Chapter 8

Breeding for Crop Improvement

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Introduction

Cassava has been evolving as a food crop ever since it became important in the second and third millennium BC (Reichel-Dolmatoff, 1965; Lathrap, 1973), but its adaptation to African and Asian conditions did not begin until post-Columbian times. In the Americas, Africa and Asia, progress towards improved adaptation and quality was first through subconscious selection by farmers. A wide range of genetic diversity was generated through centuries of such farmer selection (Bonierbale *et al*., 1995). It was not until the present century that serious attempts began by national organizations to improve the crop by plant breeding. Much of this was instigated by the colonial powers and was very successful, but progress slowed considerably when countries became independent. This trend was arrested in the 1960s, when the increasing world population and the limited supply of energy foods prompted a surge of interest in the crop.

High priority was given to cassava breeding and related research when the International Institute of Tropical Agriculture (IITA) was opened in Nigeria and the Centro Internacional de Agricultura Tropical (CIAT) was opened in Colombia. For the first time breeders and associated scientists were given resources to study the crop in depth and to assess the extensive variation available. The two International Centres collaborated with existing national programmes and instigated the initiation of new ones. In India the Central Tuber Crops Research Institute (CTCRI) took on a similar role. The objectives were to increase both the yield per unit area and the area under cultivation, and also to improve root quality.

Cytotaxonomy of the Genus *Manihot*

Manihot esculenta (cassava) is placed in the *Fruticosae* section of the genus *Manihot*, which is a member of the Euphorbiaceae. The *Fruticosae* section contains low-growing shrubs adapted to savannah, grassland or desert and is considered less primitive than the *Arboreae* section, which contains the tree species. The genus occurs naturally only in the Western hemisphere, between the southwest USA (33°N) and Argentina (33°S), and shows most diversity in two areas, one in northeastern Brazil extending towards Paraguay, and the other in western and southern Mexico.

All the species so far studied have 36 chromosomes, which 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

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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. Studies with biochemical markers identified by electrophoresis support this interpretation, in that they show disomic inheritance at 12 loci, with evidence of gene duplication (Jennings and Hershey, 1985; Charrier and Lefevre, 1987).

Flower Behaviour, Hybridization Techniques and Seed Management

Cassava is monoecious. 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 branches or on different plants of the same genotype open simultaneously. The proportions of self- and cross-pollinated seed produced depends on genotype, planting design and the type of pollinating insects present.

The availability of flowers is influenced by plant habit, because branching always occurs when an inflorescence is formed (Fig. 8.1). Hence tall, unbranched plants are less floriforous 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 is 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. Another system, for example where cyclic breeding methods such as those used in other out-breeding crops are followed, is to plant a set of varieties in a specially designed crossing block (Fig. 8.2), and to remove all the male flowers from the varieties to be used as females. The separation of male and female flowers makes the control of pollination easy, but it is still laborious to produce large quantities of seed (Kawano, 1980; Hahn, 1982). Polycross designs similar to the ones used for forage crops can also be used for cassava, using a random distribution of elite genotypes replicated several times. This method does not prevent self-pollination, but it produces considerably more cross-bred seeds than controlled pollination methods (Wright, 1965).

The fertility of clones is variable and can be very low. An average of one seed per fruit is commonly achieved through controlled pollination from a maximum of three from the trilocular ovary. The genotype of the female parent is more important in determining success than that of the pollen (Jennings, 1963).

Newly harvested seeds are dormant and require 3–6 months storage at ambient temperatures before they will germinate. Germination can be hastened by carefully filing the sides of the seed coat at the radicle end and by temperature management. Ellis *et al.* (1982) found that

Fig. 8.1. Flowering cassava showing the association of branching with inflorescence development.

Fig. 8.2. Cassava crossing block at IITA in which all male flowers were removed from parents being used as females and the resulting fruits from cross-pollinated flowers are being collected in muslin bags before they dehisce explosively.

few seeds germinated unless the temperature exceeded 30°C for at least a part of the day and the mean temperature exceeded 24°C; the best rates occurred at 30–35°C. A dry heat treatment of 14 days at 60°C was also beneficial for newly harvested seeds. If temperatures permit and irrigation is available the easiest method is to sow the seeds directly into the soil. This is successful at IITA because temperatures from January to March range from 30 to 35°C (Hahn *et al.*, 1973). 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. Seeds for storage should be kept at 5°C and 60% relative humidity (IITA, 1978) because they lose viability rapidly during a year's storage at ambient temperature (Kawano, 1978).

Breeding Strategy

The worldwide emphasis of the breeding work at the international centres implied that the objectives would be broad and that a large and variable number of characteristics would be required to achieve them. To meet all the local needs, improvements at IITA and CIAT would first have to be incorporated into broadly based breeding populations which would then be subjected to further selection at national centres. Previously, it was sufficient to make crosses between the best local varieties.

The policy adopted was to create improved populations into which exotic germplasm from several sources could be introgressed, while retaining the desirable gene complexes already present and allowing sufficient inbreeding for the expression and elimination of recessive ones (Hahn *et al.*, 1973, 1979; Hershey, 1981, 1984; Hahn, 1982). It was desirable to minimize inbreeding and to restore heterozygosity fully to avoid inbreeding depression (Kawano *et al.*, 1978a). The improved germplasm generated was distributed either in the form of elite genotypes transferred *in vitro*, or as populations of recombinant seeds (full-sibs or half-sibs) (Bonierbale *et al.*, 1995).

Breeding strategy for Africa

The germplasm of the original importations to Africa was inevitably narrowly based, but natural intercrossing among highly heterozygous varieties and subconscious selection among the resulting self-sown seedlings made possible the rapid progress towards local adaptation. Natural crossing with the introduced *M. glaziovii* (Ceara rubber) produced the 'tree cassava' and may have broadened the genetic base of the crop

in Africa. All the introduced germplasm was probably as highly susceptible to cassava mosaic disease (CMD) as most of the present American germplasm, but a level of tolerance was achieved quickly, and acceptable yields were usually obtained.

Nevertheless, the effects of CMD were often so devastating that most national programmes concentrated on breeding for resistance to it. At IITA, Hahn and his co-workers set out to create base populations by cyclic selection and recombination, and to upgrade them with a range of new high-yielding germplasm which included some highly CMD-susceptible germplasm from the Americas. They used a system based upon half-sib test-crosses, in which the selections and local, virus-resistant tester varieties were grown in isolation blocks. Three-plant plots of each of the selections and the local varieties were planted in several replications to favour random crossing, and the local varieties became universal pollinators when the male flowers from the other parents were all removed. The resulting progenies were grown in replicated trials which were sometimes duplicated in contrasting environments.

Improved cultivars have been released in several African countries (Table 8.1) although their adoption has sometimes been disappointing.

Both controlled and open-pollinated methods of breeding are used currently at IITA, but the scale of the latter procedure being followed in the 1980s can be seen from the following:

- Year 1: Up to 100,000 seedlings were raised from field-sown seed and screened for resistances to CMD and cassava bacterial blight (CBB). At harvest, selection was for compact roots with short necks, stems branching at about 100 cm and for low HCN in the leaves.
- Year 2: Up to 3000 of the selections from year 1 were grown in small non-replicated plots. Further selection was for disease resistances, yield potential and dry matter content of the roots, and the HCN in the roots was assayed enzymatically.
- Year 3: Up to 100 of the selections from year 2 were tested in replicated trials at three locations and consumer acceptance was assessed.

Table 8.1. Cassava cultivars released by National Programmes in Africa.

Country	Variety
Benin	TMS 30572, TMS 4(2) 1425, TMS 30572A, Ben 86052
Burundi	40160–1, 40160–3
Cameroon	8034, 8017, 8061, 820516, 1005, 658, 244
Ivory Coast	TMS 30572, TMS 4(2) 1425
Gabon	CIAM 76–6, CIAM 76–7, CIAM 76–13, CIAM 76–33
Gambia	TMS 60124, TMS 4(2) 1425
Ghana	TMS 30572, TMS 50395, TMS 4(2) 1425
Guinea Conakry	TMS 30572, TMS 4(2) 1425
Guinea Bissau	TMS 4(2) 1425, TMS 60142
Liberia	CARICASS 1, CARICASS 2, CARICASS 3
Malawi	Mbundumali, Gomani, Chitembwe
Mozambique	TMS 30001, TMS 30395, TMS 42025
Nigeria	N. C. idi-osi (TMS 30572), N. c. savannah (TMS 4(2) 1425), TMS 91934, TMS 90257, TMS 84537, TMS 81/00110, TMS 82/00661
Rwanda	Gakiza, Karana, TMS 30572
Sierra Leone	ROCASS 1, ROCASS 2, ROCASS 3, NUCASS 1, NUCASS 2, 80/40, 80/61
Togo	TMS 4(2) 1425, TMS 30572
Uganda	NASE 1 (TMS 60142), NASE 2(TMS 30337), MIGYERA (TMS 30572)
Zambia	LUC 133
Zaire	Kinuani, F100, 4023/3, 02864, Lwenyi/3

Source: Mahungu *et al.* (1994).

- Year 4: Selection was continued for up to 25 selections in larger trials at more locations.
- Year 5: Five of the best selections were tested on farms.
- Year 6: The final selections were multiplied and distributed.

It was found that germplasm from the Americas gave populations with large yield improvements, but that two generations of breeding with parents resistant to CMD and CBB were necessary to achieve acceptable resistance levels. Hybrids between East African selections and Nigerian varieties were the best sources of the two resistances. Seeds from the improved material were used to establish new populations in other parts of Africa. The new environments imposed new requirements, but the wide genetic base of the parental populations allowed for the selection of new traits, including resistance to diseases not prevalent at IITA. Ideally, where resources permitted, the best selections were intercrossed to produce new populations for progressive improvement in their adaptation to the local ecologies.

Breeding strategy for the Americas

Although cassava is indigenous to the Americas, the first breeding did not start there until 1948 at Campinas in Brazil, and little work elsewhere was started until the 1970s (Normanha, 1970). The main reason was that there was no single widespread and devastating disease of overriding importance as in Africa, and the crop was considered to have few problems requiring plant breeding work: diseases and pests were plentiful but the problems were not too serious and essentially local ones. Also, the crop never enjoyed a high priority within the research plans formulated by National Programmes. However, the intensification of production accentuated the need for better varieties, and, soon after its inception, CIAT began a programme which was based upon crop improvement by controlled breeding.

The objective of the breeding at CIAT was to provide germplasm for environments extending throughout both the American and Asian tropics and subtropics. The diversity in climates, soils, pests and diseases presented such a broad array of objectives that they could not easily be achieved by selection within a single population or at a single site. The breeders (Hershey, 1984) therefore classified the areas into seven so-called edapho-climate zones, each characterized by a set of soil and climatic conditions which differentially affected the performance of cassava genotypes and determined the incidence and severity of pests and diseases. Each zone had its own adaptation requirements and the landraces grown in each of them had almost certainly persisted because they were suitably adapted to them. Any attempt to improve them, therefore, had to be through selection in the particular ecosystem and from germplasm adapted to the particular stresses present. A gene pool for each zone was therefore created by intercrossing among genotypes selected for good performance in each zone.

The zones were differentiated first by temperature and then by rainfall and soil type. They included four ecosystems in the lowland tropics: humid, subhumid, acid soil savannahs and semiarid; areas of medium altitudes; highlands; and the subtropics. Priority for germplasm development was related to the importance of the crop in the different ecosytems worldwide. The distinct populations were created by both controlled and open-pollination methods, and were continually upgraded by recurrent selection and the introduction of new germplasm. Hershey (1984) describes the following procedure being followed in the 1980s:

- Year 1: Up to 50,000 seedlings were established at CIAT in groups based upon the adaptation of the parents to particular edapho-climatic zones. After 6 months, low selection pressure was applied for plant and root type to give about 25,000 selections; one cutting from each was used for testing in the zone for which the population was planned and another was retained at CIAT. Further selection was then made, including selection for disease and pest resistances.
- Year 2: Up to 3000 selections from year 1 were tested further in non-replicated plots for the above characteristics, plus root dry matter and HCN contents, both at CIAT and at one of the other target sites.

- Year 3: Up to 300 selections from year 2 were further tested in yield trials at several sites.
- Year 4: Up to 100 selections from year 3 were tested in larger trials at several sites.
- Year 5: Up to 20 selections were further tested in a Colombian trial network.
- Year 6: Promising selections were distributed for evaluation to national centres, usually in similar edapho-climatic zones.

From this programme a very broad range of improved genetic diversity was produced and distributed to cassava breeding programmes all over the world from CIAT in Colombia (Bonierbale *et al.*, 1995).

Breeding strategy for Asia

CIAT provides support to Asia in the form of seed for local selection, and IITA provides germplasm segregating for resistance to mosaic disease to India. India has a distinct form of this disease but is the only Asian country affected. The main cassava breeding activity in Asia was developed jointly by CIAT and the Field Crop Research Centre in Rayong, Thailand. According to Kawano *et al.* (1998) the basic scheme consisted of:

- Year 1: Up to 5000 seedlings from up to 100 crosses between Asian and Latin American parents were sown and transplanted in seedling trials in Rayong. After 8–10 months a low selection pressure was applied for plant and root type to give about 700 selections.
- Year 2: Up to 700 selections from year 1 were tested further in Rayong in non-replicated single rows of ten plants for the above characteristics, plus root dry matter and root yield. Special emphasis was given to selection for high harvest index.
- Year 3: Up to 80 selections from year 2 were further tested in a preliminary yield trial at Rayong, consisting of two replications of 50 plants each. The same traits were evaluated as in the single row trial.
- Year 4: Between 20 and 25 advanced selections were tested in larger yield trials in at least three locations: Rayong, Khon Kaen and Mahasarakarm, that represent a wide range of production conditions.
- Year 5–6: Between six and eight elite genotypes were planted in regional trials in at least seven locations for 2 years.
- Year 7: Selected genotypes were distributed for evaluation in other national programmes (Vietnam, China, Indonesia and Philippines), and were multiplied for further testing and distribution to farmers in Thailand.

Kawano *et al.* (1998) commented that over a period of 14 years, some 372,000 genotypes from 4130 crosses had been evaluated in their programme in Thailand, but only three genotypes had passed the tests for official release. These improved varieties occupied almost 400,000 ha in 1996, generating an economic impact estimated at around US$278 million.

Breeding for High Yield

High yield is achieved first by selecting plants that have both a genetic structure and a plant structure which maximizes performance, and then by bringing together resistances or tolerances to the factors which limit yield. Hybrid vigour through heterozygosity is the main requirement for the genetic structure of new varieties and this is a major objective of the strategies described above. The genetic base of the material imported into Africa was necessarily narrow; hence the considerable hybrid vigour obtained at IITA from crosses with new germplasm from the Americas. Elsewhere, vigour has been maintained by keeping the genetic base as wide as possible.

It may be beneficial to enlarge the genetic base further by making interspecific crosses with some of the many shrub species of the Fruticosae section of *Manihot*. Jennings (1959) obtained considerable hybrid vigour by crossing cassava with *Manihot melanobasis*, but this species may be a misnamed form of *M. esculenta* (Rogers and Appan, 1970, 1973). The subgenus has other as yet untried candidates that have tuberous roots and may provide new opportunities for increasing heterozygosity. These might include *Manihot aesculifloia*, *Manihot rubricantis*, *Manihot augustiloba* and *Manihot priuglei* that are mentioned by Rogers and Appen (1970), as well as the subspecies, *M. esculenta flabellifolia* and other species discussed in Chapter 4.

Models for high yield; the significance of plant habit, leaf longevity and disease resistances

Cassava plant habits are so variable that efforts have been made to discover which of them is best equipped for giving high yields: essentially the ability to convert solar energy into starch and store it in the roots. As physiological information became available, computer modelling was used to estimate the effects of the many variables, including those associated with stress and disease (Hunt *et al.*, 1977; Cock *et al.*, 1979).

The hypothesis followed was that crop growth rate increases at a decreasing rate as leaf area increases, whereas the dry matter required for stem and leaf production increases linearly with the leaf area index (LAI, a function of the rate of leaf formation, leaf size and longevity). Hence root growth rate, which is the difference between the total growth rate and that of the tops, increases up to a certain level of LAI and then decreases. Thus there is an optimum LAI for yield, and manipulations of the components of LAI can bring it closer to this optimum and maximize yield.

It turns out that root growth declines at values of LAI above 4, apparently because the resources required to form and maintain a higher LAI increase approximately linearly with LAI and leave less material for root growth. Leaf and stem growth have preference over root growth and the latter receives only the carbohydrate remaining after the requirements of the tops have been met (Gijzen *et al.*, 1990). The size of the roots rarely limits yield, and it is the LAI and not the root sink that determines it. Indeed, the roots can accept much more carbohydrate than is normally available (Lian and Cock, 1979; Pellet and El-Sharkawy, 1994).

These findings have a profound effect on selection criteria, selection procedure and even selection priorities. Among existing varieties, branching habit affects LAI the most. Varieties that branch 6–8 weeks after planting and six to eight times a year with four branches formed on each occasion, allocate too little of their resources to the roots. Their total dry matter production may be high and they compete well in unimproved 'extensive' agriculture, but their distribution of dry matter to the roots is too low for high yield. The models show that branching should be delayed until 30 weeks, leaf size to 500–600 cm^2 and leaf life prolonged to 15–20 weeks (Cock *et al.*, 1979).

Plants with delayed branching are desirable not only for high yield but also because they facilitate mixed cropping with other crops, which leads to the maximum food yield per land unit. Leaf longevity was not previously considered important, but it prolongs dry matter production without using resources (El-Sharkawy, 1993). Hence resistance to diseases that cause premature leaf fall are important too.

The models aid selection procedures because they explain why there is no correlation between the yields of plants in mixed populations and their yields in single row trials ($r = 0.068$). This is because the former is determined by competitiveness, for which the optimum LAI is higher than the optimum for the latter. Selection cannot therefore be done for yield itself in the mixed populations present in the early stages of breeding, but it can be done on harvest index, which is the root weight expressed as a proportion of total plant weight. Not only is this value correlated in the two kinds of population ($r = 0.608$), but it is highly correlated with root yield ($r = 0.763$), and has a high heritability, the harvest indices of progenies being highly correlated with the means of their parents ($r = 0.745$). Hence, Kawano and Thung (1982) and Kawano *et al.* (1998) reported high correlation and regression coefficients for harvest index with root yield and demonstrated the effectiveness of using the trait at all stages of selection as an indirect selection for root yield. In practice, plant competition is minimized by wide spacing (e.g. 1 m apart in rows which are 2 m apart), and the selection criterion is for plants whose main stem does not branch until it reaches about 1 m (Fig. 8.3; Kawano *et al.*, 1978b; Hahn *et al.*, 1979). Taller plants with higher branching are also less productive (Fig. 8.4). but are often preferred by smallholders because they facilitate mixed cropping with other food plants.

The models also explain the genotype interactions with temperatures that are encountered when breeding is for adaptation to altitudes above 2000 m, where average temperatures often fall to 17°C. Most varieties yield badly in these situations because they produce an inadequate LAI, but the LAI may become excessive if temperatures rise and the top growth of the plant

Fig. 8.3. Cassava with plant habit ideal for high root yield, showing branching at an intermediate height. Courtesy of CIAT.

Fig. 8.4. Tall cassava with high branching and relatively low root yield. Courtesy of CIAT.

increases. A common situation is for the most vigorous genotypes to yield the most at 20°C and the least at 28°C, and for the least vigorous ones to yield the least at 20°C and the most at 28°C. The optimum phenotype (plant type) is the same, but the genotypes that achieve it change with temperature (Irikura *et al.*, 1979).

By determining the consequences of malfunction in each organ, the models help to decide breeding priorities, i.e. they show which diseases are sufficiently damaging for resistance to them to be given a place in the programme (Cock, 1978). It turns out that priority cannot be justified for diseases which cause plant death on a moderate scale, small decreases in tuber number or small decreases in leaf size. Breeding emphasis is justified for all disorders that reduce leaf life or photosynthetic efficiency, cause stem damage or high levels of early plant death. However, although traditional farming systems often require only limited disease control, the lack of resources for purchased inputs by small farmers often forces breeders to take account of host resistance. In any case, the priorities of disease control will increase as progress is made towards an optimum plant habit, when leaf area, for example, will become more important, or if a change towards more densely planted monocrops aggravates the existing disease problems.

Breeding for Root Quality: Starch and Dry Matter Content

Cassava is used for diverse purposes and so most of the criteria for quality are also diverse, but

high starch content and quality is always required. Starch content is usually estimated from dry matter percentage, to which it is highly correlated ($r = 0.810$; IITA, 1974; CIAT, 1975), but a quicker method is to determine the root's specific gravity, which is related to both dry matter and starch content. A calculation can be obtained from the specific weight of a sample (3–5 kg) of unpeeled roots in air and water, or by passing samples through a series of sodium chloride solutions of increasing specific gravity to find the one with the lowest specific gravity in which the sample will float (Hershey, 1982).

A high dry matter content is not necessarily ideal because, for reasons unknown, it is associated with postharvest deterioration. This can be serious for commercial outlets, but not where roots are used immediately as in subsistence agriculture. Dry matter content is not associated with fresh root yield and it is still uncertain whether a high level can be maintained when yields are high: progress in one may require sacrifice in the other (CIAT, 1981; Iglesias *et al.*, 1994). Similarly, substantial progress towards a capacity for prolonged postharvest storage may be difficult, but genetic differences have been identified (Kawano and Rojanaridpiched, 1983). More recently, Iglesias *et al.* (1996) showed that it was possible to break the association between high dry matter and high postharvest deterioration, and that the heritability of the trait is high enough for considerable progress through conventional breeding.

Starch quality is influenced by the amylose content, which for good cooking varieties is 21%, for industrial varieties (more waxy types) is 15% and for multiple-purpose varieties is 17% (IITA, 1977). Wheatley *et al.* (1992) found a range of 15–28% amylose in the roots of plants in the CIAT germplasm collection. No waxy (zero amylopectin) mutants have been detected, but variations in the ratio of amylose to amylopectin could open new markets for cassava starch in future. Most of the efforts in the early days of the international centres were devoted to cassava as a human staple, but nowadays, knowledge of the genetic variation available in terms of root and starch quality may provide opportunities for marginal regions to expand into global markets.

Breeding for Low Content of Cyanogenic Glucosides

Hydrocyanic acid (HCN) forms when two cyanogenic glucosides (linamarin and lutaustralin) are hydrolysed by endogenous enzymes. Mutant acyanogenic varieties which lack genes for the production of either the glucosides or the enzymes would be ideal, but no such mutants have been found in germplasm collections or segregating progenies, probably because they are likely to be recessive and difficult to discover in cassava because of its polyploid make-up. Breeders therefore select for low HCN content, which is conferred by a complex of recessive minor genes (Hahn *et al.*, 1973). Recent studies have suggested a role of cyanide in the resistance of cassava to pests (Bellotti *et al.*, 1999). The possibility of confining the cyanogenic glucosides to non-edible plant parts in order to maintain pest resistance therefore needs to be explored, in parallel to efforts to decrease cyanide in the edible parts.

Since the glucosides are synthesized in the leaves and translocated to the roots, a common practice was to screen leaves semi-quantitatively using a sodium picrate test. However, a more accurate enzymatic analysis (Cooke, 1978) has been automated for rapid determinations (Hahn, 1984). Root analyses are now preferred because the correlation ($r = 0.36$) between leaf and root results is very low, probably because of the high variation detected for the trait in both tissues. Reports of independent synthesis of linamarin in the roots (Makame *et al.*, 1987) would certainly reduce the correlation between the occurrence of cyanogens in the leaves and roots. The correlation between the two tissues is better if determinations are confined to young leaves and root peel (CIAT, 1982). Most breeding programmes screen at the early stages with the sodium picrate test (Cooke *et al.*, 1978; CIAT, 1982), sometimes using modifications suggested by O'Brien *et al.* (1994) and Yeoh *et al.* (1998), and then evaluating advanced selections using enzymatic methods.

There appears to be no obstacle to combining low HCN with the other desirable root qualities sought, but it is difficult to reduce levels to below 10–20 p.p.m. Hahn (1984) produced a low HCN population by continuous selection and

recombination. Selections from such material have a special role where leaves are eaten as a vegetable. Leaves are rich in protein and provide a dietary complement to the roots. However, HCN is not the only factor responsible for bitterness in the roots, though roots with levels below 10 mg 100 g^{-1} are generally considered to be sweet.

Breeding for High Content of Protein and Other Nutritional Elements in the Root

The primary function of cassava roots is to store starch, and attempts to enhance the protein content could well have adverse effects on this function. It is probably better to obtain protein from other sources. Nevertheless, cassava germplasm with high root protein content is available, and attempts to use it in breeding have been made.

Several Indian varieties have a high protein content (Hrishi and Jos, 1977), as well as some related species, notably several from Brazil (Nassar and Costa, 1977; Nassar 1978), *Manihot saxicola* (Bolhuis, 1953) and *Manihot melanobasis* (Jennings, 1959). The protein contents of the interspecific hybrids derived from the last two species tended to decrease as backcrossing proceeded, however.

Selection for increased vitamin and mineral content was recently initiated at CIAT, targeted toward regions with severe deficiencies, mainly in vitamin A. Considerable improvement in carotene content can be achieved within the existing cassava germplasm (Iglesias *et al*., 1996, 1997).

Breeding for Resistance to Cassava Mosaic Disease (CMD) and to Cassava Brown Streak Disease (CBSD)

CMD is caused by whiteflyborne cassava mosaic geminiviruses (CMGs) and occurs in Africa and India (Chapter 12).Variation in virus virulence occurs and an exceptionally virulent variant found in Uganda is probably a hybrid of the two African forms (Deng *et al*., 1997). There is a wide but apparently continuous range in the expression of host resistance. There is no evidence that the resistance is specific to particular virus forms, and the highest levels of resistance available are needed to control the most virulent virus forms encountered. Hence the resistant germplasm bred in East Africa was used to initiate resistance breeding at IITA in Nigeria (Beck, 1982; Jennings, 1994) and selections from the IITA programme were successfully used as resistance donors in India (Hahn *et al*., 1980a).

After many years of limited progress in breeding with cassava germplasm at many African centres, a change of emphasis occurred when the programmes at Amani (Tanzania) and Lac Alaotra (Madagascar) began the task of transferring resistance from the tree species *M. glaziovii* Muell-Arg, *Manihot dichotoma* Ule, *Manihot catingae* Ule, *Manihot pringlei* Watson (which was probably misnamed; Rogers and Appan, 1970) and 'tree cassava', which was thought to be a natural hybrid of *M. glaziovii* and cassava. It took three or four backcrosses, made over 15 years, to restore tuberous roots and lose the tree-like characteristics of the donor species (Fig. 8.5). Only the hybrids with *M. glaziovii* were ultimately successful (Nichols, 1947; Cours, 1951; Jennings, 1957).

The resistance of the best backcross hybrids from Tanzania was adequate for most but not all situations. Resistance was later shown to be multi-genic and recessive in inheritance (Jennings, 1978; Hahn *et al*., 1980b). Intercrossing among resistant selections began in 1953, and was successful probably because it concentrated the recessive genes from different sources and made them homozygous. The material obtained from this intercrossing was much more resistant than the backcross hybrids and was the origin of the resistant parents used at IITA later.

Resistance was assessed by the proportion of symptom-bearing plants or branches present, and by the symptom intensity. The two estimates were correlated ($r = 0.43$ and 0.48 in two trials) and the efficiency of assessment was improved by 38% when both aspects were considered together (Jennings, 1957, 1994).

Experiments and observations in Tanzania (Jennings, 1957, 1960a), Madagascar (Cours-Darne, 1968) and Nigeria (IITA, 1980; Rossel *et al*., 1988) lead to the following concept of resistance (Jennings, 1994): when exposed to infection, an unknown proportion of resistant

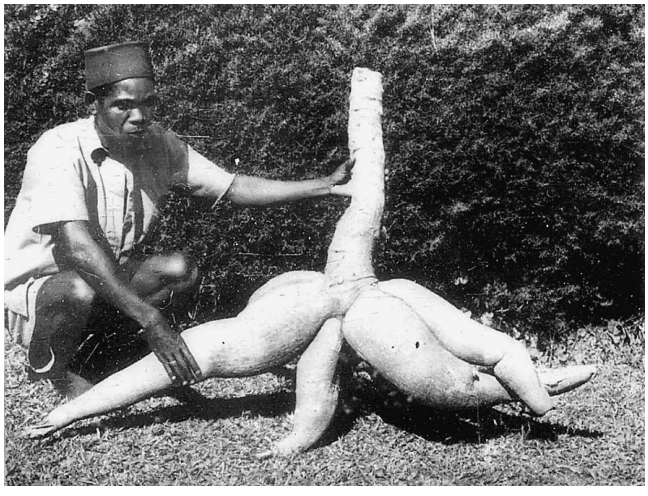

Fig. 8.5. Vigorous second backcross hybrid of *Manihot glaziovii* to cassava with large tuberous roots but of only intermediate quality produced at Amani, Tanzania.

plants (which could well be 100%) become infected at one or more of their stem apices. Some of them localize the virus at their bases and either remain symptom-free or show only transient symptoms, while others become symptom-bearing and remain so. Similarly, a proportion of plants derived from infected cuttings become symptom-free. There is thus a dynamic situation in which new infections are occurring and previously symptom-bearing plants are becoming symptom-free. A point of equilibrium is reached which depends on the resistance level of the host and the virulence and inoculum pressure of the virus, and is influenced by the management practices used.

Almost 100% of Latin American germplasm introduced into Africa has shown extreme susceptibility to CMD. Limited improvements occurred in F1 crosses and first generation backcrosses to African germplasm (Porto *et al.*, 1994). A molecular map for cassava was developed at CIAT from a cross between a Latin American susceptible variety and a genotype resistant to CMD (Angel *et al.*, 1993; Fregene *et al.*, 1997). The objective was to locate genes for resistance to CMD on the molecular map because the discovery of close linkages between genes for resistance and molecular markers would make it possible to breed for resistance to CMD in the absence of the disease.

Brown streak virus disease occurs only in coastal areas of East Africa and in Malawi, (Chapter 12) but breeding for resistance to it has been done only in Tanzania, where the resistance was considered almost as important as resistance to CMD.

The important sources of resistance to CBSD were *M. glaziovii*, *M. melanobasis* and several cassava varieties of Brazilian origin (Jennings, 1957, 1960b). Symptoms can occur in mature leaves, leaf bases on the stem and in the roots, and are best scored in mature plants at harvest time. Symptoms can be transient in resistant plants when the old leaves are shed and the necrotic tissues of the stems and roots are occluded by new symptom-free growth. The observation that symptoms in resistant plants tend to be confined to the roots suggests that the resistance mechanism may involve a localization of the virus to the lower parts of the plant as postulated for resistance to CMD (Jennings, 1960b).

Breeding for Resistance to Cassava Bacterial Blight (CBB; *Xanthomonas campestris* pv. *manihotis*)

Resistance to CBB has always been an important requirement for several of the zones considered by CIAT, but the disease did not become prevalent in Africa until the late 1970s. American varieties show a continuous range of resistance but at CIAT only 15 out of 2800 clones tested

were rated highly for resistance. The inheritance of resistance is by recessive, mainly additive genes and there is a good correlation between the mean resistance of parents and that of their hybrids ($r = 0.549$), resulting in a heritability of 48% (CIAT, 1978; IITA, 1978).

An important finding at IITA was that resistance to CBB in progenies derived from the crossing of cassava with *M. glaziovii* is associated with resistance to CMD. Hahn *et al.* (1980b) found phenotypic and genetic correlation coefficients between the two resistances in half-sib families of 0.423 and 0.899, respectively. They attributed the result to the occurrence of linked recessive gene complexes on one or more of the chromosomes inherited from *M. glaziovii*. Random transmission of some of the parental chromosomes of this species into backcross progenies with cassava has in fact been observed (Magoon *et al.*, 1969). Jennings (1978) found a discontinuity for this joint resistance both in populations of *M. glaziovii* itself and in its backcross progenies with cassava. He suggested that this discontinuity was conferred by some kind of genetic unit that was not invariably present in *M. glaziovii*, but had been retained through seven generations of breeding following the interspecific cross with this species.

These results mean that selection for one resistance should lead to an increase in the other. Studies of genetic variances and heritabilities for the two resistances suggest that genetic gain would be greater for CMD resistance in response to a first selection for CBB resistance, than in the reciprocal order, but a first screening for resistance to CMD is always preferred for practical reasons (Hahn *et al.*, 1980b). Recently, both ORSTROM in France and CIAT have studied the pathogen's genetic diversity and its implications for breeding. Restrepo and Verdier (1997) found considerable diversity of the pathogen in Latin America, but a restricted range in Africa. A set of strains representing different genetic groups is being tested to evaluate CIAT's germplasm and to select the most appropriate ones to use in screening for resistance. Clearly, the variability found in the pathogen will affect future breeding efforts in Latin America and has implications for other regions if virulent strains of the blight pathogen, presently confined to Latin America, spread to other parts of the world.

Breeding for Resistance to Fungal Diseases

Fungal diseases vary in importance in different zones, and resistance breeding for them has rarely been a primary objective of major programmes until recently. Resistances conferred by minor genes invariably occur in the landraces obtained from affected areas and are always non-race specific when physiological specialization of the pathogen occurs (CIAT, 1976). Notable examples are resistances to super-elongation disease (*Elsinoe brasiliensis*; Kawano *et al.*, 1983) and anthracnose (*Colletotrichum* species; Ezumah, 1980). For the high rainfall lowland tropics, leaf spot diseases caused by species of *Cercospora*, *Cercosporidium*, *Phaeoramularia* or *Phoma* can reduce yield by reducing the efficiency and longevity of the leaves; control of *Cercospora henningsii* in susceptible varieties, for example, improved yield by 10–23% (Teri *et al.*, 1978).

Technical problems have hindered breeding for resistance to root pathogens, but inoculation techniques under high humidity conditions have now identified moderate to strong resistance to *Diplodia* species and given reliable screening of progenies. Resistances to *Phytophthora drechsleri* and *Phytophthora nicotianae* var. *nicotianae* have been identified by a technique in which plugs of infected tissue are inserted into harvested roots which are then incubated in plastic bags for 2 weeks (CIAT, 1990). Stable, high resistance to *Phytophthora* species combined with resistance to *Fusarium* species have been identified in three Brazilian varieties which are being widely used as parents in breeding (Hershey and Jennings, 1992). Recent studies of genetic diversity for resistance to isolates from different *Phytophthora* species have revealed a number of germplasm accessions tolerant to all of them (Alvarez *et al.*, 1999). However, the strategy for breeding for resistance should still be based on an initial diagnosis of the predominant pathogen present in order to choose the most appropriate resistance genes.

Breeding for Resistance to Mites, Mealybugs and Whiteflies

Resistance to the green cassava mite (*Mononychellus tanajoa*), which caused devastation

when it arrived in Africa in the 1970s, was discovered in several African varieties (Nyiira, 1975; IITA, 1980) and appeared to be associated with plant vigour and leaf pubescence (Fig. 8.6; Leuschner, 1980). In Colombia, Byrne *et al.* (1982) identified both tolerance and antibiosis resistance mechanisms, both having high heritability. Bellotti and Byrne (1979) and Kawano and Bellotti (1980) considered that prospects for resistance breeding were good following systematic surveys of American germplasm for resistances to the three important mites, namely, *M. tanajoa*, *Tetranychus urticae* and *Oligonychus peruvianus*.

Resistances to the mealybugs *Phenacoccus manihoti* in Africa and *Phenacoccus herreni* in the Americas are important where there are extended dry periods. On both continents it is closely correlated with the pubescence of leaf buds and unexpanded leaves (van Schoonhoven, 1974; Ezumah, 1980; Hahn, 1984) and is therefore easily identified in the absence of the pest and will probably be durable. It is evaluated either by scoring the density of the pubescence or by counting the hairs on the underside of an unexpanded leaf. More recent breeding at CIAT has emphasized selection for resistance of the antibiosis type and for tolerant types that recover from pest damage (Hershey and Jennings, 1992).

Cassava is one of the few crops for which high levels of resistance to whiteflies have been detected. A high frequency of resistance is found in accessions from Ecuador. The national programme in Colombia is about to release two whitefly-resistant varieties for regions where the pest causes considerable direct damage and also acts as a vector for viruses (Bellotti *et al.*, 1999).

Breeding for Efficient Use of Basic Resources

Cassava is commonly grown in marginal regions under water stress and in soils that are low in nutrients, particularly phosphates. Hence the efficient use of these resources often reduces stress and may result in production stability, even under marginal conditions. The plant characteristics which confer tolerance to prolonged water stress are complicated, but selection under natural drought conditions is effective in improving at least some of them (Hershey and Jennings, 1992; El-Sharkawy, 1993; Tafur *et al.*, 1997).

Varieties for areas with a short rainy season are specialized in that they must produce a crop in 6 months. To do this they must distribute a very high proportion of their dry matter to the roots, and consequently they produce an insufficient photosynthetic source for a longer growing season (Cock, 1976).

Breeding for tolerance of low phosphate nutrition is possible by comparing yields on high and low phosphate plots. Large differences have

Fig. 8.6. Subglabrous (left) and pubescent (right) shoot tips and unexpanded leaves associated respectively with genotypes susceptible and resistant to mites and mealybugs. Courtesy of CIAT.

been detected, and the most tolerant types, which use the phosphates most efficiently, have been used as parents for progenies which are grown for selection on low phosphate soils (CIAT, 1981; Hershey and Jennings, 1992). Ideally, genotypes should tolerate low fertility but must also respond to improved fertility by increasing their root yield. This requires the separate evaluation of selections under high and low phosphate conditions. Plant architecture is important for efficient nutrient use. Genotypes with a short or intermediate plant habit may use 20% less nutrients than taller ones to produce a unit of yield at similar productivity levels (CIAT, 1997).

Iglesias *et al.* (1994) applied sensitivity analysis across a set of environments and showed that this method could contribute to the overall objective of improving dry matter production under poor growing conditions, while maintaining the capacity of the crop to respond to favourable environments.

Cassava Breeding in the Future: the Role of Biotechnology

The classical methods of breeding described here have produced plants capable of large advances in root yield. Hershey and Jennings (1992) reported improvements of over 200% for the period from 1976, when work began at CIAT, until 1990 for material tested at two trial sites and subjected to stress conditions. Similar advances have been made at IITA. However, the rate of improvement in average national cassava yields in the most important production countries has not paralleled the progress at experimental level, except for some Asian countries (Kawano, 1978).

Progress in the future will be aided by new biotechnology tools such as gene transfer from other species and molecular marker assisted selection (Chapter 10). Genetic engineering has a special role for improving heterozygous, clonally propagated crops such as cassava, because genes can be introduced into popular varieties without changing their positive attributes. All the quality combinations which make these varieties preferred by farmers could be maintained, allowing a higher rate of adoption of improved genotypes.

For many years this particular advantage was precluded because it was not possible to regenerate plants from transformed single cells or somatic tissues: routine regeneration was possibly only from embryogenic tissue. However, it should now be possible to achieve it by producing somatic embryos or 'artificial seeds' from somatic tissues (Stamp and Henshaw, 1982; Schoeple *et al.*, 1996; Taylor *et al.*, 1996).

Work on both *Agrobacterium tumefaciens* mediated and particle gun methods for transferring DNA to cassava is making progress (Calderon-Urrea, 1988; CIAT, 1991; Raemakers *et al.*, 1997). The DNA transfer which has the potential for the most valuable improvement is the transfer of part of the CMV genome, possibly the part which codes for coat protein, which would be expected to inhibit the synthesis of virus particles and either reduce or prevent symptom expression (Fauquet and Beachy, undated; Padidam *et al.*, 1999). In readiness for this development, the sequence has been determined of the two genomic components of the virus, which has two circular, single-stranded DNAs (Stanley and Gay, 1983). Other prospects are the transfer of a trypsin inhibitor gene from potato, which is expected to confer broad-spectrum insect resistance (Hershey and Jennings, 1992).

Research at the University of Bath and CIAT aims to identify key genes activated during the root deterioration process, with the objective of altering them in the future. Genes that have key roles have been identified by Beeching *et al.* (1994), and are now the target for genetic modification.

In the area of starch quality, the genes for anti-sense construct for granule bound starch synthase isoforms I and II, branching enzyme and ADP-glucose pyrophosphorylase isolated by Munyikwa *et al.* (1997) are being incorporated into cassava genotypes, to generate waxy genotypes and other starch variants that could open new markets for industrial uses.

The genetic control of cyanogen production is being studied at several institutions in the world and recent developments in gene identification have opened promising avenues for control of cyanogen synthesis and accumulation (Koch *et al.*, 1994).

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