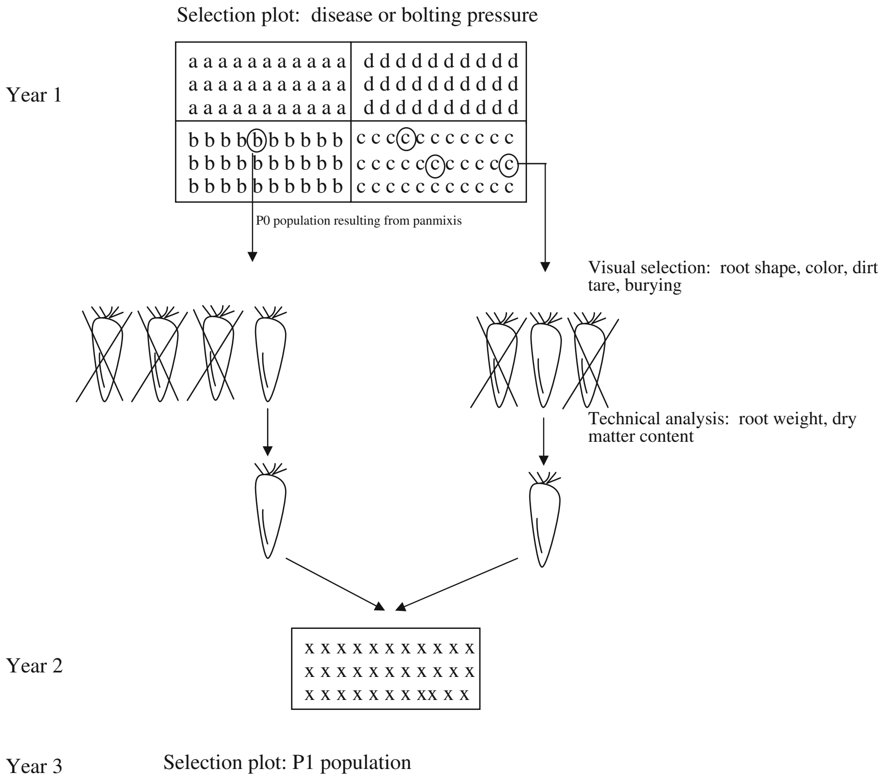

Fig. 7.4 Mass selection

a high heritability (Bosemark, 1993). For fodder beet, it was used successfully for the characteristics controlled by a few genes or with strong additivity, namely, root form, bolting resistance, resistance to rhizomania, and dry matter content. For yield, which is a characteristic controlled by many genes, with low heritability, the effectiveness of this method has not been demonstrated. Moreover, with the negative correlation between root yield and dry matter content, strong mass selection for the latter often results in a large decrease in root yield, leading to excessively sugar-rich types of varieties for animal feeds. In addition, mass selection for both yield and dry matter content involves significant drops in the intensity of selection and leads to the creation of intermediate rather than improved types. Nevertheless, at the beginning of selection, when starting from wild beets, for example, it can be effective in eliminating negative characteristics and enabling rapid progress.

7.2 Progeny Testing and Breeding Lines

For characteristics with weak heritability, such as root yield, the best way of distinguishing the effect of a micro-environment from a genetic effect is to test the

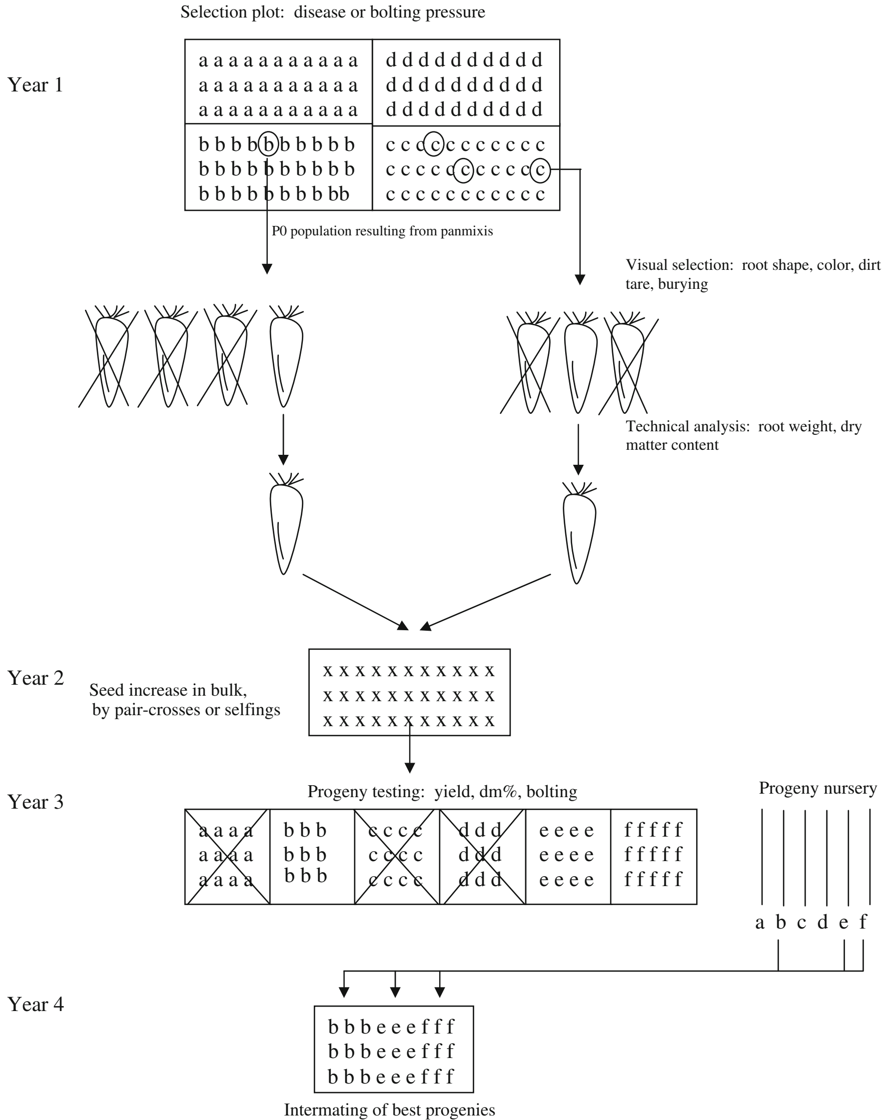

Fig. 7.5 Progeny selection

progeny of the selected plant. This method consists of selecting plants based on the performances of their descendants (Fig. 7.5). The offspring families can be obtained from inbreeding and selection if the material is self-fertilizing, or from full-sib or half-sib family selection. The start of the selection cycle is identical to that of mass selection:

– Individual selection of roots in the field by morphological characteristics
– Inter-pollination of the selected plants or selfings or pair crossings

– Harvesting each plant individually
– Individual testing of each offspring family for bolting, yield, dry matter content, etc.
– Intermating of the best families the following year

This method, which was often used by Louis de Vilmorin in the middle of the nineteenth century for sugar beet breeding programs, is undoubtedly more effective than mass selection, although in fodder beet programs an increase in root yield is limited because of the inbreeding depression and use of tetraploids. The method can be improved by the use of selfings, which makes it possible to increase the degree of homozygosity to allow easy elimination of lethal types and facilitate easy separation of the various varietal types, but this involves a reduction in vigor. The selfings are simple to use for the female breeding pathway in which the *Sf* gene was introduced on a large scale, but for the male breeding pathway, many materials today are still self-sterile and only pair crossings make it possible to improve the material.

7.3 Hybrid Breeding Methods and Development of Hybrid Varieties

To obtain more uniform and vigorous cultivars, the hybrid method was chosen for fodder beets after the discovery of the cytoplasmic-genetic male sterility system. As described in the Introduction, a fodder beet hybrid is the first generation of a crossing between more or less fixed parents of different genotypes. Each parent is initially created according to the methods described previously. The favorable hybrid combination results from parents chosen both for their per se value and for their ability to produce a vigorous hybrid. The term “combining ability” is important in this regard. The best method is to produce a simple F1 hybrid as this has the advantage of allowing a fast accumulation of dominant genes. However, for fodder beet, in order to promote seed productivity, one often produces a three-way hybrid, using the combination of a simple hybrid as female and a line as male parent. Indeed, as fodder beet is very susceptible to inbreeding depression, the use of a simple hybrid as a female makes it possible to restore the vigor of the male sterile seed-bearer and so increase the seed production.

In fodder beet breeding, the general combining ability of a line can then be investigated at different levels:

– Between the male sterile line and the OT maintainer
– Between the F1 male sterile hybrid and the pollinator

There are various ways of evaluating the general combining ability, the simplest being crosses of various males or females to a general common tester. The lines identified as having the best general combining ability can then be used in a diallel

set of crosses to identify the best hybrids which will exploit specific as well as general combining ability.

The evaluation of progenies in the field is generally carried out using multilocation tests to characterize any genotype × environment interactions and to identify the parental lines whose offspring are the most stable and vigorous in a broad range of environments.

8 Integration of New Biotechnologies in Breeding Programs

Progress based on traditional genetics has been very important and has led to the current varieties. However, the barriers between species do not allow all of the transfers of genes which it would be interesting to realize. Furthermore, variation in the chloroplast and mitochondrion genomes is not exploited because of their maternal transmission. Fixing characteristics is also a long and difficult process in spite of the use of the *Sf* gene, as it is for combining in the same genetic background the various sources of resistance to a disease. Cellular and molecular biology have made all of these possible and have also brought solutions to additional problems. The contributions of biotechnologies to traditional selection are numerous, including:

- Decreasing the breeding duration
- Exploiting genetic diversity by facilitating interspecific crossings
- Helping us to understand the genome and control the contribution of new characteristics

8.1 Micropropagation

In vitro micropropagation makes it possible to preserve and multiply an interesting genotype. The meristematic tissues, which are useful in this technique, are generally pest free, in particular of viruses. It is possible to regenerate healthy stocks from infected plants by being careful to take only the meristematic tissues. The management of the seeds of the parental lines of a variety is thus facilitated.

8.2 Haploid Production

Since it is difficult to fix genetic material by selfing, the process can be facilitated by the use of haploid cells from the male or female gametes. It is this last method which is used for beet. Colchicine treatment is used to produce doubled haploids which are completely and immediately fixed at all genetic loci. Unfavorable recessive genes can be definitively eliminated by this method (e.g., recessive susceptibility for the rhizomania gene). The haplo-diploidization fixes the various possible combinations (and recombinations due to crossing-over) between the characteristics

of the parental lines, and the choice between the doubled haploids will also be more precise because the material is genetically homogeneous. Moreover, this method makes it possible to observe the expression of recessive genes as well as dominant genes.

8.3 Gene Mapping

Fodder beet was used in crosses with sugar beet to maximize variability for gene mapping. DNA markers provide information about the genotype of an individual and are not modified by the environment. They can be used throughout an experiment and are observable at any developmental stage of the plant and in any organ. The most used are RFLP (restriction fragment length polymorphism), RAPD (random amplification of polymorphic DNA), AFLP (amplified fragment length polymorphism), and microsatellite (SSR) markers. Thanks to these DNA markers, it is possible to establish the genetic fingerprint of each individual, and to highlight and to follow genes implied in the expression of characteristics of agronomic or technologic interest. Thus, for resistance to rhizomania, markers for the Holly *Rz1* gene were developed for sugar beet by various breeding companies. The demonstration of a connection between molecular markers and this major gene was invaluable for the marketing of the first fodder beet rhizomania-resistant varieties. As a fragment of leaf is enough to obtain the DNA necessary to establish the genetic fingerprint of a plant, early and precise selection can be carried out at the beginning of the growth of the plants. This is a great time-saver since it is not necessary to await the phenotypic visualization of a gene. In addition, by means of some of these markers, it is possible to identify the heterozygous plants carrying a recessive allele whose phenotype is not observable. This method has been widely used with fodder beets for 10 years, since the registration in France of rhizomania-resistant varieties, developed thanks to the availability of resistance in sugar beet germplasm and its quick introduction into fodder beet by backcrossing. This technique also makes it possible to follow the introduction of several genes if necessary.

8.4 Genetic Transformation

This is a technique that is available for sugar beet and which could be extended to fodder beet. Since 1989, fodder beet varieties tolerant of glyphosate have been developed as a result of cooperation between DLF-Trifolium, Monsanto, and Danisco Seed. Monsanto provided the glyphosate tolerant gene, while DLF-Trifolium and Danisco were responsible for developing and breeding glyphosate tolerant varieties in Denmark. In 1998, a fodder beet variety from this technology was put on the market by DLF-Trifolium/Monsanto/Danisco. But, since the market for this species is restricted mainly to Europe, it is a field of application which has not been followed further for the moment.

On the whole, the minor importance of fodder beet does not support the use of biotechnology in Europe, unless it can profit from techniques developed for sugar beet, such as gene mapping and cloning.

9 Seed Production

In Europe, fodder beet seed production is undertaken by only a few breeding or seed companies. The production of hybrid fodder beet seeds is complex because it is necessary to maintain and multiply the three lines contributing to the final hybrid, produced from the male sterile plants (plants used as a female parent and often resulting from not very vigorous lines) and the pollinators (plants used as male parents). Moreover, it is necessary to be sure of exclusive pollination of the male sterile female by the chosen male. It is thus necessary to take precautions to avoid uncontrolled pollination especially from wild beet.

The principal fodder beet seed production areas are in the southwest of France, in a zone next to the sugar beet production area. The creation of a new variety requires much time, but the life span of the fodder varieties is relatively long compared to many sugar beet varieties, for example. The majority of these varieties are triploid hybrids resulting from the crossing between selected basic seeds as shown in Fig. 7.6.

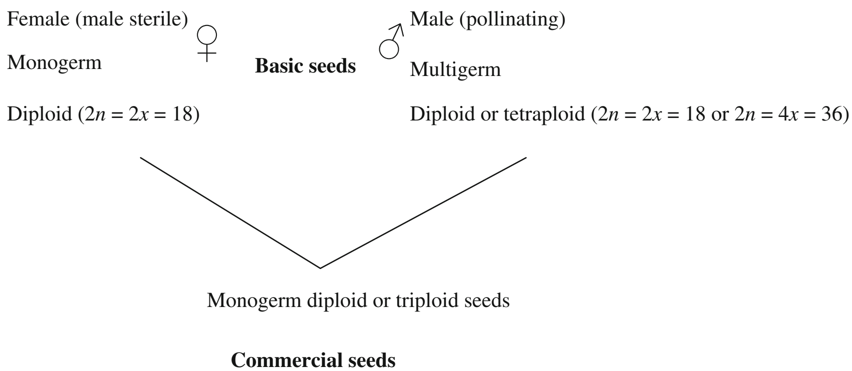

Fig. 7.6 Diagram of the production of a commercial fodder beet variety

The basic seeds are provided by the breeder to the producer. The multiplication thus consists of planting the basic seeds in an isolated field in order to obtain commercial seeds. The traditional method of multiplication of a fodder beet variety, known as the indirect method or transplanting method, takes place in two phases.

The first phase consists of sowing the basic seeds in a nursery in order to obtain healthy, vigorous seedlings of homogeneous size. These nurseries are sown in August. As the fodder beet is very susceptible to frost, the stecklings are harvested in November and stored in a cold room until the following spring. The second phase starts in March, by transplanting the stecklings in order to establish the seed crop in

the best possible conditions. The crop then continues in a traditional way. Shortly after stalk elongation, the female plants are topped to support the multiple branching and sometimes to synchronize flowering between the two parental lines. The destruction of the pollinators in July facilitates sorting. Harvest starts in July according to the precocity of the material and the climatic conditions. Stalks are laid out in swath for around 10 days. When the moisture level is quite low, the plants are threshed and the harvested seeds are processed and cleaned in a factory.

For fodder beet, control of foreign contamination as well as checking the male sterility of the female parental line is essential, even more so than for sugar beet. Any contamination from foreign pollen can lead to heterogeneity in the root color of the final variety and problems in marketing the whole seed batch due to non-conformity.

Acknowledgments Section 8 "Integration of New Biotechnologies in Breeding Programs" was mainly written regarding a personal communication from Michel Desprez and Bruno Desprez (2009) with their authorization.

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Chapter 8
Swedes and Turnips

Stuart Gowers

1 Introduction

1.1 Botanical Classification and Reproductive Biology

Swedes (*Brassica napus* var. *napobrassica* (L.) Rchb. syn. *B. napus* ssp. *rapifera* (Metzg.) Sinsk.) and turnips (*Brassica rapa* L. var. *rapa* L. syn. *Brassica campestris* ssp. *rapifera* (Metzg.) Sinsk.) are root crops which can be used for human or animal consumption. For example, in Scotland swede crops can be grazed in situ or lifted as required for housed stock throughout autumn and winter. Swedes are also the third most widely grown vegetable in Scotland, after vining peas and carrots, with production for supermarkets primarily handled by four large growers and packers. Although swede is the name used in most of the UK, they are usually called turnips or neeps in Scotland, which is confusing. Swede is also the name used in New Zealand, Australia, and India, but in North America they are called rutabagas. With the botanical names, the Sinskaja terminology has often been used in the literature as being more scientifically correct, but *B. napus* var. *napobrassica* and *B. rapa* var. *rapa* were the names adopted by UPOV (International Union for Protection of New Varieties of Plants) and are now commonly used.

B. rapa/campestris is one of the basic diploid species in the brassica family; it is the 'a' genome ($2n = 2x = 20$) of the triangle of U (U, 1935). *B. napus* is one of the three derived allotetraploids and is from the cross of *B. rapa* and *B. oleracea* (c genome; $2n = 2x = 18$); *B. napus* has the genome constitution 'aacc' with $2n = 38$ (Fig. 8.1).

B. rapa, like *B. oleracea*, is an outbreeder in which self-pollination is largely prevented by a sporophytic incompatibility system (Mackay, 1977; Sakamoto and Nishio, 2001). The sporophytic incompatibility system can operate in *B. napus*, but in practice most *B. napus* plants are self-fertile and tolerant of inbreeding (Gemmell et al., 1989).

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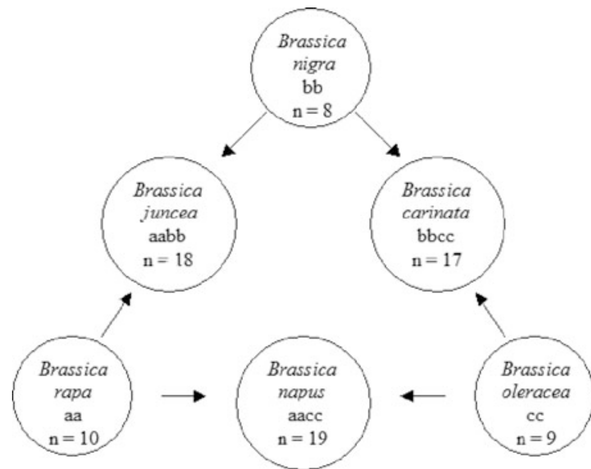

Fig. 8.1 Triangle of U – diagram to show the relationship between the main brassica species

Turnips and swedes are biennials and require vernalization to induce flowering. Turnips do not have a juvenile growth stage, and germinated seed can be vernalized in a refrigerator at 4°C. Swedes do not have such a distinct juvenile period as kale or other *B. oleracea*, but they do have a growth period where induction of flowering is delayed (Gowers and Gemmell, 1988c). There appears to be a period during early growth when a cold shock will induce premature flowering or 'bolting.' Precise vernalization requirements are cultivar dependent, but Gowers and Gemmell (1988c) found that a period of 8 weeks vernalization at 5°C and 12-h day length would induce flowering in almost all swedes when no pre-vernalization growth other than 2 days at 18°C for germination was used.

1.2 Developmental Plant Anatomy

Although plant storage organs are usually produced from swollen stems or roots, swedes and turnips are mainly enlarged hypocotyl, particularly in the case of turnip. Anatomically, the 'bulbs' are composed mostly of secondary xylem comprising unlignified parenchyma cells. The secondary phloem outside the cambium is also thin walled. Lignification occurs only after reproductive development begins, when hardening and progressive withdrawal of food reserves make the bulbs unpalatable (Langer and Hill, 1991). With swedes, the top and bottom of the bulb are derived more from stem and root tissue, respectively, than is turnip. This leads to leaf scars around the top of the swede bulb and the presence of adventitious roots on the bottom of the bulb. The degree of side root production can vary considerably and in the worst cases leads to very 'fangy' bulbs. Although providing good anchorage for grazing in situ by sheep and cows, fangy roots are not desired for vegetable swedes as they are unattractive and need more trimming to make them presentable for the market.

The top of the swede merges into a stem, or neck, which bears the leaves, whereas turnip leaves are usually borne in a tight rosette on top of the bulb. The presence of a neck is considered to be one of the main distinguishing characters between swedes and turnips; however, there are swedes with virtually no neck, and there are turnips with necks. Under certain conditions, especially with high plant stands and good fertility, some cultivars of swedes can produce very long necks. Although cows do not have much difficulty with long necks, and they may even make for a more balanced diet, sheep do have difficulty eating them and long necks are, therefore, an undesirable character.

There are differences in leaf color and morphology between the two species. Color is the most obvious, with turnips having a yellow-green leaf in comparison to the blue-green color of swedes. Swede leaves have a cuticle and a wax layer which is absent in turnips. In swedes there are 2–5 layers of collenchyma cells between the epidermis and the vascular bundles of the leaf blade and petiole, whereas in turnips the collenchyma is absent or consists of 1–2 layers of cells (Koresheva, 1974).

The flowers of swedes and turnips are borne on racemes on the main stem and axillary branches. Flowering starts at the bottom of the raceme and works upward. The racemes are indefinite, with no terminal bud, and flowering continues for several days or even weeks with large swede plants. A main distinction between turnips and swedes is the timing of flower opening. In turnips the flower opens before the stalk at that point has extended, while the pedicel extends and curves upward so that the flower opens over the top of the raceme. In swedes the stalk extends before the flower opens so the open flowers are below the unopened buds. Flowers open early in the morning by rapid expansion of the petals, and the anthers dehisce when they have dried sufficiently, which is usually later in the morning. Turnips and swedes have typical crucifer flowers – four petals and sepals in the shape of a cross, with a central style surrounded by six stamens. There are four long and two short stamens. There are four nectaries, but only the two at the base of the short stamens may be active. The style comprises two transverse carpels with a double row of (pendulous campylotropous) ovules on the perietal placenta; it becomes bipartite by the production of a septum down the center.

The stigma is two lobed, slightly bulbous, and covered with papillal cells, which control self-incompatibility when present. The papillal cells are covered by a pellicle onto which pollen grains adhere. In a compatible pollination the pollen grain germinates and the pollen tube penetrates the papillal cell wall, grows down through the central tissue of the style, and fertilizes an ovule. In an incompatible pollination, the pollen may not germinate or, if it does, the pollen tube is unable to penetrate the papillal cell wall.

The stigmas of toria and sarson (*B. campestris* ssps. *toria* and *sarson*) were fully receptive for the first 2 days after opening but, thereafter, receptivity decreased and by the fifth day very few seeds were set (Mohammed, 1935). This also appears to be the case with swedes and turnips. Petals and sepals were shed on the 4th and 5th days but pollen remained viable for 7 days. A similar result was found by Pierre and Renard (2002), who found that rape pollen remained viable for up to 8 days at low temperatures.

After fertilization the endosperm grows rapidly, followed more slowly by embryo development. The endosperm is absorbed by the developing embryo, and food reserves for seedling development are stored in two cotyledons folded on either side of the embryo. Turnip embryos grow slightly faster than swede embryos and reach full size in about 3 weeks, whereas swedes take about 4 weeks. However, it then takes a similar period for the seed coat and the siliqua to mature. The pod size depends on the number of developing seeds, which depends on the number of ovules and degree of fertilization. A fully fertilized style produces between 20 and 30 seeds. Full-sized pods of turnip are 3- to 5-mm wide and up to about 8 cm long, with swede pods slightly larger at 4- to 6-mm wide and up to 10 cm long. The pods dehisce when ripe; this is passive in nature and depends on wind or physical contact to shatter the pods and scatter the seed.

Seed size also varies with health and fertility; diploid turnip seed is about 1.5 mm in size; swede and tetraploid turnip seed about 2.0 mm in size. Seed weights are in the region of 400 and 300 seeds/g.

Bulb shape, skin color, and flesh color are important for consumer appeal and differ for turnips and swedes. Swedes usually have yellow flesh and turnips white flesh, although there are white swedes and yellow turnips. For vegetables use in most countries, swedes need to be globe shaped with red/purple skin on top of the bulb and yellow flesh, whereas turnips have white flesh and red/purple tops or no skin color, whereupon they appear completely white. Although skin color is not important for fodder, shape can be important for utilization. The Dutch tankard-type turnip has very little root anchorage and is easily pulled out of the ground; this makes it easy to pull by hand or for cows to eat, but sheep have great difficulty eating them if the bulbs pull out of the ground and roll around. Good root anchorage is needed for in situ grazing by sheep, and globe turnips and swedes usually provide this.

1.3 Chemical Composition

Swedes contain more nutrients and calories than turnips. They are an excellent source of vitamin C, folate, potassium, dietary fiber, calcium, iron, niacin, and vitamin A. Turnip roots are high in dietary fiber, vitamins C and B_6, folate, calcium, potassium, and copper, but do not contain vitamin A. However, the greens are an excellent source of vitamins A and C, as well as a good source of calcium, iron, and riboflavin (Herbst, 2001). Vitamin C in swedes has a high degree of stability during cooking and storage (Bukin et al., 1934).

The bulbs of swedes and turnips have low dry matter contents. Swede dry matter (DM) contents range from 9 to 12.8% (Bradshaw and Griffiths, 1990). Turnips are lower in DM content; the fast-growing white-fleshed turnips have about 8% DM content, are not frost resistant, and do not store well. Yellow-fleshed turnips have about 9% DM content, mature more slowly, keep better, and are more like swedes (Langer and Hill, 1991).

The leaves of swedes and turnips are high-quality feed, and DM digestibilities can be over 90%. Turnip leaves have higher DM protein content than swede leaves,

with turnip leaves having 24–27% crude protein in comparison to swede leaves with 17–18% (Jung et al., 1986). The bulbs have much lower protein levels, although Jung et al. (1986) found turnip bulbs had crude protein levels from 14% up to nearly 18%, but with swedes in the region of 11–12% crude protein. These values may be on the high side, as Vipond et al. (1990) stated that crude protein levels of swede bulb range from 8 to 10%. Lower levels have been recorded in Moldova (Popovici et al., 1992) and toward the end of winter in New Zealand crude protein levels of less than 6% have been recorded in swede bulbs (Gowers unpublished). The situation is even worse because only about 80% of total N is accounted for by amino N (Kay, 1971); Livingstone et al. (1977) found that swedes with a crude protein level of 12.8% actual had a true protein content of only 5.0%. Also the balance of essential amino acids, particularly tryptophan, is not good, and the high arginine content may interfere with the efficient absorption of lysine (Kay, 1971). The high level of non-protein N may make swedes more suitable for ruminants, but they are not suitable for large pigs (Livingstone et al., 1977).

Metabolizable energy (ME) contents of swede and turnip bulbs are very high; values of 13.9 MJ ME/kg DM (Dewey and Wainman, 1984) up to 14.9 MJ ME/kg DM (Nichol et al., 2003) are quoted for swedes. The ME values for turnip leaves and bulbs were consistently above 11.5 and 13 MJ ME/kg DM, respectively (Jacobs et al., 2004). The high energy content and digestibility of swede bulbs are largely due to high levels of simple sugars. Bradshaw and Griffiths (1990) found total sugar contents of 19 cultivars of swede ranged from 53.5 to 61.9% DM. Fructose and glucose made up most of this content, with means of 22.5 and 32.8% DM, respectively, out of total sugar mean of 58.8% DM. A more detailed analysis by Gemmell et al. (1990) of six cultivars/breeding lines over nine harvest dates found large interactions from July to October, but little variation from November to March when average values were fructose 20.3%, glucose 31.9%, and total sugars 54.6%. Slightly lower results were obtained by Davik (1992), who analyzed 20 cultivars and breeding lines of swede for sugar and glucosinolate content. The mean total sugar yield was 52.1% DM, with sucrose and glucose comprising 48% DM. Total sugar content ranged from 254.1 to 463.5 kg/ha. A total of 12 glucosinolates were identified, with progoitrin (2-hydroxybut-3-enylglucosinolate) comprising 42.9% of the mean total glucosinolate content. The goitre fraction, progoitrin, and gluconapoleiferin (2-hydroxypent-4-enylglucosinolate), and the total glucosinolate content varied from 2.4 to 10.6 and 9 to 18.5 mol/g DM, respectively. Griffiths et al. (1991) also found large differences between six cultivars and breeding lines in both the goitre fraction and total glucosinolate content. Results indicated the potential to select for high sugar and lower goitre and total glucosinolate content.

Analysis of gene bank material at the N.I. Vavilov Research Institute of Plant Industry (VIR), USSR, showed swede bulbs to have 10–16% DM, 5–10.2% sugars, 0.6–2.0% protein, 0.4–2.1% ash, 23–69.4 mg ascorbic acid/100 g, and some carotene in yellow varieties. Swedes were similar to white cabbage in vitamin C content, but richer in sugars and minerals. Turnip bulbs had 4.7–12.6% DM, 3.8–6.4% sugars, 0.7–1.5% ash, 0.4–2.1% protein, 19–63 mg ascorbic acid/100 g, and 0.08–0.12 vitamin B1/100 g (Pivovarova, 1979).

For human consumption, the nutritional composition of swede per 100 g edible portion (thinly peeled, 73% of product as purchased) was quoted by Holland et al. (1991) as water 91 g, energy 100 kJ (24 kcal), protein 0.7 g, fat 0.3 g, carbohydrate 5.0 g, dietary fiber 2.4 g, Ca 26 mg, Mg 4 mg, P 11 mg, Fe 0.1 mg, Zn 0.1 g, carotene 350 μg, thiamin 0.15 mg, riboflavin trace, niacin 1.2 mg, folate 31 μg, and ascorbic acid 31 mg. A review by Herrmann (1998) covers the constituents of swedes and turnip; reference is made to minerals, trace elements, vitamins, dietary fiber, acids, carotenoids, nitrates, phenols, crude fats, and flavor and aroma compounds.

As vegetables, swedes and turnips also contain valuable phytochemicals; they contain high amounts of anti-cancer and anti-carcinogenic glucosinolates. Of particular interest is gluconasturtiin (2-phenylethylglucosinolate), which is found in roots of brassicas but not usually in leaves (Bradshaw et al., 1984; Griffiths et al., 1991). Selections of rape for use as biofumigants have been produced with high levels of gluconasturtiin (Gowers, unpublished) and these could easily be transferred to swedes. Considerable variation of this compound was found in turnips, with a negative correlation with the quantity of progoitrin. As progoitrin produces a goitrogen on hydrolysis, low levels are wanted for vegetable and fodder use. Some Tokyo Market-type turnips have been found to contain no progoitrin (Carlson et al., 1981) and transferring this to fodder cultivars would be desirable.

Another interesting sulfur compound found in Brassicas is *S*-methylcysteine sulfoxide (SMCO). It causes hemolytic anemia in ruminants (Smith et al., 1974) but may be beneficial to non-ruminants, including humans, as it has been shown to lower cholesterol levels in plasma and liver (Itokawa et al., 1973). Griffiths et al. (1991) found that bulb content in swede rose from 5.9 g/kg DM in July to a maximum of 9.9 g/kg DM in January, with a range from 7.2 to 9.3 in six cultivars and breeding lines averaged over nine harvests.

The Chinese traditionally roasted turnips because the high temperatures increased the sweetness by converting starch to pyrodextrins in the crisp outer flesh. In France they are braised, fried, or glazed and have become a traditional accompaniment to certain dishes. The Japanese often pickle them, but young turnips can be eaten raw, or simply boiled.

Turnip greens or turnip tops are the leaves of turnips, and sometimes swedes, used as vegetables. Swede tops are the traditional 'turnip tops' in Newfoundland. Forage turnip rapes, or leafy turnips with little or no bulb, have also been developed for animal feed.

1.4 Areas Grown

In a review of swedes and turnips, McNaughton and Thow (1972) gave areas grown as published by FAO in 1950 and 1960, and commented that they remained important as fodder crops in Northern Europe, Russia, and New Zealand. Such figures are no longer available. However, the UK areas for swedes and turnips in 1960 were 183,725 ha, but now appear to be down to about 30,000 ha. In Scotland, swedes and turnips are still important crops for animal feed and 5,540 ha were grown in Scotland

for this purpose in 2008; a further 1,803 ha were grown for human consumption (Anon, 2008). In contrast, in New Zealand the areas of turnips and swedes grown in 1960 were around 95,000 and 75,000 ha, repectively. Over the next 25 years the area decreased to half that with a reduction in stock and use of all-grass wintering. However, all-grass wintering proved too unreliable and there has been a large increase in dairying in the South Island. Recent figures are not readily available, but the areas grown now seem to be back to 1960 levels or even higher.

The elimination of fodder swedes in North America led to a decline in the commercial area but this stabilized by 1990 at about 4,100 ha (Shattuck and Proudfoot, 1990). These authors gave a review of swede breeding focused more on human consumption than previous reviews. Pink (1993) also did a brief review of swede and turnip breeding. Comprehensive reviews on swedes and turnips have been produced by Shebalina (1974) and Shebalina and Sazonova (1985) but, unfortunately, these are in Russian. The present review is intended to summarize recent results and progress in breeding methodology. For information on the growing, harvesting, and storage of swedes and turnips, the review by McNaughton and Thow (1972) is still relevant.

2 Origin and Domestication

Turnips have been grown for a long time; they are first known to be mentioned by Theophratus about 300 BC (Toxopeus, 1974). Several types were grown by the Romans, many of which had Greek names suggesting that they originated in ancient Greece. Originally grown as vegetables, the importance of turnips in agriculture is much more recent. Although used earlier to some extent, it was only with the development of the four-field rotation system that turnips became important. Turnips were an integral part of this system (wheat, turnips, barley, and clover being a typical four-course rotation) which, originating in Flanders, was introduced into Britain by Lord ‘Turnip’ Townshend about 1730. This led to the British Agricultural Revolution of the eighteenth and nineteenth century, which enabled the population base for the Industrial Revolution to take place. Traditional fodder turnips are faster growing than swedes and can be sown later (e.g., early June rather than early May in Scotland), used earlier, and grown at higher elevations on less fertile soils. Quick-growing white turnips are used as a universal catch crop with sowing in southern Britain from April to May for mid-summer grazing or June to August in cereal stubble for autumn grazing. They are also known as stubble turnips and Dutch turnips.

The origin of *B. napus* is uncertain, but it appears to have arisen several times in recent history to produce swedes and both oilseed and forage forms of rape (Song and Osborn, 1992). Swedes were first recorded in Europe in 1620 by the Swiss botanist Caspar Bauhin, although they probably existed earlier than this (Boswell, 1949). It is assumed that swedes were introduced into UK from Sweden – hence ‘Swedish turnips’ and, also from etymology, that they were introduced directly into

North America from Sweden because of the name ‘rutabaga,’ derived from the Swedish ‘rutabagge.’ However, swedes may not have originated in Sweden, but may have come from Finland and possibly Ingria Province (Ahokas, 2004). The swede is said by Sinclair, in the account of the system of husbandry in Scotland, to have been introduced into Scotland about 1781–1782, and a quotation in the Gardeners’ Chronicle says it was introduced into England in 1790.

The rapid expansion in the variety of swedes in a relatively short time is probably accounted for by them not being recognized initially as a new species, distinct from turnips. The two species cross readily together and, although the triploid hybrid is highly infertile on self-pollination, it backcrosses easily to both parent species.

Before the potato was introduced into Europe, the turnip was a very important vegetable which provided a reliable source of food at times when other vegetables were scarce. Many areas selected their own regional variations, and there are numerous named varieties of vegetable turnips available around the world (although many of them are synonyms). Turnips were introduced to Canada in 1541 by Jacques Cartier, but not into the US (Virginia) until 1609 apparently.

In continental Europe, swedes acquired a bad reputation during World War I, when it became a food of last resort. In the German *Steckrübenwinter* (swede winter) of 1916–1917, large parts of the population were kept alive on a diet consisting of swedes and little else, after grain and potato crop failures had combined with wartime effects. After the war, most people were so tired of swedes that they came to be considered ‘famine food’ or ‘peasant food’ and they have retained this reputation. As a consequence, they are rarely planted in German speaking regions as vegetables; kohlrabi seems to be the equivalent vegetable of choice.

There is little documentation on the recent breeding of turnips in Europe, but the major feature has been the production of tetraploid turnips. Several tetraploids were produced and tested from the 1940s to 1960 at Svalöf. Results summarized by Olsson and Ellerstrom (1980) showed tetraploids having up to 22% higher dry matter yields than their respective diploids. However, the only release was a ‘mixed F_1’ strain marketed as ‘Svalöf’s Tetraploid Sirius Turnip.’ Since then, tetraploid turnip breeding seemed to become the standard breeding method in the Netherlands for some time. Out of 17 cultivars on the 1972 Rassenlijst, five were tetraploids. There appeared to be no advantage as far as total yield was concerned, and the top two lines were diploid. Tetraploids also have lower dry matter contents which may result in reduced animal intake (McNaughton, 1995). De Roo (1962) compared the diploid cultivars ‘Waaslander’ and ‘Meetjeslander’ with their corresponding tetraploids and found the dry matter yield was 6% lower for the tetraploids. Gowers (1977) compared diallel crosses of five cultivars at both the diploid and tetraploid levels. In all cases the tetraploids were lower yielding than their respective diploids. The conclusion was that the relative yield of diploid and tetraploid turnips depends on the cultivars used or that there are large genotype × environment interactions.

3 Varietal Groups

Varietal groups of turnips and swedes are mainly based on leaf characters and bulb shape and color. Turnips are usually white fleshed and swedes usually yellow fleshed, but there are yellow-fleshed turnips and white-fleshed swedes. White flesh is controlled by a single dominant gene (3:1 ratio in F_2) in turnip and duplicate dominant genes (15:1 ratio in F_2) in swedes (Davey, 1931). Flesh color in both species is pleiotropic with flower color; yellow flesh is associated with buff yellow flowers and white flesh with bright yellow flowers (Davey, 1931). Again the basic situation in turnips is simpler than in swedes but there are other modifying factors. In turnips, there is a pale yellow flower color, which is recessive to bright yellow, but which still has white flesh. In swedes there appear to be several shades of yellow for flesh color, but corresponding variations in flower color have not been noted.

Skin color in turnips is relatively simple in comparison to swedes. Red color in the superficial cells covers an underlying layer with green pigmentation, with each being controlled by single, independent, dominant genes (Davey, 1931). If both color genes are present, the skin appears purple in color, and if neither are present the skin is transparent, so the color is white or yellow depending on that of flesh underneath. Hence when a true breeding purple line is crossed with a white one, in the F_2 generation one sees purple, red, green, and white bulbs in a 9:3:3:1 ratio. However, it is not quite that simple; in the cross between red (raphanusin, pelargonidin glucoside) and white plants, F_1 hybrids were all purple (rubrobrassicin, cyanidin glucoside), and F_2 progenies were segregated into three groups – purple, red, and white in the ratio of 9:3:4, respectively (Hoshi, 1975). This indicates recessive epistasis (two loci A and B, A-B-purple, A-bb red and aa – white, where white → red → purple), as previously found in the case of radish. Nazeer and Tanki (1994) consider that there are two independent dominant genes controlling purple color, with the single genes giving pink and partially purple color when the other is recessive and, in the absence of both, the skin is colorless.

Although there may have been swedes with no skin color, and it should certainly be possible, there do not appear to be any in existence at the moment. Otherwise, given that the skin color genes are present in both ancestral genomes, the inheritance of color in swedes is more complicated. Apart from variation in purple/red color from nearly black to pale pink, there are intermediate combinations with green color, usually termed bronze. The density of color is strongly affected by light, and bronze bulbs can be quite dark purple on the sunny side while completely green on the shaded side.

The shape of turnips is more variable than swedes, presumably because selection has been carried out over a far longer period. Most swedes are basically globe shapes, but with variations depending on the widest part of the bulb; there are some slightly flattened globes and some tankard types, but nothing as wide as the range available in turnips (Fig. 8.2). Bulb shape is largely governed by additive gene action in swedes (Grant et al., 1982) and is generally low in heritability in turnips (Hoen, 1968).

Fig. 8.2 Variations in shape of turnips, ranging from long, tankard type (*left*) through round globe (*center*) to flat globe (*right*)

Several attempts have been made to classify types of turnip, the oldest of which appears to be Pliny the Elder, circa 77 AD. Pliny wrote that he considered the turnip one of the most important vegetables of his day, stating "it should be spoken of immediately after corn, or the bean, at all events; for next to these two productions, there is no plant that is of more extensive use." Pliny says that there are five kinds: "the Corinthian, Cleonaeum, Liothasium, Boeoticum, and the Green. The Corinthian, the largest, with an almost bare root, grows on the surface and not, as do the rest, under the soil. The Liothasium, also called Thracium, is the hardest. The Boeoticum is sweet, of a notable roundness and not very long as is the Cleonaeum."

More recent classifications are those of Sinskaia (1928) and Shebalina (1968). Sinskaia classified seven groups: Teltow turnips; West European turnips with dissected leaves; Asia Minor and Palestine types; Russian Petrovskij types; Asiatic/Afghan-type turnips; Japanese turnips with entire glabrous leaves of Asian origin, and European types with entire pubescent leaves. The latter two seem to have a major distinction between them, with a difference in the seed coats of the two kinds (Shibutani and Okamura, 1954). With plant breeders crossing them together the distinction may have blurred somewhat. Otherwise the classifications are mainly based on combinations of leaf shape and glaucosity with bulb shape and color.

As far as bulb shape and color are concerned there are several distinct types of vegetable turnip available. The most generally grown is the quick-growing white globe 'snowball' type or 'May' turnip. The Japanese equivalent is the 'Tokyo Market' type, but this is considered stronger in taste, and more like radish. The 'Milan'-type turnip is a white-fleshed flat globe, with or without a red skin on top. Other very distinctive turnips are the French 'long black' type and the very long Japanese 'Hinona Kabu' – a 40 × 200 mm or longer white turnip with red shoulders. A completely red-skinned turnip, *atsumi-kabu*, has long been cultivated in some areas of Japan and is used for the production of pink, pickled turnips. Tests on the anthocyanins from the skin of these turnips gave significant improvement in the cholesterol levels of rats (Igarashi et al., 2008).

Teltow turnip is a yellow and somewhat carrot-shaped turnip that has a distinct taste. However, various turnips were being sold as Teltow turnips that did not conform to the original form and taste. From morphology and tasting tests, a true Teltow-type turnip was reselected (Brueckner et al., 2007). A Bavarian turnip, also considered to have special and very distinct characters, was commonly grown until 1900, but only four farms north of Munich were known where it is still grown and maintained (Reiner et al., 2005).

In general, swedes are relatively uniform in morphology and have little to differentiate them. Shebalina (1974) classified swedes into Northern European, Western European, and Siberian types, and there seems to be some difference between these based on electrophoretic fractionation of seed proteins. Danish swedes are classified into Wilhelmsburger and Bangholm types, but this differentiation seems to be based solely on Wilhelmsburgers being green skinned and Bangholms purple skinned.

4 Genetic Resources

New variation can be introduced into swedes and turnips from crosses with related species or subspecies. The turnip group has a wide range of oriental vegetable subspecies, and the cabbage group (*B. oleracea*), a wide range of occidental subspecies, with the latter having an incredible range of morphological variation (cabbage, broccoli, sprouting broccoli, cauliflower, Romanesco, Brussels sprouts, kohlrabi, marrow-stem kale, thousand-head kale, curly kale, and kalin). *B. napus* has a relatively restricted range, with swede, forage rape, and canola (oilseed rape). The possibility of extending the morphological range of *B. napus* is quite large, but improvements to swedes are more limited. Crosses of *B. rapa* and *B. oleracea* are highly infertile because of endosperm abortion, but *B. napus* is reasonably easy to produce using embryo rescue techniques.

Besides wild turnip (*B. rapa* ssp. *sylvestris*) and the oriental vegetable relatives of turnips, there are also oilseed types of *B. campestris* (for a comprehensive list see Stewart, 2002). A lot of research and breeding is being carried out on oilseed turnip rape (*B. campestris* ssp. *oleifera*). Toria (*B. campestris* ssp. *toria*) and sarson (*B. campestris* ssp. *dichotoma* and *trilocularis*) are important oilseed crops in India and Pakistan. All these types appear fully fertile with turnips, and any desired character in those types could be transferred to turnip.

Swedes are fully fertile with all forage and oilseed rape forms of *B. napus*. Characters can easily be transferred from turnip to swede, so it is assumed that any characters from the *campestris* subspecies could also be transferred to swede. Some characters may also be transferred in the reciprocal direction. The ease with which this happens depends in which genome the character resides. With a gene on the ‘a’ genome transfer from swede to turnip should be easy, but with a ‘c’ genome gene it would be much more difficult as this would require translocation to the ‘a’ genome.

The initial cross between turnips and swedes could be made either way. The cross is more fertile with swedes as female parent and sets better seed (Calder, 1937).

However, the hybrids look like swedes (Honda and Niiuchi, 1966) so it would be difficult to tell if there were any accidental selfs with swede as the female parent. Using turnips as the female parent it is obvious if any of the offspring are not hybrids.

Several methods have been used to transfer characters from *B. rapa/campestris* to *B. napus*. Swede × turnip hybrids are highly infertile on self-pollination but they will set some seed in bag isolation (Davey, 1959). Lammerink (1970) transferred clubroot resistance from turnip to swede by using honeybee pollinations of swede × turnip hybrids in a glasshouse to produce seed and then backcrossed the progeny to swedes. Shiga (1970) reported that workers in Japan had made backcrosses between oilseed *B. napus* and *B. campestris* hybrids with *B. napus* for many years. To produce many F_2 plants, hybrids were open pollinated among rape plants to promote backcrossing. This worked well with turnip × swede hybrids in isolation with swede pollinators (Gowers, 1974a). The backcross progeny was screened cytologically and 12% was found to have 38 chromosomes. Screening progeny from the first backcross for the desired character, and then screening the second backcross for chromosome number was considered a more efficient way of identifying balanced *B. napus* plants (Gowers, 1982). However, chromosome screening is not necessary. Selecting the largest seed from the backcross gives a high proportion of *B. napus* like plants (Shiga, 1970). Using the triploid hybrids as male parents in the backcrosses means there is natural selection for euploid pollen. Screening for pollen shape and size can be used to identify fertile plants, simply by observing dry pollen under a microscope. After a second backcross, and certainly by a third backcross, fully fertile swedes can be obtained and are usually obvious by their seed set, although this may be confounded with self-incompatibility.

If the gene concerned is in the ‘a’ genome, then gene transfer works equally well in the reciprocal cross. This way round, of course, turnips should be used as the female parent in the backcrosses. Herbicide resistance has been transferred from *B. napus* to turnips in this way (Gowers unpublished).

Transfer of characters from swede to turnips is unusual, as turnips and relatives have the greater range of variation available. One other instance of transfer from swede to turnips is known and that was the transfer of TuMV resistance (Palmer, 1983).

The transfer of characters from *B. oleracea* to *B. napus* can be carried out in a similar manner but is much more difficult. Although fertilization takes place readily, endosperm abortion occurs and the embryo dies. A few instances of naturally produced seed have been recorded, but embryo rescue is usually required (Quazi, 1988). The hybrid is highly sterile, but backcrossing to *B. napus* may produce some seed. There also seems to be a tendency for male sterility in the hybrids. In this situation especially, the embryo rescue technique of Christey (Gowers and Christey, 1999) is advantageous. A mass of fused leaf tissue is produced from which normal shoots develop and these are sub-cultured so that several plants can be obtained from one embryo.

Transfer of characters from both *B. rapa* and *B. oleracea* can be achieved by resynthesis of *B. napus*, although the hybrid is highly sterile. Of four hybrids

obtained by U (1935) one was haploid *B. napus* and one was a spontaneously doubled diploid *B. napus*. By crossing tetraploid parents, Frandsen (1947) obtained 37 diploid *B. napus* from 3,000 crosses; Olsson (1963) also had good success using this technique. Namai and Hosoda (1968) produced artificial swedes by pollinating grafted plants at relatively low temperatures (below 20°C) and doubling the hybrids with colchicine. Starting with a synthetic *B. napus* produced by crossing a tetraploid kale (*B. oleracea*) with an autotetraploid form of ECD04 (*B. rapa*) (see Section 7.4.1), and using embryo culture (Snell, 1978), Bradshaw et al. (1997) produced the clubroot-resistant swede 'Invitation.' Embryo rescue techniques are usually used these days to obtain hybrids, from crosses made with diploid parents.

The other related species that is highly compatible with turnips and swedes is *B. juncea*. Producing hybrids from these crosses appear easy; Sharma and Singh (1992) obtained 3.99 and 2.96 seeds per pollination from crossing with swede and turnip, respectively. However, the situation is similar to transferring characters from swede to turnip – only genes carried by the 'a' genome will transfer easily. If the gene is on the 'b' genome then translocation to the 'a' genome is needed.

Radish (*Raphanus sativus*) is a close relative of the brassicas, and several workers have crossed *B. napus* and *B. rapa* with radish (Voss et al., 2000; Kaneko et al., 2001). *Brassicoraphanus* has also been used as bridging cross with rape to try to transfer nematode resistance (Lelivelt et al., 1993).

A number of other species have been crossed with *B. campestris* and *B. napus* by using embryo rescue. Some of the more recent ones are *B. maurorum* (Chrungu et al., 1999), *Orychophragmus violaceus* (Li and Heneen, 1999), *Sinapis arvensis* (Snowden et al., 2000), *B. tournefortii* (Choudhary and Joshi, 2001), and *Diplotaxis siifolia* (Ahuja et al., 2003). In all cases where the gene required is not on the 'a' genome, or alternatively on the 'c' genome for *B. napus*, translocation onto the native genome is necessary.

A large amount of genetic material for brassica breeding is now stored in gene banks. The Centre for Genetic Resources, the Netherlands (CGN), co-ordinates the data for the 32 European and Russian gene banks cooperating in the project. Apart from CGN itself, which holds a collection of turnip material, the other main gene banks of interest for swedes and turnips are the Genetic Resources Unit, Warwick HRI, England; the Nordic Gene Bank, Alnarp, Sweden; and the N.I. Vavilov Research Institute of Plant Industry (VIR), St. Petersburg, Russia. The gene bank at the Asian Vegetables Research and Development Centre (AVRDC) at Shanhua, Taiwan is an excellent source of *B. campestris* subspecies.

5 Major Breeding Achievements

5.1 Standard Breeding

One of the early breeding achievements with turnips and swedes, and many other crops, was the 'Scientific Farm Plant Breeding' work of John and Robert Garton in the UK in the late 1800s. From what appears to have been the first major systematic

plant breeding program, Gartons Limited released five swede cultivars and three turnip cultivars by 1900. Greentop Scotch Yellow and Mammoth Purpletop were two of the turnips, and these are still being sold today; the latter is the second commonest grown turnip in Australia (Jacobs et al., 2001). Of the other swede cultivars released over the following years, Superlative (1905) and Acme (1914) are still available for vegetable use today.

During the period 1900–1930 the chemical composition of swedes was determined with a view to improving their feeding value (Bradshaw and Griffiths, 1990). However, opinions differed over the relationship between chemical composition and feeding value, and dry matter yield per unit area became the main selection criterion in breeding programs, rather than dry matter percentage, soluble solids, or sugar content. Indeed, a major achievement has been the breeding of forage swedes with reliable, high dry matter yields. One of the main indicators of progress in the UK was the recommended lists of varieties produced by the National Institute of Agricultural Botany (NIAB) and the Scottish Agricultural Colleges up until 1993. Several new cultivars were first listed in the 1970s which showed an improvement in yield over older cultivars still on the recommended list. In comparison to Acme and Doon Major, the 1985 NIAB list showed Wilhelmsburger Sater Ǿtofte, Ruta Ǿtofte, and Marian producing over 15% higher dry matter yield. In turn, the cultivars listed in the 1980s and early 1990s (Angus, Melfort, Angela, Airlie, and Ruby) showed a further 8% improvement on Ruta Ǿtofte, Marian, and Magres (1993 NIAB Recommended List). Cultivar Kenmore, National Listed in 1994, is the highest yielding Scottish one to date (Bradshaw and Wilson, 1993; Bradshaw et al., 2009) (Table 8.1).

The reliability of dry matter yield depends on disease resistance. In Scotland the main diseases affecting swede yields are clubroot (*Plasmodiophora brassicae*) and powdery mildew (*Erysiphe cruciferarum*). The first cultivar to combine resistance to these diseases was 'Invitation,' released in 1995 (Bradshaw et al., 1997), followed by 'Gowrie' and 'Lomond' in 2006 (Bradshaw et al., 2009) (Table 8.1). Breeding

Table 8.1 A summary of trial results from SCRI, Dundee, averaged over the years 2003, 2004, 2006, and 2007

	Mildew 1 susceptible 9 resistant	DM%	DM yield t/ha	Year of release
Ruta Ǿtofte	4.34	12.34	10.55	1972
Marian	5.67	11.69	10.00	1976
Magres	7.66	13.19	11.28	1980
Melfort	5.00	14.80	11.02	1982
Angela	2.67	11.48	10.13	1984
Ruby	7.83	12.67	11.43	1992
Kenmore	9.00	11.97	13.44	1994
Invitation	8.83	13.64	11.44	1995
Gowrie	8.83	11.84	12.59	2006
Lomond	8.83	12.07	11.98	2006
SED	0.939	0.187	0.370	

for powdery mildew resistance can be considered a major success as the susceptible cultivars Acme and Doon Major have been replaced with resistant ones in which the resistance is controlled by partially recessive genes and is likely to prove durable (Bradshaw et al., 1989). In contrast, breeding for clubroot resistance can be considered only a partial success as major genes were used which have proved useful in the UK, but are unlikely to be durable in the long term (Bradshaw et al., 1997).

In New Zealand the main diseases affecting swede yields are clubroot and dry rot. The first cultivar to combine resistance to these diseases was 'Tina,' released in 1982 (Lammerink and Hart, 1985). However, the yield of 'Tina' was relatively low, so a program to correct this was carried out. From a cross of 'Tina' with the high-yielding cultivar 'Highlander,' the new cultivar 'Winton' was produced. A summary of trials carried out to assess 'Winton' was published by Gowers et al. (2006) (Table 8.2). While having levels of dry rot and clubroot resistance similar to 'Tina,' 'Winton' produced over 30% higher bulb and total dry weight yield than 'Tina' and the four old cultivars in trial.

Table 8.2 A summary of results from trials of 'Winton' swede showing bulb and total dry matter yields in comparison to older cultivars

Cultivar	Date of release	Bulb Fresh yield (t/ha)	Bulb Dry matter (%)	Bulb Dry yield (t/ha)	Bulb proportion (%)	Total DM yield (t/ha)
Winton	2000	87.3	11.4	10.5	68.6	13.6
Major plus	1997	105.0	9.2	9.9	74.0	11.9
Highlander	1987	92.4	10.4	10.0	68.9	12.8
Tina	1981	64.0	10.4	7.1	66.4	9.5
Kiri	1978	73.8	10.3	8.0	68.2	10.4
Doon major	1959	91.2	9.3	8.7	72.8	10.6
Calder	1950	67.3	10.4	7.4	66.3	9.9
Crimson king	1943	70.5	10.8	8.0	70.3	10.2
Mean SED		3.5	0.1	0.33	0.9	0.74

The only winter hardy, Scottish-type turnip produced for many years is the cultivar 'Massif' (Bradshaw et al., 2002). Starting from seven selected cultivars, the program was based on half-sib family selection on a biennial cycle. Progenies were assessed in replicated yield trials and observation plots, from which plants were selected for the next cycle. The program demonstrated the value of population improvement in an outbreeding crop species and how the data collected could be used to allocate future resources to improve the response to selection. Six generations of selection resulted in a population with a yield that was 25% higher than the mean of the initial seven cultivars. This was remarkably close to the predicted superiority of the population, despite a significant discrepancy in one generation. It was concluded that the greatest response to selection per year would be achieved by selecting eight families from 128 assessed for 1 year, in trials at two or three sites, with an overall total of six replicates, given a resource limit of 800 plots.

A new development in the 1970s was forage turnip rape, or leafy turnips with little or no bulb. 'Appin' (released 1979) was bred at the Scottish Plant Breeding Station from a cross between tetraploid Dutch turnip 'Tigra' and oriental salad vegetable *B. campestris* ssp. *nipposinica.* (McNaughton et al., 1975): it had resistance to premature leaf senescence and improved root anchorage to facilitate grazing. However, in comparison to rape, Appin gave poor animal performance (Young et al., 1982). Likewise, 'Tyfon' and 'Perko' were produced by continental breeders at the tetraploid level. Much more successful in recent years in New Zealand has been the diploid cultivar Pasja (Vandijke Semo BV).

A recent development in swede and turnip breeding is herbicide-resistant lines. A chlorsulfuron-resistant gene was obtained by mutagenesis of a rapid cycling *B. napus* by Conner et al. (1994). This has been transferred to swedes and turnips by a simple backcrossing program in both cases (Gowers unpublished). Both bulb and leafy turnip types have been developed.

Another achievement has been the production of more uniform crops of swede for mechanical harvesting and for culinary use. Although swedes are insect pollinated, they are usually self-fertile and inbreeding depression is mild (Davey and Lang, 1938). Davey (1941) developed a pedigree inbreeding method of breeding which was used at SCRI for over 50 years to produce high-yielding inbred line cultivars. These were more uniform than older cultivars produced by selection of attractive looking bulbs which were then seeded in isolation, but where cross-pollination could occur between the genetically different selections. More recently high-yielding and uniform inbred line cultivars have been produced by single seed descent (Bradshaw et al., 2009) and microspore culture (Hansen and Bratberg, 2003). The alternative way of producing high-yielding and uniform cultivars of both swedes and turnips is through F_1 hybrids, and this is another achievement.

5.2 *F_1 Hybrid Breeding*

Josefsson (1948) and Frandsen (1958) reported F_1 hybrids exhibited considerable increase in root weight and dry matter yield. Similar results from a small trial at SPBS (McNaughton and Munro, 1972) initiated a program to breed hybrid swedes. At the time, there did not seem to be any suitable cytoplasmic male sterility in *B. napus*. Self-incompatibility was examined, and experimental F_1 hybrids have been produced using this outcrossing system.

Although *B.napus* is generally considered self-compatible, there did appear to be evidence of self-incompatible (SI) plants in the species, particularly from crosses involving the parent species (Olsson, 1960a, b). Davey found an SI line in swedes (see Gowers, 1974b) and another one was obtained from cultivar Gullaker III used in out crossing tests (Gowers, 1981b). A range of SI lines was obtained from various sources, and the origins of 19 lines are given in Gemmell et al. (1989); all types of dominance relationships were found within these lines (Gowers, 1989). As self-incompatibility (SI) is dominant to self-compatibility (SC), its backcrossing into

agronomically desirable inbred lines of swedes with good combining ability was straightforward but laborious (Bradshaw and Wilson, 1993).

As opposed to kale and turnips, where inbred lines suffer severely from inbreeding depression, a relatively high percentage of sibs could be tolerated in hybrid swede cultivars. Especially in comparison to vegetable hybrids, production systems that produce lower proportions of hybrids would be feasible. A combination of SI and self-compatible sister lines to produce a double-cross hybrid was suggested as the most practicable method (Gowers, 1975). With a multiplication factor of ×1,000, two multiplications will produce 1 tonne of hybrid seed from 1 g of inbred seed. Only small quantities of inbred seed are, therefore, needed to produce commercial quantities of hybrid seed. In a comparison of CMS and modified SI systems, the main differences are that two self-sterile lines need to be maintained with SI but only one for CMS, as opposed to the whole F_1 crop being sown and harvested together with SI, whereas pollinator rows have to be sown separately and then cut out with CMS (Fig. 8.3).

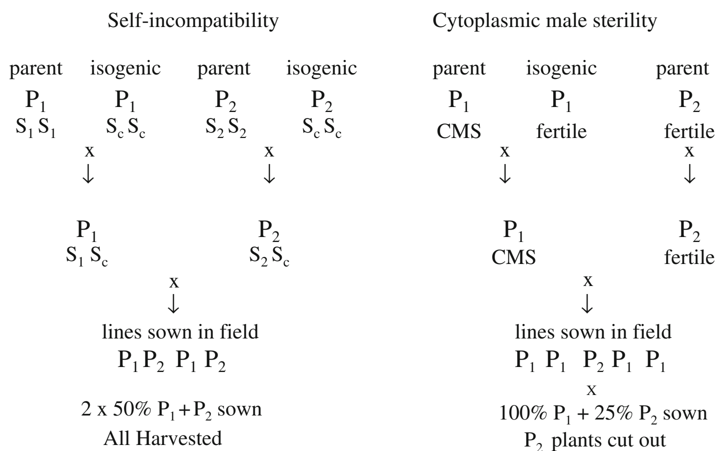

Fig. 8.3 Simplified diagram to show the main differences between hybrids produced by a modified double cross using self-incompatibility and a F_1 hybrid using cytoplasmic male sterility, from parental lines P_1 and P_2. Two self-sterile lines (S_1S_1 and S_2S_2; both dominant to S_cS_c fertile, self-compatible lines) have to be produced by CO_2-enhanced pollination with self-incompatibility but only one sterile line with CMS; however, a greater area of crop has to be sown for the final seed production with CMS and the pollinator line cut out, whereas the whole crop is simply sown out and harvested with self-incompatibility. As only a vegetative crop is required, a restorer line is not needed with CMS

Several workers have confirmed high levels of heterosis in F_1 hybrids while examining the genetic basis of the heterosis. In all cases dry matter content has been shown to be mainly due to additive genetic variance, but results for yield have been rather contradictory. Denton and Whittington's (1976) analysis showed overdominance for root fresh weight, which would mean that F_1 hybrids would be superior to inbred lines. Grant et al. (1982) found that the variance component of fresh weight

was mainly additive, with partial dominance at the remaining loci. An initial diallel analysis by Ramsay et al. (1994a) appeared to show partial dominance for yield, but the only definite conclusion was that a simple additive/dominance model was inadequate to describe the data. Two augmented triple test crosses were carried out (Ramsay et al., 1994b), and the result showed that the heterosis was due to dispersion of dominant and partially dominant genes and not due to overdominance or epistasis. Theoretically, this means that inbred lines with equal or better yield than the F_1 hybrids could be obtained. However, Davik (1997) found that the probability of obtaining an inbred line that would outyield the F_1 hybrid was usually very low; mostly probabilities were below 0.01 and in several crosses the *P*-values were around 0.001. However, there was one outstanding cross where the probability was 0.34, which must surely show that the results obtained are highly dependent on the parents involved.

To compare hybrid breeding with inbred breeding, Bradshaw and Wilson (1993) produced lines by both methods from similar parents. An inbred line cultivar 'Kenmore' was produced that was higher yielding than the F_1 hybrid from which it was derived by pedigree inbreeding with selection. They considered that similar amounts of effort had been deployed in the two methods and that inbred line breeding was, therefore, the better method. However, the work was an experimental study and involved the production of several hybrids. If a particular F_1 hybrid was identified and a simple backcrossing program carried out to produce that particular hybrid, it would be considerably less effort than would be involved in a single seed descent program with hundreds of progenies each generation, for example.

The breeding requirements in most cases, however, have other selection criteria than just high dry matter yield. Disease or pest resistance is also usually wanted in a new cultivar. A simple, single dominant gene resistance, such as can be the case with clubroot resistance, would not be much of a problem in producing a hybrid. When the inheritance is more complex, as seems to be the case with powdery mildew and dry rot resistance, then producing a resistant hybrid is much more difficult.

However, since the initial work on producing F_1 hybrid swedes, a considerable amount of work has been carried out on CMS in oilseed rape and much more reliable systems are now available. Two F_1 hybrid swedes, 'Tweed' and 'Tyne,' have now been produced by Elsoms in the UK using CMS (Kennedy, personal communication). Uniform, inbred lines were first produced using single seed descent. The OGRA male sterility was licensed from INRA and backcrossed for at least six generations to obtain fertile and sterile lines for hybrid production. Several hybrids were evaluated before deciding on two that met the market criteria for shape, uniformity, color, and good pack-out yield for the supermarket.

With turnips, the SI system for producing hybrid cultivars is already present. However, the strength of *S* genes varies somewhat, so selecting strong *S* genes is a pre-requisite for good hybrid production. Workers in Japan have been particularly successful at producing F_1 hybrid turnips. For example, the whole range of nine turnips marketed by the Musashino Seed Co. Ltd. are F_1 hybrids. Cytoplasmic male sterility is also available in *B. rapa*, either derived from cytoplasms used in oil seed

rape (Hu et al., 1997; Sovero, 1988; Verma et al., 2000) or from new cytoplasms (Okawa, 1985; Matsuzawa et al., 1999; Deol et al., 2003).

6 Current Goals of Breeding

The main objectives are still reliable, high yields of swedes and turnips that have high nutritional value, and are highly acceptable to animal or human. Disease and pest resistance remain objectives to increase the reliability of crops while minimizing the use of chemical protectants, both for environmental sustainability and to reduce costs. Some problems such as clubroot (*P. brassicae*) are universal, but others may be more localized. In swedes, for instance, powdery mildew (*E. cruciferarum*) is a significant problem in the UK, but not in New Zealand; conversely, dry rot (*Leptosphaeria maculans/Phoma lingam*) is a major problem in New Zealand but not in the United Kingdom. More discussion of priorities for disease and pest resistance will be found in Sections 7.3 and 7.4.

Swedes and turnips are cool temperate crops used for winter vegetables or animal feed, and winter hardiness is very important. If the crops are lifted and stored, then the bulbs need to be resistant to storage rots and diseases (Shattuck and Proudfoot, 1990).

Chemical composition is very important, both from a nutritional point of view and for acceptability of taste, but is difficult to translate into breeding goals. Glucosinolates (or their hydrolysis products), disulfides, and sugars are probably the major factors affecting taste, but what some consider to be desirable flavor is what others dislike about the taste of swedes and turnips. The complexity of these compounds and the difficulty and cost of analysis are a major reason why no laboratory-based selection has been carried out on taste. Glucosinolate analysis by HPLC is very expensive, but some of the major ones in swedes and turnips are isothiocyanate releasing, and analysis of these by gas chromatography is much cheaper. As mentioned in Section 1.3, lower levels of progoitrin are desirable, as it produces a bitter tasting goitrogen on hydrolysis, and hence lower levels are unlikely to have an adverse effect on flavor. Manipulation of levels of other glucosinolates could have both desirable and undesirable effects on pest resistance, particularly if concentrations were changed in leaf tissue.

The other major antinutrient in brassicas is *S*-methyl cysteine sulfoxide (SMCO); in ruminants this causes hemolytic anemia from conversion to dimethyl disulfide (Smith et al., 1974). Nevertheless, swedes and turnips and generally considered to be safe crops and the bulbs are usually quite low in SMCO, although under some growing conditions they may contain enough SMCO to cause problems for ruminants (Griffiths et al., 1991). However, swede tops can be relatively high in SMCO especially in mid-winter (Griffiths et al., 1991). Griffiths modified the automated technique of Gosden (1979) for measuring SMCO, but a simplified, non-automated version was developed by Gowers and Shaw (1999). Hence SMCO concentrations would be easy to determine in a breeding program, but it is not clear if lower levels are really necessary. Indeed, for non-ruminants, including humans, as mentioned

earlier (Section 1.3), SMCO may be beneficial as it has been shown to lower cholesterol levels in plasma and liver (Itokawa et al., 1973).

For animal feed, the digestibility and energy content of swedes and turnips are very good and higher levels are not needed. For body weight maintenance of adult animals overwinter this is all that is needed, but for increasing weight in younger animals higher protein is necessary. In this respect, ‘Tina’ swede may be a good resource as it usually has about 3% higher crude protein than other cultivars (Gowers unpublished); true protein content is not known.

Low dry matter swedes that are softer and easier to slice and dice are preferable for human use. Softer swedes may also be beneficial for sheep grazing, but higher dry matter is better for animal feed intake. Vipond et al. (1989) found sheep had a higher intake with the low dry matter, softer swede ‘Doon Major,’ but lower liveweight gain (LWG) than with the high dry matter, harder ‘Dryden.’ The intermediate hardness ‘Melfort’ had a similar intake to ‘Doon Major,’ but the highest LWG as it is one of the highest dry matter content cultivars (Table 8.1). Softer swedes are thought to be better for tooth loss when sheep are grazing swedes. However, the initial cause of premature incisor loss was found to be invasive peridontitis (Spence et al., 1980), although the cause was unclear, and grazing on swedes may only exacerbate the problem.

Blaxter (1971) suggested that, even without an increase in dry matter yield, it would be advantageous if the dry matter content (DMC) of swedes was doubled. From the results of cultivar trials, Frandsen (1958) calculated that the heritability of DMC was 94%, but considered it rather difficult to increase DMC because of the small variability of this character. However, working within cultivars, Gowers (1979) found a heritability of only 40%. To start a breeding program for high DMC, several high DMC lines that produced reasonably high DM yields were wanted. Selections from the ten highest DMC cultivars available were made. Frandsen (1958) found a negative correlation between DMC and fresh weight yield, so selfed progeny were selected on deviations from the regression of DMC on fresh weight yield. The negative regression of DMC was found to be generally correct, but in half of the cultivars examined the regression was not significant. With a second generation of selfing and selection, however, the negative regression was highly significant. Although Bangholn Wilby was one of the lowest DMC cultivars tested (Gowers and Gemmell, 1988b), a line from this cultivar was outstanding for high DMC. Submitted for National List trials under the name of Dryden, this line had a dry weight yield of 99% of the mean of control cultivars and a DMC of 30% higher; 20% higher than the highest standard cultivars (Gowers and Gemmell, 1988b). However, because the dry weight yield was only average, the fresh weight yield was low and the bulbs looked small; the reaction of the seed trade was not positive and the line was not released as a new cultivar.

When the cultivar ‘Pentland Harvester’ was released, it became apparent that it suffered more than most cultivars from ‘brown heart’ caused by boron deficiency. For the production of new cultivars, brown heart resistance became a breeding objective at SPBS/SCRI. The usual method of screening was to take a diagonal core sample from the bulb (as taken for DM sampling). Selected bulbs were then

transplanted outside to overwinter for seed production in spring. Apart from brown heart selection, having a hole through the bulb also tended to select for disease resistance and winter hardiness.

7 Breeding Methods and Techniques

7.1 General Methodology

Turnips and swedes differ in breeding methodology because they are outbreeders and facultative inbreeders, respectively. As such, turnips are bred using outbreeding techniques, whereas swedes can be bred to some extent by either outbreeding or inbreeding techniques. In older breeding programs, swedes were treated as outbreeders and the general method used for most swede and turnip breeding has been inter-crossing of desirable parents and then simple mass selection or progeny selection (Frandsen, 1958; Shattuck and Proudfoot, 1990; Davik, 1997). More modern turnip breeding was dealt with in Section 5.1 where population improvement, leading to an open-pollinated cultivar 'Massif', and F_1 hybrid breeding in Japan, were covered. Also covered in Section 5.1 was the drive to produce high-yielding and uniform swede cultivars. This was achieved both through inbred line cultivars, produced by pedigree inbreeding with selection, single seed descent (SSD) or by doubled haploids, and F_1 hybrid cultivars. Here more theoretical and practical information is provided on breeding methods in swedes which involve self-pollination.

Swedes are predominantly self-pollinated under field conditions, with over 80% selfing (Gowers, 1981b). This means swede cultivars are generally a mixture of partially inbred lines, with an overall inbreeding coefficient of about 0.69 at equilibrium. It also means that if the yield of a new cultivar is partly reliant on residual heterosis, then up to 70% of that heterosis could be lost on multiplication before release. It seems reasonable to assume that a pedigree breeding or SSD program should be taken to F_5 or F_6 before final yields can be assessed with any degree of reliability.

Alternatively, there is the possibility of maintaining the heterosis of swede lines by converting them to outbreeders. Self-incompatibility is now available in *B. napus* (Gemmell et al., 1989) and its introduction into swede cultivars would maintain heterosis. By selfing a population of known inbreeding coefficient, an estimate of the yield of the base population with no inbreeding can be calculated (Gowers, 1988). Using the cultivar 'Bangholm Dima' as an example, it was estimated that an increase in yield of 28% would be obtained by converting it to an outbreeder.

Swedes are generally tolerant to inbreeding, and Davey and Lang (1938) found that a third of inbreds was definitely inferior to the original cultivar from which they were obtained, a third was equal, and a third was slightly better. More detailed work on inbreeding in swedes was carried out by Gowers and Gemmell (1988a). Plants of an old cultivar were selfed and their progeny produced from 22% less to 23% more dry matter yield than the parental cultivar. A further generation of selfing produced

a family of lines with a mean dry matter yield of 12.5% higher than the original cultivar. This knowledge was used at SCRI to develop inbred lines for breeding and genetic studies, but it also led to two commercial releases. A line from cultivar 'Criffel' is being grown in New Zealand as an improved cultivar, 'Highlander,' and a superior line from the shopping swede 'Acme' is being marketed in the UK as an improved stock.

Davey (1954) tried breeding swedes by selfing and selection after making intercrosses, but found that after about four generations lines became fixed, not only for desirable characters but also for faults which could no longer be removed by selection. He concluded that inbreeding put the lines at a disadvantage compared with commercial varieties, and he then attempted to breed strains that did not become too inbred. However, as long as yield can be maintained during inbreeding, producing uniform lines without undesirable characters is just a case of testing enough lines. With the use of a mechanical harvester and larger trials, later work at SPBS/SCRI used inbreeding and progeny selection as the standard procedure. From crosses made in 1967, 'Angus' and 'Melfort' were released in 1982, and another four cultivars were released using this methodology in the next 13 years: Airlie (released in 1992), Kenmore (1994), Brora (1995), and Invitation (1995).

The program at Scottish Crop Research Institute (SCRI) in the 1980s was probably the largest there has been. The resources put into the production of one cultivar 'Kenmore' were (Bradshaw and Wilson, 1993):

F_2 – several thousand plants overwintered for selection
F_3 – 204 lines, three replicates of single drill plot
F_4 – 94 lines, three replicates of single drill plot
F_5 – 14 lines, three replicates of two drill plots
F_6 – 23 lines, two replicates of two drill plots
(single drill = 10×0.75 m)

Alpha designs (Patterson et al., 1978) were used to grow and analyze the early generation trials, as this was considered the best statistical method available for the large number of plots involved.

When Government funding for brassicas breeding at SCRI ceased, further work had to be commercially funded. A cheaper method than the biennial inbreeding program was wanted. Single seed descent had been used on a small scale for experimental work and had produced the cultivar 'Virtue' (Bradshaw et al., 2009), so it was decided to use this method for the commercially funded project (Bradshaw et al., 2009). It involved the production and trialling in 1999 of 1,037 F_6 families from 15 crosses made in 1993. Fifty F_6 families were advanced to F_7 in a glasshouse in 2000 and assessed in 2001. Six F_7 families were mass multiplied in polythene tunnels in 2002 and trialled in 2003. Two cultivars, 'Gowrie' and 'Lomond,' from the cross between Airlie and Invitation, were produced from the program and entered NL trials in 2004. The SSD was traditional in the sense that each advanced family was descended from a different F_2 plant without selection. However, it was

not possible to grow a large number of plants at really high density because the inflorescences needed to be covered with Glassine bags to prevent cross-pollination. Bradshaw et al. (2009) recommended a modified SSD breeding scheme in which family selection is practiced at F_3.

The fastest way to produce inbred lines is to use microspore culture, which Hansen and Bratberg (2003) used to produce the improved cultivar 'Vigod' from the old Norwegian cultivar 'Vige.' This technique depends on having the facilities and expertise to culture microspores and produce diploids from them. Inbreds are produced immediately, but again it may take several years to select and multiply a line for commercial production.

There is little possibility of running a breeding program with full yield trials in the field on an annual basis in Scotland or Norway, for example. In New Zealand, however, with late sowing and a longer growing season, an annual program has been developed. Initially using heated glasshouse space that was available (Gowers and Armstrong, 1986), the method is now being used with an unheated glasshouse. The system depends on swede seed not having a dormancy period and being able to germinate immediately after being harvested. Seed is harvested by the first week of December and sown during the second week of December. Trials are sown in normal fields and also in a clubroot-infested field. After yield assessments from the normal fields in June or July, resistant selections are taken from the clubroot-infested plots and potted up into an unheated glasshouse. The plants flower and set seed from September through to November and enable an annual cycle. The system has been further modified in that the selections are also injected with dry rot (*L. maculans/P. lingam*) spores in the glasshouse. An annual cycle of self-pollination and progeny selection for yield, clubroot, and dry rot resistance has been achieved.

When yield data are not needed, an annual cycle can be carried out in most places where swedes are grown by growing plants in a glasshouse. Under Scottish conditions, seed can be obtained in 8 months by sowing in January and growing the plants in an unheated glasshouse (Gowers and Gemmell, 1988c). For a simple backcrossing program, a 6-month generation time can be obtained using controlled environment cabinets. Swedes are unusual in that there is a juvenile period of several weeks when it becomes harder to vernalize plants and then a maturity phase where after it becomes easier to vernalize plants (Gowers and Gemmell, 1988c). If swedes are germinated for 2 days and then placed under vernalizing conditions for 8 weeks, plants will flower about 8 weeks later; a generation time of less than 6 months can, therefore, be achieved.

A considerable part of the generation time is taken with seed development and ripening, which can take up to 2 months. If necessary, seed can harvested before the pods are fully ripe and then air dried. As long as 4–5 weeks have elapsed since pollination, green pods can be harvested and dried; smaller but viable seeds can be obtained (Shattuck and Proudfoot, 1990). The seed is usually fully developed by 30 days after pollination, and germination in the pod is inhibited by the seed coat; if the seed coat is removed at this stage embryo germination can take place.

7.2 Yield Trials

Trial procedures depend on the time, space, facilities, available labor, and generation. When potential swede cultivars are assessed in yield trials, multiple-row plots are used and the outside rows discarded in order to minimize competition effects between cultivars in adjacent plots (Kimber, 1977). This is common practice in yield trials of crop plants but is not always possible in the early stages of a breeding program (Bradshaw and Wilson, 1993). A uniformity trial (Gowers, 1976) showed that a plot size of 4 rows by 10 m was a good compromise between area of trial and low coefficient of variation (COV). Harvesting the two center rows of such plots, with the outer rows as guard rows, had a higher COV, similar to two row plots without guard rows. However, Bradshaw (1989) found competition effects between plots, and guarded plots may be necessary. For earlier generation material, two replicates of double row plots were considered preferable to four replicates of single row plots. Similar work by Davik (1994) in Norway did not find any interplot competition for yield. The difference may be due to technique, with transplants used instead of direct seeding, but it may also have been due to less competition between plots as some cultivars were harvested relatively early.

The main selection criterion for swedes is usually high dry matter yield. Estimates of dry matter (DM) content are usually made from 10 mm diameter diagonal core samples (Davey, 1932). Such samples contain a far greater proportion of pith than the true proportion and underestimate DM content by about 0.8% (Dyson, 1980). A good representation of the whole bulb is obtained from a segment (as in an orange). However, such samples are very laborious to obtain in comparison to core samples, and for comparative purposes between breeding lines core samples should be adequate. Another factor affecting the accuracy of DM estimates is the drying regime. Davey (1932) recommended 60°C for 48 h for drying, as higher temperatures lead to caramellization of sugars and loss of weight; this is the drying treatment for root crops specified in the MAFFS ‘Analysis of Agricultural Crops’ procedures handbook. Work at SPBS found no significant difference between freeze drying and oven drying at 60°C (Gowers and Barclay, 1978). However, there were significant differences with higher temperatures, and a significant interaction with cultivar; drying at 100°C is definitely not recommended. However, with a limited amount of oven space and a large throughput of samples required, 80°C for 24 h may be a reasonable compromise for early generation material. A sample size of six cores per row or 10/12 per plot has been found adequate.

With the relatively low plant stands used for growing swedes and turnips, poor seed germination can lead to very unevenly spaced plots. Because of this, it might be considered that adjustment of yield with covariance on plant stand would be advantageous. In an experiment on inbreeding within a swede cultivar, Gowers (1979) found that heritability of dry weight yield was 0.24; when covariance adjustment on plant stand was made, the heritability was not significantly different from zero. Lang and Holmes (1965) found that there was no difference in swede yields with plant density between 36.5 and 125 plants per hectare, and variable spacing also made no difference. It appears, therefore, that yield compensation covers most situations,

and that covariance causes adjustments that are not necessary and make matters worse.

When a machine is used to harvest swedes the tops are discarded. This gives a suitable estimate of yield for crops lifted and stored overwinter, when only the bulb weight is wanted. If the crop is grazed in situ then the tops are a valuable contribution to the feed value – especially when the protein content of the bulb is very low. Hand harvesting may be the only suitable way to get bulb and top yields, but then much smaller plots or much smaller samples can be handled. A comparison of machine and hand harvesting was made by England (1977). He concluded that results obtained by either method were similar; interaction effects were small in comparison to varietal effects and coefficients of variation were similar, so both methods seemed equally precise. However, the same size plots were used in both cases, and it is assumed that smaller plots harvested by hand would be less precise.

Although DM yield is the main selection criterion in yield trials, other traits are considered, particularly any defects. Typically, skin color, root shape, regularity of shape, neck length, skin finish, and proportion of roots with splits and rots would be recorded before harvest. At harvest, a sample of roots (six per row or 10/12 per plot) would be cut open to check for internal defects such as browning and to record flesh color (Bradshaw et al., 2009). Furthermore, the overwintering of plants for seed production the next year would result in selection for adequate winter hardiness.

A serious problem with sheep grazing swedes is tooth loss. Softer swedes are wanted to alleviate this problem. Laboratory machines to test hardness are available, but these would need swedes to be lifted and carted inside for testing. A portable and reliable technique for testing hardness in the field was required, so that bulbs could be tested at the time trials are being harvested. Gowers (1981a) developed a device whereby a blunt 4-mm needle with a cylindrical weight on the end was dropped vertically down a tube to penetrate the swede bulb. A notch at the top of the weight ensures the needle drops from a constant height. The depth to which the needle penetrates gives a measure of hardness. A high correlation between penetrometer reading and dry matter content was found ($r = 0.86$) but there were exceptions that suggest that softer bulbs with higher dry matter content may be selected.

7.3 Other Techniques

With knowledge of simple, single dominant genes, breeding populations can be produced without hand pollinations. Stewart (personal communication/unpublished) allowed plants with single gene dominant and recessive marker genes to flower together and grew out offspring from the recessive parent. Hybrids were chosen from the progeny by selecting plants with the dominant character. These were allowed to pollinate together and sown out, whereupon the recessive character was selected; a true breeding population for that character was, therefore, established. Further selection and breeding by progeny selection could then be carried out for yield and other characters.

7.4 Disease Resistance Breeding

The three most important fungal diseases of swedes are clubroot, dry rot, and powdery mildew; clubroot is a worldwide problem, whereas the latter two appear more regional problems. Turnip mosaic virus (TuMV) can be a problem with some swede cultivars but there are resistant cultivars available. TuMV is a major problem in turnips in some areas. Turnip crinkle virus and Beet western yellows virus can also cause problems. Other fungal diseases that affect swedes and turnips are damping off (*Pithium* ssp.), downy mildew (*Peranospora parasitica*), and several leaf spot diseases. Black crater (*Rhizotonia solani*) does not usually cause problems for fodder swedes, but can have a marked effect on culinary swedes. Storage diseases are associated with several bacterial diseases, including black rot (*Xanthomonas campestris*), bacterial soft rot (*Erwinia carovora*), and *Pseudomonas flourescens* (Shattuck and Proudfoot, 1990). Differential reaction of cultivars to most of these minor diseases has been noted and should, therefore, be amenable to breeding and selection, but they do not appear to have caused sufficient problems to warrant specific breeding programs being undertaken.

7.4.1 Clubroot

Clubroot, caused by *P. brassicae*, is one of the most important diseases of brassicas (Fig. 8.4). Although usually referred to as a fungus, *Plasmodiophora* is a myxomycete, a member of the small group of Protista which do not have cell walls and are commonly called slime molds. What they do have are very long lived resting spores that can survive in the soil for many years. Control of clubroot by chemical means is very difficult, and plant resistance is by far the best means of control.

Clubroot resistance can be tested either in the field or in the glasshouse tests. Setting up field testing would be relatively easy if a suitable, evenly infested field was available. However, testing would be restricted to the particular population in

Fig. 8.4 Clubroot disease of swede, showing severe galling and reduction in yield

that field. Setting up a uniformly infested field for testing is rather laborious and involves considerable effort. However, once set up, a clubroot testing field allows screening for clubroot resistance and selection for good bulb characters at the same time (Fig. 8.5). If a field is used continuously for clubroot testing, then yield is likely to be affected after some years. Trials in a normal, uninfected field are probably needed for yield assessment.

Fig. 8.5 Clubroot testing of swedes in a specially infested field, with susceptible line in *center* and resistant breeding lines at the sides (photo: Stewart Armstrong)

Greenhouse tests are much quicker to carry out and allow specific populations of clubroot to be tested. However, clubroot resistance may be the only character of worth in the selected plants. Sib testing in the field must simultaneously be carried out for agronomic characters, unless the testing is part of a SSD program and all resistant plants are later evaluated in the field.

A number of methods have been tried out for greenhouse tests. The basic one is to prepare a mixture of clubbed tissue and soil (Nieuwhof and Wiering, 1961) or a spore suspension with soil (Williams, 1966) and grow seedlings in the mixture. The soil should be slightly acid, adequately moist and warm (26°C). The slurry method of Toxopeus and Janssen (1975) is essentially similar to this technique. The other main technique used has been a dipping method, whereby a suspension of spores is produced and the roots of young seedlings are dipped in the suspension before being repotted (Nieuwhof and Wiering, 1961; Johnston, 1968). A survey of techniques and conditions used by various workers was published by Dixon (1976).

As well as a range of techniques being used, several workers used different sets of differential hosts to distinguish different strains of clubroot. An attempt was made to rationalize the situation and an internationally agreed set of differential hosts was produced. This comprised four differential hosts and a susceptible line for each of *B. rapa/campestris, B. napus*, and *B. oleracea* and was called the European Clubroot Differential set (ECD). Seed of the 16 differentials is available from the Genetic Resources Unit, Warwick HRI. A review of this and other aspects of breeding for clubroot resistance was written by Crute et al. (1980).

When it is wanted to test plants for clubroot resistance and the plants are still needed if they are susceptible, a technique for screening rooted leaf cuttings was

developed by Williamson (1981). The disease incidence in cuttings was lower than in seedlings tested, but the relative incidence was similar and gave a good indication of resistance.

There have been several cultivars of turnips which were reputed to have resistance in their own locality, but most of these lines were found to be severely attacked by clubroot races in the Netherlands (Schreijgrond and Vos, 1954). The only turnips they found with resistance were the Belgium selections Meetjeslander and Waaslander. Where the resistance of these two cultivars came from is unknown. The resistance in the two cultivars differed, because Wit and van de Weg (1964) found a differential reaction with two populations of clubroot. The cultivar Novitas appeared to combine these two resistances. Wit (1966) suggested that three dominant genes were involved in the resistance to the three races studied. Inbred lines were selected from Waaslander that must have had all three. It appears that this is the derivation of ECD 04, which was the most resistant turnip available. However, even this is overcome by some strains of clubroot. Working with such a strain, when trying to isolate the three postulated resistance genes, Williamson and Hodgkin (1987) obtained resistance to the strain from ECD 03.

The clubroot resistance obtained so far in swedes has been single dominant genes obtained from turnips, as in cultivar 'Invitation' (Bradshaw et al., 1997). This type of resistance is prone to be overcome by the development of more pathogenic strains of the disease (Gustafsson and Falt, 1986; Gustafsson and Gummeson, 1988). Although clubroot is a soil-borne disease and will not spread as quickly as an air-borne disease, there are races that attack the most resistant turnips available. Resistance in kale (*B. oleracea* ssp. *acephala*) is 'horizontal'-type resistance (Bradshaw and Williamson, 1991). It would, therefore, be desirable to transfer such resistance to *B. napus*. Material from rape × kale (Gowers and Christey, 1999) has been found to have clubroot resistance, and this resistance is being examined and transferred to swede.

7.4.2 Dry Rot

Dry rot in swedes is caused by the same fungus that causes blackleg or stem canker in rape (*L. maculans*/*P. lingam*). Although apparently not a major problem in the Northern Hemisphere, in New Zealand it can be the most serious disease of swedes.

Initially a cotyledon test was examined in the greenhouse as a means of selecting for resistance to dry rot (Gowers and Armstrong, 1998). However, the results did not correlate well with field trial scores. Also, infection of the bulb itself can occur later, although this is not as serious as the disease developing in the early stages through infection of the cotyledons or young leaves. A method where the mature bulb is inoculated with spore suspension has been used, and this should select an adult plant-type resistance (Fig. 8.6).

Two races of dry rot that attacks cultivar 'Tina' are used, and these are maintained on agar plates. A drop of spore suspension (1×10^6 spores/ml) is placed over holes in the bulb made with a hypodermic needle, and the plants are grown overwinter in a glasshouse.

Fig. 8.6 Dry rot testing of swedes – bulbs susceptible to dry rot race injected on the *right-hand side* but resistant to that injected on *left-hand side* (*arrows* indicate injection sites)

'Tina' was the first swede produced with good resistance to dry rot and clubroot (Lammerink and Hart, 1985). However, because of its good disease resistance, some farmers grew this cultivar continuously in the same field for several years, and within about 10 years the dry rot resistance of 'Tina' had broken down. In a search for new sources of resistance, swedes from the gene banks at Warwick HRI Genetic Resources Unit and FAL Braunschweig were tested (Gowers and Armstrong, 1998). The cultivars 'Heikenborsteler' and 'Niko' were found to have good resistance to the dry rot races tested, and this resistance has been introduced into more agronomically desirable lines.

7.4.3 Mildew

The situation with powdery mildew (*E. cruciferarum*) in swedes is the opposite to that of dry rot. Whereas mildew can be a serious problem in the UK, it has only been scored once in over 20 years in swede cultivar trials in the south of New Zealand. In the UK, large increases in yield have been obtained by controlling mildew with fungicides (Jenkyn and Rawlinson, 1977; Brain and Whittington, 1979; Williamson, 1984). Mildew resistance is, therefore, highly desirable to produce higher yields of swedes without the use of fungicides.

The inheritance of mildew resistance was studied by Bradshaw et al. (1989) from a diallel cross of 11 inbred lines, chosen to include the most resistant and susceptible parents. The most resistant parents were from cultivars 'Bangholm Dima' and 'Bangholn Magres.' Their resistance was due to the association of partially recessive genes and should be more durable than the race-specific major genes found in cereals. Scoring of mildew resistance was carried out in the field from the beginning of August, but because natural infection can be late in Scotland, the center row of each plot was inoculated at the end of July with a spore suspension prepared from infected plants grown in a glasshouse. Resistance has been successfully incorporated into cultivars 'Kenmore' (Bradshaw and Wilson, 1993), 'Invitation' (Bradshaw et al., 1997), and 'Gowrie' and 'Lomond' (Bradshaw et al., 2009).

7.4.4 Turnip Mosaic Virus

TuMV is a widespread and important virus infecting brassica crops. It is spread by aphids in a non-persistent manner which means it is not controlled by aphicides. TuMV can cause serious epidemics in some years, but in other years it is not a problem. In 1975 there was extensive infection of swede crops in Britain and in 1985 an epidemic in swedes caused severe losses in Ontario. Unusually, early season swede plantings in Ontario escape virus infection whereas late plantings are prone to being infected (Shattuck and Proudfoot, 1990). With turnips in New Zealand it is usually the other way round. TuMV was a problem in New Zealand in the 1950s, and a breeding program was set up to produce a resistant cultivar. This was successful with the release of cultivar 'Kapai' in 1966 (Palmer, 1983), whereupon TuMV virtually disappeared for many years, only to return with a major epidemic in 1998 (Fig. 8.7). It has remained a serious problem since then, and 'Kapai' is not resistant to the present strain.

Fig. 8.7 Total devastation of a turnip trial by turnip mosaic virus, except for the resistant swede cultivar 'Winton' which was included

Several New Zealand swede cultivars seem to be resistant to TuMV (Palmer, 1983), but this may be natural selection as much as anything, because there does not seem to have been a program aimed at breeding for resistance. 'Sensation' and 'Calder' were found to be highly resistant to the Ontario strains of TuMV, and the resistance appears to involve several major genes (Shattuck and Proudfoot, 1990). Resistance in swedes has also been found in European cultivars by Tomlinson and Ward (1982). They also found that commercial swede cultivars were often heterogeneous for resistance. By inbreeding and selection they produced two lines (165 and 181) from cultivar Ruta Ǿtofte with fully heritable immunity, i.e., symptomless and no virus detected. Shattuck and Stobbs (1987) found that Ruta Ǿtofte line 165 was very resistant, but not immune, to a virulent Canadian strain of TuMV. They showed that when crossed with a susceptible Canadian cultivar 'Laurentian', the resistance was inherited as a single dominant gene.

At the moment, there does not appear to be any suitable resistance in bulb turnips to the TuMV strain presently infecting New Zealand crops. Selection in the field

from natural infection is rather unpredictable and, although some degree of field tolerance may have been obtained, this material is not resistant in glasshouse tests (Gowers unpublished). Field material can be manually inoculated with TuMV, but it is much easier to inoculate plants in a greenhouse. Inoculation procedures and detection methods are described by Stobbs and Shattuck (1988).

The most promising source of resistance to TuMV for turnips is from Chinese cabbage (*B. campestris* ssp. *pekinesis*). Several workers have found resistance in Chinese cabbage and these have been examined by Hughes et al. (2002) and Walsh et al. (2002). The most promising resistances appear to be those obtained by Liu et al. (1996). From a survey of more than 3,000 accessions tested against 19 major TuMV isolates, eight lines were found that were immune or highly resistant to all TuMV isolates. This material has been incorporated into a whole range of new cultivars in China. The inheritance of some of these lines has been studied (Jenner et al., 2003) and one line has been found to exhibit broad spectrum resistance to TuMV (Rusholme et al., 2007). This line shows resistance to the New Zealand strain of TuMV, and another two lines with good resistance have recently been obtained from the gene bank at AVRDC (Gowers unpublished).

7.5 Insect Pests

A number of insects attack swedes and turnips, but little breeding work has been done on them. Several insects around the world attack young seedlings at emergence, for example, flea beetles (*Phyllotreta undulata* and *P. nemorum*) cause damage to cotyledons. Various leaf eating caterpillars cause various amounts of damage in various parts of the world (cabbage white butterfly, *Pieris rapae*; diamond back moth *Plutella xylostella*; cabbage looper *Trichoplusia ni*; purple cabbage worm *Evergestis pallidata*) but not usually causing sufficient damage to invoke chemical control or a breeding program. Aphids, as well as being vectors of viruses, can cause crop damage. In fodder crops, the cabbage root fly (*Delia radicum*) and the turnip root fly (*D. floralis*) may cause some damage but it is not obvious, whereas in vegetable crops the damage detracts seriously from market appeal. In Scotland such crops are now covered with fleece or Wondermesh, because chemical control is no longer available. When the cultivars 'Angus' and 'Melfort' were released, they had higher DM content than other cultivars on the market and appeared relatively resistant to turnip root fly. To see if there was a relationship, Gowers et al. (1984) carried out a small trial but found there was no consistent association of the two factors. They did find that the resistance appeared to be dominant. In detailed field and glasshouse studies of the resistance of cultivars 'Angus' and 'Melfort,' Birch (1988) found that egg-laying antixenosis was the major component of resistance, and that root antibiosis against larval feeding was a second but less important component. A field cage method for assessing turnip root fly resistance was developed by Birch (1989) which involved the controlled release of adult flies, but cultivars with better resistance have not been bred. The best resistance found to date is in the curly kale cultivar 'Fribor' which Ruuth (1988) reported to be very resistant to turnip root

fly, mainly through a high level of antixenosis resulting in few eggs being laid and consequently little larval damage to roots. However, a major breeding effort would be required to transfer the resistance to *B. napus*.

8 Integration of New Biotechnologies in Breeding Programs

It is not very likely that much in the way of new biotechnologies will be integrated into swede and turnip breeding in the near future. There is a lack of basic genetic studies in these crops on which to base such work, and swede and turnip breeding is still at a relatively low level. Molecular genetic techniques are expensive, and the swede and turnip programs that still exist do not have a great deal of funding or resources. However, there is a tremendous amount of work being carried out on the related oil seed crops, and this work should easily be transported into swede and turnip breeding. Molecular marker maps have been established for both *B. rapa* (Kim et al., 2006, Mun et al., 2008) and *B. napus*; because of its economic importance, at least six independent maps of *B. napus* have been produced (Quiros and Paterson, 2004). An ultradense genetic recombination map for *B. napus* was more recently produced by Sun et al. (2007). A short review on molecular mapping of *B. rapa* was written by Hirai and Matsumoto (2007). Details on progress in brassica genomics can be found on the Multinational Brassica Genome Project website at http://www.brassica.info.

The use of molecular markers to breed for recessive traits of disease and pest resistance is likely to be of most use. If the polygenic resistance of *B. oleracea* to clubroot is transferred to *B. napus*, then RAPD markers (Grandclément and Thomas, 1996) or RFLP/AFLP markers (Voorrips et al., 1997) could be available to help select resistant lines.

TuMV is another disease that would benefit from molecular marker techniques. A *B. rapa* genetic map based on 231 marker loci was established by Rusholme et al. (2007) and two TuMV resistance genes were mapped. Quantitative trait loci (QTL) analysis for TuMV was carried out by Zhang et al. (2008) using a total of 376 molecular markers of various types. Two QTLs were found associated with resistance at the seedling stage and two with resistance at the adult plant stage.

Other recent disease work of interest is the identification and mapping of three loci for blackleg/dry rot resistance in canola (Yu et al., 2008) and localization of a major QTL for seedling resistance to downy mildew in Chinese cabbage (Yu et al., 2009).

Although most work is on related species, and usually on seed characters, there has been some work involving turnips. Hirai et al. (2004) showed that turnips have at least three clubroot resistance loci, and Lu et al. (2008) conducted work to identify and map QTLs affecting root morphological traits in *B. rapa* by using molecular markers.

The other technique that could provide great improvements in pest and disease resistance, and in other desirable characters, is genetic engineering. Again much has been done with oil seed rape that could easily be transferred to swedes and turnips. Swedes have been transformed with *Agrobacterium tumifaciens* (Li et al., 1995)

and both turnips and swedes have been transformed with *Agrobacterium rhizogenes* (Christey and Braun, 2004). However, there is still enormous public pressure against the release of transformed material in many places. Genetic engineering of brassicas has ceased in New Zealand and it could be a long time before it recommences.

9 Seed Production

A particular aspect of turnip seed production is where the climate is so severe that plants grown outside overwinter are killed by the cold. Commercial turnip seed production in the Netherlands uses artificial vernalization of germinated seed. After soaking in water at 20°C for 4–5 h, the seed is germinated at 20°C for 17 h, or until 80% of the seed has germinated. Room must be left to allow the seeds to swell as water is imbibed. The germinated seed must be dried off rapidly and must be dry enough to pour. The seeds in trays are covered with plastic sheet and kept at 0 to −1°C for 35 days up to 15 March for sowing. For later sowing extra days must be spent at low temperature, but it is not recommended to go beyond 45 days. After treatment, the seed must be sown directly with the minimum amount of time.

Although relatively little work may have been done on swedes and turnips themselves, considerable work has been done on other brassicas, especially their oil seed relatives. It is assumed in most cases that results applicable to rape will be very similar to those for swedes, if not identical, and similarly with turnip and its related subspecies. Many of the comments made on seed production are made on these related species.

Initial breeding lines are usually produced by hand pollination, either by self-pollination or by inter-crossing plants to produce hybrids. Several techniques to carry out pollination are used. For large numbers of pollinations, a small paint brush or bee-sticks (Williams, 1980) can be used to transfer pollen. For smaller numbers, a pair of fine forceps is simple to use, by removing an anther and brushing it across the receptor stigma. Forceps are also easy to sterilize with alcohol between crosses; hands should also be swabbed to kill pollen. For cross-pollinations, the anthers should be removed before pollen dehisces and causes self-pollination; this involves opening buds before anthesis and pinching out the anthers; usually the sepals and petals are removed with forceps to do this.

After pollination, the flowers are covered with a tissue paper bag, or similar material, that allows excess water vapor to dissipate. It is advisable to do this even in an insect-proof greenhouse; not only does it prevent accidental pollination by human or insect but also it stops the immature pistils from drying out on hot, sunny days. Pollen will germinate at 98% RH (Ferrari et al., 1981). Water availability to the pollen grain is controlled by the pellicle, and if this dries out too much poor pollen germination and poor fertilization can occur.

Within a species, any plants used for making crosses are usually flowering at the same time and pollen is readily available. If this is not the case, then pollen can be stored successfully. Fresh cabbage pollen will remain viable for a month at 4°C but is killed within a day in a deep freezer (Chiang, 1974). Pollen dried and stored with a silica gel dessicant will keep for several weeks at room temperature, several

months at 4°C, and over a year at –20°C (Ockenden, 1974). However, the storage ability of different cultivars may vary; Cunningham (1981) found pollen from turnip ‘Civasto’ did not keep well, whereas ‘Ponda’ and ‘Marco’ remained viable for up to a year at −20°C.

For self-pollination of swedes, all that is usually required is to place an inflorescence, or even the whole plant, in a bag. In a greenhouse a large, tissue paper bag can enclose the flowering stem and frequent shaking effects pollination; if the bags are not shaken regularly, seed set is only a third to half normal set (Jenkinson and Glynne-Jones, 1953). If the greenhouse is insect proof, the bags can be left open (see Fig. 8.8) but they ensure that plants do not cross-pollinate. For outside seed production from swedes, starched cloth bags were initially used at SPBS to cover the flowering shoots, but these were replaced by enclosing the whole plant in a polythene tube with a gauze top to allow ventilation.

Fig. 8.8 Seed production of swede breeding lines on an annual cycle in New Zealand by transplanting into a glasshouse in winter and isolating plants with tissue paper bags during flowering in spring (photo: Stewart Armstrong)

For self-incompatible brassica plants, techniques other than open pollination have to be used to effect selfing. For many years bud pollination was carried out, which was very laborious. This depends on the flowers being protogynous (receptive before the flower opens), and also on the fact that the incompatibility reaction sets in only just before the flower opens. The largest buds should be discarded, as they will be self-incompatible, and the next smaller buds that are big enough to handle will usually set seed on selfing. The buds can just be prized open at the top to allow pollination, but snipping the top off the bud with forceps makes actual pollination easier.

Several techniques have been described to overcome this problem. Monteiro et al. (1988) used salt solution to produce seed set in *B. campestris*; a drop of 15 g/l NaCl applied shortly before pollination gave good seed set. With self-incompatible swedes, removing the stigma and pollinating the cut end of the style produced similar quantities of seed to bud pollination (Gowers, 1986). However, the simplest and easiest method seems to be covering open-pollinated flowers with a polythene bag and blowing into it (Gowers, 1994). Concentrations of CO_2 over 4% for 6 h

after pollination are very effective in overcoming self-incompatibility (Nakanish and Hinata, 1973) especially at high humidity. Human breath has a CO_2 concentration of about 4% and high humidity, and if you hold your breath for as long as possible this increases the CO_2 concentration. The polythene bag should be deflated before blowing into it, and a plastic tube inserted into the bag makes it easier to displace any remaining air inside. The bag should be removed early next day, as the humidity becomes too high because water from respiration is trapped in the bag.

On a field scale CO_2 enrichment is obviously not possible, but on a glasshouse scale it can be used. Commercial equipment is available for CO_2 enrichment, for tomato growing, for instance, but modification would probably be needed because concentrations used are much lower. For small enclosures, injection of 1 kg CO_2/ 10 m^3 gives a concentration of about 5% and, if the there is little loss of gas, the concentration should remain above 4% for long enough to ensure fertilization takes place.

In the field, the only practical means of overcoming self-incompatibility has been the use of salt solution (NaCl). Spraying 5% NaCl solution every 3–5 days was found to be the optimum concentration for overcoming self-incompatibility of rape in the field (Fu et al., 1992). Tao and Rui (1986) used 3% NaCl every second day with Chinese cabbage (*B. campestris* ssp. *pekinensis*). It is assumed that similar results would be obtained with swede and turnips.

During breeding programs different sizes of insect-proof multiplication plots are required to produce increasing quantities of seed as the lines progress toward cultivar status. Excluding natural pollinators means that pollinators have to be introduced to cross-pollinate turnips. Swedes will usually set seed without pollinators, but Williams et al. (1987) found seed set in rape was greatly enhanced by using honeybees. As well as increasing seed yield, pollinating with bees leads to the production of more viable seed with better germination (Kevan and Eisikowitch, 1990).

Honeybees are good pollinators, but are not suitable for small cages because even the smallest size of hive is too large. Blowflies have been found to be excellent pollinators in these conditions (Smith and Jackson, 1976). Blowfly maggots were readily available in the UK, where they were sold as fishing bait, but if they cannot be purchased they can be reared (Jones and Emsweller, 1934; Kuckuck and Kobabe, 1962). Alternatively, hives of bumble bees may be available commercially. In this case, a number of bees are released into a cage and they will usually live reasonably happily for some time in the absence of the parent hive and continue pollinating for several days. Today at SCRI small hives of bumble bees are used in polythene tunnels.

In the production of hybrid Brussels sprouts, honey bees can selectively pollinate the inbred lines (Faulkner, 1971) whereas blowflies appear to pollinate at random. Methods of hybrid swede production were compared using blowflies in polythene tunnels with isolation plots using natural pollinators (Gowers, 2000); blowflies produced higher levels of outcrossing when different flower colors were used, but lower levels when the same flower color was used.

Small glasshouses are ideal for multiplying seed, but are expensive to build and maintain. In warmer climates, flat-topped cages covered with insect-proof mesh are suitable, but these had several problems in Scotland; cold, wet conditions in late summer gave poor ripening, late snowfalls caused serious damage and styles could grow through the mesh and possibly allow contamination. Tunnel houses have since been used at SCRI, based on a 4.3 m wide framework and adding 1.5 m long sections as required, with 12 m long tunnels used for pre-basic seed production.

Clear polythene is usually used to cover tunnel houses, but on hot, sunny days temperatures get too high for plants and pollinators. Smith and Jackson (1976) found that blow flies only live for a few days at temperatures over 21°C. Covering materials were examined by Gowers (1984) using a single 1.5 m long section, with a 2 m × 1 m mesh panel in one side providing entry and ventilation. Seed yield of swedes was considerably lower under clear polythene than under white polythene and other shade materials. The temperature was slightly lower under white polythene, but the contributing factor was thought to be fly activity. On hot, sunny days, with the more diffuse light under white polythene the flies remained constantly active, whereas with clear polythene the flies sheltered in the shade and carried out little pollinating activity. MacFarlane Smith and Newbould (1988) did further work on covering material and fertilizer application in tunnel houses. They used mesh panels on both ends of polythene covered tunnels, and with 1.5 and 3.0 m tunnels found little difference between clear and white polythene, although white polythene was slightly better for swedes. A white, porous acrylic/polyester sheet was also tried that gave improved conditions and some better results with rape and kale, but little difference in yields for swedes. Condensation and disease may be improved under this material, but it is more expensive at about 2.5 times the cost.

When it comes to full-scale multiplications in the field, the isolation distance between crops is important. As a matter of course, breeders and maintainers want to keep sufficient distance between crops so that there is no cross-contamination. In the case of Government Certified seed, the isolation distances required between crops are usually specified, as is the length of time since any previous crop of a different cultivar. The distance specified may depend on the stage of multiplication the material is at and on the level of purity required. These regulations may vary somewhat; but in the UK and New Zealand the specified isolation distance between seed crops for swedes and turnips is 400 m for basic seed and only 200 m for final multiplication to produce certified seed.

Brassica pollen is relatively heavy and is usually considered not to be blown very far by wind. However, Timmons et al. (1995) found air-borne pollen density at 360 m was 10% of that at the field margin. Pollen counts of 0–22 grains/m^3 were observed 1.5 km from source fields and in sufficient numbers to allow seed set on emasculated bait plants.

The degree of outcrossing and isolation distance is mainly dependant on the foraging behavior of the main pollinators and the distance they can travel. Honey bees were regularly found to visit rape fields 3–4 km from the hive (Waddington et al., 1994). Honey bees are usually the most important pollinators of brassicas, but other

species can be important. A review of nectar production and bee foraging is given by Free (1970).

A knowledge of inter-crossing between the various species is important. As far as swede and turnips are concerned, they should be regarded as fully compatible with each other and with rape. The other species that is important for swede and turnip multiplications is *B. juncea*. Co-cultivation experiments under field conditions by Bing et al. (1996) found some contamination with *B. campestris* and from 3 to 5% contamination with *B. napus*; again it is assumed that results for turnip and swede would be comparable. Most other species are of no concern as far as standard cultivar production is concerned. For the production and maintenance of pure breeding stocks, it is advisable that these be produced under insect-proof netting. A review of factors affecting commercial brassica seed production has been written by Stewart (2002).

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