CHAPTER 13

Selection methods Part 5: Breeding clonally propagated crops

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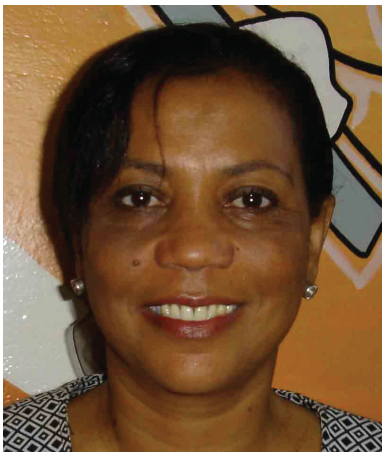


13.1 INTRODUCTION

The literature about participatory breeding of clonally propagated crops is very limited. This makes it difficult to write this chapter exclusively about how farmers have bred, how they breed—with or without support of scientists—and how they should breed clonally propagated crops. There are today clear definitions for participatory plant breeding (PPB), participatory variety selection (PVS) (see Chapter 9) and indigenous plant breeding (IPB; the selection process of farmers for more than 60 centuries). Breeding by scientists and economic entities has been redefined as formal plant breeding (FPB), consisting of breeding carried out on-station, linked with multi-location trials, and assisted by quantitative genetics, selection theory and biotechnology (with or without recombinant DNA technology). A variety developed through FPB is termed a modern variety (MV), in contrast to a farmer-bred variety (FV), which is developed by IPB. Especially in clonally propagated crops, FVs continue to dominate crop production in many developing world regions. Obviously FPB has not been so successful, because farmers decided to continue to grow FVs instead of adopting MVs (Friis-Hansen, 1992; Witcombe *et al.,* 1996). Definitions are helpful, providing a common term for the same technique or method. Here we need to bear in mind two points when considering such definitions and the issues associated with them:

- the every-day formula used to predict the response to selection (with all its extensions to several selection steps and traits) may well be the most useful tool given to breeding by statistics; and
- breeders have to adapt a crop to human needs and they must pay adequate attention to the needs of clients.

13.2 AN OVERVIEW OF CLONALLY PROPAGATED CROPS

What are clonally propagated crops?

Standard textbooks list a surprisingly large number of crops: all important root and tuber crops, many forage crops, nearly all types of fruit and wooden ornamentals, many cut flowers and pot plants, as well as forest trees. The definition of a clonally propagated crop is that the material to cultivate and maintain a variety is obtained by asexual reproduction, regardless of how different the plant material used for propagation is within and between species, encompassing tubers, roots, stem cuttings and corms, as well as asexually developed seeds (seeds developed without meiosis). It should be remembered that if crops such as maize (bred as an open-pollinated or hybrid crop) or beans (bred as a cross-fertilized, self-fertilized or hybrid crop) were to be propagated by stem cuttings or asexually developed seeds, they would be clonally propagated crops. In contrast, in breeding clonally propagated crops, the breeding techniques and methods that are usually associated with cross-fertilized and hybrid crops can be very useful. An example is the selection of parents in potato, cassava and sweet potato breeding, which are recombined in open-pollinated polycross nurseries to create new genetic variation. It is almost certain that techniques and methods from breeding cross-fertilized and hybrid crops will become much more important in the future of clone breeding.

What is the general principle in breeding clonally propagated crops?

It appears to be simple: to break the normal clonal propagation by a crossing step, and thus develop sexual seeds and genetic variation from which to select new clones. All propagation steps from the first to the last

selection step are again 'normal' asexual reproduction (Simmonds, 1979). Hence, the finally selected clone is genetically identical with the original seed plant from which the selected clone is derived. In other words, each seed plant is a potential variety. Roots and tubers, fruit and tree plant species have been used by human since long before the dawn of agriculture. They have been domesticated by IPB (Simmonds, 1979) and several made a substantial yield progress by FPB in some regions of the world. However, in other regions of the world there is not much yield progress, and in these regions there appears to be a clear need of PPB for progress. We wish to illustrate this by two examples: potato and sweet potato.

An example of the needs and requirements of clonally propagated varieties can be found in potato (*Solanum* spp.). There are about 200 wild potato species (Huamán and Ross, 1985). They usually contain glycoalkaloids, which give tubers a bitter taste and which are toxic when consumed in large quantities (Zitnak and Filadelfi, 1985). It is nearly certain that 100 to 130 centuries ago indigenous knowledge in the Andes and along the Pacific coast of South America was those sites where it was possible to collect wild potato tubers where species and mutants were growing that had low alkaloid content. Although these tubers were very small, the man, or more probably a woman, made life much easier by growing and maintaining desirable types by cloning close to their homes. This happened more than 8000 years ago, and most likely independently at several places (Ugent, Pozorski and Pozorski, 1982; Ugent, Dillehay and Ramirez, 1987). Those types were preferred that were easier to maintain, easier to harvest (shorter stolons) and had larger tubers compared to other types. The result was the domestication of *pitiquiña* (*Solanum stenotomum*), which was most probably selected from *S. leptophyes* or *S. canasense*. From the view-point of the knowledge of the twenty-first century it is not surprising that suddenly potato plants with larger leaves and larger tubers were found. Potato spontaneously changes its polyploidy level by unreduced gametes and recombination. Polyploid potatoes are more vigorous than their diploid ancestors. The result was the domesticated of polyploid *andigena* (*S. tuberosum* subsp. *andigena*). Andigena is the ancestor of the commercial potato in long-day temperate climates—the so-called *Irish potato* (*S. tuberosum* subsp. *tuberosum*) (Hawkes, 1979, 1981). This IPB of potato and introductions of FV of potato into the Northern Hemisphere changed the world both socio-economically and politically (Hobhouse, 1985).

Today, eight species of potato are still cultivated in the Andes, variously diploid, triploid, tetraploid and pentaploid:

(i) cultivated diploid potatoes are pitiquiña (*S. stenotomum)*, its close relatives *phureja* (*S. phureja)* and *limeña* (*S. goniocalyx*), and *ajanhuiri* (*S. ajanhuiri*), which evolved from interspecific recombination of diploid pitiquiña and the diploid wild potato species *S. megistacrolobum*;

(ii) cultivated triploid potatoes are *chaucha* (*Solanum* × *chaucha*), a hybrid between diploid pitiquiña and tetraploid andigena, and *rucki* (*Solanum* × *juzepczukii*), a hybrid between diploid pitiquiña and the tetraploid wild potato species *S. acaule;*

(iii) cultivated tetraploid potatoes are andigena and Irish potato; and finally

(iv) the cultivated pentaploid potato is a hybrid species (*Solanum* ×

curtilobum), which evolved between tetraploid andigena and triploid rucki, and unfortunately is also called rucki (Hawkes, 1981; NRC, 1989).

The andigena is the best known potato in the Andes (with about 2500 known FVs). It is cultivated in tropical mid-elevation valleys and mountainsides. The second most important potato is phureja, which is cultivated on the warm and moist eastern slopes of the Andes (with about 500 known FVs), followed by *limeña*, ajanhuri and rucki. Limeña or *papa amarilla* is grown in the temperate areas of the Andes and still achieves high market prices due to its taste and flavour. Ajanhuri and rucki are the most frost resistant cultivated potato species and cultivated up 4200 masl. The former is used as an insurance crop in cases where andigena fails due to unpredictable hail and frost (some ajanhuri varieties are bitter and must be processed). The latter are usually only eaten after having been processed into *chuño*, the famous storable food product of the Incas. Many of these potatoes have clearly better taste and flavour compared with what is considered potato in the Northern Hemisphere (Huamán 1983; NRC, 1989; De Haan, 2009). However, taste is a variable characteristic; it changes from person to person, from family to family, and from society to society. Moreover, many of these IPB potatoes are clearly superior in protein and micronutrient concentration in their tubers (pro-vitamin A, calcium, magnesium, iron and zinc) compared with MVs (Ochoa, 1990; Morris *et al.*, 2004; Burgos *et al.*, 2008), and are useful as genetic resources or directly as FVs to alleviate malnutrition in the mountain regions of the world.

The potato and the Andes were chosen as an example to give an impression of an aspect of breeding that is as least as important as taste, flavour and nutrient content, namely the importance of adaptation of a crop and its varieties to the local environment. They who know the Andes also know that is unrealistic to breed a widely adapted potato variety for this region of the world. Temperature, rainfall, soil conditions (including salinity and drought) and pest and disease pressures change from microclimate to microclimate from sea level at the Pacific coast up to 3500 to 4500 masl in the Andean highlands (mid-elevation valleys and plateaus), and again down into the warm tropics, where the Andes meet the Amazon. Breeding potatoes in this region of the world was and can only be successful by decentralization and with farmer participation (Johns and Keen, 1986; Gabriel and Torrez, 2000). Admittedly this is an extreme example, but such situations can be found in less extreme form in nearly all regions of the world. In the Southern and Northern Hemispheres, potatoes generally must be day-neutral; in South-west and Central Asia, potatoes must be very quick to mature, with a short crop duration of 80 to 90 days; in Europe and Northern America, more than 30 quality characteristics combine to determine tuber quality for market needs; and finally in the UK, a potato variety must be white fleshed, whereas in Germany it must be yellow fleshed, otherwise it is not eaten (CIP, 1984; Levy, 1984; Tarn *et al.*, 1992). An additional major factor for adaptation and acceptance of potato varieties is their tolerance and resistance to diseases and pest. In all temperate and moist climates, potato farmers have to fear Late blight (*Phytophthora infestans*), which is not important at temperature above 25°C, but then Early blight (*Alternaria solani*) takes over. In tropical lowlands, the farmer has to fear Bacterial wilt (*Pseudomonas solanacearum*), and in all warm dry regions

the potato crop can be lost because of Colorado beetle (*Leptinotaras decemlineata*) (CIP, 1977, 1980; Radcliffe, 1982; Rich, 1983).

A simpler example for the needs and requirements of clonally propagated varieties can be found in sweet potato (*Ipomoea batatas*). Sweet potato was domesticated in the Americas more or less during the same prehistoric period as the potato (O'Brien, 1972). The evolution of sweet potato was not as complex and diverse as that of potato. There are about 500 *Ipomoea* species, but only the *I. batatas* species was domesticated (Austin and Huamán, 1996). Again polyploidy was important. Sweet potato is hexaploid and its closest relative is *I. trifida* (di- and tetraploid). It is certain that sweet potato contains the *I. trifida* genome, but obviously it is not simply a multiple copy. Two-thirds of the sweet potato genome corresponds to the *I. trifida* genome and one-third to an ancestor very closely related to *I. trifida* (Shiotani and Kawase, 1989). Within diploid *I. trifida* accessions (seed families) it is also possible to find plants that form small storage roots (Daniel Reynoso, pers. comm.). However, sweet potato has been found in the ruins of the so-far oldest city in the Americas, *Caral* on the Pacific coast of central Peru (Solis, 2004), and the crop reached Pacific Polynesia and parts of South-East Asia (naturally or by early seafarers) before Columbus. It was primarily the Portuguese that introduced it into Europe, Africa, South Asia and East and South-East Asia (Yen, 1976).

Although the taste of sweet potato in FVs and MVs differ tremendously, two major types can be distinguished: (i) the orange-fleshed, moist, low dry matter (DM) and sweet type, which has a soft mouth feeling; and (ii) the white- or pale-yellow-fleshed, high DM, low-sweet or bland type, which has a dry mouth feeling. The first type, also called the dessert type, has extremely high pro-vitamin A concentrations (Huang, Tanudjaja and Lum, 1999) and a 50 g piece of fresh storage roots can meet the daily requirements of a pre-schooler (Low *et al.,* 2007). Moreover, sweet potatoes with high pro-vitamin A concentrations have high protein and mineral concentrations (Grüneberg, unpublished). In the United States of America, the dessert type is generally the desired sweet potato to meet market and consumer needs. In the Caribbean, low DM orange-fleshed sweet potatoes (OFSP) are consumed, but as a staple, so a dryer mouth feel and less sweet flavour is preferred. These white- or yellow-fleshed varieties are known as *bonitos* or *ricos* (Baynes, 1972). Along the Pacific coast of South America we observed that sweet potatoes are mainly pale orange fleshed and less sweet. However, locally, white- and purple-fleshed sweet potatoes are consumed, which clearly have different taste, texture and flavour compared to OFSP. In Brazil, the sweet potato storage roots must clearly have a high DM concentration (28 to 30% DM), and usually this is a white-fleshed sweet potato; however, locally, high DM OFSP can be found (Amauri Buso, pers. comm.).

The taste preferences in sub-Saharan Africa are similar to those in Brazil, perhaps because the Portuguese introduced the sweet potato into Africa. All FVs are nearly exclusively white- or yellow-fleshed and have high DM concentrations; however, a few pale- to medium-orange-fleshed FVs can be found with high DM concentrations (Tumwegamire, unpublished). These local OFSP FVs are very promising for alleviation of vitamin A deficiency in sub-Saharan Africa (e.g. FVs such as 'Ejumula', 'Carrot

C', 'Carrot Dar es Salaam' or 'Zambezi'). In eastern Africa, storage root DM contents must be >30 percent (Mwanga *et al.*, 2003). In southern Africa, storage DM concentration between 26 and 29 percent are accepted (Laurie Sunette, pers. comm.), whereas in West Africa, sweet potato must be non-sweet, very high in DM concentrations (between 30 and 35% DM) and with a texture and flavour tentatively similar to yam (*Dioscorea* spp.) (IITA, 1981). In contrast, in India, where sweet potato consumption has been very low in the past, today people prefer sweet potatoes with high DM, high sugar content, dark orange flesh and a storage root shape that is cylindrical but tapering at both ends (Sreekanth Attaluri, pers. comm.).

In addition to regional and local preferences for storage-root colour, DM, texture and taste, the acceptability of sweet potato varieties is mainly determined by pest and disease pressures. However, the number of pest and diseases in sweet potato are considerable lower than in potato. Generally, sweet potato varieties must have a certain degree of tolerance to Sweet potato virus disease (SPVD). The disease occurs after infection by two viruses: the Sweet potato feathery mottle virus (SPFMV) and the Sweet potato chlorotic stunt virus (SPCSV). The SPCSV is the more problematic component of SPVD, because yield losses due to SPFMV, in the absence of SPCSV co-infection, are low and SPFMV resistance in sweet potato breaks after the plant is infected by SPCSV (Gibson *et al.*, 1998; Karyeija *et al.*, 2000). SPVD often causes serious yield losses in high-virus-pressure zones of sub-Saharan Africa, and American OFSPs have failed in many regions of sub-Saharan Africa due to insufficient SPVD tolerance. Although the virus pressure of SPVD along the Pacific coast of South America is not extreme, farmers have not adopted MVs (e.g. cv. INA100, which is a high yielding OFSP and fits consumer needs very well), because of insufficient SPVD tolerance. Farmers became disappointed with new MVs and after a few growing seasons returned to FVs such as cv. Jonathan and cv. Huambachero. OFSPs from the Americas with elevated DM (e.g. cv. Jonathan) are partially successful in southern Africa, and in south-west and central Asia, provided that weevil pressure is not extreme. Weevil damage is associated with drought-prone regions (Central and South America, sub-Saharan Africa and south-west and central Asia); however, weevil species differ: *Cylas formicarius* in all parts of the tropics, *C. puncticollis* additionally in Africa, and *Euscepes postfasciatus* in the West Indies. On-station and farmers' field experiments show that there are significant differences in weevil tolerance among sweet potato genotypes (Hahn and Leuschner, 1981), but this tolerance appears to be less pronounced or inexistent on-farm. At the same time, farmers in drought-prone regions of Malawi want sweet potatoes in which storage roots are formed deep in the soil and which are clearly tapering at the top, because they associate this with less weevil damage (Ibrahim Benesi, pers. comm.). Moreover, latex in the storage root skin has been associated with less weevil damage by farmers, and varieties like Santo Amaro from Brazil clearly have considerably less weevil damage than other sweet potato varieties (Rafael Vasquez Martinez, pers. comm.).

The International Potato Center (CIP) is promoting OFSPs to alleviate vitamin A deficiency in the world (Low *et al.*, 2007; Pfeiffer and McClafferty, 2007). However, introductions from the Americas failed in the high-SPVD-pressure zone of eastern Africa (as did the FV Jonathan). To a

certain extent this was associated with the storage root flesh colour and taste. At the same time, local African OFSP FVs, such as Ejumula, Carrot C, Carrot Dar es Salaam and Zambezi, and locally-bred OFSP MVs, such as NASPOT5 (Mwanga *et al.,* 2003), have been adopted after awareness campaigns on the vitamin A deficiency problem (Regina Kapinga, pers. comm.). For this reason, CIP puts emphasis on decentralized sweet potato breeding, and has recently started to recommend incorporation of at least one participatory selection step in the breeding process.

In sweet potato breeding for human consumption, decentralization is characterized by a general overall goal: that of developing more OFSP varieties that meet local needs and consumer preferences, to alleviate hunger and malnutrition and to improve public health. The emphasis is on organizing OFSP breeding in eastern and southern Africa, with national OFSP breeding programmes starting recently in West Africa (Ghana and Nigeria) and south-west and central Asia (India, Bangladesh and Sri Lanka). Breeding is almost exclusively carried out by national agricultural research system (NARS) breeders and on NARS breeding stations, with currently 12 NARS and two sweet potato breeders from the CGIAR system involved. NARS breeders are provided with funds for parental recombination and to consider the quality trait of storage root flesh colour in the breeding process. Main emphasis in breeding is given to: (1) material exchange at the seed and clone level, (2) exchange of information, knowledge and results from breeding trials by annual meetings, reports and backstop visits, and (3) a sweet potato breeding research and training build up on the needs shaped among discussions between NARS and CGIAR breeders. This has resulted in an additional aim to build up regional platforms for sweet potato breeding in eastern, southern and West Africa, with a focus on dual purpose OFSP (human consumption and animal feed), drought tolerant OFSP, and non-sweet high DM OFSP. PPB has so far mainly been a research component in the organization of sweet potato breeding.

There are strong indications that PPB in early selection steps of the sweet potato breeding process increases the efficiency and minimizes the risk of making wrong selection decisions. In contrast to PPB, PVS is tentatively a form of on-farm evaluation (in the frame of a larger number of multi-location trials) and cannot be as efficient as PPB, because there is considerably less genetic variation, and, for highly heritable traits, there is nearly no genetic variation at later breeding stages among clones. Not surprisingly, akin to the role of IPB in potato crop evolution, it has been shown that farmers have the ability to manage selection stages in sweet potato (Gibson *et al.,* 2008). This is consistent with results for potato (Gabriel and Torrez, 2000) and cassava (Manu-Aduening *et al.,* 2006). Farmer selections are mainly made by visual screening. This includes quality characteristics, diseases and pests, as well as the growth type, which is to a certain extent associated with drought adaptation in sweet potato (see below on selection in early breeding stages). Most importantly, farmers use more criteria and characters to select sweet potato clones than do breeders in FPB (Gibson *et al.,* 2008). In such a situation, the risk of FPB is to ignore characters that are important for good overall performance of a clonal variety. However, in the study of Gibson *et al.* (2008) in three provinces in Uganda, the most important characteristics for selection by farmers in early selection stages were common to those used by

breeders, as were their relative weighting of characters, namely: (i) good root yield and big roots > (ii) SPVD tolerance or resistance > (iii) tolerance to drought, attractive root colour prior to cooking, straight root shape and orange- or yellow-flesh storage-root colour, and finally > (iv) tolerance to weevils. Characters of storage roots after cooking were not determined. It should be noted that in later selection stages (FVS) the priority list of farmers or relative character weights changed, namely: (i) good root yield, big roots, drought tolerance, sweet and mealy roots after cooking > (ii) early root maturity, continuous root yield for piecemeal harvesting, and weevil tolerance > (iii) long root storage in the soil, extensive foliage, tolerance to caterpillars (*Acraea acerata*), marketability, attractive colour of storage roots prior to cooking, and non-fibrous roots after cooking > (iv) followed by a group of characteristics with very low weights, such as good root yield in poor soils, good vine establishment, tolerance to rats and other vertebrates, non-sappy and no loss of taste in storage roots prior to cooking, soft texture, nice looking at the table, nice flavour and easy or quick to cook storage roots. For some characters (mainly biotic pressures, i.e. SPVD and weevil tolerance; Gibson *et al.,* 2008) there were clearly different weights given to characters in different provinces, which might reflect local biotic challenge. Moreover, farmers used more attributes (51 attributes) than scientists and breeders (11 attributes) to describe and distinguish varieties. To what extent this is important is not clear; however, it might show the importance to farmers of distinguishing varieties.

To summarize:

(i) not surprisingly, farmers have the ability to select successfully both in the early and later breeding stages of a breeding programme (Gabriel and Torrez, 2000; Manu-Aduening *et al.,* 2006; Gibson *et al.,* 2008);

(ii) selection by farmers, mainly by visual screening, is more efficient in earlier stages than in later stages of the breeding programme, which can also be explained by the larger genetic variation in early selection stages compared with later stages in breeding clonally propagated crops; and

(iii) so far, selection by farmers at early selection stages has only been applied to a sample of the genetic variation generated by FPB in crossing programmes and it must be more efficient to expose the full genetic variation to farmer selection in the breeding process.

In Sections 13.2 and 13.3 we suggest how this can be done in a cost-efficient way and without losing time in the breeding process. However, doubts remain as to whether farmers can efficiently use and treat large amounts of true seeds and true seed plants, which often appear in quite different amounts per cross combination and have quite different performance compared with plants grown from vegetative plant parts. It might be more useful that plant breeders germinate seeds and multiply for each family a reasonable numbers of clones so that farmers can select clones in small plots comprising a few plants (2 to 4 per genotype). A further advantage of this is that the breeder can use the frequency of selected clones per family by the farmer as additional information to identify appropriate parents for recombination. However, we think that the selection of parents in breeding clonally propagated crops should have a participatory component, but should be mainly carried out by the breeder due to the genetics (see Section 13.2) and statistics

(see Section 13.5) involved in appropriate choices of parents in breeding clonally propagated crops.

To consider the range of needs, preferences and adaptation requirements for the large number of clonally propagated crops is out of scope in this chapter. Here we want to give the principles of breeding clonally propagated crops and how PPB can be carried out or linked into these breeding programmes. The breeding objectives and methods will be considered for four agricultural crops in more detail at the end of this chapter, namely: potato, sweet potato, cassava, and banana or plantain. Table 13.1 gives the plant parts used for propagation, the world production and the area harvested, as well as the polyploid level of the most important clonally propagated crops in agriculture. Obviously, quality characteristics determined by consumer preferences and market needs are key characteristics for breeding clonally propagated crops, because many of these are eaten fresh, or are only boiled or roasted, and when they are processed this is often carried out at the household level. Exceptions are sugar cane, fruit crops used for the juice industry, and to certain extent root and tuber crops (potato, cassava and sweet potato) when they are used for the starch, alcohol or biofuel industries. In resource-poor environments, yields and yield stability with low input are a priority, in addition to consumer acceptability. As has been mentioned above, a major factor that determines yields, yield stability and adaptation are pests and diseases. The most important pests and diseases of important clonally propagated crops in agriculture by eco-geographical region are given in Table 13.2, together with the most important quality characteristics.

Most clonally propagated crops are polyploid (Table 13.1). An exception is cassava, which can be considered as a polyploid behaving like a diploid (see below). Polyploidy is an important aspect in crop

TABLE 13.1
Data on the 11 most important clonally propagated crops on a global basis

Species	Planting material	World production†	Area harvested†	Polyploidy
Potato (*Solanum tuberosum*)	Sprout tubers	315 $\times 10^6$ t	18.8 $\times 10^6$ ha	2x, 3x, 4x, 5x
Cassava (*Manihot esculenta*)	Hardwood cuttings	226 $\times 10^6$ t	18.6 $\times 10^6$ ha	2x
Sweet potato (*Ipomoea batatas*)	Sprout cuttings	124 $\times 10^6$ t	9 $\times 10^6$ ha	6x
Yam (*Dioscorea* spp.)	Root tubers	51 $\times 10^6$ t	4.6 $\times 10^6$ ha	3x–10x
Taro (*Colocasia esculenta*)	Corms	12 $\times 10^6$ t	1.8 $\times 10^6$ ha	4x
Sugar cane (*Saccharum officinarum*)	Cane stalks	194 $\times 10^6$ t ‡	20.4 $\times 10^6$ ha	8x
Banana and Plantain (*Musa* × *paradisiaca*)	Corms	105 $\times 10^6$ t	9.6 $\times 10^6$ ha	3x
Citrus fruit (*Citrus* spp.)	Bud stick grafting on rootstocks	89 $\times 10^6$ t	5.6 $\times 10^6$ ha	2x, 3x+1, 4x-3
Grapes (*Vitis vinifera*)	Hardwood cuttings	69 $\times 10^6$ t	7.4 $\times 10^6$ ha	6x
Apple (*Malus pumila*)	Bud stick grafting on rootstocks	64 $\times 10^6$ t	4.8 $\times 10^6$ ha	2x, 3x
Strawberry (*Fragaria grandiflora*)	Adventitious shoots	4 $\times 10^6$ t	0.26 $\times 10^6$ ha	8x

NOTES: † FAOStat 2006 at faostat.fao.org, ‡ Sucrose production.

TABLE 13.2
Quality characteristics, pests and diseases by production zone of the 11 most important clonally propagated crops in agriculture and horticulture

Major production zones	Quality characteristics	Pest and diseases
	Potato (*Solanum tuberosum*)	
Tropical highlands	Various fresh consumption traits, high iron and zinc contents, adaptation to various micro-climates	Late blight (*Phytophthora infestans*), cutworms (*Agrotis* spp.), potato tuber moth (*Phthorimaea* spp.)
Tropical lowlands	More uniform fresh consumption traits, high iron and zinc contents, extremely short crop duration (<80 days)	Bacterial wilt (*Pseudomonas* spp.), Early blight (*Alternaria solani*), Root-knot nematode (*Meloidogyne* spp.), viruses (Potato leaf roll virus (PLRV), Potato virus Y (PVY), etc.), year round aphid pressure
Temperate zones	Various fresh consumption traits, high starch for industrial use, various processing traits (chips, French fries)	Late blight, cyst-forming nematodes, (*Globodera* spp.), potato virus diseases (PLRV, PVY, PVX, etc.)
	Cassava (*Manihot esculenta*)	
Humid tropics	Cooking quality, elevated provitamin A content for human consumption with low HCN content, high DM for industrial uses	Bacterial blight (*Xanthomonas axonopodis*) in Asia, Africa and the Americas, Frogskin disease (CFSD) in the Americas
Drought-prone tropics	Cooking and processing (fried cassava, gari, fufu) quality, elevated provitamin A content for human consumption with low HCN content, high DM for industrial uses	African cassava mosaic (CMD) virus and Cassava brown streak disease (CBSD) in Africa, Green mite (*Mononychellus tanajoa*) and mealybugs (*Phenacoccus* spp.) in Africa and the Americas
	Sweet potato (*Ipomoea batatas*)	
Humid tropics	High DM WFSP and OFSP	Extreme Sweet potato virus disease (SPVD), especially in eastern Africa
Drought-prone tropics	Elevated DM WFSP and OFSP, and clearly non-sweet in West Africa	Sweet potato weevils (*Cylas* spp.) and SPVD to a lesser extent
Tropical highlands	Elevated DM	*Alternaria* spp. and SPVD to a lesser extent
Temperate zones	OFSP with low DM and WFSP with high DM (both with medium sugar content)	Root-knot nematode (*Meloidogyne* spp.) and SPVD to a lesser extent
	Yam (*Dioscorea* spp.)	
Humid tropics	Thirteen species with regional importance (main species *D. rotundata*), majority in wet hot tropics, but *D. abyssinica*, *D. alata* and *D. esculenta* also in dryer regions due to dormancy of tubers; growing time and taste varies extremely among species (some are poisonous and must be cooked)	Yam tuber beetles (*Heteroligus* spp.) and Anthracnose (*Colletotrichum* spp.), especially in West Africa, Yam nematode (*Scutellonema bradys*), Root-knot nematode (*Meloidogyne* spp.) and Shoe string virus disease
	Taro (*Colocasia esculenta*)	
Humid tropics	*Colocasia* cultivar groups: (1) one large corm with few cormels; and (2) several small cormels. Genotypes have very different shelf lives (dormancy period) and some require excessive processing before edible	Corm and root rots (caused by *Pythium* spp., *Phytophthora* spp., *Rhizoctonia* spp. and *Erwinia* spp.) and Dasheen mosaic virus (DMV) across world regions, Taro blight (*Phytophthora colocasiae*) and Taro beetle (*Papuana* spp.), especially in the South Pacific
	Sugar cane (*Saccharum officinarum*)	
All regions	Weight of canes, sugar content, juice purity, short or long vegetative times and adaptation to photoperiod (non-flowering)	In the past, virus diseases were most important; today they play a subordinate role due to resistance breeding and virus-free planting materials Pineapple disease (*Ceratocystis paradoxa*), Red rot (*Colletotrichum falcatum*), Smut (*Ustilago citaminea*), Shoot and Internode Borer (*Chilo* spp.) in nearly all regions
Humid tropics		Yellow leaf spot (*Cercospora* spp.), Scale insect (*Melanaspis glomerata*), Pyrilla (*Pyrilla purpusilla*)
Drought-prone tropics and subtropics		Eye spot (*Drechslera sacchari*), Whitefly (*Aleurolobus barodensis*)
Tropical highlands		Leaf scald (*Xanthomonas albileneans*), Wilt (*Cephalosporium sacchari*)

Major production zones	Quality characteristics	Pest and diseases
	Banana and plantain (*Musa x paradisiaca*)	
Humid tropics and subtropics	Bananas have a lower DM and higher sugar contents (very narrow genetic variation in triploid gene pool – ca. 30 cvs.) compared with high DM and starchy plantains (larger genetic variation in triploid gene pool – ca. 125 cvs.). Plantains are important staples in Central Africa and some parts of South America.	Banana wilt (*Fusarium oxysporum*) especially in the Americas, Yellow sigatoka (*Mycosphaerella musicola*), Black sigatoka (*M. fijiensis*) especially in Asia, Moko disease (*Pseudomonas solanacearum*), Bunchy top virus, nematodes such as *Radopholus similes*, banana root borer (*Cosmopolites sordidus*)
	Citrus fruit (*Citrus* spp.). Cultivated citrus may be derived from as few as four species: Key Lime (*C. aurantifolia*), Pomelo (*C. maxima*), Citron (*C. medica*) and Mandarin (*C. reticulata*). All other "species" are hybrids	
All regions	Very different tastes and fruit sizes (oranges — ca. 1100 cvs.; mandarins, lemons, pomelo). Citrus trees hybridize very readily and new hybrids easily maintained by apomixis.	Strong rootstock influence (*C. jambhiri, C. reshni, Poncirus trifoliata*) in adaptation to cold and resistance to *Phytophthora* root rot and virus diseases such as Tristeza, Porosis and Exocortis
Subtropics		Citrus canker (*Xanthomonas citri*), Foot rot (*Phytophthora* spp.), Melanose (*Diaporthe citri*), Blue and green mould (*Penicillium* spp.), Tristeza virus, nematodes such as *Tylenchulus semipenetrans*, fruit fly (*Bactrocera* spp.)
Drought-prone tropics		Foot rot (*Phytophthora* spp.), Gummosis (*Phytophthora* spp.), Citrus scab (*Elsinoe fawcetti*), Tristeza virus, Porosis viruses, fruit fly (*Bactrocera* spp.), citrus psyllid (*Diaphorina citri*), moth species such as *Ophideres, Sphingomorpha,* etc.
	Grapes (*Vitis vinifera*) North American species: *V. aestivalis, V. labrusca, V. rotundifolia*	
Temperate zones	The North American species are of interest for the summer rainfall regions in the tropics because of their disease resistance and minimal chilling requirement – especially in crosses with *V. vinifera* (better taste, better texture of berries)	Bunch rot (*Botrytis cinerea*), Downy mildew (*Peronospora sparsa*), Powdery mildew (*Erysiphe necator*), vine moths (*Eupoecilia ambiguella, Lobesia botrana*), eriophyid mite (*Calepitrimerus vitis*)
Drought-prone tropics and subtropics		Downy mildew, powdery mildew, Anthracnose (*Elsinoe ampelia*), beetles such as *Popillia japonica*, thrips (*Scirtothrips dorsalis, Thrips hawaiiensis* and *Rhipiphorothrips cruentatus*), grape root borer (*Vitacea polistiformis*), bugs such as *Lygocoris inconspicuous*, Grape mealybug (*Pseudococcus maritimus*)
	Apple (*Malus pumila*)	
Temperate zones	There are large differences in vernalization need among cultivars and several can be grown very successful in Mediterranean climates. Some cultivars in higher places in the equatorial region if the leaves are removed before beginning of bud dormancy (stripping off, or chemical defoliation)	Fireblight (*Erwina amylovora*), Apple rust (*Gymnosporangium* spp.), Apple scab (*Venturia inaequalis*), Plum curculio (*Conotrachelus nenuphar*), Apple maggot (*Rhagoletis pomonella*), Codling moth (*Cydia pomonella*)
Subtropics		Apple scab, Powdery mildew (*Podosphaera leucotricha*), Crown rot (*Phytophthora cactorum*), Apple crown gall (*Agrobacterium tumefaciens*), Bitter rot (*Glomerella cingulata*), root rots (*Phytophthora* spp.), Woolly apple aphid (*Eriosoma lanigerum*), Apple sawfly (*Hoplocampa testudinea*), Green apple aphid (*Aphis pomi*)
Tropical Highlands		Fireblight, Crown rot, Woolly apple aphid
	Strawberry (*Fragaria ×ananassa*)	
Subtropics and temperate zones	Ancient cross of *F. virginiana* (8x) from eastern North America and *F. chiloensis* (8x) from Chile	Grey mould (*Botrytis cinerea*), Powdery mildew (*Sphaerotheca macularis*), Strawberry blossom weevil (*Anthonomus rubi*), European tarnished plant bug (*Lygus rugulipennis*)

Sources: Kranz, 1978; Rehm and Espig, 1984; Mandal, 2006.

evolution (as we have already seen in potato) and has important consequences in breeding clonally propagated crops. It is important to note that all 'breeding lines' or varieties of clonally propagated crops are homogenous (clone lines and varieties are genetically fixed and as homogenous as non-segregating breeding lines or hybrids from breeding self-fertilized or hybrid crops). The homogenous clones are exact genetic copies of their mother plants, if mutations are ignored. This is more or less obvious in potato, cassava or sweet potato field plots, or in fruit and tree plantations, provided no genotype mixtures are observed. What is not directly obvious to an observer is that each clone line or variety in the field or plantation is a highly heterozygous hybrid (clone lines and varieties are highly heterozygous hybrids comparable with heterozygous hybrids developed in hybrid breeding). It should be noted that due to polyploidy, clonally propagated crops are usually more heterozygous than those diploid crops in which hybrid breeding is applied. The difference between "clone hybrids" and "seed hybrids" such as maize is that the first are propagated by asexual reproduction and the latter are developed by sexual reproduction.

13.3 POLYPLOIDY

General knowledge about polyploidy is required to get an understanding of breeding clonally propagated crops. Polyploidy has a strong effect on the performance of clones as well as the parent–offspring correlations. A polyploid genotype contains more than two homologous sets of chromosomes in the nucleus of somatic cells. According to the number of chromosome sets in the nucleus we distinguish different polyploid types: triploid (three sets; 3x), tetraploid (four sets; 4x), pentaploids (five sets; 5x), hexaploids (six sets; 6x) (Tate, Soltis and Soltis, 2005) – and species with even higher polyploidy levels are known (Table 13.1). The haploid level (one set; 1x) does not occur as a normal stage in the life cycle of a crop. However, haploid plants occur by spontaneous mutations, wide crosses and anther culture (e.g. diploids are developed from tetraploid potatoes by pollination with specific clones of *S. phureja* and haploids by anther culture). Haploids are occasionally used in FPB of clonally propagated crops, especially potatoes (Hermsen and Verdenius, 1973; Wenzel and Foroughi-Wehr, 1984). In crop evolution different polyploidy levels originated from genome mutations and by hybridization between very closely related species. Autopolyploids and allopolyploids include wheat (*Triticum durum* and *T. aestivum*), canola (*Brassica napus*) and cotton (*Gossypium* spp.), and nearly all clonally propagated species are autopolyploids. The homologous chromosomes in autopolyploids are similar enough that multivalents of the same homologous chromosomes are formed. Doubling of chromosomes occurs if the spindle poles are not developed when the nucleus is dividing chromosomes in mitotic and meiotic cell division. There are several possible outcomes of abnormal meiosis. Natural formation of 2n gametes was most important in evolution of cultivated *Solanum* species, and the formation is mainly determined by one recessive gene (Watanabe and Peloquin, 1988), so this character can be used in breeding potato. Polysomic inheritance is sensitive to disorders and therefore autopolyploids often have reduced fertility, and occasionally they are completely infertile and propagate only asexually.

Multiple chromosome sets occur spontaneously in nature from 2n gametes and can be induced artificially by colchicine (an alkaloid of autumn crocus, *Colchicum*

autumnale). In the case of diploid plants (2x), this leads to tetraploid plants (4x). An example is the evolution of *S. tuberosum* spp. *andigena* (4x) from cultivated *S. stenotomum* (2x) (Hawkes, 1979). Hybridization of diploid and tetraploid plants forms triploid plants (3x) and by a further doubling of chromosomes hexaploid plants (6x) are formed. An example is hexaploid *I. batatas*, which probably evolved by genome mutation and hybridization, because the sweet potato genome (6x) consists of two closely related sets of chromosomes ($B_1B_1B_2B_2$), of which one is duplicated ($B_1B_1B_2B_2B_2B_2$) (Shiotani and Kawase, 1989; Austin and Huamán, 1996). Many important clonally propagated crops are triploids (3x), such as the economically important genotypes of banana and plantain (*Musa* × *paradisiaca*). The triploid banana and plantain groups evolved in two different ways by genome mutation and hybridization: in the case of banana, from one diploid wild species *M. acuminata* (AA) to form the triploid banana group (AAA), and in the case of plantain, from two diploid wild species: *M. acuminata* (AA) and *A. balbisiana* (BB), forming the triploid plantain group (AAB) (Simmonds, 1962). Many FVs in the banana and plantain group evolved only by somatic mutation, because triploid banana and plantain are infertile. However, breeding triploids is possible by working with two gene pools, one which is diploid and the other which is tetraploid, such as using gene pools of *M. acuminata* and *M. balbisiana* on a diploid and tetraploid polyploidy level to develop new triploid banana and plantain varieties.

In contrast to autopolyploids, the genome in allopolyploids differs so much between hybridized species that only bivalents of homologous chromosomes of the parental genomes can be formed. The breeding behaviour of allopolyploids is very similar to diploids. The formation of bivalents or multivalents appears to be genetically determined, e.g. without the gene (ph) on chromosome 5B in polyploid wheat (*Triticum durum* and *T. aestivum*), homologous chromosomes form multivalents. This gene is relatively new in the evolution of wheat (Dhaliwal, 1977). Among clonally propagated crops, cassava (*Manihot esculenta*) is considered to be a diploidized allotetraploid, which also was formed recently in the evolution of plants (Nassar, 2000). Indications for this are: (i) the high chromosome number (2x = 36) of all *Manihot* spp. (other *Euphorbia* have basic chromosome numbers within the range of six to eleven); (ii) natural hybridization occurs among *Manihot* species and crossing barriers appear to be weak; and (iii) *M. esculenta* shows meiotic irregularities, such as terminal non-pairing, multivalent associations and repetition of chromosome types, which results in low fertility of parental combinations.

Polyploid plants usually have larger plant cells, larger and stronger plant organs, greater height and increased biomass production. In nature, polyploid plants tend to succeed in new habitats. In breeding, the tallest and best thriving plants are selected, so that, unintentionally, many crops have been bred to a higher level of ploidy. However, as chromosome number increases, the increase in biomass production becomes successively less, and production decreases above a specific optimum biomass. This optimum differs from species to species. In autopolyploids, this advantage of increased vigour is associated with the disadvantage of increased meiotic disorders during the formation of multivalents. This is the reason why the harvest in many important autopolyploid

crops is represented by vegetative plant parts. Most polyploids display heterosis relative to their parental species, as well as relative to inter-gene-pool crossings within a species. A polyploid population contains three, four, five, six or more alleles at each locus. Hence, considerably more effects due to dominance and epistasis are possible, and the genetic variation due to dominance and epistatic effects in polyploidy crops is very large compared with the genetic variation caused by dominance and epistatic effects in diploid crops. For this reason, the performance of clonally propagated crops is mainly determined by heterosis. Usually in breeding of clonally propagated crops, an F_1 clone hybrid is crossed with another F_1 clone hybrid, so that the offspring shows extremely extensive segregation. In parent–offspring studies it is possible to determine mid-parent and mid-offspring heterosis, as well as the best-parent mid-offspring heterosis (similar to the assessment of heterosis in a hybrid breeding programme of diploid crops—see Chapter 11 in this volume). In polyploids, more than one allele per locus is transferred in gametes to the next generation, so that, in contrast to diploids, the genetic variation due to dominance determines the response to selection in population improvement as long as the population is not in equilibrium (after recombining parental material in controlled crossings, a population is usually not in equilibrium). In tetraploid potato populations that are not in equilibrium, one-third of the dominance variance is exploitable for selection progress when selection takes place on the female and male sides (Wricke and Weber, 1986; Gallais, 2004). The exploitation of the dominance variance in population improvement, in combination with the selection for different levels of ploidy (using the inheritance of 2n gametes), has been proposed for breeding tetraploid potatoes (Ortiz, 1998). Polyploidy, heterozygosity and heterosis make the selection of good parents in population improvement of clonally propagated crops very difficult. A good parent generates large genetic variation around a high family mean. Cross-prediction and inter-gene-pool crosses are very important in population improvement of clonally propagated crops. This aspect of clonal breeding is often neglected and this might be the reason for the low level of breeding progress in many clonally propagated crops. In contrast to population improvement (selection of superior parents – see Section 13.7 below), selection within a given genetic variation for variety development is relatively easy in clonally propagated crops (discard inferior material). All the genetic advantages of clonally propagated crops can be used for variety development, and the genotype finally released is in the hands of the breeder immediately after the initial crossings.

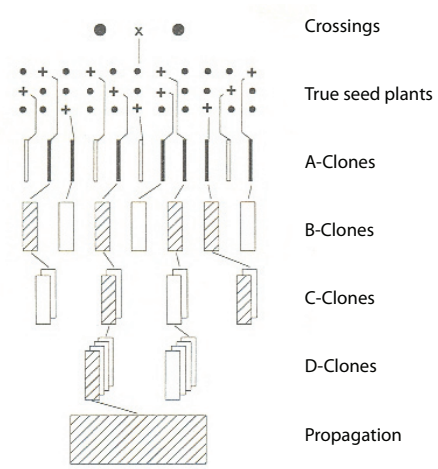
A clonally propagated crop that has no, or nearly no, sexual reproduction is close to a dead end in evolution and breeding. Genetic variability can only accumulate by mutations. However, this source of new variation has often been used to find enhanced types of fruits and ornamentals (van Harten and Broertjes, 1988). Nevertheless, the main source of generating new variation in clonally propagated crops is sexual reproduction. Owing to a more or less regular meiosis in polyploids with an even number of chromosome sets (4x or 6x), sexual seed production and generation of new genotypes is possible. Nearly all clonally propagated crops, e.g. potato, sweet potato and cassava, are cross-fertilized crops in combination with self-incompatibility. Incompatibility alleles are the reason why specifically sought after

cross combinations are difficult to realize, and seeds from controlled crossings can have a very high value in clonally propagated crops.

13.4 GENERAL BREEDING SCHEMES

The general principle of breeding clonally propagated crops is to break normal clonal propagation by introducing a crossing step, which culminates in sexual seed production and genetic variation. After the genetic recombination, all subsequent propagation steps are asexual in nature and done by clonal propagation. Nearly all clonally propagated crops are polyploid and cross-fertilized species. A more or less regular meiosis is possible in polyploids, if the number of chromosome sets is even, as in tetraploids (4x) and hexaploids (6x). The parents in cross combinations are highly heterozygous hybrids, with the exceptions of inbreeding lines generated by self-fertilizations or doubled-haploid and doubled-triploid production. The populations developed from seeds are again formed by very different and highly heterozygous genotypes, which do not exchange genetic material. Each seed plant grown in the so-called seedling nursery can be considered a potentially new variety. This is the basis for selection. The selection between clones is described most often in plant breeding textbooks as a process conducted in several steps (Figure 13.1).

FIGURE 13.1
The general breeding scheme for clonally propagated crops



Source: modified from Becker, 1993.

The breeding scheme illustrated in Figure 13.1 is straightforward and it is most often interpreted as requiring clonally propagated crops to be bred sequentially in several steps over several years. The diagram implies that there are two parents being crossed, followed by five subsequent selection steps in time (one selection step in seed plants and four selection steps in clone plants). This is misleading. First the breeder must work with many parents (further details about number and size of crosses are given in Section 13.5). Second, there is no further genetic development in clonally propagated crops as one moves between selection steps. The selected D-clone in Figure 13.1 is genetically identical to the true seed plant the selected D-clone derives from. Provided that the true seed plant can be cloned in large quantities, it is theoretically possible to test the population with adequate accuracy in the first year to select the 'best' genotype.

Selection among true seed plants is made for tolerance and resistance to pathogens. However, often no selection between plants grown from seed is made by the breeder. Nevertheless, natural selection occurs during germination, and should not be completely avoided, because genotypes difficult to germinate delay the breeding programme. The main reasons for no selection in the seedling nursery are: (i) plants

grown from seed often differ considerably from plants raised from vegetative planting material, (ii) the plants raised from seeds are normally grown in pots in greenhouses, and for most traits this is not representative of field conditions, and (iii) a single plant evaluation is usually not appropriate, with the exceptions of susceptibility to highly aggressive pathogens. In field crops, an important factor is interplant competition. A genotype must be tested under conditions that simulate the field conditions in practice. For this reason several plants of each clone are tested in plots in blocks under homogenous field conditions. The aim is an unbiased comparison of genotypes within blocks. The number of plants per plot and the plot size depends on the crop as well as the breeding stage. Fruit trees and perennial shrubs are tested in larger plots with fewer plants than potatoes, and these again are tested in larger plots than cut flowers. Early selection stages (A-clones and B-clones) are tested in smaller plots than later selection stages (C-clones and D-clones). The amount of planting material at each breeding stage is determined by the propagation coefficient of the crop. For example potato has, among clonally propagated crops, a very low propagation coefficient of about 10, whereas sweet potato has a relatively high propagation coefficient of between 30 and 90 (depending on the field propagation method used). This is one factor why potato breeding is relatively slow (about 8 to 10 years from cross to variety release).

Breeders do not breed for a single environment; they breed for a range of environments. Hence, the field evaluations must simulate the range of target environments. For this reason, and depending on the propagation coefficient, the clones are tested in plots, in homogenous blocks, at several locations and for several years. It is obvious that the wide range of quality preferences and the numerous pests and diseases in each clonally propagated crop and their interaction with genotypes justifies decentralization and participatory approaches. However, the better simulation of the final target environment realized with FPB justifies a stronger PPB approach. Many advocate PPB because the stress and marginal field conditions of resource-poor farmers are not adequately simulated by FPB (see also below). In this context the two clear advantages of breeding clonally propagated crops should be pointed out: (i) no genetic changes occur in genotypes after seed has been produced; and (ii) the total genetic variation of genotypes (comprising the genetic variances due to additive, dominance and epistatic effects) can be exploited by selection. For these reasons, only the genotype×environment (G×E) interaction and the plot error must be considered (and reduced by testing in several environments) to identify the best clone.

13.4.1 Early breeding stages and PPB

In the general breeding scheme (Figure 13.1) each surviving seed plant is cloned to be raised as A-clones in observation plots (visual screening of general clone performance), or evaluation plots (recording of data on specific traits of each clone). Figure 13.2 shows the planting of sweet potato A-clones. The plot size of A-clones is usually a single-row plot comprising 3 to 5 plants. The trial is conducted with no replications. It is open to discussion whether A-clones should be evaluated at two locations. Selection theory results show that it is nearly always the best resource allocation to test as many clones as possible at one location, without replications (Wricke and Weber, 1986). Many breeders use only one location at the early breeding stages

FIGURE 13.2
Planting early selection stages of sweet potato for the accelerated breeding scheme in San Ramon (one of four locations)

due to the restrictions of the propagation coefficient and breeding budget. However, there are several reasons to test A-clones at two locations: (i) a trial at one location can be lost (e.g. extreme weather conditions) and then a full breeding step and population is lost; (ii) trials at only one location are of little value (the G×E interaction cannot be separated from the genotypic effect); and (iii) the response to selection is still very close to the optimum in a wide range of scenarios, including the scenarios where A-clones are tested at two locations (Grüneberg *et al.*, 2004). Moreover, information from contrasting environments can be combined if the breeder tests A-clones at two locations. For example, clones that clearly fail in a marginal or hot-spot environment (for drought, salinity, biotic challenge, etc.) can be discarded, or at least considered with caution in good environments.

A-clones are only selected for highly heritable traits such as general performance (growth type; tuber, root or fruit size, shape and colour), resistance to pests and diseases, harvest index, dry matter and nutritional quality. Breeders nearly always conduct a visual selection at the A-clone breeding stage. However, it can be questioned if the A-clones selected by the breeder match farmer needs and would be selected by farmers. With two locations, one location can be easily evaluated by farmers in a PPB approach, while the other location is used by the breeder. It should

be noted that visual selection of general performance can also be an efficient indirect selection for yield. In sweet potato, we observed among several thousand A-clones grown in 1 m-row plots at three locations a heritability for yield of about $h^2 \approx 0.4$ (harvesting and recording all A-clones for yield at all three locations). It was considered as 'useless work', because a visual selection at the first location resulted in a nearly common set of selected clones and a heritability for yield of about $h^2 \approx 0$ in the selected fraction. This was demonstrated for two different breeding populations grown in two different seasons, so that the breeding scheme was changed. Only those clones that have passed the visual selection step at location 1 are harvested and considered for storage root quality evaluations at location 2 and 3. However, relying on visual selection in early breeding stages requires a person who is very experienced with sweet potato. We think farmer participation at the visual selection stage in early breeding stages is essential to avoid genotypes entering later breeding stages with characteristics (storage root size, shape, form, colour, etc.) unacceptable to farmers. As described above, farmer preferences vary substantially both within and between regions, and the visual selection can be conducted by independent farmer groups. The advantages of PPB are very obvious in the early selection stages of clonal breeding, in which large numbers of fixed genotypes must be screened for many highly heritable traits. PPB in the early selection stages has been successfully applied in potato (Gabriel and Torrez, 2000), cassava (Manu-Aduening *et al.,* 2006) and sweet potato (Gibson *et al.,* 2008), and by working with 2 or 3 locations it can be linked into FPB, in which selection is conducted for traits that cannot be evaluated by farmers, such as nutritional quality (starch, vitamins and micronutrients by fast through-put analysis methods) (Hartmann and Buning-Pfaue, 1998; Lu, Huang and Zhang, 2006; Zum Felde *et al.,* 2007; Bonierbale *et al.*, 2009).

In the next season, B-clones—also called "promising clones"—are planted in larger plots in 2 to 3 rows with planting material obtained from selected A-clones. The B-clone trials are still conducted without replications, but generally at two or more locations. The B-clone stage is usually the beginning of selection for low heritability traits such as yield, biomass and yield stability. The determination of stability parameters such as the slope of the regression line and deviations from regression (Fox, Crossa and Romagosa, 1997) requires at least three locations. However, it should be noted that stability parameters from less than 6 environments are still of little value. As mentioned above, a strong justification for PBB is that stress and marginal field conditions of resource-poor farmers are not adequately simulated by FPB (Ceccarelli, 1994). Cross-over G×E interactions occur, and what appears to be good in resource-rich environments often does not perform well in resource-poor environments. This has also been clearly observed in sweet potato, and outstanding clones for resource-poor environments were discarded by FPB (e.g. the clone SR92.499-23; Grüneberg *et al.,* 2005). Usually, but not always, the response to selection in poor production environments is smaller than in good production environments. The genetic variance is smaller while interaction and error are larger, so that the performance of individual clones becomes more difficult to distinguish. However, outstanding genotypes with different growth types adapted to resource-poor environments cannot display their full potential if FPB does not test

in such environments. Taking sweet potato breeding as an example again, yield stability is associated with harvest index (Grüneberg *et al.,* 2005). Under drought stress, good performing sweet potato clones have a harvest index of about 0.5 (on the basis of fresh matter storage root yields and total fresh matter biomass yields). Vine production is of considerable importance to farmers to obtain sufficient planting material for the next growing season. Clones performing well in resource-rich environments usually fail in drought-stress environments due to insufficient vine production rather than to unacceptable storage root production. At the same time, outstanding clones in drought-stress environments show a strong increase in vine production with medium storage root yields when grown in environments with good water supply (Andrade, unpublished). The selection of genotypes with desired growth types or desired sink–source allocations in marginal environments requires that breeders evaluate the breeding population in such an environment; this characteristic cannot be determined in a resource-rich environment. Here we suggest linking the evaluation in a marginal environment with the visual selection in early breeding stages. All clones that fail in the marginal environment (i.e. extreme reduced storage root production or vine production) are eliminated from all other selection steps.

13.4.2 Later breeding stages and PPB

At the beginning of the C-clone and D-clone selection stages the breeding population has been reduced to between 30 and 300 clones. While the number of clones in later selection stages is further reduced, those selected clones are tested in more environments and in replications. The plots for C-clones and D-clones are 3- to 5-row plots. All important agronomic traits are determined, including taste and post-harvest characteristics. Furthermore, it merits determination of the above-mentioned stability parameters: (i) slope of the regression line, and (ii) deviations from regression, as well as conducting an Additive Main Effect and Multiplicative Interaction (AMMI) Analysis in those cases where the regression model does not fit (Fox, Crossa and Romagosa, 1997). Usually, a clone is considered to have stable performance if the slope of the regression line is close to 1, and the deviations from the regression line are small. An important question in later breeding stages is that of how many locations and how many replications to use. With more locations and more replications, the estimation of the yield performance of clones is more reliable. At the same time, for a given testing capacity, increasing the number of locations and replications results in fewer clones being tested. Generally, the gain from increasing the number of replications is less than that obtained by increasing the number of genotypes and locations. Investigations of this problem in selection theory have led to a recommendation to conduct advanced clone trials still with no replications but in the maximum number of environments that can be managed by the breeder (Utz, 1969, cited in Wricke and Weber, 1986). However, many scientists are still very reluctant to conduct trials without replications. Since the fixed costs of experimental stations are high, it is usually an advantage to (i) create 'artificial environments' on experimental stations (by running part of a station without fertilizer or with less irrigation) and (ii) to go on-farm to evaluate clones with farmers, i.e. PVS. However, nearly all of the initial genetic variation in the breeding population has been discarded at later breeding stages,

so that specific characteristics needed by farmers and consumers are often no longer present in advanced or elite clones if they had not been considered at earlier breeding stages.

13.5 MODIFICATIONS OF THE GENERAL BREEDING SCHEME

The general principle for breeding clonally propagated crops presented above is very simplified. In practice, it is more or less modified. The differences can be large, depending on the crop, country and breeder. For example, resistance or tolerance can already be determined at the true-seed plant stage by eliminating infected plants from the seed nursery. Potato breeders usually try to obtain only a single tuber from each true seed plant to start selection with single plant tests. Clone selection in shrubs and fruit trees uses fewer plants per row and fewer selection stages. Potato breeding uses more selection stages due to the low propagation coefficient of potato. However, there is a common question in all the different breeding schemes: How many genotypes should be selected at each selection stage? In selection of breeding clonally propagated crops this can be easily determined using selection theory. There is an optimum number of clones, locations and replications at each selection step for a given test capacity. Fortunately, the area around the optimum is flat and deviations from the optimum do not have large effects, as long as the deviations are not strong. To select between 5 and 20 percent of the total number of clones at each step is still close to the optimum. However, in the wide range of practical breeding situations, the optimum has always been found in the direction of higher selection intensities, more so than most breeders intuitively realize. It is important: (i) to increase the number of genotypes at the first stage, to the maximum of the available breeding capacity; (ii) to use a high selection intensity; and (iii) to use as many environments as can be managed at each breeding stage (Wricke and Weber, 1986). Replications are of minor importance and should only be used at the final breeding stages. These characteristics of the optimum in multistage selection for clonally propagated crops led to the suggestion of using an accelerated breeding scheme (ABS) for clonally propagated crops in sweet potato breeding (Grüneberg, unpublished).

ABS responds to the frustration that it takes on average 7 or 8 years from a cross until variety release. Donors are also reluctant to invest in breeding when concrete outputs take so long to materialize. ABS uses the simple fact that in breeding clonally propagated crops each true seed plant is already a potential variety, with the advantages of sweet potato having a very short crop duration (3 to 4 months) and a high propagation coefficient (up to 90 cuttings per plant within 3 to 4 months). ABS overturns the general principal breeding scheme of clonally propagated crops by: (i) crossing and multiplication; (ii) early selection stages; and (iii) late selection stages. Everything that can be implemented simultaneously in these three stages and years is done simultaneously in different environments. However, to reduce labour, every clone that has not met a desired target for a character in the first environment is discarded and not considered (harvested) in the second environment, and the same for characters evaluated in the second environment, and so forth. In selection theory, this multi-trait selection procedure is designated 'independent culling' and it is the procedure also used to optimize multistage selection procedures (Cochran, 1951; Wricke

and Weber, 1986). In ABS, independent culling is conducted: (i) in a poor resource environment where clones undergo visual selection; (ii) only those clones passing the first selection step are harvested in environments 2 and 3 to determine yield and quality of selected good performance clones over all traits and environments (index values are determined by the Pesek-Baker index (Pesek and Baker, 1969) to assist the breeder in their selection decisions); and (iii) only those clones that have passed the second selection step are harvested in environment 4, where clones were already planted in season 2 in a farmer's field under high SPVD pressure in a third selection step to select for SPVD tolerance. About 300 sweet potato clones enter the later breeding stages. In two subsequent seasons and two selections steps, 4 to 5 clones are finally selected for variety release (1st season: 300 clones, 3 environments, two plot replications and 5-row plots; 2nd season: 40 clones, 16 environments, two plot replications and 5-row plots). This is carried out in cooperation with NARS and farmer groups.

13.6 MAINTAINING VARIETIES AND S-CLONE MULTIPLICATION

As a result of clonal propagation, maintaining varieties should not be difficult. Genetic changes in varieties do not occur by undesired crossings nor by segregation, and mutations are rare. However, the opposite is the truth, and maintaining clonally propagated varieties is a difficult and expensive part of the breeding operation. The main reason is that in clonal propagation through vegetative plant parts, many more diseases can be transmitted compared with seed propagation. A new variety will have no impact in practice, and even can be lost (a clone hybrid developed from two hybrids cannot be reproduced by crossing the hybrids again) without a system that maintains and provides at least some healthy planting material.

Numerous viruses, bacteria and fungi are transmitted by vegetative planting material. Viruses are particularly important, because viral diseases cannot be controlled chemically. Viruses are spread by vectors, most often aphids and whiteflies. The traditional maintenance of varieties and production of healthy planting material includes protecting the base plants of varieties in greenhouses or under nets, and to prevent the development of a vector population by intensive use of insecticides. The base material is also termed 'mother plants'. However, under these conditions, only 20 to 200 plants of each variety can be maintained, and planting material must be produced in the field. These clones in the field for producing healthy planting material are the so-called S-clones, because planting material is usually called seed in clonally propagated crops. Healthy S-clone production is supported by (i) application of insecticides against vector populations (monitoring by yellow cards); (ii) choosing locations for S-clone production that are out of range of vector populations (i.e. locations close to the sea or in cool highlands); and (iii) removing all visibly infected plants from S-clone fields.

The detection of virus infections has been simplified by use of the enzyme-linked immunosorbent assay (ELISA) procedure. The principle is a reaction between the viruses in plants and antibodies against these viruses. The reaction is made visible by an enzymatic colour formation. In practice, some leaf sap is pressed out and the colour reaction is assessed on special test plates coated with antibodies. In the case of sweet potato, the plants tested

negative for viruses are further grafted on an indicator plant such as *Ipomoea setosa* to confirm the absence of viruses for sweet potato viruses. In this way all maintained mother plants of a variety are routinely screened, and only virus-free mother plants are used for further propagation steps. Recently, techniques have been developed to detect viral DNA and RNA directly by real-time polymerase chain reaction (PCR) (Mumford *et al.,* 2006).

However, the best option for maintaining clone genotypes is to start from absolutely virus-free material. This is obtained by *in vitro* propagation of plants under sterile conditions, and these *in vitro* plantlets are the starting point for greenhouse and field propagation. *In vitro* plantlets are replacing mother plants in the greenhouse, often by eliminating all greenhouse plants. If no virus-free material is available, new virus-free plantlets can be obtained by thermotherapy and meristem culture. Meristems of very-fast-growing infected plants are virus free following proper heat treatment, because viruses only start to enter older plant cells. However, this process requires considerable time (at least 18 months for sweet potato, and depends on the virus titre of the infected source plants). In breeding, virus-free material can be achieved by germinating true seed *in vitro* and maintaining these true-seed plantlets *in vitro* until the final selection decision has been made.

Distribution channels for clonally propagated crops are well developed in temperate regions of the world. However, they are almost non-existent in most tropical and subtropical countries, although the pest and disease pressure is considerable higher than in temperate regions. S-clone production in resource-poor environments is nearly all in the hands of farmers, and the health status of planting material is a key factor in high farm yields. Without a certain discipline in S-clone production on farm, the yield level remains low, although virus-tolerant varieties with good overall performance are available. The most important factors for S-clone production on farm are: (i) separating S-clone production from cultivation for production; (ii) removing all visibly infected plants in S-clone field areas; and (iii) obtaining new, healthy planting material at least occasionally from private or public sources. Nevertheless, the private and public seed sectors are an important factor in production of clonally-propagated crops, but this topic belongs to integrated crop and pest management (Salazar, 1996). The breeder's role in this context is to maintain and provide virus-free starter material for the private and public seed sectors.

13.7 SELECTION OF PARENTS AND PREDICTION OF CROSS OUTCOMES

The choice of parents is perhaps the most important step in a breeding programme. Many breeders make several hundred crosses each year and it is often observed that in later steps of the breeding programme the best clones derive from one or only a very few crosses. Hence, there is a desire to predict which cross combinations are most promising. If this were possible, the efficiency of a breeding programme could be increased by reducing the number of cross combinations and increasing the number of genotypes from good cross combinations (produce more genotypes from within the best families). In the situation where not much is known about the performance of a cross, the number of combinations should be increased to the maximum of the breeder's capacity and the number of genotypes per cross should be kept small. The rationale underlying this is based on selection theory, which shows that if "the breeder

has no prior knowledge on the cross … the breeder has to make as many crosses as possible", which is also minimizing the risk of raising genotypes with poor performance (Wricke and Weber, 1986). As mentioned above, most clonally propagated crops are polyploid and highly heterozygous, so that dominance and epistatic effects contribute considerably to clone performance. For this reason, it should be assumed that not much is known about the value of a cross combination until it has been made and tested. This is in agreement with our observations in sweet potato, where the correlation between mid-parent and mid-offspring yields is low ($r \approx 0.5$). We currently recommend raising 10 to 20 genotypes per cross combination, while increasing the number of cross combinations to the maximum possible with the resources available. However, after clones of these crosses have been evaluated, the good crosses should be repeated on a large scale. An optimum for the number and size of crosses can determined if estimations are available for the genotypic variance between crosses and within crosses, and the non-genetic variance components (Wricke and Weber, 1986). Breeders often generate a large number of seeds in polycross nurseries, but in these only the female parent is controlled. The correlation between parent and mid-offspring in breeding populations derived from polycross nurseries is half of mid-parent–mid-offspring correlation in controlled crosses.

Often the parents are chosen due to their performance *per se*. For theoretical reasons, this cannot be very secure in clonally-propagated crops. Clone varieties are highly heterozygous hybrids and usually polyploids, so that segregation in crossings is almost unpredictable. Therefore, for a long time now, suggestions have been made for better assessment of parents; however, they are rarely used in practice. One suggestion is to determine the value of a parent on the basis of the offspring performance from test crosses. Another suggestion is to work on a reduced polyploidy level, which has been especially proposed for breeding tetraploid potatoes (Ross, 1986). However, the latter has been little applied in practice for parental selection, but has often been used to incorporate germplasm of wild *Solanum* species into advanced breeding populations (Tarn *et al.,* 1992). Parental selection on the basis of test crosses are made on a large scale in potato breeding programmes for long-day, temperate climates (150 to 500 cross combinations per breeding programme, cited by Ross, 1985). It has been observed that specific combining ability is nearly as large as general combining ability, and in some cases specific combining ability has been observed that is clearly larger than general combining ability (Sanford, 1960; Mullin and Lauer, 1966; Tai, 1976; Killick, 1977; Veilleux and Lauer, 1981; Gaur, Gopal and Rana, 1983, cited by Tarn *et al.,* 1992; Gopal, 1998; Kumar, 2004; De Galarreta *et al.,* 2006). This is not surprising as long as potato breeders do not work with two clearly separate gene pools for variety development. In potato breeding for tropical and subtropical regions, heterosis and high general combining ability have been observed between *andigena* and *tuberosus* gene pools in tuber-propagated potatoes and in true-seed potatoes (Enrique Chujoy, pers. comm.). However, as long as these gene pools are not improved on the basis of general combining ability separately from the complementary gene pool, such effects cannot be exploited in the long term.

In sweet potato experiments we observed a mid-parent–mid-offspring heterosis of 84 percent among 48 cross combinations (or 184 percent if the mid-parent value is set to

100 percent). This is a clear indication that the design of breeding schemes using the combining ability of two gene pools merits investigation. Two breeding gene pools are available for sweet potato to test heterosis: the Jewel Gene pool, developed mainly from North American varieties, and the Zapallo-SPK Gene pool, developed mainly from South American and African FVs.

The value of a parent is nearly always determined by several characteristics. In general, parents should be recombined with a good combining ability and good performance over all traits. The PPB study in Uganda (Gibson *et al.,* 2008) underlines how many characteristics are important for good performance over all traits. Moreover, FPB also has the aim of improving nutritional quality, especially pro-vitamin A, iron and zinc concentrations (Pfeiffer and McClafferty, 2006) in potato, sweet potato, cassava, plantain and other crops. With an increasing number of characters, breeders operate with larger breeding populations, as in potato and sweet potato. Aiming at only 30 genotypes finally selected, and assuming 10 characters each, selected in sequential selection steps with a selected fraction of 10 percent (1 out of 10), then 300 000 000 000 genotypes would be needed in the original base population. Populations of this size cannot be established in practice. Moreover, even if the population size is extremely large, some desired combinations probably do not exist, such as sweet potato genotypes with high yield, high SPVD tolerance, high DM and high pro-vitamin A, iron and zinc concentrations). Often, breeding can only approach the desired genotype in several steps of recombination and selection. In practice, some characters are selected sequentially (especially where there is clearly a lowest acceptable value (tuber size, shape and colour, as well as pest and disease resistances), while others are selected simultaneously by aggregating characters into an index (often an intuitively formed index, such as score values for overall performance).

A parent appears to have a good overall trait performance if no trait is below the population average. However, only in those cases where trait associations are close to zero or positive can it be expected that parents with good performance over all traits produce offspring in which each character has been improved. In parental selections, negative trait associations can be very critical. Table 13.3 gives an example for sweet potato, in which DM shows a strong negative trait association with pro-vitamin A, iron and zinc concentrations, as well as a moderate negative trait association with storage root yield. The associations in the example are strong enough that under various scenarios of multi-trait selection the breeding population is improved for yield, pro-vitamin A, iron and zinc, whereas the DM of the population decreases.

In other words, the DM is changing in the wrong direction even though it was selected for improvement. These surprising undesired effects in the case of sweet potato and DM improvement in connection with pro-vitamin A, iron and zinc improvement was also observed for the Williams selection procedure and this index selection procedure (Williams, 1962) comes very close to intuitive selection procedures used by breeders in which a weight is assigned to each trait on the basis of its economic importance. The only selection procedure that can monitor the response to selection in each trait is the Pesek Baker index (Pesek and Baker, 1969). However, this index requires estimations of genetic variance and co-variances, but the procedure ensures that parents are selected that

TABLE 13.3
Estimations of genetic correlations for yield, dry matter, total carotenoids, iron and zinc in sweet potato storage roots of 24 megaclones and 26 advanced breeding clones grown in at two locations in two replications

	Storage root yield	Dry matter	Total carotenoids	Iron
Megaclones (orange and white fleshed)				
Dry matter	-0.49			
Total carotenoids	-0.06	-0.54		
Iron	-0.26	-0.23	0.94	
Zinc	-0.22	-0.39	0.93	0.74
Advanced breeding clones (only orange fleshed)				
Dry matter	-0.54			
Total carotenoids	0.55	-0.71		
Iron	-0.24	-0.07	0.14	
Zinc	0.39	-0.20	0.37	0.53

develop populations in which traits are improved according to a ratio of desired genetic improvements (so-called desired genetic gains) given by the breeder.

An alternative is the Elston index (Elston, 1963), in which the breeder can raise the threshold for the trait at risk by modifying the lowest acceptable value for each. This index can be easily applied in each replication and environment, so that index mean values for each genotype can be calculated together with other statistical parameters (Grüneberg *et al.*, 2005).

We are aware of only one case in which PPB has been applied for the selection of parents in clonally propagated crops. In the Cochabamba region of Bolivia, farmers selected potato parents in an *andigena* population, which had been improved for agronomic performance and Late blight tolerance. Selected clones in this population were used as parents with the regionally grown FV 'Waycha' (Gabriel and Torrez, 2000) and the PPB approach included hand-crossing by farmers. We think that the ability of farmers in the selection of parents is limited beyond a selection of clone performance *per se*. Test crosses, general combining ability, specific combining ability and improving gene pools on the basis of general combining ability values (called reciprocal recurrent selection in maize breeding) are the most difficult tasks in breeding; however, they can greatly increase yield gains. At the same time, we think that the visual selection of potential parents in a PPB approach should be used as additional information by the breeder. It should be noted that the work plan for both the selection of parents for the next cycle of selection and the early selection stages for variety development are always to a certain extent in common. In sweet potato breeding at CIP we use a combination of sequential and simultaneous index selection in early selection stages (see also above): (i) visual selection by eliminating all genotypes that do not meet the lowest acceptable values for each trait (this lends itself to PPB); (ii) in the remaining selected fraction (about 2500 clones), apply index selection for yield and nutritional quality traits using the Pesek-Baker index, with the square roots of variance components as desired genetic gains; and (iii) selecting for pest and disease tolerance (mainly SPVD) in the remaining selected fraction (about 300 clones) by visual selection (this lends itself to PPB) and

ELISA. The remaining 100 to 200 clones enter later breeding stages, but are also used as parental material for the next cycle of recombination and selection. In such a breeding system, with one population, two PPB steps can easily be applied. However, a PPB approach is feasible also in inter-pool crosses linked with general combining ability improvement. Farmers select in families (derived from recombining the two gene pools) in early generations for variety development (as described above). The interesting information for the breeder provided by farmers could be the numbers of selected clones per family. With this information the breeder can focus only on those parents in the improvement of the separate gene pools, which for the farmer results in interesting cross combinations with the other gene pool. On top of this, the breeder can use the opportunity to apply the general combining ability concept. This would be a very elegant PPB approach for selection of parents and cross prediction. Although Hull (1945), in his fundamental paper on reciprocal recurrent selection, proposed this for breeding clonally propagated crops, this method of clonal breeding is rarely found in practice.

The topic has been considered in breeding clonally propagated trees (e.g. Baudouin *et al*., 1997; Kopp *et al*., 2001; Pâques, 2004) and recently discussed by Miles (2007) in the frame of apomixis for cultivar development in tropical forage grasses. The proposed "evolutionary breeding approach" for *Musa* spp. (Ortiz, 1997) is also in the narrow sense a reciprocal recurrent selection scheme. However, subsequent application of reciprocal recurrent selection is rarely found in practice, although we think that this is the way ahead to exploit heterosis and achieve more breeding progress in clonally propagated crops.

13.8 APOMIXIS

As mentioned earlier, the principle advantage of breeding clonally propagated crops is that each clone variety is fixed and maintainable. However, this is associated with the disadvantage of vegetative propagation. Diseases are easily transmitted and the maintenance of varieties and the production of healthy planting material are expensive. The ideal propagation system for clone varieties would be vegetative propagation by seeds. This ideal propagation system exists in nature, and is called apomixis (Nogler, 1984). Apomixis is the formation of seeds without meiosis, and two forms are distinguished: (i) agamogenesis (also called gametophytic apomixis), in which the asexual embryo is formed from an unfertilized egg; and (ii) adventitious embryony, in which the asexual embryo is formed from nucellus tissue. Apomictically produced seeds are genetically identical with the parent plant. The breeding work on apomictic species is very difficult and requires developing population improvement by sexual reproduction and subsequent variety development by apomixis. Apart from some forage (Miles, 2007) and citrus species (Soost and Roose, 1996), apomixis is not used in plant breeding.

The difficulty in breeding apomictic crops is the development of genetic variation. However, in populations with a high frequency of apomictic plants, both facultative apomicts and completely sexual plants can usually be found, and such genotypes can be used to develop new genetic variation. For breeding, it is important to find or develop a system in which both apomixis is maintained (variety development) and sexual reproduction is restored (population improvement) so as to be able to develop new genetic variation. This can be compared to male sterility systems used in

hybrid seed production. It is interesting that apomixis is distributed across many plant families. It appears not to be controlled by a complex genetic system. An example is Guinea grass (*Panicum maximum*), in which the sexual tetraploids are recessive homozygous (aaaa), whereas apomictic genotypes carry a dominant allele and are heterozygous (Aaaa) (Savidan, 1983). So far, studies on apomixis have been mainly made in tropical grasses, but more and more attention is being paid to rice and maize. There are opinions that apomixis systems will become available to breeders, and in this context gene isolation and an 'apomixis gene' have been mentioned (Savidan, 2000). However, so far there is no such apomixis system usable in breeding programmes. The major problem is that plants with the same genotype can express different degrees of apomixis.

13.9 PROPAGATION OF POTATOES BY SEED

Finally, the option that clonally propagated crops can be propagated by sexual seeds is considered. In many countries there have been research projects in which potatoes were cultivated by seed. These are potatoes that are sown instead of planted. Since the planting material of clonally propagated potatoes is often called a 'seed' potato, the term 'true potato seed' (TPS) was introduced. The use of TPS has two principle advantages: the most important potato diseases cannot be transmitted in true seed, and only a few hundred grams of TPS are needed to cultivate a potato field, where usually several tonne of tubers are needed (Simmonds, 1997). This is associated with two disadvantages: potatoes grown from seed are weak in vigour and are sensitive to many factors, and the breeding method and advantages of breeding clonally propagated crops can no longer apply. Moreover, breeding TPS potato as a cross-fertilized crop will not lead to completely homogenous varieties. This is the reason why only a few TPS varieties have been developed in the Northern Hemisphere. All these have been exclusively used for home garden production. However, we think that by using hybrid selection schemes and inbreeding in two separate gene pools it should be possible to develop more and more homogenous and attractive TPS varieties.

The advantages of TPS are mainly of interest in tropical and subtropical regions of the world. Under these climatic conditions, the production, storage and transportation of potato planting material is difficult. Moreover, potato yields are considerable lower in tropical and subtropical regions of the world than in the Northern Hemisphere, so that about 20 percent of the harvest is needed as planting material. Hence TPS varieties in the tropics can have 20 percent lower yields compared to clonally propagated potato varieties and remain competitive. About 20 TPS varieties have been developed. Most interesting are those varieties developed from recombination of the *andigena* and *tuberosus* gene pools. However, the original idea of raising seedlings in nurseries and then planting seedlings into the field by hand has not been adopted. What has been adopted is to raise TPS varieties in seedling nurseries to obtain healthy planting material, and then to cultivate these TPS varieties for several growing seasons as a clonally propagated crop, and to request true seed again after yield declines are significant due to declining health status (Fuglie, 2001). However, today, not more than 10 000 ha of TPS are grown, mainly in Asia, which trace back to about eight TPS varieties. The future of TPS is debatable. From the

breeding perspective, the future of TPS will mainly depend on working with two gene pools, in which a certain extent of inbreeding is applied, with subsequent use of general combining ability to improve these two gene pools.

13.10 POTATO

Breeding potatoes has been reviewed by Tarn *et al.* (1992). The andigena potato (*Solanum tuberosum* subsp. *andigena*; autotetraploid with 48 chromosomes) originated in the highlands of South America about 5000 BC, while today two-thirds of world potato production is in temperate latitudes. Following introduction into Europe, andigena evolved into the Irish potato (*S. tuberosum* subsp. *tuberosum*), which is mainly characterized by day-length neutrality, uniformity of tuber shape, shorter crop duration and higher harvest index than andigena. Andigena remains the predominant cultivated potato in the Andes, whereas the Irish potato is the potato of commerce in long-day temperate climates. Potatoes introduced into other, tropical, regions of the world trace back to breeding populations derived from crossings between andigena and Irish potato. However, in the Andean region, seven other potato species are still in cultivation; most important are phureja (*S. phureja*; diploid with 24 chromosomes), limeña, ajanhuri and rucki. In addition to these cultivated species, 160 wild potato species are known (Hawkes, 1979 and 1981; Spooner and Hijmans, 2001), so that potato might have the largest gene pool among crops. Wild and indigenous species are important resources of pest and disease resistance for andigena and Irish potato. The evolution of the potato was described in the introduction of this chapter. Asia and Europe are the world's largest potato producing regions, with annual production of about 130 and 128 million tonne, respectively, followed by the Americas (41 million tonne) and Africa (16 million tonne) (FAO, 2006). The top 20 potato producing countries are China (22%), The Russian Federation (12%), India (8%), Ukraine (6%), United States of America (6%), Poland (3%), Germany (3%), Belarus (3%), Canada (2%), France (2%), United Kingdom (2%), Turkey (2%), Netherlands (1%), Bangladesh (1%), Brazil (1%), Romania (1%), Peru (0.8%), Spain (0.6%), Nepal (0.5%) and Pakistan (0.5%).

Breeding objectives

Characteristic of potato breeding is the large number of breeding objectives. For the Irish potato, quality traits are at least as important as yield. Moreover, breeding for resistance against numerous pest and diseases, e.g. numerous viruses (potato leafroll virus (PLRV), Potato virus Y (PVY) and Potato virus X (PVX)), Late blight (*Phytophthora infestans*), dry rots (*Fusarium* spp.), Soft rot and Blackleg (*Erwinia* spp.), cyst-forming nematodes (*Globodera rostochiensis* and *G. pallida*) have major importance in long-day temperate as well as in tropical temperate climates. For the andigena potato, these pests and diseases are of nearly similar importance (i.e. Late blight can destroy the whole crop in cool, high-altitude regions, especially when the weather is wet).

There are clear differences between tropical temperate and tropical hot climates. At temperatures above 25°C, Late blight and cyst-forming nematodes decline rapidly in importance, but Early blight (*Alternaria solani*) and root-knot nematodes (*Meloidogyne* spp.) take over, and Bacterial wilt (*Pseudomonas solanacearum*) is widespread in tropical

lowlands. The phureja potato (for PVY and Late blight) and the wild species *S. acaule* (for PLRV, PVX and both *Globodera* spp.) and *S. demissum* (for PLRV, PVY, and late blight) are important resources in breeding for tolerance and resistance. It should be noted that the pests and diseases presented represent only the most important species. Ross (1985) provides a list of resistance sources in wild potato species.

However, it is possible to find tolerance or resistance genes in cultivated and wild potatoes against nearly all potato diseases. An exception is Bacterial wilt, for which so far no useful tolerance or resistance have been found for breeding purposes. Today, all new Irish potato varieties contain one or more resistance genes from wild and other cultivated potato species. For the Northern Hemisphere, yield, crop duration, tuber size, shape and flesh colour, eye depth, starch content, storability, cooking characteristics, taste and suitability for mechanical harvesting, as well as processing characteristic for chips (crisps) and French fries (chips) are the most important quality breeding objectives. For tropical regions, yield, regional adaptability, crop duration, storability, cooking characteristics, taste and nutritional quality are the most important quality breeding objectives. Outside of the Andes, crop duration is one of the most important traits (i.e. in south-west and central Asia there is a requirement for potato varieties with less than 80 days crop duration). Recently, focus has been given to improve pro-vitamin A, iron and zinc concentrations in tubers to alleviate micronutrient malnutrition in tropical regions (potatoes have comparatively high iron and zinc concentrations). This has resulted in a separate breeding programme for phureja, which has the highest iron and zinc contents among potatoes, together with considerable levels of total carotenoids, including pro-vitamin A.

Breeding methods

Crossing is relatively easy. In nature, crossings occur easily by open pollination by insects. For breeding purposes the flower architecture of the potato allows easy emasculation and controlled hand pollination. A fruit with about 200 seeds develops from each successful pollination. In commercial breeding, controlled crossings are usually made (both parental genotypes are clearly defined). Genotypes with good performance over all traits and with a certain degree of genetic distance are recombined. The value of a cross combination is usually determined in test-crosses with 100 to 200 seeds per combination. Occasionally plant breeding text-books recommend combining parents with complementary traits. However, many breeders find that this results in potatoes breeding in a 'wild' segregation, so that finally only genotypes can be selected with moderate performance over all traits. Each year, breeders plant 10 000 to 200 000 seeds, which trace back to 150 to 200 cross combinations. Crossings are made with flower sprouts obtained from cuttings of field plants, grown in greenhouses in nutrient solutions. Frequencies of successful crosses differ tremendously between parental combinations and about one-eighth to one-quarter of all parental combinations cannot be recombined due to no flowering, low pollen quality, no fruit formation or genetic incompatibility. For an overview of overcoming crossing barriers in potatoes, the reader is referred to Jansky (2006).

Pre-breeding crosses are important when one requires to improve one or two traits in an enhanced breeding gene pool (e.g. shorter crop duration). Pre-breeding

is usually made in two or three cycles of recombination and selection in which the desired traits are incorporated in a genetic background that is more close to the enhanced breeding gene pool. This is generally done for resistances genes from a wild parent or exotic variety. It often involves an additional selection step at a different polypoidy level. The re-synthesis of tetraploids by mitotic duplication of diploid genotypes (colchicine treatment of seed, axillary buds, tuber germs, leave explants or callus) is usually not recommended, because mitotic tetraploids have considerably lower yields than meiotic tetraploids. Meiotic tetraploids occur naturally in crossings between tetraploid and diploid potatoes (4x × 2x) due to meiotic anomalies that result in unreduced gametes (Rowe, 1967; Jacobsen, 1980).

Breeding potato for tropical regions of the world aims mainly at improvement of four gene pools: (i) the andigena (A) gene pool for short-day high altitudes; (ii) the andigena × tuberosus (AT) gene pool for short- and long-day temperate regions, with emphasis on selection for Late blight tolerance and PVY and PLRV resistance; (iii) the tuberosus × tuberosus (TT) gene pool for short- and long-day warm regions, with an emphasis on selection for short crop duration; and the (iv) phureja gene pool (P), with emphasis on nutritional quality. In the A, AT and TT gene pools at CIP about 60 parents are recombined by controlled crossings to raise about 20 000 seedlings, whereas in the P gene pool the number of recombined parents is considerable lower (about 30 parents). The selections start in seedling populations for both resistance and tuber formation. In three selection steps the material is reduced to about 300 clones, which are evaluated in 2-row plots with two replications. Later selection stages include evaluation at several locations in replicated plots. However, the propagation coefficient in potato is very low (≈ 10) so that it takes about 8 to 10 years before final selections enter the variety release and dissemination stage.

Today there is little investment in TPS in the original sense. However, selection of families that are to a certain degree homogenous in crop duration, tuber form and shape, with some genetic diversity in tuber yield and specific adaptation, are of interest. The reason is that these can be disseminated as seeds and farmers have the option to exploit genetic variation for specific adaptation in a PPB approach. PPB has been successfully applied in Bolivia in early breeding stages (Gabriel and Torrez, 2000) and in later breeding stages (FVS) in Ecuador (Bonierbale, pers. comm.).

13.11 SWEET POTATO

Breeding sweet potatoes has been reviewed by Martin and Jones (1986), Laurie and van den Berg (2002) and Grüneberg *et al.* (2009). The sweet potato (*Ipomoea batatas*, Convolvulaceae, hexaploid with 90 chromosomes) is also known as *batata, camote* or yam (United States of America). The crop was domesticated in tropical America about 6000 BC and reached the Pacific and southeast Asian islands naturally or by early seafarers before Columbus. The number of wild species in the genus *Ipomoea* is large (more than 500 species). However, no wild form of *I. batatas* has been found. It is assumed that *I. batatas* developed from an interspecific cross between a diploid and a tetraploid *Ipomoea* species in the *I. trifida* complex. It is possible to re-synthesize new *Ipomoea* hexaploids by hybridization of diploid *I. leucantha* and tetraploid *I. littoralis* (Nishiyami, Miyazaki and Sakamoto, 1975.). The Spanish intro-

duced sweet potato in the sixteenth century into the Philippines, whence it spread to other islands and the east Asian mainland. Portuguese seafarers introduced the crop into Europe, Africa and India. Today it is cultivated in 117 countries in all tropical and subtropical regions of the world. Asia is the world's largest sweet potato producing region, with about 107 million tonne of annual production, followed by Africa and the Americas, with approximately 15 and 3 million tonne, respectively. The top 12 producing countries are China (80%), Nigeria (2.8%), Uganda (2.2%), Indonesia (1.5%), Viet Nam (1.2%), United Republic of Tanzania (0.9%), Japan (0.8%), India (0.8%), Burundi (0.7%), Kenya (0.6%), Rwanda (0.6%) and United States of America (0.6%). Further important sweet potato producing countries are Angola, Argentina, Bangladesh, Brazil, Cuba, Egypt, Ethiopia, Egypt, Haiti, Korea (Democratic Republic of), Madagascar, Peru, the Philippines and Papua New Guinea, with annual production between 0.3 and 0.5 million tonne (FAO, 2006). Nearly half of the sweet potato produced in Asia is used for animal feed, with the remainder primarily used for human consumption, either as fresh or processed products. In Africa, the crop is cultivated almost exclusively for fresh consumption.

Sweet potato is a perennial vine, propagated by cuttings, and usually cultivated as an annual crop. The planting distances in fields vary. In Africa, planting distances are usually 1 m between rows and 30 cm within rows. In China, recommended planting distances are 75 cm between rows and 20 cm within rows. The crop duration is very short (4 to 6 months) and the crop is even cultivated in northern China. It produces more edible energy per hectare per day than wheat, rice or cassava, and is well adapted to salinity, drought and marginal soil conditions (Woolfe, 1992).

The crop has recently received more interest due to the very high levels of pro-vitamin A (concentrations of up to 700 ppm DM) in OFSPs, and hence as a vehicle to reduce vitamin A deficiency problems in the world (Huang, Tanudjaja and Lum, 1999; Low, 2007). We observed up to 1200 ppm β-carotene on a DM basis in clones with variety potential in our breeding population 'Jewel II' (this corresponds to 30 mg β-carotene in 100 g fresh sweet potato storage roots. A preschooler needs 4.8 mg β-carotene per day, and it merits discussion as to what extent OFSP should be recommended as baby and weaning food. Moreover, storage roots provide medium levels of iron and zinc (Woolfe, 1992). Recent finding of about 50 ppm DM iron and 40 ppm DM zinc in deep orange fleshed sweet potato storage roots (Burgos and zum Felde, pers. comm.) merits further investigation.

The stems and leaves can have spinach-like taste and some varieties are used in China specifically as a green vegetable. Stems and leaves have on DM basis about four times more protein, iron and zinc than storage roots. It appears that stems and leaves must be cooked to reach an acceptable iron bioavailability, but investigations into iron bioavailability of sweet potato tops is very limited.

There is new demand for purple-fleshed sweet potato due to the health-promoting effects of anti-oxidant anthocyanin substances, and cell lines for a potentially ongoing production for the food industry have been established (Konczak, 2006). However, much more important appears to be the demand for non-sweet sweet potatoes, but few genotypes are non-sweet (Kays, 2006). There is a very large genetic

variation for DM, starch and sugars in sweet potato, and a strong positive correlation has been observed for DM and starch, whereas a strong negative correlation was found between sugars and DM and starch (Grüneberg *et al*., 2009). This is nearly ideal for the breeding target of a non-sweet high-DM sweet potato type, and we think that the development of non-sweet sweet potatoes should not be too difficult.

Breeding objectives

FPB started very late for sweet potato. One of the first breeding programmes was established at Louisiana State University in the 1920s. Today there are several strong national breeding programmes (e.g. China, Japan, South Africa, Uganda, United States of America and Uruguay) and one international breeding programme, at CIP (Peru). Four major breeding objectives can be clustered: (i) breeding of OFSP for consumption of storage roots and leaves; (ii) breeding for high DM and extractable starch; (iii) breeding for biofuel production, which has started in China (Dai Fu Ma, pers. comm.); and (iv) breeding of purple-fleshed sweet potatoes for consumption. In breeding for consumption, it has to be considered that people in different regions have very different taste preferences; the extremes are low DM content, moist mouth feel, very sweet taste and deep orange flesh colour, versus high DM, bland, dry mouth feel, low sweet taste and white, yellow or orange flesh colour. In breeding for human consumption, focus is more on high DM OFSP varieties with elevated iron and zinc concentration and a dry and less-sweet mouth taste. This breeding is hampered by a strong negative genetic correlation between storage root DM and storage root pro-vitamin A, iron and zinc contents. The breeding for human consumption includes the use of the crop as animal feed and folder. The breeding for high DM and extractable starch is relatively easy: the target is a high starch yield per hectare. However, currently, in many regions of the world the price of sweet potato starch currently cannot compete with the price of cassava starch. Only in large regions where the growing period is too short for cassava within the cropping system (e.g. China) is there an economic demand for sweet potato varieties for starch production. Breeding for biofuel production is in its initial stages, and so far variety recommendations for this purpose are made on the basis of screening existing successful varieties. The breeding of purple-fleshed sweet potatoes as a separate breeding programme is a relatively new trend, and so far only carried out on a small scale in Japan, Indonesia and Peru. Future targets are the non-sweet sweet potato, and quick cooking features (cv. Quick Sweet) (Katayama *et al.*, 2006), as well as suitability for processing into chips, puree, juice, weaning and baby food, and bread on the basis of a wheat-sweet potato flour mixture (Woolfe, 1992); these trends appear nearly exclusively in east Asia, and for recent developments the reader should consult proceedings, such as Liu (2008).

Major constraints on high yields are pests and diseases, especially Sweet potato chlorotic stunt virus (SPCSV) and the sweet potato weevils. The prevailing diseases and insects affecting sweet potato vary from region to region. There are about 35 bacterial and fungal diseases, more than 20 viruses or virus-like agents, 20 nematodes and 20 insect species known to affect sweet potato (Martin and Jones, 1986).

Currently there are only four important pest and diseases: SPVD, Alternaria, sweet potato weevils and the root-knot nematode. The most important virus is whitefly-

transmitted SPCSV, which often occurs in co-infection with Sweet potato feathery mottle virus (SPFMV – aphid-transmitted). Clear synergistic disease effects are seen with SPFMV and SPCSV (the so-called SPVD virus complex). Generally, all varieties need a certain degree of tolerance to SPVD, and there is genetic variation for SPVD (Mwanga, Yencho and Moyer, 2002). Very high tolerance or resistance is needed in eastern Africa. Currently, it is assumed that SPFMV and all other sweet potato viruses (except SPCSV) are not important, because sweet potato has an effective virus defence system, which is broken by SPCSV (I. Barker, pers. comm.).

The major fungal disease in subtropical America is Fusarium wilt (*Fusarium oxysporum* f.sp. *batatas*) and in the African highlands the main problem is Alternaria storage root, leaf spot and stem blight (*Alternaria* spp.). Although there are many bacterial and fungal diseases with a wide distribution, high levels of tolerance or resistance are frequently found. This is also true for resistance to nematodes.

There has been recurrent success in breeding for root-knot resistance against new races of *Meloidogyne* spp. (Martin and Jones, 1986). However, in regions with a pronounced dry season, the greatest constraints are sweet potato weevils (*Cylas formicarius elegantulus* in all parts of the tropics, *C. puncticollis* and *C. brunneus* in Africa, and *Euscepes postfasciatus* in the West Indies). It has been an objective to find weevil resistance for more than 50 years, but differences in weevil attack probably depends on preference factors of the weevil. It is believed that dense storage roots developed deep below the soil surface are less susceptible than less dense, moist-fleshed storage roots. No effective weevil resistance has been found so far. For this reason, a transgenic approach using *Bt* genes has received attention. Recent findings of compounds in the latex of the storage root skin and the effect of these on weevils might of interest for breeding (P.C. Stevenson, H. Muyinza, D. Hall and R. Mwanga, unpubl.).

Breeding methods

True seed set occurs easily in nature by cross pollination (by insects, mainly bees), and for breeding purposes the flower architecture of sweet potato allows easy emasculation and controlled hand pollination. A skilled technician can make 200 crossings per day, with a success rate of 25 percent. From each successful cross, 2 or 3 true seeds are obtained. Not all sweet potato parents flower readily, but flowering can be easily induced by grafting on *Ipomoea nil* (2n = 30 chromosomes). It should be noted that frequencies of successful crosses differ tremendously between parental combinations, and about one-third of all parental combinations are incompatible, with no seed formation. The sweet potato seed has a hard coat and needs to be scarified with concentrated sulphuric acid to obtain even and rapid germination. In a well managed breeding nursery, after 3 months it is possible to obtain 40 to 60 cuttings from a true seed plant if the plant is grown in the field, and 20 to 30 cuttings if the plant is grown in a pot in a greenhouse. The extreme genetic make up of the crop (hexaploid, highly heterozygous, open-pollinated by insects with true seed set occurring easily), the short crop duration (4–5 months), and the rapid propagation (40 to 60 cuttings from one plant) permits the design of a very efficient and rapid breeding system.

The recombination of parents is still usually carried out in polycross nurser-

ies by open pollination. Polycrosses have been considered as very efficient in sweet potato breeding (Martin and Jones, 1986). However, theoretically controlled crosses must be more efficient, provided that high selection intensities can be reached, which depends on technical skills and costs. Only a few breeding programmes are making (at least to any major extent) controlled crosses (e.g. in China, Mozambique, Peru and Uganda). The numbers of recombined parents vary between 20 and 120, and the number of genotypes raised per population (true-seed plants) varies between 5000 and 30 000. Selection of parents is almost exclusively carried out on the parental performance *per se*. In China, Uganda and at CIP in Peru, the information from progeny test crosses is used to repeat good cross combinations on a larger scale (2000 to 3000 genotypes per cross). In recent years, CIP has established two genetically divergent populations to test heterosis and general combining ability in applied breeding material. There are plans to change from a selection of parents by parental performance *per se* to a reciprocal recurrent selection scheme based on general combining ability. Selection of genotypes for variety development is usually carried out as described in the section of the general breeding scheme for clonally-propagated crops. Starting with recombining parents, it takes on average 7 to 8 years until variety release. At CIP, Peru, an accelerated breeding scheme is used in which temporal variation of test environments are replaced by spatial variation of test environments. This accelerated breeding scheme takes on average 3 to 4 years until variety release. It appears that there are funding opportunities to implement this breeding scheme in Africa, particularly in Ghana, Uganda and Mozambique.

13.12 CASSAVA

Breeding cassava has been reviewed by Byrne (1984), Bonierbale *et al.* (1994) and Ceballos *et al.* (2004). Cassava (*Manihot esculenta*, Euphorbiacaea, diploid with 36 chromosomes) originated in South America. The crop is also known as *manioc* and *yucca*. Wild *Manihot* species—weedy sub-shrubs, shrubs and trees—are principally found in dry regions of Mesoamerica and South America. The highest density of diversity is found in west-central Brazil. Many wild *Manihot* species show considerable tuber production and it is assumed that *M. esculenta* was selected from one or several of these wild species in the northern part of South America or in west-central Brazil. The crop was disseminated by tribal migrations and its variability increased by selection for agronomically preferred types and further hybridization with wild species. Cassava was introduced in the fifteenth century into West Africa by the Portuguese from Brazil, and from there it spread to eastern Africa, Madagascar and southern India. Moreover, it was introduced in the sixteenth century into the Philippines by Spanish traders from Mesoamerica. Today the crop is cultivated worldwide in lowland tropics. World production of cassava root was estimated to be about 226 million tonne in 2006, with most production in Africa, where 122 million tonne were grown, while 67 million tonne were grown in Asia and 37 million tonne in Latin America and the Caribbean (FAO, 2006). The top ten cassava producing countries are: Nigeria (18% of world production), Brazil (12%), Thailand (10%), Indonesia (9%), Democratic Republic of the Congo (8%), Ghana (5%), United Republic of Tanzania (4%), India (4%), Mozambique (3%), and Angola (3%).

Cassava adapts to a wide range of eco-

logical conditions and is known for its tolerance of low soil fertility, drought and pests. The growing period is long, between 7 and 18 months. The yields are very high (about 30 to 40 t/ha under commercial practice). However, the protein content of cassava is low (<3% DM), which makes the crop ideal for starch production. Cassava is often grown in low input production systems, particularly when it is grown as a food crop. Planting material is easily obtained from plant stems available from the farmers' own or neighbouring fields. About 70 percent of cassava is grown by small-scale producers for direct human consumption. The crop tolerates more drought, lower soil levels of nitrogen, potassium and phosphorus, lower pH and higher aluminium levels than most other crops. Under these conditions, yields are about 7–10 t/ha. Cassava is often found in mixed stands, together with a variety of other food or cash crops. Estimates indicate that at least one-third of the cassava grown worldwide is intercropped (Cock, 1985).

Breeding objectives

FPB started in isolated programmes in the early 1900s when cultivation was extended by several colonial governments as a safeguard against famine, and breeding new clones with resistance against cassava mosaic disease (CMD) was required. Cassava breeding programmes started in Brazil (in the 1930s), India (in the 1940s), Indonesia (in the 1950s) and at two international institutions: CIAT, Colombia, (in the 1970s) and IITA, Nigeria, (in the 1970s). These institutions have developed a very successful cassava breeding network.

In cassava breeding, three diseases have been the highest priority for decades: (i) Cassava mosaic disease (CMD), which is a whitefly-transmitted virus widespread in Africa and India; (ii) Cassava brown streak disease (CBSD); and (iii) Cassava bacterial blight (CBB), caused by *Xanthomonas campestris* pv. *manihotis*, which can have devastating effects on yield in Africa. Of regional importance in Latin America and the Caribbean is Frogskin disease, suspected to be caused by a virus. Aside from these, cassava is much less affected by disease than other tropical crops, the only other two of importance being Cassava anthracnose disease (*Colletotrichum manihotis*) and root rots (*Phytophthora drechsleri* and *Rhizoctonia* spp.) (CIAT 2001; Hillocks and Wydra, 2002).

The major pests of cassava are nematodes (*Meloidogyne* spp.), whiteflies as a vector of CMD, Cassava green mites (*Mononychellus* spp. and *Tetranychus* spp.), cassava mealybug (*Phenacoccus* spp.), and the grasshopper (*Zonocerus elegans*). Pests and diseases, together with poor cultural practices, combine to cause yield losses as high as 50 percent. In the late 1980s, a new strain of CMD occurred in Uganda that made the virus more harmful. This mutated virus has been spreading and is now found throughout Uganda, Burundi, Cameroon, the Democratic Republic of Congo and Rwanda (Thresh and Cooter, 2005). Next in importance in breeding are more short and thick storage roots with high starch content. This is important for mechanical harvesting, but makes also manual harvesting easier. It is desirable for the roots to be as far as possible horizontal in the soil and near to the soil surface. Breeding selects for plants with lower height and higher harvest index. In cassava breeding for human consumption, the focus is on yield and quality such as low fibre, low levels of cyanogenic glucosides, high protein, elevated pro-vitamin A, iron and zinc concentration in the storage roots, reduced post-harvest physiological

deterioration and regional preferences for the peel of the roots (Ceballos *et al.*, 2004). Cassava varieties are often categorized as either 'sweet' (actually 'not bitter') or 'bitter', signifying the absence or presence of toxic levels of cyanogenic glucosides. The so-called 'sweet' cultivars can produce as little as 20 mg/kg cyanide in fresh roots, while 'bitter' ones may produce more than 50 times as much. Additionally, an important breeding objective is to develop more clones with high adaptation to drought-prone environments. The genetic variation in cassava for pro-vitamin A concentrations is small. However, breeding for yellow cassava genotypes with a pro-vitamin A concentration of 15 ppm appears to be possible. Additionally, a transgenic approach is used to introduce the β-carotene pathway into cassava (J. Tohme, pers. comm.). Breeding for commercial production also selects for plants with shorter height and higher harvest index – giving more stability and resistance against storms – and extensive branch formation, quickly forming a full canopy of leaves not too close to the soil (Byrne, 1984).

Breeding methods

Cassava is a monoecious, highly heterozygous plant. All 36 chromosomes show regular bivalent pairing at meiosis. However, in both cassava and *Manihot glaziovii* (sect. Arboreae) there is evidence of polyploidy from studies of pachytene karyology. There are three nucleolar chromosomes, which is high for true diploids, and duplication for some of the chromosomes. This indicates that *Manihot* species are probably segmental allotetraploids derived from crossing between two taxa whose haploid complements had six chromosomes in common but differed in the other three (Nasser, 2000). Cassava shows self-fertility with strong inbreeding depression and wide segregation in cross progenies. Time of flowering depends on the genotype. There are types in which flowering starts about 2 months after planting, as well as types that do not flower until after 24 months or more. This makes planned recombination difficult. Earlier and more abundant flowering is obtained by foliar application of indole acetic acid (IAA) and naphthalene acetic acid (NAA). The female flowers are large, nearly always located at the base of the inflorescence, and open first. The female flowers normally open 10–14 days before the males on the same branch, but self-fertilization can occur because male and female flowers on different plants of the same genotype can open simultaneously.

The proportions of self- and cross-pollinated seed produced depends on genotype, planting design and the type of pollinating insects present (5 percent self-pollination occurs naturally). Both the stigma and the pollen are sticky and pollination is easily carried out by honey bees. In the Northern Hemisphere, cassava usually flowers from July to January, with a peak between September and November. In the Southern Hemisphere, it usually flowers from January to July, with a peak between March and May. Tall plants with less branching are less floriferous than highly branched, low growing ones. To make a controlled cross between two parents, unopened flowers are first enclosed in muslin bags and the chosen pollen applied to the stigmas as soon as the female flowers open. The muslin bags are then replaced with netting bags to catch the seed when the ripe fruits dehisce explosively. The fertility of clones is variable and can be very low; an average of 1 or 2 seeds per fruit is common in controlled pollination. Seed matures 70 to 90 days after pollination. The fruits are collected when the coat begins to

shrivel and are sun dried until they shatter, releasing hybrid seeds that are ready for germination. Cassava seed have a very short dormant period and germinate quickly. No scarification is necessary. Few seeds germinate unless the mean temperature exceeds 24°C, with a temperature exceeding 30°C for at least part of the day; the best rates occur at 30–35°C. A dry heat treatment of 14 days at 60°C is also beneficial for newly harvested seeds. If temperatures permit and irrigation is available, the easiest method is to sow the seeds direct into the soil. This is successful at IITA because temperatures from January to March range from 30° to 35°C. At CIAT, seeds are frequently planted in a screen house and the emerging seedlings held until they reach 20–25 cm before being transplanted to well prepared soil with good moisture conditions.

Since many national programmes do not have a continuous cassava crossing programme, they rely on distribution of preselected clones from the two international institutions, CIAT and IITA. The improved germplasm generated is distributed either in the form of elite genotypes transferred *in vitro*, or as populations of recombinant seeds (full-sibs or half-sibs). Cassava breeding operates with larger populations than potato or sweet potato.

In West Africa, up to 100 000 true seed plants are raised from field-sown seed, which are screened in a first selection step for resistances to CMD and CBB. At harvest, selection is for compact roots with short necks, stems branching at about 100 cm, with low HCN in the leaves. In the second selection step, about 3000 clones are grown in small, non-replicated plots. Further selection is made for disease resistances, yield potential and root DM content, and the HCN in the roots is assayed enzymatically. For the third selection step, ca. 100 clones are tested in replicated trials at three locations, and consumer acceptance is assessed. Final selections are multiplied and enter dissemination in year 6.

In eastern and southern Africa, 10 000 to 50 000 true-seed plants are raised for the first selection step and screened for resistance to major diseases and pests at 1, 3, 6 and 9 months after sowing, namely East African cassava mosaic disease (EACMD), African cassava mosaic disease (ACMD), Cassava brown streak disease (CBSD) and CBB. In a second selection step, 2000 clones are planted in single-row plots (3 to 5 plants) at 1×1 m spacing. The observations made in the first year are repeated again at 1, 3, 6 and 9 months after planting. Each clone is scored for yield, and agronomic characteristics assessed, such as branching height and angles, canopy and number of stems per plant. In a third selection step, 20 to 50 clones are grown in preliminary yield trials in single rows with 10 plants per clone and three replications at one to three locations. In year 6, the final selections are taken on-farm and into national variety release trails.

In the Americas and Asia, cassava improvement is closely linked with the institutions EMBRAPA (Brazil), FCRI Rayoung (Thailand) and CIAT (Colombia). In contrast with Africa, there are no extremely devastating diseases. CIAT established 50 000 seedling selections for particular climatic zones. Up to 20 parents from each gene pool are disseminated for evaluations to national centres in similar edapho-climatic zones. From this programme a very broad range of improved diversity has been developed and distributed worldwide.

Generally, MVs in Asia can be traced back to 100 crosses between Asian and American parents (Kawano, 2003). Recent findings show that the general combining ability for cassava fresh root yields are

clearly larger than the specific combining ability across contrasting environments (Ceballos *et al.*, 2004). This is a clear indication that heterotic gene pools in cassava can be formed and exploited by improving two gene pools with a reciprocal recurrent selection scheme.

13.13 BANANA OR PLANTAIN

Breeding bananas and plantains has been reviewed by Rowe (1984), and Jain and Swennen (2001) have edited recent proceedings on banana improvement, with a main emphasis on biotechnology. Banana and plantain (*Musa* × *paradisiaca*, Musaceae, usually triploid with 33 chromosomes) originated in Southeast Asia. The term plantain is used for those bananas that are palatable only when cooked. The crop was introduced into Africa about 3000 BPE. Introduction into the Americas came after 1500 AD. Today the crop is cultivated worldwide in the tropics. Bananas and plantains evolved from two diploid wild species, *Musa acuminata* (AA) and *M. balbisiana* (BB) in the Eumusa series (x = 11) of the genus *Musa*. An exception is the small group of 'Fehi ' bananas in the Pacific, which have their origin in the Australimus series (x = 10) of *Musa*. All export fruit bananas are triploids (AAA) and originated from *M. acuminata*. All plantains and several locally preferred fruit bananas are hybrids between *M. acuminata* (AA) and *M. balbisiana* (BB). The higher dry matter (about 5–8 percent) and higher starch content of plantains compared to pure *M. acuminata* cultivars is attributed to the BB genome. The AAB cultivars have long curved fruits and appear like an oversized export banana. They are important food crops in south India, eastern and central Africa and tropical America. The ABB cultivars have thick straight fruits, which are much shorter than the AAB types (Simmonds, 1976; Ortiz, 1995). They are a staple in Samoa, the Philippines, south India and the West Indies. Around 87 percent of all bananas and plantains grown worldwide are produced by small-scale farmers for home consumption or for sale in local markets. About two-thirds of world production is dessert bananas and one-third plantains. The fruit export market comprises only one-sixth of total world production. The banana is the number one fruit crop in the world, with about 70.5 million tonne produced annually. The top ten producing countries are India (24%), Ecuador (9%), Brazil (9%), The Philippines (8%), China (8%), Indonesia (5%), Costa Rica (3%), Mexico (2%), Thailand (2%) and Colombia (2%). Plantains are grown as a staple food in 52 countries worldwide with a total production of 34 million tonne. The top ten plantain producing countries are Uganda (30%), Colombia (9%), Rwanda (8%), Ghana (7%), Nigeria (6%), Peru (5%), Cote d'Ivoire (4%), Congo (4%) and Kenya (3%) (FAO, 2006).

Bananas and plantains are one of the very few crops in which breeders are still trying to find an appropriate conventional breeding method to develop new MVs. Nearly all cultivars are FVs and have been selected from genetic variation developed by natural evolution. In cases of crop failure due to new pathogens and diseases, FPB still focuses on identifying alternative cultivars within existing genetic variation (collections and large screening programmes). Hence, an important source for identifying 'new' cultivars are germplasm collections held in trust in genebanks, such as the International Musa Germplasm Collection in Leuven, Belgium. Spontaneous mutants in *Musa* have played a very important role in banana and plan-

tain breeding, including the replacement of the export banana cultivar 'Gros Michel' (susceptible to Panama disease or Fusarium wilt (*Fusarium oxysporum* sp. *cubense*) by 'Cavendish' banana cultivars, which are resistant to most fusarium wilt pathogens, and the replacement of the plantain cultivar 'Horn plantain' (AAB) (susceptible to Black sigatoka (*Mycosphaerella fijiensis*)) by the 'Laknau' cultivar (AAB), which is tolerant to Black sigatoka and closely resembles the Horn plantain (Stover, 1972). However, the cooking qualities of Laknau are inferior to Horn plantain. Owing to the low level of occurrence of spontaneous mutations, mutagenic agents and mutation breeding have often been used to generate new genetic variation in bananas and plantains, followed by screening programmes for plants with resistance or tolerance to pest and diseases, coupled with desirable agronomic qualities (e.g. tolerance to Panama disease; tolerance to the toxin of *Mycosphaerella fijiensis*; short; larger fruit size; and earliness). The FPB programmes for bananas and plantains started in the early 1900s, to develop new AAA cultivars for the export market, with resistance against Panama disease or Fusarium wilt (*Fusarium oxysporum* f.sp. *cubense*). Despite continued breeding efforts, no new banana and plantain cultivar acceptable by farmers and consumers was bred until the 1980s (Roux, 2001). Nevertheless, by the end of the twentieth century, efforts to improve *Musa* started to focus on the use of diploid and tetraploid gene pools to develop triploid and tetraploid bananas and plantains. To date, the first improved cultivars (AAA, AAAA, AAB, AAAB and AABB), developed at *Fundación Hondureña de Investigación Agrícola* (FHIA) in Honduras through the International Musa Testing Program (IMTP), have been widely distributed. However, for several of these FHIA cultivars, taste and cooking qualities are still problematic (Roux, pers. comm.). Further breeding programmes have been set up at the *Empresa Brasiliera de Pesquisa Agropecuaria* (EMBRAPA) in Brazil, the *Instituto de Investigaciones en Viandas Tropicales* (INIVIT) in Cuba, the *Centre Africain de Recherches sur Bananiers et Plantains* (CARBAP) in Cameroon, the International Institute of Tropical Agriculture (IITA) in Nigeria, and the National Research Centre on Banana (NRCB) in India.

Breeding objectives

In breeding, resistance against Panama (Fusarium wilt) and sigatoka diseases are in the foreground. In the first half of the twentieth century, Panama disease destroyed approximately 40 000 ha of bananas in Central and South America. Fortunately, resistant Cavendish cultivars could substitute for the predominantly grown Gros Michel variety. However, Cavendish cultivars are not resistant to all fusarium wilt pathogens (i.e. race 4). It should be noted that Panama disease cannot be controlled chemically, so that use of resistant varieties is the only way to maintain production in regions with challenge from this disease. The leaf spot diseases caused by *Mycosphaerella musicola* (Yellow sigatoka) and *M. fijiensis* (Black sigatoka) are costly pathogens and must be regularly controlled by fungicides. Cultivars with an AAA genome are very susceptible to both sigatoka diseases. The Horn plantain is resistant to Yellow sigatoka, but susceptible to Black sigatoka. The latter disease threatens continued cultivation of the plantain food crop. Triploid cooking bananas of the ABB type, such as 'Chato', 'Pelipita' and 'Saba', are highly tolerant to the Black sigatoka

pathogen. However, Chato is susceptible to bacterial wilt or Moko disease caused by *Pseudomonas solanacearum* and to race 2 of *Fusarium oxysporum* f. sp. *cubense*, while Pelipita does not meet flavour and fruit-shape preferences, so that currently only Saba remains as a possible substitute for the Horn plantain. Moreover, nematodes, mainly the burrowing nematode (*Radopholus similis*), are a major constraint to bananas in monoculture, and outside of the Americas the Bunchy top virus is widely distributed, which is transmitted by the banana aphid (*Pentalonia nigronervosa*). Many diploid accessions of *M. acuminata* subsp. *malaccensis* and *M. a.* subsp. *burmannica* are resistant to races 1, 2 and 4 of Panama disease. Sources of resistance to Yellow sigatoka are available in several subspecies of *M. acuminata*, while *M. a.* subsp. *burmannica* is highly tolerant to the Black sigatoka fungus. The tolerance in *M. acuminata* accessions to sigatoka diseases is apparently controlled by several dominant genes. Resistance to the burrowing nematode has been found in the 'Pisang Jari Buaya' group of diploid accessions. The resistance is controlled by one or very few dominant genes and has been incorporated into diploid and polyploid progenies. Today, several FHIA varieties are resistant to burrowing nematodes (Kalorizou, Gowen and Wheeler, 2007).

Among agronomic qualities, dwarfness is most important in bananas and plantains, because they are often grown in areas with periodic strong winds. Dwarf and semi-dwarf mutants have been found in many diploid and triploid bananas and plantains. Examples are 'Highgate' (a dwarf mutant of Gros Michel) and the Cavendish cultivar 'Grand Nain'. In dwarf diploids, the dwarfness character is controlled by a single dominant gene. After this in importance are fruit characteristics and tillering capacity (Ferwerda and Wit, 1969; Rowe and Richardson, 1975; Persley and De Langhe, 1987).

Breeding methods

Triploid bananas and plantains are vegetatively parthenocarpic, i.e. no pollination is necessary for fruit development. In diploids, pollination often results in seeded fruits. Diploids are not suitable as varieties since fruit size and plant vigour are low. However, diploids are the basis for crop improvement. In the initial stages of breeding efforts, a few seeds per bunch in some triploid varieties were used when these had been pollinated by diploid genotypes. The reason for this seed production and genetic variation is the formation of unreduced triploid gametes in some triploid female parents after pollination within diploid male parents, which produces reduced haploid gametes. The progenies of these crosses are tetraploid. This method was used to generate genetic variation with the female banana parent Gros Michel and the female plantain parent Laknau (AAB), which closely resembles the Horn plantain. Tetraploid hybrids (AAAA) from crosses with Gros Michel were resistant to Panama disease and closely approached commercial acceptability, but the inferior agronomic characteristics of the diploid parents were also present in the hybrids. Triploid hybrids derived from crosses between these tetraploid hybrids and diploid genotypes were useless. Unfortunately, the cooking qualities of hybrids derived from Laknau were also inferior to those of the Horn plantain. No seeds have been produced from Cavendish clones and no other suitable triploid parents—except Gros Michel and Laknau—for seed production by unreduced gametes have been found. This

breeding method has not succeeded in creating acceptable new varieties. However, the major finding of this work was that it is necessary to improve the diploid male parent gene pool to increase the chances of developing either new tetraploid or new triploid varieties.

Today, banana and plantain breeding aims at producing tetraploid and triploid varieties on the basis of diploid accessions resistant to various diseases, and the continuous improvement of this diploid gene pool for agronomic qualities (i.e. plant height, fruit characteristics and tillering capacity) as well as high pollen production. Crossings within the diploid gene pool are complex: the diploid 'SH-2095', which was later successfully used in tetraploid variety development, was derived from a four-way cross of three diploid cultivars and one wild accession (('Sinwobogi' × 'Tjau Lagada') × ('Guyod' × a wild *Musa acuminata* subsp. *malaccensis*)). Nevertheless, the genetic basis of diploid pollen parents with improved agronomic performance is considerably wider than in the past. The currently best diploids are continually crossed on triploid Highgate and Laknau, which produces unreduced triploid gametes, as well as on seed-fertile tetraploids with good agronomic performance. The first results in new potentially tetraploid varieties and the later in new potentially triploid varieties. The advantage of triploids in variety development is that they are female-sterile due to the uneven number of chromosome sets. In contrast, the even number of the chromosome set in tetraploids requires an additional selection step for female sterility in variety development. Several improved FHIA cultivars (AAA, AAAA, AAB, AAAB and AABB) have been developed by this breeding method, and farmers participate in the final breeding stages in acceptability studies of these tetraploid and triploid varieties (i.e. PVS) (Ssemwanga, Thompson and Aked, 2000; Ludger, 2005; Kalorizou, Gowen and Wheeler, 2007). However, to our knowledge, no PPB has been applied in early breeding stages. Most likely the reason for this is that almost no diploid clone in its performance *per se* would achieve acceptability by farmers. Nevertheless, the future of banana and plantain breeding, as in other clonally propagated crops, should be seen in testing the combining ability between two gene pools and in the improvement of two gene pools on the basis of the general combining ability and reciprocal recurrent selection. In banana and plantain breeding, such a breeding system can be established by a seed-fertile diploid gene pool with high pollen production and a seed-fertile tetraploid gene pool, which is used as the male parent. In such a breeding programme, PPB could easily be incorporated. However, the important information provided by the farmers would not be seen in the evaluation of clone performance *per se* in the diploid and tetraploid gene pool, but in the numbers of acceptable clones per cross combination and family between genotypes of the diploid and tetraploid gene pool, as described above in the section on selection of parents and cross prediction.

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