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Cassava breeding: opportunities and challenges

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Abstract

Although cassava is a major food crop, its scientific breeding began only recently compared with other crops. Significant progress has been achieved, particularly in Asia where cassava is used mainly for industrial processes and no major biotic constraints affect its productivity. Cassava breeding faces several limitations that need to be addressed. The heterozygous nature of the crop and parental lines used to generate new segregating progenies makes it difficult to identify parents with good breeding values. Breeding so far has been mainly based on a mass phenotypic recurrent selection. There is very little knowledge on the inheritance of traits of agronomic relevance. Several approaches have been taken to overcome the constraints in the current methodologies for the genetic improvement of cassava. Evaluations at early stages of selection allow for estimates of general combining ability effect or breeding values of parental lines. Inbreeding by sequential self-pollination facilitates the identification of useful recessive traits, either already present in the *Manihot* gene pool or induced by mutagenesis.

Abbreviations: AEZ, agro-ecological zone; AYT, advanced yield trial; CBSD, cassava brown streak disease; CET, clonal evaluation trial; CG, cyanogenic glucosides; CMD, cassava mosaic disease; DH, doubled-haploids; DMC, dry matter content; FRY, fresh root yield; GCA, general combining ability; HI, harvest index; IITA, International Institute of Tropical Agriculture; LAC, Latin America and the Caribbean; PPD, post-harvest physiological deterioration; PT, plant type or architecture; PYT, preliminary yield trial; RT, regional trials; SCA, specific I combining ability; SED, rating for superelongation disease; SI, selection index

Introduction

Cassava (*Manihot esculenta* Crantz), along with maize, sugarcane and rice, constitute the most important sources of energy in the diet of most tropical countries of the world. The species originated in South America (Allem, 2002; Olsen and Schaal, 2001), and was domesticated less than 10 000 years ago. Early European sailors soon recognized the advantages of the crop and carried it to Africa. From there, traders later introduced it to Asia. Until recently, cassava and its products were little known outside the tropical and subtropical regions where it grows. No major scientific efforts had been made to improve the crop. However, with the creation of the International Institute of Tropical Agriculture (IITA) in Nigeria and the International Center of Tropical Agriculture (CIAT) in Colombia in the early 1970s a new era began for cassava with the implementation of successful breeding projects, modernization of cultural practices and develop-

ment of new processing methods (Cock, 1985; Jennings and Iglesias, 2002). National research centers in Brazil, Colombia, Cuba, India, Thailand and Vietnam among many other countries have conducted successful research on cassava as well.

Plant breeding has one of the highest rates of return among the investments in agricultural research. Fehr (1987) reported that the remarkable increase in the productivity of many crops during the twentieth century was due to genetic gains achieved through crop breeding. Cassava has also benefited from technological inputs in the area of breeding (Kawano, 2003). New varieties in Africa, Asia, and Latin America and the Caribbean satisfy the needs of farmers, processors, and consumers, bringing millions of dollars in additional income to small farmers. New technologies in the area of tissue culture, genetic transformation and molecular biology have also made positive contributions (Calderón-Urrea, 1988; Fregene *et al*., 1997 and 2000; Puonti-Kaerlas *et al*., 1997; DeVries and Toenniessen, 2001).

Currently cassava is an important crop in regions at latitudes between 30°N and 30°S, and from sea level up to 1800 m above sea level. Although its most common product is the starchy root, cassava foliage has an excellent nutritional quality for animal and human consumption and offers great potential. Cassava is the fourth most important basic food after rice, wheat and maize and is a fundamental component in the diet of millions of people (FAO/FIDA, 2000). Scott *et al*. (2000) estimated that for the year 1993, annual production of cassava was about 172.4 million tons, with a value of approximately $US 9.31 billion. Between 1961–1963 and 1995–1997, cassava production increased at a rate of 2.35%/year (Scott *et al*., 2000), a trend comparable to that found in other crops such as wheat (4.32%), potato (4.00%), maize (3.94%), yams (3.90%), rice (2.85%) and sweet potato (1.07%). Between 1994 and 2005 cassava productivity will increase by 1.1%/year.

Cassava is a very rustic crop that grows well under marginal conditions where few other crops could survive. A large proportion of cassava varieties are drought tolerant, can produce in degraded soils, and are resistant to the most important diseases and pests. The crop is naturally tolerant to acidic soils, and offers the convenient flexibility that it can be harvested when the farmers need it.

Cassava: the plant and its uses

Cassava is a perennial shrub. Basically every part of the plant can be utilized, but the starchy roots are by far the most commonly used product. Cyanogenic glucosides (CG) are found in every tissue except in the cassava seed. The most abundant CG is linamarin (about 85%), with lesser amounts of lotaustralin. The CG, synthesized in the leaves and transported to the roots, is broken down by the enzyme linamarase to produce HCN, a volatile poison (Du *et al*., 1995; McMahon *et al*., 1995; Wheatley and Chuzel, 1993; Andersen *et al*., 2000). Linamarin and linamarase accumulate in different parts of the cell, thus preventing the formation of free cyanide. However, most processing methods disrupts the tissues, allowing the enzyme to act on the substrate for a rapid release of cyanide. CG accumulation varies with genotypes, environment, agronomic practices, age of the plant and plant tissue, being highest in the leaves and peel of roots (Cock, 1985).

The starchy roots are a valuable source of energy and can be boiled or processed in different ways for human consumption. Roots can also be used for obtaining native or fermented starches and as dried chips, meal or pellets for animal feed. Cultivars with less than $50\,mg\,CG\,kg^{-1}$ fresh weight in the roots are considered 'sweet'. Above this level cassava roots are considered 'bitter'. Depending on the processing methodologies, bitter or sweet clones may be preferred. In addition to the cyanogenic potential other relevant traits for the roots are dry matter content, percentage of amylose in the starch composition, protein and carotenoid contents. There is variation in starch quality in relation to its amylose percentage with a mean around 15% (Wheatley *et al*., 1993). Cassava roots are low in protein content with an average of about 2–3% (dry weight basis). There have been, however, some preliminary results suggesting that protein content in the roots can be considerably higher (6–8%) in some landraces, particularly from Central America (CIAT, 2002). Yellow cassava roots have considerable amounts of carotenes (Iglesias *et al*., 1997; Chávez *et al*., 2000).

Cassava roots are not tubers and, therefore, cannot be used for reproductive purpose. A major consequence of this situation is their short shelf life (Beeching *et al*., 1998): within one or two days after harvest there is a rapid initiation of post-

harvest physiological deterioration (PPD). To date, little useful genetic variation to delay or reduce PPD has been found, and the solution to this problem remains one of the most important goals for cassava research.

Cassava stems are the most important source of planting material to propagate the crop. Cassava foliage is not widely exploited in spite of its high nutritive value, although consumption of leaves by human populations is relatively common in certain countries of Africa and Asia. Foliage is also used for animal feeding. Crude protein content in leaves typically ranges from 20% to 25% dry weight (Gomez *et al.*, 1983; Buitrago, 1990; Babu and Chatterjee, 1999), but levels as high as 30% have been identified (Buitrago, 1990). Content of cyanogenic glucosides in the foliage is markedly higher (3800–5900 mg HCN kg^{-1} fresh weight), than in roots (4–113 mg HCN kg^{-1} fresh weight). Exploitation of foliage in cassava is expected to increase because of the recent developments and testing of mechanical harvesters and alternative cultural practices to exploit it (Cadavid Lopez and Gil Llanos, 2003).

Reproduction in cassava

Cassava can be propagated either by stem cuttings or by sexual seed. However, the former is the most common practice used by farmers for multiplication and planting purposes. Propagation from true seed occurs occasionally in farmers' fields and, as such, is a starting point for the generation of useful genetic diversity (Alves, 2002). Most breeding programs generate seed through crossing, as a mean of creating new genetic variation. Occasionally botanical seed has also been used in commercial propagation schemes (Rajendran *et al.*, 2000).

Cassava is monoecious, with female flowers opening 10–14 days before the male ones on the same branch. Self-pollination can occur because male and female flowers on different branches or on different plants of the same genotypes can open simultaneously (Jennings and Iglesias, 2002). Flowering depends on the genotype and the environmental conditions. Branching occurs when an inflorescence is formed. Because erect, non-branching types, are frequently preferred by farmers, the crossing of elite clones in certain regions may become more difficult because of the scarcity of their flowers. Synchronization of flowering remains a difficult issue in cassava breeding. Some clones flower relatively early at 4 or 5 months after planting whereas others flower only at 8–10 months after planting. Because of this, and the time required for the seed to mature, it takes generally no less than a year to obtain seeds of a planned cross. On average, between one and two seeds (out of the three possible formed in the trilocular fruit) per pollination are obtained. Several publications illustrate the procedures for controlled pollinations in cassava (Kawano 1980; Jennings and Iglesias, 2002). Seeds often have a dormancy period for a few months after maturity, and they require relatively high temperatures (30–35 °C) for optimum germination (Ellis *et al.*, 1982).

Breeding objectives

Breeding objectives depend on the ultimate use of the crop. Productivity plays a major role in industrial uses of cassava (i.e. starch production and dried roots for animal feed), whereas stability of production will be fundamental in the many regions where cassava is the main subsistence crop. Industrial uses of cassava require high dry matter content as the main quality trait for the roots, whereas human consumption will frequently emphasize cooking quality or starch characteristics over productivity, as a determining trait. Good cooking quality is usually associated with other morphological traits such as the color of the peel of the roots, the leaf petiole or the shoot. Farmers frequently reject any change in such morphological traits, although they may have little or no correlation with actual cooking quality. Because of those types of farmers and consumer preferences, participatory research and breeding approaches had to be developed for cassava breeding (Gonçalvez Fukuda *et al.*, 2000; DeVries and Toenniessen, 2001; Gonçalvez Fukuda and Saad, 2001).

Other root quality traits relevant to different cassava breeding programs of the world are the cyanogenic potential in the roots (Dixon *et al.*, 1994), early bulking capacity, higher protein content in the roots and reduced post-harvest physiological deterioration. Unfortunately, the genetic variability for the latter two traits is limited in *M. esculenta* and, therefore, interspecific crosses

with other *Manihot* species to introgress useful alleles have been attempted.

Stability of production is associated with resistance or tolerance to major biotic and abiotic stresses, the emphases vary with the target environments. In Africa cassava mosaic disease (CMD) and cassava brown streak disease (CBSD) are important constraints. A disease similar to CMD is also present in southern India. In certain regions of Latin America and the Caribbean (LAC), frogskin Disease causes roots to become 'corky' and commercially unusable. The causal agent has not yet been identified, although it has been suspected for many years that it may be a virus. Bacterial blight, induced by *Xanthomonas axonopodis* pv. *manihotis* (also known as *X. campestris* pv. *manihotis*), is found in Asia, Africa and LAC, and can have devastating effects on yield and the availability of planting material, particularly in Africa and LAC (Hillocks and Wydra, 2002). Several fungal diseases also may affect cassava productivity. Super elongation disease, induced by *Sphaceloma manihoticola* (Teleomorph: *Elsinoe brasiliensis*) is widespread in the Americas, from Mexico to Southern Brazil. In tropical lowlands with high rainfall *Cercospora*, *Cercosporidium*, *Phaeoramularia* or *Colletotrichum* species can affect cassava productivity (Jennings and Iglesias, 2002). Phoma species cause leaf and stem lesions in the tropical highlands. Several species of *Phytophthora* induce root rot but also different species of the genera *Sclerotium*, *Armillaea* and *Fusarium*. There are sources of genetic resistance to most of these diseases (CIAT, 2001; Hillocks and Wydra, 2002).

Several arthropod pests feed on cassava and can reduce productivity. The green mite (*Mononychellus tanajoa*) devastated cassava fields upon its introduction in Africa in the 1970s (Nyiira, 1975, IITA 1980). Other mites important for cassava are *Tetranychus urticae*, *T. cinnabarinus*, *Mononychellus caribbeanae* and *Oligonychus peruvianus* (Bellotti *et al.*, 2002). The mealybugs *Phenacoccus manihotis* and *P. herreri* feed on cassava fields of Africa and LAC, respectively. Thrips (particularly *Frankliniella williamsi* and *Scyrtotrips manihoti*) considerably reduce yields of susceptible genotypes. Clones with pubescent leaves in their early stages of development offer excellent levels of resistance to these insects (Belloti, 2001), and this trait has been broadly incorporated into improved varieties.

Whiteflies are among the most widespread pests in cassava. *Aleurotrachelus socialis* is the predominant species in northern South America, where it causes considerable crop damage through direct feeding. *Bemisia tabaci* is widely distributed in tropical Africa and several Asian countries. Until 1990 *B. tabaci* biotypes found in the Americas did not feed on cassava. The major effect of *B. tabaci* is as a vector of the devastating CMD disease in Africa. Several other species of whiteflies affect cassava in different regions. Genetic resistance to whiteflies in cassava has been found particularly for *A. socialis* in several germplasm accessions from the CIAT collection (Bellotti, 2001). Based on breeding work at CIAT, Colombia released the first whitefly-resistant variety of any crop, in 2002, targeted toward the Tolima Valley, where whiteflies typically devastate plantations.

There are several other arthropod pests affecting cassava roots, foliage and/or stems, particularly Lepidoptera, Diptera and Hemiptera. There is little or no genetic resistance to those pests and their management is commonly achieved through biological control measures. Recently, attempts to produce transgenic cassava have succeeded with the introduction of cry genes encoding insect-specific endotoxins (Bt toxins) from *Bacillus thuringiensis* (Ladino *et al.*, 2001; Fregene and Puonti-Kaerlas, 2002). For further information consult Taylor *et al.* (this issue).

There are a variety of abiotic factors limiting cassava productivity. The crop is frequently grown in drought-prone regions and/or on low fertility soils. It can also be found in alkaline or acidic soils, most frequently the latter. Some traits associated with adaptation to these conditions have been suggested (Jennings and Iglesias, 2002), such as: leaf longevity (El-Sharkawy, 1993; CIAT 2001; Fregene and Puonti-Kaerlas, 2002), optimum leaf area index (Lian and Cock, 1979) and ideal plant architecture (Hanh *et al.*, 1979; Kawano *et al.*, 1998; Kawano, 2003). The capacity of the stems to withstand long storage periods (sometimes up to two months) from harvest to planting affects final density of established plants and is an important trait for areas with relatively long dry spells or erratic rainfall, because the storage period may extend to the point it compromises their viability. While there is known genetic variation for stem storability, it has not been a major breeding objective of any program so far.

Breeding scheme

As in most crop breeding activities, cassava genetic improvement starts with the assembly and evaluation of a broad germplasm base, followed by production of new recombinant genotypes derived from selected elite clones. Scientific cassava breeding began only a few decades ago and, therefore, the divergence between landraces and improved germplasm is not as wide as in crops with a more extensive breeding history. As a result, landrace accessions play a more relevant role in cassava than in other crops. Parental lines are selected based mainly on their *per se* performance and little progress has been made to use general combining ability (Hallauer and Miranda, 1988) as a criterion of parental selection. Crossing can be by controlled pollinations, done manually, to produce full-sib families or else in polycross nurseries where open pollination results in half-sib families.

For open pollinations a field planting design developed by Wright (1965) is followed to maximize the frequency of crosses of all the parental lines incorporated in the nursery. Knowledge about flowering capacity is important in order to select a group of materials with synchronized flowering. When there are considerable differences in flowering habit a delayed planting and/or pruning of the earliest flowering genotypes may be required. At harvest the seed harvested from each clone are bulked to form a half-sib family. Seeds from full-sib families can be obtained in isolated open pollination plots were two clones are planted together and one of them, chosen to act as female progenitor is emasculated. Alternatively, several male-sterile clones have been identified, which can act as female parents.

The botanical seed obtained by the different crossing schemes may then be planted directly in the field (as done at IITA) to take advantage of the availability of irrigation and high temperatures. At CIAT, seeds are germinated in greenhouse conditions and the resulting seedlings transplanted to the field when they are about 20–25 cm tall (Jennings and Iglesias, 2002). Root systems in plants derived from botanical seed or vegetative cuttings may differ considerably. The tap roots in seedlings store fewer starches than those from vegetative cuttings (Rajendran *et al.*, 2000). Because of this, it is difficult, if not impossible, to correlate the root yield of clones at later stages in the evaluation/selection process with early results from the plants obtained from botanical seeds. However, when seeds are germinated in containers and later transplanted, the tap root often does not develop, and the seedling-derived plant may be more similar to subsequent stake-derived plants in terms of starchy root conformation.

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

Table 1 illustrates a typical selection cycle in cassava. It begins with the crossing of elite clones and ends when a few clones surviving the selection process reach the stage of regional trials across several locations. There is some variation among the different cassava-breeding programs in the world, regarding the numbers of genotypes and plants representing them through the different stages; however, the numbers presented in Table 1 are fairly common and illustrate the different stages required to complete a selection cycle and the kind of selection pressures generally applied.

The first selection is conducted the second year on the nurseries with plants derived from botanical seed (F1 in Table 1). Because of the low correlations between the performance at this early stage of selection and when the genotypes reach replicated trials the early selections are based on high-heritability traits such as plant type, branching habits and reaction to certain diseases (Hahn *et al.*, 1980a,b; Hahn, 1984; Hershey, 1984; Iglesias *et al.*,

Table 1. A typical selection cycle in cassava, beginning with the crossing of elite clones, through the different stages of the selection process (adapted from Jennings and Iglesias, 2002).

Year	Activity	Number of genotypes	Plants per genotype
1	Crosses among elite clones	Up to 100 000	
2	F1: Evaluation of seedlings from botanical seeds. Strong selection for ACMV in Africa	100 000[a], 50 000[b], 17 500[c]	1
3	Clonal evaluation trial (CET)	2000–3000[a,b]; 1800[c]	6–12
4	Preliminary yield trial (PYT)	100[a], 300[b], 130[c]	20–80
5	Advanced yield trial (AYT)	25[a], 100[b], 20–18[c]	100–500
6	Regional trials (RT)	5–30[a,b,c]	500–5000

Figures for the cassava breeding at [a] IITA (Ibadan, Nigeria); [b] CIAT (Cali, Colombia) and [c] CIAT-Rayong Field Crops Research Center (Thailand). Averages from data in Kawano (2003).

1994). At IITA, combining selection for resistance CMD and bacterial blight (*Xanthomonas axonopodis* pv. *manihotis*) begins with about 100 000 seedlings and only about 3000 genotypes survive this first stage of selection, which is based on single plant performances. At CIAT, Colombia, smaller F1 nurseries (up to 50 000 seedlings) have typically been planted since there is no single trait, such as CMD resistance, which so drastically reduces the number of selections.

The second stage of selection is called clonal evaluation trial (CET). The few surviving genotypes from the single-plant selection conducted during the F1 stage, produce the 6–10 vegetative cuttings required for this second stage. The capacity to produce this number of cuttings is in fact another selection criteria utilized at the F1 stage. CETs usually range from 2000 to 3000 clones (>1000 in Thailand). Within a given trial, however, the same number of plants is used to avoid the confounding effects between number of plants and genotypic differences. Because the competition between neighboring genotypes in the CET may favor more vigorous plant architectures, selection at this stage will rely heavily on high heritability traits such as harvest index (Kawano *et al.*, 1998; Kawano, 2003). Plant type is an important selection criterion at early stages of selection: plants whose main stem does not branch until it reaches about 1 m are preferred (Kawano *et al.*, 1978; Hahn *et al.*, 1979). Other selection criteria at this stage include high dry matter and cyanogenic potential (Iglesias and Hershey, 1994). Between 100 and 300 clones will survive the clonal evaluation trial (>100 in the case of Asia). A common feature in the first two stages of selection for some programs is that selection is frequently visual with no data recording, in order to manage a larger number of materials at lower costs.

Preliminary yield trials (PYT) are based on the evaluation of 20–40 plants either in single plots, replicated trials in a single location or even including several locations. Only with the initiation of replicated trial does the emphasis of selection shift from high-heritability traits to those of low heritability such as yield. Starting with PYT and increasingly during the advanced yield trials (AYT) and the regional trials (RT) there will be a greater weight on yield and its stability across locations. Cooking quality, 'poundability' (IITA), and 'farinha' quality (Brazil) trials will also began at these stages, when the number of genotypes evaluated has been reduced to a manageable size.

The clones that show outstanding performance in the RTs are released as new varieties and, often, incorporated as parents in the crossing nurseries. This completes a selection cycle and a new one begins. It should be pointed out that the selection scheme described above has the following characteristics:

(a) The process is indeed a mass phenotypic recurrent selection, because no family data are involved in the selection process.
(b) Few data are taken in early stages of selection, especially on genotypes that can be readily discarded by visual evaluation. Therefore, no data regarding general combining ability effects ($\approx$ breeding value) are available for a better selection of parental materials.
(c) There is no proper separation between general (GCA $\approx$ additive) and specific (SCA $\approx$ heterotic) combining ability effects. The outstanding performance of selected materials is likely to

depend substantially on positive heterotic effects, which cannot be transferred to the progenies sexually derived from them.

(d) Inbreeding was intentionally omitted in the breeding scheme. Therefore large genetic loads are likely to remain hidden in cassava populations and useful recessive traits are difficult to detect.

(e) Two or more stages of selection may be based on unreplicated trials. A large proportion of genotypes is eliminated without the proper evaluation set up.

Because of the foregoing reasons there are some clear opportunities to further improve the efficiency and effectiveness of cassava breeding. Kawano *et al.* (1998) mention that during a 14-year period, about 372 000 genotypes, derived from 4130 crosses, were evaluated at CIAT-Rayong Field Crop Research Center. Only three genotypes emerged from the selection process to be released as official varieties. Nonetheless, it should be mentioned that these varieties have achieved remarkable success in Asia, with more that one million hectare planted. Similar experiences have been observed at IITA, CIAT – Colombia and Brazil. The resulting increases in productivity account for a higher income (about one billion $US annually) to the poor farmers who grow the improved germplasm (Kawano, 2003).

An approach for estimating general combining ability of parents involved in cassava breeding

One of the major decisions taken by any breeder is the selection of parents used to produce a new generation of segregating progenies. In cassava, this decision has been mainly based on the *per se* performance of each clone. Nonetheless, some empirical knowledge on the quality of progenies produced by different parents could be produced. This lack of organized information on the breeding values of parental lines used in the breeding projects was partially due to the fact that no data was taken and recorded during the first stages of selection (CET and PYT in Table 1) or else, they were incomplete. Some modifications for overcoming this drawback have recently been introduced at the breeding project at CIAT – Colombia. These modifications can be summarized as follows:

All the clones from a given family (either full- or half-sib) are separated in three groups. Each third of a family is randomly allocated to one of three 'blocks' for the CET trials.

(a) Data are taken and recorded on every genotype, regardless of whether or not they are selected to pass to the following stage.

(b) Selection of clones at the CET stage is conducted within each 'block'. These trials are relatively large (1.5 ha) and the division of 'replications' allows for a stratified phenotypic selection (Gardner, 1961) with all the reported advantages of this system over mass selection.

(c) Each family is divided in three groups. The clones making up each group will obviously be different (just as plants within half- or full-sib families in maize will be different from one replication to another). However, replicated information can be derived for each family. This information can then be used to derive the relative values of the parents that generated these families.

(d) A selection index integrating the most relevant variables is used to facilitate selection. To avoid the problems related to the magnitudes used to measure different variables, the index is constructed using standardized deviation units (Steel and Torrie, 1960). A typical selection index for the acid soil savannas environment of Colombia is

$$\mathrm{SI} = (\mathrm{FRY} * 10) + (\mathrm{DMC} * 8) + (\mathrm{HI} * 8) - (\mathrm{PT3}) - (\mathrm{SED3}),$$

where SI is the selection index; FRY = fresh root yield; DMC = dry matter content; HI = harvest index; PT = rating for plant type or architecture; and SED = rating for superelongation disease. Negative signs are used for those variables where lower values represent most desirable phenotypes. The weights associated with each variable reflect the subjective importance given to it by the breeder. Harvest index has been consistently favored as one relevant variable to be included in early stages of selection such as CET trials (Kawano *et al.*, 1998). Plant architecture also plays an important role in early stages of selection (Hahn *et al.*, 1979). Since the SI is estimated using the standardized values, a positive SI means a performance better

than the average, while a negative one means a poor performance.

Table 2 provides information from the CET conducted in the acid soil savannas in the Meta Department of Colombia. There were a total of 49 families of which 10 were full-sib and the rest half-sib families. A variable number of genotypes represented each family, ranging from 4 to 60 with an average of about 25. Results are averages across the three 'blocks'. In some families, no clone was selected, whereas in a few more than 30% of sibling clones performed well enough to pass to the next stage of selection. The percentage of selected clones is closely associated with the family mean SI value, which ranged from −22.47 to +15.97. The overall mean SI value should be 0.00 (and they are for each of the three 'block') but deviates slightly when averaged across the entire experiment. Sharp differences among families suggest large differences in the genetic value of the parents that produced them. Moreover, the origin of these differences can be understood by analyzing individual traits such as disease reactions, harvest index, dry matter content, etc.

Similar results were obtained for other regions as well. Table 3 presents the results for the sub-humid conditions in the northern coast of Colombia where the main problems are the short rainy season, low soil fertility and different species of mites and/or thrips. A total of 50 families were evaluated with an average of about 44 genotypes per family. Pressure from mites and thrips was relatively low during this evaluation. In addition to the usual traits, leaf retention was measured from 5 to 7.5 months after planting, using the score of 1 = 10% or less of the stem with leaves to 9 = 90% or more of the stem with leaves attached.

To illustrate the efficacy of the analysis the best three families in Table 3 were derived from germplasm developed by CIAT and Thailand for Asian sub-humid conditions, which are relatively homologous to those found in the northern coast of Colombia. Out of 50 families, this analysis could place these three families on top. Therefore, it can be concluded that the progenitors that gave rise to these families (Rayong -5, -60, and -90, and Kasetsart University 50) should be preferentially selected as parents in the crossing nurseries for this type of environment. Leaf retention score shows a high within-family variation, but average values are similar for best and worst families.

This approach will also allow for the identification of useful germplasm for particular traits. The best progenitors for resistance to diseases, insects and different types of abiotic stress can now be identified much more precisely, not only based on the *per se* performance, but more importantly, on the performance of the progenies they produce.

Table 2. Results of the four best and four worst families of a clonal evaluation trial for the acid soil savannas agro-ecological zone (Meta Department, Colombia), harvested in May 2003. Averages across three 'blocks' in which a total of 49 families were evaluated.[a]

	Family size (no. of clones)	Selected (%)	Plant type (1–5)	FRY (t ha^{-1})	HI (0–1)	DMC (%)	SI	SED (1–5)
Mean	25.2	13.3	3.4	21.5	0.49	31.4	−1.3	2.9
Minimum	4	0	2.4	16.9	0.39	27.5	−22.5	1.8
Maximum	60	37.5	4.4	25.3	0.58	34.8	16.0	4.3
Family rank	Family averages							
1	24	37.5	2.5	22.6	0.54	33.3	9.4	1.8
2	15	33.3	3.5	24.1	0.56	30.5	3.5	3.1
3	46	32.6	2.8	24.2	0.52	33.4	12.9	2.2
4	14	28.6	2.7	23.0	0.54	31.3	8.9	1.9
46	15	0	4.2	19.0	0.49	29.0	−16.1	3.9
47	9	0	3.8	16.8	0.39	31.3	−16.7	3.3
48	4	0	3.3	17.8	0.43	27.5	−18.1	3.5
49	22	0	4.3	17.1	0.48	27.8	−22.5	3.5

[a] Selected = percentage of selected clones within a given family; plant type score 1 = excellent to 5 = unacceptable; FRY = fresh root yield expressed in t ha^{-1}; HI = harvest index (fresh root biomass/total biomass); DMC = dry matter content expressed in %; SI = selection index; and SED = score for super-elongation disease where 1 = very low damage and 5 = high damage.

Table 3. Results of the four best and four worst families of a clonal evaluation trial for the sub-humid agro-ecological zone (Atlántico Department, Colombia), harvested in May 2003. Averages across three 'blocks' in which a total of 50 families were evaluated.[a]

	Family size (no. of clones)	Selected (%)	Thrips (1–5)	FRY (t ha^{-1})	HI (0–1)	DMC (%)	SI	Leaf (1–9)	Ret.
Mean	44.38	13.0	1.3	13.7	0.46	26.4	−0.7	4.8	
Minimum	10	0.00	1.0	9.6	0.36	21.5	−16.0	3.2	
Maximum	83	61.6	2.8	21.4	0.60	30.5	23.5	7.0	
Family rank	Family averages								
1	73	61.6	2.0	21.4	0.58	30.5	23.5	4.7	
2	32	53.1	1.8	20.7	0.60	28.4	20.6	4.3	
3	32	40.6	2.8	17.5	0.56	30.1	16.4	5.2	
4	22	36.4	1.0	17.2	0.49	27.0	7.8	6.4	
47	56	0.0	1.0	12.4	0.47	23.9	−7.7	5.1	
48	53	0.0	1.0	11.1	0.36	21.9	−16.0	5.3	
49	33	0.0	1.5	11.9	0.43	26.2	−5.5	4.2	
50	35	0.0	1.3	11.9	0.41	25.4	−10.4	5.2	

[a] Selected = percentage of selected clones within a given family; thrip score 1 = excellent to 5 = unacceptable; FRY = fresh root yield expressed in t ha^{-1}; HI = harvest index (root biomass/total biomass); DMC = dry matter content expressed in %; SI = selection index; and leaf retention score 9 = 90% or more of stem with leaves attached and 1 = 10% or less of the stem still with leaves.

Progress in understanding the genetics of important traits in cassava

Knowledge on the inheritance of traits of agronomic relevance in cassava is limited. Very few studies have been conducted and published and therefore the cassava breeder has to work without the advantages of a clear understanding of the way the traits to be improved are inherited.

To fill the vacuum of knowledge in this regard three diallel studies have been conducted one for each of three contrasting agro-ecological zones (AEZ) in Colombia (acid soil savannas, sub-humid environment and mid-altitude valleys). The study involved nine or ten parents, and 30 clones representing each F1 cross. Two locations with three replications each were used for the field evaluation. Each clone was represented by six plants, which were planted in the three replications at each of the two locations. Therefore, for each F1 cross the analysis of the thirty clones can also involve the within family segregation, since replications for each individual genotype is available.

The study allowed estimation of two genetic parameters for the set of genotypes involved: (a) the average performance of parents in crosses, which estimates the breeding value of a given genotype due to additive gene effects, known as general combining ability (GCA); and (b) the deviation of individual crosses from the average performance of parents, due to specific allelic combinations or dominance effects, or specific combining ability (SCA). This kind of study also allows for the estimation of the relative importance of epistasis.

Table 4 presents information on GCA and SCA for fresh root yield at each location. In every case, both GCA and SCA were significant at the 1% probability level, with the exception of SCA effects for the higher fertility soils at CORPOICA – Porcinos (acid soil savannas), where SCA reached significance at the 5% probability level. These results demonstrate that not only additive effects are important in determining the performance of derived progenies but also that there is a large component of dominance effects that translate into significant heterosis for traits such as fresh root yield of cassava. GCA effects were larger than SCA effects in all cases, and much larger in most cases. Results from other traits showed similar behavior (CIAT, in press).

Alternatives for improving cassava breeding

There are many opportunities for improving cassava breeding. Most of them should address one or

Table 4. Relative importance of general (GCA) and specific (SCA) combining ability effects for fresh root yield on diallel cross evaluations conducted in three contrasting environments in Colombia.

Source of Variation	Acid soil savannas[a]		Mid-altitude valleys[b]		Sub-humid region[b]	
	Native savannas	CORPOICA Porcinos	Palmira	Jamundí	Santo Tomás	Pitalito
Genotypes	0.500**	0.61**	2.15**	1.71**	0.70**	0.81**
GCA	1.65**	1.05**	5.20**	1.95**	1.77**	2.00**
SCA	0.20**	0.49*	1.25**	1.64**	0.38**	0.45**
Error	0.09	0.29	0.42	0.47	0.16	0.15

*,** Significant (by *F*-test) at 5% and 1% probability level, respectively.
[a] Ten-parent diallel. Degrees of freedom for genotypes, GCA, SCA and Error were 44, 9, 35, and 88, respectively.
[b] Nine-parent diallel. Degrees of freedom for genotypes, GCA, SCA and Error were 35, 8, 27, and 70, respectively.

more of the following current restrictions on the efficiency:

(a) Because no inbreeding is carried out, a sizeable genetic load (undesirable or deleterious genes) may prevent large and sustained genetic gains. Lack of inbreeding also reduces the probabilities of detecting useful recessive traits.

(b) Since there are no clearly defined populations (quantitative genetics sense), allelic frequencies cannot be efficiently modified.

(c) Because the crop is highly heterozygous, dominance effects are likely to play an important role in the performance of materials being selected (as suggested by data from the diallel studies). The current scheme can exploit dominance effects because, once an elite clone is identified, it can be propagated vegetatively (therefore carrying along the dominance effects). However, if the same elite clones are frequently selected as progenitors for the production of new segregating material, the current procedure has a bias because the breeding value of these clones are unlikely to be well correlated with their performance *per se*, precisely because of the distorting effects of dominance.

(d) Production of recombinant seed is cumbersome in cassava. Only 0.6 viable seeds per pollination are produced on average. It takes about 12–18 months from the time a given cross is planned until an adequate amount of seed is produced.

(e) When a desirable trait is identified, it is very difficult to transfer it from one genotype to another (even for a single gene controlled trait). The backcross scheme, one of the most common, successful and powerful breeding schemes, is not feasible in cassava, because of the constant heterozygous state used throughout the breeding process.

The genetic improvement of cassava could benefit from introducing inbreeding into the process. However, because inbreeding would require about 9–10 years to attain acceptable levels of homozygosity, an efficient protocol for production of doubled-haploids (DH) through anther or microspore culture is critical. Inbreeding in cassava is desirable because:

(a) Selection among DH would not be affected by dominance effects which could be exploited through reciprocal recurrent selection;
(b) Additive effects among DH are twice as large as in the current array of evaluated genotypes;
(c) Homozygous lines are genetically fixed, and therefore, their genetic superiority (as progenitors) can be better exploited, than genetically unstable heterozygous parents;
(d) Germplasm exchange based on botanical seed is much easier than that of vegetative cuttings (Iglesias *et al.*, 1994);
(e) Cleaning planting stocks from viral or other pathogens could be achieved without the need of meristem culture;
(f) Mutation breeding would be more easily implemented;
(g) The identification of useful recessive mutants would be greatly facilitated;
(h) The production of genetic stocks for basic and applied research would be feasible;

(i) For those projects exploiting polyploidy in cassava breeding (Sreekumari *et al*. 2000), the availability of haploids and DH is also desirable;
(j) The backcross breeding scheme could be implemented for the transfer of useful genes from one cassava inbred to another, or from an heterozygous clone to an inbred. In summary, DH would allow designing better performing hybrids compared to less efficient systems based on heterozygous parents.

The introduction of inbreeding in cassava, therefore, offers several advantages. However, inbreeding is likely to induce a drastic reduction of vigor in the first few cycles of selection. This phenomenon is known as inbreeding depression. Maize was severely affected when it was first subjected to inbreeding. However, inbred maize lines yielding as much as 4 t/ha have now been developed (Duvick, 1999). Tolerance to inbreeding depression can be built in crops and fifth-generation inbreds have been developed at IITA (DeVries and Toenniessen, 2001). However, it is accepted that tolerance to inbreeding in cassava needs to be improved before full homozygosity can be attained through the production of DH lines. Developing families with tolerance to inbreeding is currently also an ongoing activity at CIAT, through recurrent selection schemes that involve inbred families (S_1 or S_2).

Adequate screening of genetic available variability

Genetic variability available within *Manihot* has not been fully explored and screened. Therefore, this genetic wealth has not been fully exploited, and should offer interesting possibilities for the future. In part, the limited evaluation of cassava genetic variability is because the collection and maintenance of cassava germplasm is difficult, cumbersome and expensive. Compared with other crops, a relatively smaller number of accessions is maintained in the germplasm collections (5728 and 32 445 for cassava and beans germplasm collections at CIAT, respectively). The remarkable genotype-environment interaction shown by cassava (Bueno, 1986), complicates the interpretation of evaluations across different environments. Furthermore, detection of some of the economically important traits in the roots is difficult. For instance, the many different starch mutants in maize (popcorn, sweet, floury, waxy corn, etc.) are more or less easily recognizable. No equivalent mutant has been reported for cassava.

Nutritional quality factors studied to date also show relatively low variation, with the exception of the high carotene levels found in yellow cassava roots (Iglesias *et al*., 1997). Very little success has been obtained for increasing protein content in the roots. Few *M. esculenta* accessions from Meso America have been found to have higher levels of proteins in the roots (CIAT, 2002). An introgression of genes from related *Manihot* species into the *M. esculenta* gene pool may have occurred but these findings require further analysis and confirmation.

Three approaches (apart from genetic transformation) can be followed to overcome the limited variation for starch, root and nutritional traits in cassava:

(a) aggressive evaluation of crosses with related *Manihot* species;
(b) induced mutations;
(c) conduct more extensive germplasm collections in areas not yet covered in germplasm collections. As an example, Carvalho *et al*. (this issue) reported the finding of cassava landraces from the Amazon basin with novel starch and sugar contents in their roots.

Perspectives

During the past 30–40 years, significant progress has been achieved in the initial phase of the scientific genetic improvement of cassava. In a way it could be said that the adaptation of the crop to more intensive cultivation systems has been completed. This process involved assembling major traits such as improved yield (mainly through a higher harvest index), low cyanogenic content (when desirable), improved plant architecture and resistance/tolerance to the major diseases and pests.

Future activities involve an increasing emphasis on complex traits such as higher yield and dry matter content in the roots, early bulking, etc., which are more difficult to improve. It is critical for cassava that efficient methods for the improvement of these complex traits are found to

maintain the competitive edge that this crop currently has in tropical regions as an alternative to imported carbohydrate sources from temperate regions. Several approaches have been taken to address this situation in recent years. Modifications of the breeding scheme have been implemented for a more dynamic recurrent selection system and for obtaining valuable information on the breeding value of parental lines. Biotechnology tools have been adapted to cassava and are currently incorporated in different projects for its genetic improvement. A molecular map has been developed and marker assisted selection is currently used for key traits. Genetic transformation protocols are available and have been used successfully for the incorporation of different genes. Tissue culture techniques can also benefit cassava through the production of doubled-haploid lines.

One of the challenges for the crop is for a more extensive exploration to increase the germplasm collections and to develop approaches that will allow for an efficient evaluation of such germplasm. In this regard, tools for rapid identification of novel starch types are needed. The lack of genetic variability for overcoming the problem of post-harvest physiological deterioration remains a major bottleneck for cassava utilization and commercialization.

The inherent potential of cassava, its capacity to grow in marginal environments and the incorporation of new, powerful biotechnology tools as described in several articles from this special issue, offer a bright perspective for the crop and the people that depend on it.

References

Allem, A.C. 2002. The origins and taxonomy of cassava. In: R.J. Hillocks, J.M. Tres, and A.C. Bellotti (Eds.), Cassava: Biology, Production and Utilization. CABI Publishing, pp. 1–16.

Alves, A.A.C. 2002. Cassava botany and physiology. In: R.J. Hillocks, J.M. Tres, and A.C. Bellotti (Eds.), Cassava: Biology, Production and Utilization. CABI Publishing, pp. 67–89.

Andersen, M.D., Busk, P.K., Svendsen, I. and Møller, B.L. 2000. Cytochromes P-450 from cassava (*Manihot esculenta* Crantz) catalyzing the first steps in the biosynthesis of the cyanogenic glucosides linamarin and lotaustralin. J. Biol. Chem. 275(3): 1966–1975.

Babu, L. and Chatterjee, S.R. 1999. Protein content and amino acid composition of cassava tubers and leaves. J. Root Crops 25(20): 163–168.

Beeching, J.R., Yuanhuai, H., Gómez-Vázquez, R., Day, R.C. and Cooper, R.M. 1998. Wound and defense responses in cassava as related to post-harvest physiological deterioration. In: J.T. Romeo, K.R. Downum and R. Verpporte (Eds.), Recent Advances in Phytochemistry, vol. 32. Phytochemical Signals in Plant-Microbe Interactions. Plenum Press, New York, pp. 231–248.

Bellotti, A.C. 2001. Arthropod pests. In: R.J. Hillocks, J.M. Thresh and A.C. Bellotti (Eds.), Cassava: Biology, Production and Utilization. CABI Publishing, pp. 209–235.

Bellotti, A.C., Arias V., B., Vargas H., O., Reyes Q., J.A. and Guerrero, J.M. 2002. Insectos y ácaros dañinos a la yuca y su control. In: B. Ospina and H. Ceballos (Eds.), La Yuca en el Tercer Milenio. Sistemas Modernos de Producción, Procesamiento, Utilización y Comercialización. CIAT Publication No. 327, Cali, Colombia, pp. 160–203.

Bueno, A. 1986. Avaliacão de cultivares de mandioca visando a seleção de progenitores para cruzamentos. R. Bras. Mand. 5(1): 23–54.

Buitrago, A., J.A. 1990. La yuca en la alimentación animal. Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia, 446 pp.

Cadavid López, L.F. and Gil Llanos, L. 2003. Investigación en producción de yuca forrajera en Colombia. Informe annual de Actividades CLAYUCA. Apdo Aéreo 6713, Cali, Colombia, pp. 266–275.

Calderón-Urrea, A. 1998. Transformation of *Manihot esculenta* (cassava) using *Agrobactrium tumefaciens* and expression of the introduced foreign genes in transformed cell lines. M.Sc. thesis. Vrije University, Brussels, Belgium.

Carvalho, L.J.C.B., de Souza, C.R.B., Cascardo, J.C.M., Junior, C.B. and Campos, L. 2004. Identification and characterization of a novel cassava (*Manihot esculenta* Crantz) clone with high free sugar content and novel starch. Plant Molecular biology, this issue.

Chavez, A.L., Bedoya, J.M, Sánchez, T., Iglesias, C.A., Ceballos, H. and Roca, W. 2000. Iron, carotene, and ascorbic acid in cassava roots and leaves. Food Nutrition Bull. 21: 410–413.

CIAT (Centro Internacional de Agricultura Tropical), 2001. Project IP3, Improved Cassava for the Developing World, Annual Report 2001. Apdo Aéreo 6713, Cali, Colombia.

CIAT (Centro Internacional de Agricultura Tropical), 2002. Project IP3, Improved Cassava for the Developing World, Annual Report 2002. Apdo Aéreo 6713, Cali, Colombia.

CIAT (Centro Internacional de Agricultura Tropical), in press. Project IP3, Improved Cassava for the Developing World, Annual Report 2003. Apdo Aéreo 6713, Cali, Colombia.

Cock, J. 1985. Cassava. New Potential for A Neglected Crop. Westview Press. Boulder, CO, USA, 240 pp.

DeVires, J. and Toenniessen, G. 2001. Securing the harvest: biotechnology, breeding and seed systems for African crops. Chapter 13: Cassava. CABI Publishing Oxon, UK, pp. 147–156.

Dixon, A.G.O., Asiedu, R. and Bokanga, M. 1994. Breeding of cassava for low cyanogenic potential: problems, progress and perspectives. Acta Horticult. 375: 153–161.

Du, L., Bokanga, M., Møller, B.L. and Halkier, B.A. 1995. The biosynthesis of cyanogenic glucosides in roots of cassava. Phytochemistry 39(2): 323–326.

Duvick, D.N. 1999. Heterosis: feeding people and protecting natural resources. In: J.G. Coors and S. Pandey (Eds.), Genetics and Exploitation of Heterosis in Crops. American Society of Agronomy, Inc., Crop Science Society of America, Inc. Madison, WI, USA, pp. 19–29.

Ellis, R.H., Hong, T.D. and Roberts, E.H. 1982. An investigation of the influence of constant and alternating temperature on the germination of cassava seed using a two-dimensional temperature gradient plate. Ann. Botany 49: 241–246.

Fregene, M., Angel, F., Gomez, R., Rodríguez, F., Chavarriaga, P., Roca, W. and Tohme, J. 1997. A molecular genetic map of cassava (*Manihot esculenta* Crantz). Theoret. Appl. Genet. 95: 431–441.

Fregene, M., Bernal, A., Duque, M., Dixon, A. and Tohme, J. 2000. AFLP analysis of African cassava (*Manihot esculenta* Crantz) germplasm resistant to the cassava mosaic disease (CMD). Theoret. Appl. Genet. 100: 678–685.

Fregene, M. and Puonti-Kaerlas, J. 2002. Cassava biotechnology. In: R.J. Hillocks, J.M. Thresh and A.C. Bellotti (Eds.), Cassava: Biology, Production and Utilization. CABI Publishing, pp. 179–207.

Gonçalvez Fukuda, W.M., Fukuda, C., Leite Cardoso, C.E., Lima Vanconcelos, O. and Nunes, L.C. 2000. Implantação e evolução dos trabalhos de pesquisa participativa em melhoramento de mandioca no nordeste Brasileiro. Documento CNPMF No. 92. EMBRAPA, Cruz das Almas, Bahia, Brazil.

Gonçalvez Fukuda, W.M. and Saad, N. 2001. Participatory research in cassava breeding with farmers in Northeastern Brazil. Document CNPMF No. 99. EMBRAPA, Cruz das Almas, Bahia, Brazil.

Gardner, C.O. 1961. An evaluation of effects of mass selection and seed irradiation with thermal neutrons on yields of corn. Crop Sci. 1: 241–245.

Gomez, G., Santos, J. and Valdivieso, M. 1983. Utilización de raíces y productos de yuca en alimentación animal. In: C.E. Domínguez (Ed.), Yuca: investigación, producción y utilización. Working Document No. 50. Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia.

Hahn, S.K. 1984. Progress of root and tuber improvement at IITA. In: Proceedings of the 6th Symposium of the International Society for Tropical Root Crops. Lima, Peru, 20–25 February, 1983.

Hahn, S.K., Terry, E.R., Leuschner, K., Akobundu, I.O., Okali, C. and Lal, R. 1979. Cassava improvement in Africa. Field Crops Res. 2: 193–226.

Hahn, S.K., Terry, E.R. and Leuschner, K. 1980a. Breeding cassava for resistance to cassava mosaic disease. Euphytica 29: 673–683.

Hahn, S.K., Howland, A.K. and Terry, E.R. 1980b. Correlated resistance to cassava to mosaic and bacterial blight diseases. Euphytica 29: 305–311.

Hershey, C.H. 1984. Breeding cassava for adaptation to stress conditions: development of a methodology. In: Proceedings of the 6th Symposium of the International Society for Tropical Root Crops. Lima, Peru, 20–25 February, 1983.

Hallauer, A.R. and Miranda Fo, J.B. 1988. Quantitative Genetics in Maize Breeding. Second Edition. Iowa State University Press, USA, pp. 45–114.

Hilllocks, R.J. and Wydra, K. 2002. Bacterial, fungal and nematode diseases. In: R.J. Hillocks, J.M. Thresh and A.C. Bellotti (Eds.), Cassava: Biology, Production and Utilization. CABI Publishing, pp. 261–280.

Iglesias, C.A. and Hershey, C. 1994. Cassava breeding at CIAT: heritability estimates and genetic progress in the 1980's. In: F. Ofori and S.K. Hahn (Eds.), Tropical Root Crops in a Developing Economy. ISTRC/ISHS, Wageningen, Netherlands, pp. 149–163.

Iglesias, C.A., Hershey, C., Calle, F. and Bolaños, A. 1994. Propagating cassava (*Manihot esculenta* Crantz) by sexual seed. Exp. Agric. 30: 283–290.

Iglesias, C.A., Mayer, J., Chávez, A.L. and Calle, F. 1997. Genetic potential and stability of carotene content in cassava roots. Euphytica 94: 367–373.

Jennings, D.L. and Iglesias, C.A. 2002. Breeding for crop improvement. In: R.J. Hillocks, J.M. Thresh and A.C. Bellotti (Eds.), Cassava: Biology, Production and Utilization. CABI Publishing, pp. 149–166.

Kawano, K. 1980. Cassava. In: W.R. Fehr and H.H. Hadley (Eds.), Hybridization of Crop Plants. ASA, CSSA, Madison, WI, pp. 225–233.

Kawano, K. 2003. Thirty years of cassava breeding for productivity – biological and social factors for success. Crop Sci. 43: 1325–1335.

Kawano, K., Daza, P., Amaya, A., Ríos, M. and Gonçalvez, M.F. 1978. Evaluation of cassava germplasm for productivity. Crop Sci. 18: 377–380.

Kawano, K., Narintaraporn, K., Narintaraporn, P., Sarakarn, S., Limsila, A., Limsila, J., Suparhan, D., Sarawat, V. and Watananonta, W. 1998. Yield improvement in a multistage breeding program for cassava. Crop Sci. 38(2): 325–332.

Ladino, J., Mancilla, L.I., Chavarriaga, P., Tohme, J. and Roca, W.M. 2001. Transformation of cassava cv. TMS60444 with *A. tumefaciens* carrying a cry 1Ab gene for insect resistance. In: Proceeding of the Fifth International Scientific Meeting of the Cassava Biotechnology Network, Donald Danforth Plant Science Center, St. Louis, Missouri, USA, November 4–9, 2001.

McMahon, J.M., White, W.L.B. and Sayre, R.T. 1995. Cyanogenesis in cassava (*Manihot esculenta* Crantz). J. Exp. Botany 46: 731–741.

Nyiira, Z.M. 1975. Advances in research on the economic significance of the green cassava mite *Mononychellus tanajoa* Bondar in Uganda. International exchange and testing of cassava germplasm in Africa. In: E.R. Terry and R. MacIntyre (Eds.), Proceedings of an Interdisciplinary Workshop, Ibadan, Nigeria, 17–21 November 1975. IDRC-063e, Ottawa, Canada, pp. 22–29.

Puonti-Kaerlas, J., Li, H.Q., Sautter, C. and Potrykus, I. 1997. Production of transgenic cassava (*Manihot esculenta* Crantz) via organogenesis and *Agrobacteruim*-mediated transformation. Afr. J. Root Tuber Crops 2: 181–186.

Rajendran, P.G., Ravindran, C.S., Nair, S.G. and Nayar, T.V.R. 2000. True cassava seeds (TCS) for rapid spread of the crop in non-traditional areas. Central Tuber Crops Research Institute (Indian Council of Agricultural Research), Thiruvananthapuram, Kerala, India.

Scott, G.J., Rosegrant, M.W. and Ringler, C. 2000. Roots and tubers for the 21st century. Trends, projections, and policy options. International Food Policy Research Institute (IF-

PRI)/Centro Internacional de la papa (CIP), Washington, USA, 64 pp.
Sreekumari, M.T., Abraham, K. and Nair, S.G. 2000. Triploidy breeding in cassava. Central Tuber Crops Research Institute (Indian Council of Agricultural Research), Thiruvanantapuram, Kerala, India.
Steel, R.G.D. and Torrie, J.H. 1960. Principles and Procedures of Statistics. McGraw-Hill Book Company, New York. USA, pp. 39–40.
Taylor, N., Chavarriaga, P., Raemarkers, K., Siritunga D. and Zhang, P. 2004. Development and application of transgenic technologies in cassava. Plant Molecular biology, this issue.
Wheatley, C.C., Sanchez, T. and Orrego, J.J. 1993. Quality evaluation of the core cassava collection at CIAT. In: W.M. Roca and A.M. Thro (Eds.), Proc. Of the First Intl. Scientific Meeting of the Cassava Biotechnology Network, Cartagena, Colombia, August 1992. CIAT, Cali, Colombia, pp. 255–264.
Wheatley, C.C. and Chuzel, G. 1993. Cassava: the nature of the tuber and use as a raw material. In: R. Macrae, R.K. Robinson and M.J. Sadler (Eds.), Encyclopaedia of Food Science, Food Technology and Nutrition. Academic Press, San Diego, CA, pp. 734–743.
Wright, C.E. 1965. Field plans for a systematically designed polycross. Record of Agricultural Research 14: 31–41.