

Marker-Assisted Introgression of Resistance to Cassava Mosaic Disease into Latin American Germplasm for the Genetic Improvement of Cassava in Africa

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ABSTRACT

The gene pools for breeding cassava (*Manihot esculenta* Crantz) in Africa currently contain only a fraction of the existing genetic variation found in Latin America where the crop originates. Our research aimed to broaden the genetic base in Africa by introducing Latin American (LA) germplasm. The first set of introductions comprised sexual seeds that led to the evaluation of 20,032 seedlings in Nigeria between 1990 and 1994. A second set comprised in vitro cultures, where the dominant *CMD2* gene for cassava mosaic disease (CMD) resistance was introgressed into LA germplasm through marker-assisted selection (MAS). Through MAS 156 genotypes were preselected for the gene and evaluated in Nigeria between 2004 and 2006. Initial results from the first set of introductions indicated that LA germplasm was highly susceptible to CMD, minimizing its usefulness in African cassava-breeding programs. In the second set of introductions from LA, introgression of the *CMD2* gene resulted in high CMD resistance under African field conditions. Now at advanced stages in the African breeding program, 14 genotypes combining CMD resistance and high yield are being evaluated. Marker-assisted introgression of CMD resistance into LA germplasm has improved the potential value of LA germplasm for Africa and enhanced the prospect of elite LA genotypes being released as improved varieties in Africa.

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Abbreviations: AYT, advanced yield trial; BC₁, first back cross generation; CET, clonal evaluation trial; CIAT, Centro Internacional de Agricultura Tropical; CMD, cassava mosaic disease; F₁, first filial generation; IITA, International Institute of Tropical Agriculture; LA, Latin America(n); MAS, marker-assisted selection; NARS, national agricultural research systems; NCRP, Nigerian Nationally Coordinated Research Program; NRCRI, National Root Crop Research Institute; OP, open-pollinated; PYT, preliminary yield trial; SCAR, sequence characterized amplified region marker; SI, severity index; SN, seedling nursery; SSR, simple sequence repeat marker; TMS, Tropical *Manihot* Species; UYT, uniform yield trial.

CASSAVA (*Manihot esculenta* Crantz) is the major source of carbohydrates in sub-Saharan Africa and the fourth most important tropical crop worldwide. Cassava is a major staple for millions of people in tropical Africa (El-Sharkawy et al., 1990). The crop's ability to tolerate drought and degraded soils has

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ensured its increasingly worldwide economic importance (FAO, 2000). Aside from food, cassava also has important uses in the production of animal feed, starch, and alcohol.

Native to tropical America (Olsen and Schaal, 2001), cassava was introduced to Africa by Portuguese in the sixteenth century (Cock, 1985). Although considerable diversity exists within the African *M. esculenta* accessions, it can be broadened by using the vast germplasm base from Latin America, where cassava originates, so that untapped genetic resources may be exploited fully in breeding programs in Africa. The cassava germplasm bank held at the Centro Internacional de Agricultura Tropical (CIAT) contains nearly 6000 accessions. Of these, 93% are landraces (locally selected cultivars), collected from tropical and subtropical regions of the world, but particularly the neotropics. Most are traditional cultivars that represent centuries of farmer selection and cassava cultivation in diverse habitats (Bellotti and Arias, 2001). The remaining 7% of the CIAT collection comprises hybrids from specific crosses.

Genetic improvement of crops depends on the extent of genetic variation present in available germplasm (Patterson et al., 1991). Genetic diversity provides farmers and plant breeders with useful variability that can be used to develop, through selection and breeding, crops that are resistant to virulent pests and diseases, have high yield potential, and are adapted to adverse environments. For this reason, germplasm collections are important.

Given the spread of cassava to the rest of the world, many founder events are thought to have occurred, with the concomitant effect of reduced diversity (Fregene et al., 2003). Although farmers have selected cassava varieties that are relatively tolerant of diseases and pests, cassava gene pools outside the neotropics are relatively limited for breeding purposes and possess small numbers of seedlings from which to select broad-based tolerant types. In addition, the primary method of vegetative propagation further reduces genetic diversity over time because of the accumulation of systemic pathogens and the spread of a few, vigorous, well-adapted landraces able to produce many planting stakes (Fregene et al., 2000). Breeders rely on repeated intercrossing of adapted elite materials to improve quantitative traits. All these factors lead the crop to having a narrow genetic base with elite clones sharing a similar pedigree, as in the 1990s in Africa (Miller and Tanksley, 1990; Porto et al., 1994).

The current cassava and *Manihot* collection, held in trust at CIAT, has provided access to useful agronomic characteristics. Given the need to exploit fully the germplasm in Latin America for the benefit of African breeding programs, two international centers with a mandate for cassava research, CIAT and the International Institute of Tropical Agriculture (IITA), began a project to widen and improve the genetic base of cassava in Africa. Exotic

germplasm were introduced from CIAT into Africa and integrated within IITA's breeding scheme. The introduced germplasm were derived from elite crosses from Latin America (Porto et al., 1994). Initial results indicated that LA germplasm was susceptible to cassava mosaic disease (CMD), the most widespread cassava disease of economic importance in Africa. The disease does not occur in the neotropics, and its complex of begomoviruses is unknown in the Americas. Yield losses caused by CMD are as high as 100% (Thresh et al., 1994). Legg and Thresh (2000) estimated that, of Africa's total production of 97 million tonnes in 2003, losses to CMD accounted for 19 to 27 million tonnes (FAO, 2003). Conservatively, this amounts to an annual economic loss of about US\$3 billion.

Jennings (1976) first described a source of resistance to CMD derived from the Ceara rubber tree (*M. glaziovii* Müll. Arg.) and was thought to be polygenic. It is the most widely deployed source of resistance and is represented principally in African farmer fields by clones of the Tropical *Manihot* Species (TMS) series [TMS 4(2) 142, TMS 30337, TMS 91934, TMS 30001, TMS 60142, and TMS 30572] (Hahn et al., 1980). Given the need to increase CMD resistance in LA germplasm, efforts were intensified to cross the TMS series of clones with LA germplasm, incorporating genes for resistance to CMD into the exotic germplasm. The clones were then shipped back to Africa as seeds. However, evaluation of progenies of neotropical parental genotypes crossed to parents having the older polygenic form of CMD resistance revealed that resistance was not fully transferred from the donor clones.

Additional sources of resistance to CMD were found in local cassava varieties from Nigeria, providing a means of developing multiple resistances to the disease that the pathogen would find difficult to circumvent. Both CIAT and IITA began mapping multiple sources of resistance among CMD-resistant landraces and developing molecular markers for these genes. Classical genetic analysis and molecular genetic mapping of local Nigerian varieties resistant to CMD revealed that a major dominant gene confers resistance to CMD (Akano et al., 2002). The CMD resistant gene, designated *CMD2*, is located on Linkage group R of the molecular genetic map of cassava (Fregene et al., 1997; Akano et al., 2002).

Markers associated with *CMD2* have been used to introgress the gene into LA germplasm (Fregene et al., 2006). Although CMD has not been reported in the Americas, its vector, the whitefly (*Bemisia tabaci* Gennadius) has recently been found there, and its new biotype B (also referred to as *B. argentifolia*) is now widespread. Its host range is broad and includes cassava (Polston and Anderson, 1997). This is a frightening prospect for LA cassava production, and there is a need to prepare for any accidental introduction of the CMD virus into the neotropics.

Similarly, LA germplasm bred for resistance to CMD have been transferred to Africa to help broaden the cassava genetic base. Recently, *in vitro* plants from CIAT were genotyped and preselected, using markers for CMD resistance. This strategy is meant to bring the benefits value of LA cassava germplasm, including enhanced protein and vitamin content, high dry matter yield, and resistance to pests, to breeding programs in Africa, where the crop is a major food staple.

This paper describes results obtained from transferring germplasm from CIAT to Africa. It covers several years of introduction and the use of markers to introgress CMD resistance into LA germplasm to genetically improve cassava in Africa. We also consider how results from this study can be explored in plant breeding programs to develop new and improved varieties with novel traits. The results described here are from continued efforts not only to increase the usefulness of LA germplasm in Africa but also to fast-track germplasm delivery schemes by reducing the length of time needed to develop new varieties from germplasm acquisition to release and dissemination of new varieties to farmers in African countries. For this latter work, we examine how promising germplasm can be delivered rapidly and cost effectively to African national agricultural research systems (NARS) to benefit the continent's expanding cassava industry.

MATERIALS AND METHODS

Two separate introductions were made. The first introduction consisted of sexual seeds of breeding populations that were obtained from (i) crosses between elite parents of neotropical gene pools or (ii) crosses between these parents and CMD-resistant genotypes introduced to Latin America from IITA. No selection for CMD resistance was conducted on this first set of germplasm. The second set of introductions consisted of those materials that had been selected through MAS, using markers associated with CMD resistance.

First Set of Germplasm Introductions

Elite clones were chosen from neotropical cassava gene pools selected for adaptation to agroecologies with homologues in Africa, high productivity, and quality traits. They were then crossed with each other to generate seeds for introduction to Africa. The main tool for this strategy comprised the maps of climatic homologues between South America and Africa produced by CIAT's Geographic Information System Unit. Crossing blocks and open pollination fields were established in Palmira, Colombia, between the 1990 and 1993 planting seasons. Seeds were generated from both controlled hybridization and open pollination. The controlled hybridization crosses were designated as the CM series, while the open-pollinated (OP) seeds were designated as the SM series. Attempts to incorporate resistance to CMD into the LA germplasm were initially performed by controlled hybridization of 19 introduced IITA elite clones. These clones had the older polygenic sources of CMD resistance from *M. glaziovii* (Hahn et al., 1980) and elite parental lines of CIAT cassava gene pools.

Controlled crosses were of two types: (i) crosses between CMD-resistant clones from IITA and CIAT elite parents (CI crosses), and (ii) crosses between CIAT elite clones (CC crosses). Mother plants (of parents) and the resulting seeds were tested for the presence of virus and seed treatment by thermotherapy and pesticides at CIAT. Seeds were then introduced to Africa through IITA, Ibadan, Nigeria. At IITA the healthy seeds were planted in isolation in screen houses after treatment with hot water (60°C for 20 min) to check for the presence of bacterial and fungal diseases. A total of 20,032 seedlings germinated and were evaluated in nurseries between 1990 and 1994.

Evaluation Site in Nigeria

The introductions were evaluated in IITA's research field, which lies within the humid forest-savanna transition agroecology, where CMD pressure is usually high. The institute is situated near Ibadan at 7°30' N and 3°54' E. Elevation at IITA is 240 m above sea level. Rainfall is characteristically bimodal, with peaks in June and September and a period of less precipitation in August. The main dry season runs from December through February (Moormann et al., 1985). Annual rainfall fluctuates between 800 and 1900 mm, and annual temperatures range from an average minimum of 21°C to an average maximum of 31°C. The soils are slightly acidic, contain small amounts of exchangeable Al, and have a sandy loam texture with a low percentage of silt (Moormann et al., 1985).

The germplasm shipped for evaluation in Ibadan consisted of those materials generated from parents adapted to cassava-producing regions of Latin America that have conditions similar to those found in Ibadan. That is, climates in terms of rainfall distribution and amount, and temperature; soils (acidity, nutrients, and physical characteristics); and biological agents (disease and pest pressures) were similar across the two regions.

Evaluating for Resistance to Cassava Mosaic Disease

The standard IITA breeding scheme is a multistage evaluation of genotypes that starts with a seedling nursery planted in one site per Nigerian agroecology. The introduced germplasm were first planted in a seedling nursery (SN), spaced at 1 × 0.5 m on ridges. The seedlings were planted by family and evaluated for resistance to CMD. After the first season, materials with good performance for CMD resistance, as well as other traits, were selected, cloned, and evaluated in successive trials for further evaluation in Ibadan, according to the following breeding scheme:

- For the clonal evaluation trial (CET), six plants per genotype were planted in single-row plots, no replications.
- For the preliminary yield trial (PYT), 10 plants per plot, with two replications.
- For the advanced yield trial (AYT), 20 plants per plot, with three replications.

Except for the SN where seeds were sown, planting materials—mature stem cuttings—were planted at 20 cm on ridges spaced at 1 × 1 m apart. All trials in the breeding scheme were laid out according to a randomized complete block design. Germplasm were evaluated for resistance to CMD once a month after planting.

Each genotype was scored in each season, using the highest (maximum) score observed as a measure of the plant's reaction (resistance or susceptibility) to the disease. The rating for

reaction to CMD is based on a severity index (SI) that consisted of five classes: 1 = no symptoms; 2 = mild chlorotic pattern over the entire leaf although the leaf appears green and healthy; 3 = moderate mosaic pattern throughout the leaf, narrowing, and distortion in the lower one-third of leaflets; 4 = severe mosaic, distortion in two thirds of the leaflets, and general reduction in leaf size; and 5 = severe mosaic distortion in the entire leaf.

Severity of pest damage was also rated on a scale of 1 to 5, where 1 = resistant (healthy plants) and 5 = highly susceptible (Hahn et al., 1989).

Statistical Analysis

The mean SI for CMD of each family was calculated. Disease incidence was calculated and expressed as a percentage of infected plants within the total population. Progenies of the controlled crosses were compared for CMD with those of OP sources, using a *t* test analysis done with the statistical functions of the Microsoft Excel program (Microsoft, Redmond, WA). The test was based on a two-tailed distribution, and differences between means were declared significant at $P < 0.05$. Two check varieties from IITA—TMS 30001 (resistant) and TMS 91934 (susceptible)—were planted to monitor disease pressure. They were compared with each of the two groups, using *t* test analysis, and with the introduced germplasm.

Second Set of Germplasm Introductions

We used the embryonic axes of sexual seeds of TME 3, a local Nigerian cassava variety, that possesses the *CMD2* gene. The seeds had been developed at IITA and shipped to CIAT in Colombia, in 2000. We obtained 18 first filial generation (F_1) progenies that became the source for MAS for CMD resistance, using the *CMD2* gene. They were crossed extensively to elite parents of the four cassava gene pools, as defined by agroecology, namely, subhumid lowland tropics, acid-soil savannas, mid-altitude valleys, and tropical highlands. These initial F_1 crosses were designated as the CR series.

We also developed another group of 335 genotypes—the second backcross (BC_2) generation—obtained from crossing CMD-resistant donor parents to BC_1 derivatives of a wild progenitor (*Manihot esculenta* subspecies *flabellifolia*) of cassava with resistance to the cassava green mite (*Mononychellus tanajoa* Bondar). Development of mites resistance from the wild progenitor has been described elsewhere (Fregene et al., 2006). This second set of genotypes—the AR series—combines resistance to CMD with that to the green mite.

Embryo Culture

At CIAT, Colombia, mature F_1 and BC_2 seeds, obtained as above, were treated with concentrated sulfuric acid for 50 min, then washed thoroughly and rinsed with water before soaking in water for 30 min. The seeds were surface-sterilized by immersion in 70% alcohol for 5 min, followed by immersion in 5% sodium hypochlorite and Tween 80 (0.5%) for 20 min, and then rinsed three times with sterilized water. Once washed and under aseptic conditions, the seeds were tested for viability by soaking them in water. Viable embryos were then excised by first splitting the seeds along the longitudinal axis and then removing the embryos, using sterilized forceps and scalpel. The

excised embryos were placed radicle down in 17N medium, which was prepared with mineral salts of Murashige and Skoog (Roca, 1984) and supplemented with 0.01 mg L⁻¹ NAA, 0.01 mg L⁻¹ GA₃, 1.0 mg L⁻¹ thiamine-HCL, 100 mg L⁻¹ inositol, 2% sucrose, 0.7% agar (Sigma Chemical Co., St. Louis, MO), and 25 mg L⁻¹ of a commercial fertilizer containing N–P–K at 10–52–10. The medium's pH was 5.7 to 5.8 (Roca, 1984).

The embryo cultures were then incubated in darkness for 3 d to promote radicle growth and then transferred to growth chambers with a 12-h photoperiod. The plantlets were then micropropagated in 4E medium, as described by Roca et al. (1984) and kept in the growth chamber. After 3 or 4 wk of growth, each embryonic axis-derived plantlet was micropropagated to obtain three to five plants. After another 4 wk, leaves of all the plants were removed for genotypic selection.

Genotypic Selection

All parental lines were evaluated with eight markers—simple sequence repeats (SSRs) or sequence-characterized amplified regions (SCARs)—that were associated with the *CMD2* gene. These were NS158, SSRY28, RME1, RME2, RME3, RME4, RME5, and RME6. At least two markers, polymorphic in the parents and flanking the resistance gene, were used to evaluate the progenies. Details of the markers and the MAS process have been described by Fregene et al. (2006). Based on the molecular data, genotypes with the favorable allele of the *CMD2* gene were selected. Selected genotypes were micropropagated again in vitro to obtain 10 to 20 plantlets for shipping to Africa. Selected genotypes were also evaluated in the field at CIAT for other desirable traits (e.g., yield and plant morphotype).

Hardening

For postflask management, optimal potting was achieved by using bags, as follows: plantlets were first inspected, and those found to be contaminated, broken, or malformed were eliminated. The remaining plantlets were extracted from their flasks by adding water to moisten the agar substrate and so facilitate easy extraction of the plantlet. Plants were transferred from test tubes to pots and placed where they would be protected from direct sun and insects. After extraction, the plantlets were transplanted into polythene bags, measuring 7 × 14 cm and containing a sterilized mixture of one part soil to three parts of fine sand and moistened with water. Transplanting was done at 17:00 h. After transplanting, the plantlets were watered and placed in a humidity chamber for 8–12 d. The plantlets received applications of fungicide (2 g Benlate L⁻¹ [Du Pont de Nemours & Co., Wilmington, DE] and fertilizers (3 g of Plantex L⁻¹ [Plant Products, Canton, OH]). After 2 wk, the plants received additional fertilizer (3 g of 15–15–15 N–P–K L⁻¹ and 2 g of power plant fertilizer L⁻¹ for micronutrients [Plant Products, Canton, OH]). After 4 wk, the plants were transplanted to the field. The screen houses were then disinfected and cleaned. Leaf tissues from the plants were obtained and used for the genotypic screening of the *CMD2* gene.

Evaluating for Cassava Mosaic Disease

For the humid forest agroecology, CIAT introduced 156 genotypes possessing the *CMD2* gene and other desirable traits through the National Root Crop Research Institute (NRCRI), Umudike, Nigeria. About 5 to 10 copies of each genotype were

shipped. The *in vitro* cultures were introduced through the Nigerian Plant Quarantine Service. The seeds were then tested for pathogens. Healthy *in vitro* plantlets were planted in the screen house for hardening, following the same procedure as described above. After hardening, the number of plants per genotype varied from 2 to 10 copies, depending on whether the plants survived the hardening process. According to their growth and vigor, the plants were transplanted to the field after 8 to 12 wk of hardening.

Clones were planted according to a randomized complete block design so that copies of the same genotype were randomly distributed in the field. The plants were planted on ridges, spaced at 1.0 × 1.0 m, on a farm belonging to the Nigerian Starch Mills, Opuoma, Imo State, Nigeria. Opuoma is situated at 5°24' N and 7°18' E. The soils are sandy loams of medium fertility. Annual rainfall varies from 2200 to 2300 mm. The Starch Mills are located in the humid forest agroecology where CMD pressure is very high. The germplasm was evaluated monthly.

The highest (maximum) SI observed within a season was taken as the measure of a plant's reaction (resistant or susceptible) to the disease. Plants with scores of 1 or 2 were described as resistant; plants scoring 3 were considered tolerant, whereas plants scoring 4 or 5 were categorized as susceptible. Seedlings continued to be closely inspected under field conditions.

The 156 introduced genotypes were evaluated in the 2004–2005 and 2005–2006 growing seasons. An average of 5 to 10 copies was initially evaluated in the first season, with the number of plants per genotype increasing in the second season to 30 to 80, depending on vigor and the number of copies of stakes that could be obtained from each genotype in the first year.

RESULTS

Evaluating Resistance to Cassava Mosaic Disease among the First (Non-Marker-Assisted Selection) Set of Introductions

Cassava mosaic disease had a devastating effect on the first set of introductions from LA to Ibadan (i.e., CC, CI, and SM F₁ progenies). Results indicated that all F₁ seedlings evaluated at Ibadan showed symptoms of CMD as early as 2 wk after transplanting, with the SM families (from OP seeds) being the hardest hit group. Data from 1990 to 1994 indicated that CMD incidence increased from 63% within 4 wk of planting to 100% at peak disease pressure, which coincided with heavy rainfall (Fig. 1). Disease severity dropped slightly during the dry season, when whitefly activity was reduced. However, CMD epidemiology did not vary much from year to year.

Seedlings tended to recover from the disease during the dry season, sprouting newly formed leaves that showed no or mild symptoms. The rate of recovery did not appear to differ between the CM and SM families from 1990 to 1993 (Table 1). Decline in SI from peak disease pressure in September (rainy season) to December (dry season) was between 3.3 and 2.6 for CI crosses, 3.7 and 3.0 for CC crosses, and 4.1 and 3.4 for OP seedlings (Table 1). This indicates that the decline in SI was about 0.7 in each group of seedlings (CI, CC, and OP). After the dry season, the severity of symptoms increased again.

The CM families (CI and CC crosses) recorded less expression of symptoms than did the SM families (OP seedlings), with most (95%) of the SM genotypes showing

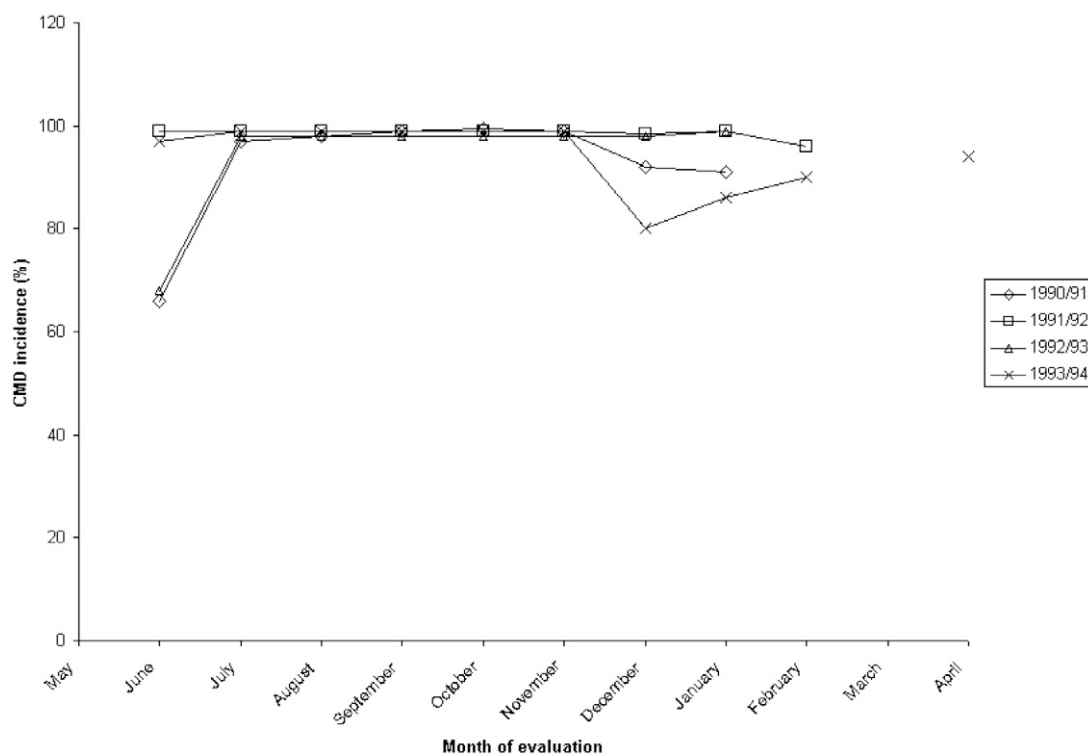


Figure 1. Incidence of cassava mosaic disease (CMD) in the seedling nursery, Ibadan, Nigeria, 1990–1994.

high susceptibility. Some seedlings with moderate resistance (mainly in the CM families) showed delayed expression of symptoms; that is, the SI was low (1 and 2) during the early stages of growth. They also showed few symptoms during the dry season, when whitefly activity was often low. However, at peak disease pressure, their SI

Table 1. Mean monthly scores[†] for severity of cassava mosaic disease over 3 yr (1990–1993) for seedlings of different backgrounds evaluated at Ibadan, Nigeria.

Month [‡]	Controlled hybridization		Open pollinated
	CIAT × IITA [§]	CIAT × CIAT	
June	1.7	1.8	1.9
July	3.0	3.2	3.7
August	3.3	3.5	3.8
September	3.3	3.7	4.1
October	3.1	3.6	4.1
November	3.1	3.7	3.9
December	2.6	3.0	3.4
January	3.1	3.2	3.3
February	2.9	3.1	3.2
March	2.8	3.0	3.3
April	3.0	3.0	3.4
Mean	2.90	3.16	3.46

[†]The cassava cropping season (usually 11–12 mo period) starts with the beginning of the rainy season at Ibadan usually from May (planting year) to April of the following year (harvesting year).

[‡]The cassava cropping season in Ibadan is usually 11–12 mo, starting at the beginning of the rainy season, usually in May (planting year), and finishing in April of the following year (harvesting year).

[§]CIAT, Centro Internacional de Agricultura Tropical; IITA, International Institute of Tropical Agriculture.

Table 2. Mean severity index of cassava mosaic disease[†] for Latin American germplasm in the seedling nursery and International Institute of Tropical Agriculture check varieties, Ibadan, Nigeria, 1991 to 1994.

Seasons	Nursery	Check variety	
		TMS 91934	TMS 30001
1991–1992	3.42	2.58	1.82
1992–1993	3.60	2.83	1.56
1993–1994	3.27	2.73	1.39
Mean	3.43	2.71	1.59

[†]Scores are based on five classes, where 1 = no symptoms; and 5 = severe mosaic distortion in the entire leaf.

Table 3. Severity rating for cassava mosaic disease (CMD) in 156 Latin American cassava genotypes selected with markers for the *CMD2* gene and introduced to Nigeria via in vitro cultures.

CMD severity index	Genotypes	
	no.	%
1	84	53.8
2	21	13.5
3	31	19.8
4	19	12.2
5	1	0.6

increased, although usually they did not score more than 3. Hence, the mean SI of the moderately resistant genotypes for the whole season was often less than 3.

However, no genotype obtained an SI of 1 or 2 for the entire cropping cycle; thus, no genotype was resistant to CMD. The average SI for the SN did not vary much from one season to another (Table 2). Similarly, the SI for the susceptible (TMS 91934) and resistant (TMS 30001) check varieties did not vary much between seasons. Over a three-season period (1991–1994), the lowest mean SI per season was 3.27 for the introduced germplasm (1993–1994), 2.58 for TMS 91934 (1991–1992), and 1.39 for TMS 30001 (1993–1994).

Peak disease pressure often occurred between August and November in Ibadan, with the highest SI being 3.3 for CI crosses, 3.7 for CC crosses, and 4.1 for OP progenies. Although CI crosses were better than CC crosses, no significant difference in CMD response was found between the two groups ($P = 0.223$). However, the CI crosses were significantly better than the OP progenies ($P = 0.024$). Again, no significant difference ($P = 0.23$) was found between CC crosses and those of OP sources.

When progenies of CI crosses were compared among themselves, most of the seedlings with moderate resistance (i.e., with a seasonal SI mean of less than 3) were observed to have derived mainly from crosses that had either TMS 30001 or TMS 30572 as a parent. Progenies from crosses involving TMS 30001 or TMS 30572 were therefore more resistant to CMD than other IITA elite clones used as CMD-resistant materials in crosses with CIAT elite clones.

Clone TMS 30001, highly resistant to CMD, showed no or few symptoms during the evaluation period, with the average SI varying from 1.39 to 1.82 between 1991 and 1994. At peak disease pressure, the highest SI for this variety was 2. However, progenies of crosses with TMS 30001 as a parent were not as resistant as TMS 30001. TMS 30001 had a significantly ($P = 0.003$) better reaction to CMD than did TMS 91934 (susceptible check). Overall, TMS 30001 ($P = 0.0004$) and TMS 91934 ($P = 0.005$) had significantly better reactions to CMD than the introduced germplasm over a 3-yr period (1991–1994).

The reaction to CMD of each genotype in the F_1 progeny is best given as the highest SI observed during the evaluation period, which then becomes a measure of the genotype's genetic potential to resist the disease. Because no genotype in the F_1 progeny had an SI of less than 3, this parameter thus indicated the LA germplasm's genetic vulnerability to CMD.

Evaluating Resistance to Cassava Mosaic Disease among the Second (Marker-Assisted Selection) Set of Introductions

The *CMD2* gene was introgressed into CR and AR genotypes, using markers. When they underwent evaluation for CMD, most (67.3%) showed resistance to the

disease with an SI of 1 or 2 (Table 3). This result agrees with findings by Fregene et al. (2006) in a pilot phase study of MAS for the *CMD2* gene. A higher percentage of the resistant genotypes showed near immunity with an SI of 1. Disease incidence was 46%, indicating that 54% of the materials were free of the disease. About 20% of the introduced genotypes showed tolerance of the disease, with an SI of 3 (Table 3). The remaining 13% were severely affected by CMD.

Highly susceptible plants with a score of 5 suffered drastically distorted leaves and poor morphology and growth. Some susceptible genotypes began showing symptoms within 4 wk of being transplanted. The CMD disease reaction of the germplasm was generally low in the dry season, when whitefly activity was low. In this period, the genotypes also showed low disease severity. For the resistant genotypes, the mean SI was 1.33 at peak disease pressure, whereas, for susceptible genotypes, the SI was 4.05.

Selecting Latin American Clones for the Breeding Scheme

Resistance to CMD is a primary criterion, together with other diseases and pests (e.g., cassava bacterial blight and cassava green mite) and productivity traits (yield, vigor, and harvest index), for selecting seedlings and clones in breeding programs in Africa. At Ibadan CMD pressure is high. Few clones from the first neotropical non-MAS germplasm were selected from the SN and yield trials because of their susceptibility to CMD. Selection in the SN was based mainly on the progenies' reaction to CMD. Selection varied from 5 to 15% between 1990 and 1993. Hence, of the 18,014 seedlings evaluated in the SN over 4 yr (1990–1993), 1480 were advanced for evaluation in the CET (Table 4).

Because of high disease incidence and susceptibility of the F_1 progenies, only plants having an SI of no more than 3 at peak disease pressure were selected. Most of the materials selected from the breeding scheme itself—from the CET through PYT to AYT—were from the CM families, especially the CI crosses, which performed better than the OP SM families. For example, in the 1995 PYT, of the seven best materials with a mean SI of less than 3, six were from CI crosses and the remaining one was from OP source (Table 5). None of the F_1 had high resistance per se. After 5 yr of evaluation, only four genotypes of the F_1 progenies were selected for AYT (two in 1993 and two in 1994). They had moderate tolerance to CMD. Some genotypes selected in the SN were observed to deteriorate further in CMD and yield at later stages in the breeding scheme.

Fertilizers were not applied in yield trials in IITA breeding program because activities were targeted to meet low-input conditions under which most peasant farmers operate. Moderate yields from 14 to 17 t ha⁻¹ at 12 mo were obtained at Ibadan in yield trials for the best LA

Table 4. The number of Latin American genotypes selected at various stages of the International Institute of Tropical Agriculture breeding scheme at Ibadan, Nigeria 1990–1994.

Trial	1990	1991	1992	1993	1994
Seedling nursery	5400	5300	3514	3800	2018
Clonal evaluation trial		538	253	117	572
Preliminary yield trial			9	10	26
Advanced yield trial				2	2
Uniform yield trial					0

Table 5. Latin American cassava germplasm showing moderate resistance in Ibadan, Nigeria, at the preliminary yield trial stage, 1995.

CIAT's code [†]	Type of cross [‡]	Female parent	Male parent	IITA's code [†]
SM 1766-1	OP	CM 5644-2		IB93/0357
CM 8060-23	CI	M Col 1505	M Nga 8	IB93/0895
CM 8213A-24	CI	CM 2772-3	M Nga 5	IB93/0290
CM 8217B-1	CI	M Nga 16	CM 523-7	IB93/0289
CM 8218B-24	CI	M Nga 10	CM 2772-3	IB93/0285
CM 8283B-99	CI	CM 2772-3	M Nga 12	IB93/0317
CM 8250-3	CI	M Nga 3	CM 2772-3	IB92/0387

[†]CIAT, Centro Internacional de Agricultura Tropical; IITA, International Institute of Tropical Agriculture.

[‡]OP = open pollinated; CI = Cross involving CIAT and IITA clones as parents.

Table 6. Yield of cassava genotypes possessing the *CMD2* gene harvested at 8 mo after planting at the Nigerian Starch Mills farm in Opuoma, Imo State, Nigeria, and advanced to uniform yield trial in 2006.

Genotypes	CMD severity index [†]	Yield
AR 38-3	1	33.2
CR14A-1	1	37
AR37-108	1	14
CR36-2	1	20
CR36-5	1	31.8
CR52A-25	1	35.4
CR41-10	1	46
CR42-4	1	33.3
CR26-1	1	34.3
AR12-45	1	40.3
AR1-82	1	33
AR15-5	1	26.7
CR52A-41	1	32
CR52A-22	1	41.3

[†]CMD, cassava mosaic disease. Scores are based on five classes, where 1 = no symptoms; and 5 = severe.

clones within the period. The yields obtained were lower than the average yield of 24 t ha⁻¹ for TMS 30572, an improved high yielding clone, generally used as standard check for yield in IITA breeding program. TMS 30572 is moderately resistant and tolerant to CMD. The moderate yields of the best LA clones obtained at Ibadan were in contrast to the high yields (27–38 t ha⁻¹ at 12 mo) reported

for the best LA clones elsewhere in zones with low CMD pressure in Nigeria (CIAT, 1996). In this study, high yields (20–46 t ha⁻¹ at 8 mo) were also observed in LA clones introgressed with the *CMD2* gene for CMD resistance in the second set of introductions. This indicates that the susceptible LA germplasm of the first introductions may not have fully expressed its yield potential at Ibadan due to high CMD incidence in this location.

Selection of genotypes for the UYT, the last trial in the breeding cycle, is based strictly on high resistance to CMD and good yield. After 5 yr, therefore, no entry from the first introductions was selected for UYT, meaning that of the 20,032 seedlings evaluated from the first introductions, none passed the test for official release in Nigeria—they were too susceptible to CMD.

About 70% of the CR and AR progenies, which possessed the introgressed *CMD2* gene, showed high resistance to CMD. They have been integrated into the NRCRI's breeding program at Umudike, Nigeria. Selected progenies have maintained consistency of response to the disease over two seasons. Genotypes with an SI of 1 or 2 were evaluated for yield potential. After 2 yr of evaluation, 14 genotypes with high CMD resistance and good root yield potential (Table 6) were selected for multisite UYT trials.

Although high yielding, one genotype (AR-37-108) appeared to be late bulking, whereas the other 13 genotypes were early maturing. Yields were high, with preliminary estimates ranging between 20 and 46 t ha⁻¹ at 8 mo after planting (Table 6). Three of the 14 genotypes (CR 14A-1, AR-38-3, and CR 41-10) are also being simultaneously evaluated in regional trials established by the Nigerian Nationally Coordinated Research Program (NCRP) and covering eight sites in diverse agroecologies. The NCRP trial is the last evaluation before the on-farm adaptation trials that precede official release. About 105 CR and AR genotypes with high CMD resistance are being used in crosses in the NRCRI cassava-breeding crossing block.

DISCUSSION

Marker-assisted introgression of CMD resistance into LA germplasm deployed to African NARS continues from CIAT's pioneering activities toward broadening the genetic base of cassava in sub-Saharan Africa. A systematic and well-coordinated approach is required to exploit fully the true potential of cassava germplasm in LA for the benefit of African cassava. Efficient use and rapid deployment of genetic resources from LA to Africa are key elements in current efforts to meet the flexibility sought for in the crop's food and industrial attributes by farmers, processors, and end users. Thus, this most versatile of crops can be enabled to provide food security, income, and new commercial opportunities for a growing population.

Important traits of interest for which useful genes are sought from introduced germplasm include increased dry

matter content, novel starch types, morphological and agronomic traits, pest and disease resistance, low cyanogenic potential, carotene content, and resistance to post-harvest physiological deterioration (PPD). The severe effect of CMD in the humid agro-ecology imposes great limitations on selecting LA cassava in yield trials, despite the presence of other desirable traits. Okogbenin et al. (1998) reported a high correlation of 0.83 between yield and CMD in LA germplasm.

Results obtained in this study indicate that the F₁ progenies introduced via seeds between 1990 and 1994 were not well adapted because of their susceptibility to CMD. This agrees with earlier findings by IITA (1975). The F₁ progenies from OP sources were the most affected, indicating the LA germplasm's high vulnerability to CMD. The increased severity in some genotypes at later stages in the breeding scheme was likely a result of the accumulation of the virus in planting materials, as cassava is normally vegetatively propagated. Disease pressure from CMD was fairly stable in the humid forest-savanna transition agroecology across seasons, as the SI for the SN and check varieties did not vary much between seasons. The severity of symptoms peaked during the rainy season, suggesting a relationship between symptom expression and high rainfall (Hahn and Howland, 1972).

The use of IITA CMD-resistant clones in crosses with LA clones improved the reaction of F₁ progenies of CI crosses to CMD. However, CMD resistance was not fully transferred from the donor clones, suggesting that CMD inheritance in the TMS series was polygenic. Nevertheless, the use of a dominant gene for resistance to CMD was more successful in transferring resistance to neotropical gene pools. The single-dominant-gene nature of the new source of resistance makes it particularly useful for breeding for CMD resistance in cassava, for which genetic improvement is limited by the crop's highly heterozygous nature, long cropping cycle, and vegetative mode of propagation.

As described above, because of their susceptibility to CMD, the F₁ genotypes of the first set of introductions could not be released as varieties. However, with respect to other useful traits of agronomic interest, the best were used in crosses with African elite cassava clones. The aim was to introgress, through recurrent selection, useful genes from introduced germplasm into the African gene pool and thus develop new varieties. Achieving the desired impact usually took 8 to 10 yr. However, clones from the second set of introductions with the introgressed *CMD2* dominant gene were resistant to CMD, making it possible to effectively use genotypic selection, using the markers linked to the *CMD2* gene. This created great potential to efficiently exploit useful traits in LA cassava germplasm for African germplasm.

Apart from the improved chances of releasing these introduced elite lines as varieties, their true potential for

other desirable traits could be evaluated and used in crosses to effectively transfer these traits to farmer-preferred cassava cultivars in a predictable and timely manner. The results from this study indicate that resistance for CMD is an important component for introducing neotropical cassava germplasm into Africa and that genes with a gene action that can easily be recovered in F_1 progenies is more efficient in transferring resistance.

Some CR and AR progenies were found susceptible to CMD. When the linked markers used for selecting CMD resistance are at a distance from the gene of interest, false positives or negatives in the screening process are likely to occur because of recombinations (crossovers) between marker and gene (Mohan et al., 1997).

The 14 genotypes from the backcrossed progenies are in advanced breeding stages at the NRCRI, with 3 of the 14 genotypes already undergoing multisite testing across Nigeria. This is an improvement over results obtained with F_1 progenies. At this rate, the AR and CR genotypes with promising yield potential have a good chance of being released shortly. By using markers to select for CMD resistance in LA germplasm and by exploiting the phenotypic data available at CIAT on desirable traits, transferring elite LA germplasm of high quality to African breeding programs is now feasible. The prospect of releasing LA genotypes as improved varieties is thus enhanced.

The MAS strategy holds great promise in fast tracking the use of neotropical germplasm materials and their possible release as new varieties in Africa. The advantage with this strategy is that markers are used to preselect neotropical cassava genotypes for CMD resistance. The selected genotypes are then evaluated for 1 or 2 yr at CIAT before being shipped to Africa, where they are evaluated for 2 yr. Those neotropical genotypes that perform well can then be rapidly evaluated in multisite trials for another 2 yr and subsequently released as varieties. Within 5 or 6 yr of the first evaluation, farmers can receive elite clones from exotic germplasm.

The reduced costs associated with this approach are another advantage for African NARS. By starting preliminary evaluations in Latin America (at CIAT), the initial cost of evaluation is not carried by African NARS, which have limited funds for their breeding programs. This approach also markedly reduces the population sizes of imported germplasm, making them more manageable for NARS.

The Centro Internacional de Agricultura Tropical also benefits, through minimized costs, from using markers to select for CMD resistance and deploying selected genotypes via *in vitro* culture. For example, the arduous task of making separate crosses for NARS where seeds are to be used is eliminated. The same genotypes can be shared between NARS programs. These materials are likewise important for CIAT's breeding program, which is devel-

oping LA gene pools for resistance to CMD on a preemptive basis to handle possible outbreaks of the disease in the neotropics. This means that CIAT and NARS will also share the same genotypes. This is important for CIAT, as it must select in the absence of the disease. Hence, CIAT can evaluate genotypes for other desirable traits because the genotypes' response to CMD can be inferred from their performance in Africa.

The MAS strategy is likely to lead to the identification of elite lines that have the introgressed CMD resistance and are also well adapted to both Latin America and Africa. That is, clones already evaluated under field conditions with CMD pressure in Africa can immediately help CIAT create CMD-resistant clones adapted to Latin America. In contrast, if germplasm materials were to be introduced as segregating seeds (with *CMD2* gene), then achieving this goal would be difficult. By shipping several copies (as *in vitro* cultures) of LA genotypes to Africa, sufficient planting materials of selected genotypes can be generated easily and quickly for evaluation in breeding schemes and dissemination to farmers. Cassava planting materials (stems) cannot be transferred easily across countries because of quarantine concerns of transmitting diseases. Introducing top cassava clones selected for CMD resistance as *in vitro* plants will therefore become increasingly important.

The aim of breeding for resistance is to produce cultivars with improved resistance that persists under a wide range of environmental conditions. The continued expansion of the CMD pandemic in Africa is a threat to production gains achieved in recent decades. Durability of resistance is therefore a priority in Africa. Identifying and pyramiding different disease-resistance genes will provide stable resistance against a broad spectrum of the CMD virus. The recent identification by IITA of five additional sources of resistance to CMD opens up possibilities for pyramiding multiple genes for durability in new varieties (Legg and Fauquet, 2004). Breeding for disease resistance in cassava is very slow and cumbersome because of the biological constraints of a heterozygous crop. Gene pyramiding in cassava will require molecular marker-assisted breeding to expedite the process.

Molecular markers are being increasingly integrated into existing plant breeding programs in Africa to allow researchers access, transfer, and combine genes at a rate and precision not previously achieved. Under the Generation Challenge Program, efforts are being made to introgress useful genes (for resistance to postharvest physiological deterioration, mites, and cassava brown streak) from wild progenitors into elite progenitors of cassava in Africa. Crosses are being performed in Latin America where most of wild *Manihot* species exist. A significant component of this activity entails improving developed germplasm for CMD resistance in breeding programs in Africa. The

CMD2 gene is being used for this purpose. Identification of markers for other traits in addition to CMD resistance can be used to choose parents more efficiently to combine different traits. Clearly, laboratory costs associated with MAS applications are decreasing, and more effective and efficient molecular markers are being developed. Such progress will make MAS more attractive and encourage its implementation as a tool for cassava breeding in sub-Saharan Africa.

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References

- Akano, A., E. Barera, C. Mba, A.G.O. Dixon, and M.A. Fregene. 2002. Genetic mapping of a dominant gene conferring resistance to cassava mosaic disease. *Theor. Appl. Genet.* 105:521–525.
- Bellotti, A.C., and B. Arias. 2001. Host-plant resistance to whiteflies with emphasis on cassava as a case study. *Crop Prot.* 20:813–823.
- Cock, J.H. 1985. *Cassava, new potential for a neglected crop.* Westview Press, Boulder, CO.
- CIAT. 1996. *Cassava program annual report 1995.* CIAT, Colombia.
- El-Sharkawy, M.A., J.H. Cock, J.K. Lynam, A.D.P. Hernández, and L.F. Cadavid. 1990. Relationship between biomass, root-yield, and single-leaf photosynthesis in field grown cassava. *Field Crops Res.* 25:183–201.
- FAO. 2000. *Cassava. Food Outlook 2:11.* Available at http://www.fao.org/docrep/004/x4910e03.htm#P1446_82916 (verified 30 July 2007).
- FAO. 2003. *Cassava production statistics, 2002.* Available at <http://faostat.fao.org/site/336/DesktopDefault.aspx?PageID=336> (verified 30 July 2007). FAO, Rome, Italy.
- Fregene, M., F. Angel, R. Gomez, F. Rodríguez, P. Chavariaga, W. Roca, J. Tohme, and M. Bonierbale. 1997. A molecular genetic map of cassava. *Theor. Appl. Genet.* 95:431–441.
- Fregene, M.A., A. Bernal, M. Duque, A.G.O. Dixon, and J. Tohme. 2000. AFLP analysis of African cassava (*Manihot esculenta* Crantz) germplasm resistant to the cassava mosaic disease (CMD). *Theor. Appl. Genet.* 100:678–685.
- Fregene, M.A., N. Morante, T. Sánchez, J. Marín, C. Ospina, E. Barrera, J. Gutiérrez, J. Guerrero, A. Bellotti, L. Santos, A. Alzate, S. Moreno, and H. Ceballos. 2006. Molecular markers for the introgression of useful traits from wild *Manihot* relatives of cassava; marker-assisted selection of disease and root quality traits. *J. Root Crops* 32(1):1–31.
- Fregene, M.A., M. Suárez, J. Mkumbira, H. Kulembeka, E. Ndedya, A. Kulaya, S. Mitchel, U. Gullberg, H. Rosling, A.G.O. Dixon, and S. Kresovich. 2003. Simple sequence repeat (SSR) diversity of cassava (*Manihot esculenta* Crantz) landraces: Genetic structure in a predominantly asexually propagated crop. *Theor. Appl. Genet.* 107:1083–1093.
- Hahn, S.K., and A.K. Howland. 1972. Breeding for resistance to cassava mosaic disease. p. 37–39. *In* E.R. Terry (ed.) *Proc. Cassava Mosaic Workshop.* International Institute of Tropical Agriculture, Ibadan, Nigeria.
- Hahn, S.K., J.C.G. Isoba, and T. Ikotun. 1989. Resistance breeding in root and tuber crops at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. *Crop Prot.* 8:147–168.
- Hahn, S.K., E.R. Terry, and K. Leuschner. 1980. Breeding cassava for resistance to cassava mosaic disease. *Euphytica* 29:673–683.
- IITA. 1975. *Annual report for 1974.* International Institute of Tropical Agriculture, Ibadan, Nigeria.
- Jennings, D.L. 1976. Breeding for resistance to African cassava mosaic. p. 39–44. *In* *African cassava mosaic: Report of an interdisciplinary workshop held at Muguga, Kenya.* IDRC 071e. IDRC, Ottawa, Canada.
- Legg, J.P., and C.M. Fauquet. 2004. Cassava mosaic geminiviruses in Africa. *Plant Mol. Biol.* 56:585–599.
- Legg, J.P., and J.M. Thresh. 2000. Cassava mosaic virus disease in East Africa: A dynamic disease in a changing environment. *Virus Res.* 71:135–149.
- Miller, J.C., and S.D. Tanksley. 1990. RFLP analysis of phylogenetic relationships and genetic variation in the genus *Lycopersicon*. *Theor. Appl. Genet.* 80:437–448.
- Mohan, M., S. Nair, A. Bhagwat, T.G. Krishna, and M. Yano. 1997. Genome mapping, molecular markers, and marker-assisted selection in crop plants. *Mol. Breed.* 3:87–103.
- Moormann, F.R., R. Lal, and A.S.R. Juo. 1985. *The soils of IITA.* Technical Bull. no. 3. International Institute of Tropical Agriculture, Ibadan, Nigeria.
- Okogbenin, E., M.C.M. Porto, and A.G.O. Dixon. 1998. Influence of planting season on incidence and severity of African cassava mosaic disease in the sub-humid zone of Nigeria. p. 388–392. *In* M.O. Akoroda and I.J. Ekanayake (ed.) *Root crops and poverty alleviation.* International Institute of Tropical Agriculture, Ibadan, Nigeria.
- Olsen, K.M., and B.A. Schaal. 2001. Microsatellite variation in cassava and its wild relatives: Further evidence for a southern Amazonian origin of domestication. *Am. J. Bot.* 88:131–142.
- Paterson, A.H., S.D. Tanksley, and M.E. Sorrells. 1991. DNA markers in plant improvement. *Adv. Agron.* 46:39–90.
- Polston, J.P., and P.K. Anderson. 1997. The emergence of whitefly-transmitted Gemini viruses in tomato in the Western hemisphere. *Plant Dis.* 81:1358–1369.
- Porto, M.C.M., R. Asiedu, A.G.O. Dixon, and S.K. Hahn. 1994. An agroecologically oriented introduction of cassava germplasm from Latin America into Africa. p. 118–129. *In* F. Ofori and S.K. Hahn (ed.) *Tropical root crops in a developing economy.* Proc. of 9th Symp. of Int. Society of Tropical Root Crops, Accra, Ghana. 20–26 Oct. 1991. International Society for Tropical Root Crops, International Institute of Tropical Agriculture, Ibadan, Nigeria.
- Roca, W.M. 1984. Cassava handbook of plant cell culture. p. 269–301. *In* W.R. Sharp, D.A. Evans, P.V. Ammirato, and Y. Yamada (ed.) *Crop species.* Macmillan, New York.
- Roca, W.M., J.A. Rodríguez, G. Mafla, and J. Roa. 1984. Procedures for recovering cassava clones distributed *in vitro*. CIAT, Cali, Colombia.
- Thresh, J.M., D. Fargette, and G.W. Otim-Nape. 1994. Effects of African cassava mosaic geminiviruses on the yield of cassava. *Trop. Sci.* 34:26–42.