BIOTECHNOLOGY RESEARCH APPLIED TO CASSAVA IMPROVEMENT AT THE INTERNATIONAL CENTER OF TROPICAL AGRICULTURE (CIAT)

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ABSTRACT

CIAT activities in developing biotechnology applications to cassava in the last decade are described. The strategies to identify research topics and implement in house capabilities and an international cooperative effort in cassava biotechnology are emphasized. Successful integration of selected biotechniques into the research on germplasm conservation, characterization and propagation at CIAT and national programs in developing countries is highlighted, and progress in developing advanced biotechnology tools for genetic mapping and manipulation at CIAT is presented. The CIAT efforts are oriented to link modern biological research with the needs of cassava farmers through the generation of low input, environmentally friendly, germplasm-based technologies.

THE CASSAVA CROP

Cassava, along with rice, sugar-cane and maize, is the greatest supplier of calories in the developing world. Globally, cassava provides food for more than half a billion people. Cassava originated in tropical America. From the Americas, cassava disseminated to West and East Africa and to South East Asia between early 1500 to mid 1800. Cassava is traditionally grown by small farmers under conditions in which climate and soil makes it difficulty to grow other crops without costly inputs. Total world production increased from 130 million tons in 1986 to 150 million tons in 1990; Brazil, Zaire, Thailand, Nigeria and Indonesia are the largest producers, with 60% of the total (De Bruijn and Fresco, 1989). In sub-saharan Africa, cassava provides dietary calories for nearly 150 million people. In Asia and America, 60% and 40%, respectively, is destined for human food (Cock, 1985). In recent years, cassava has entered the animal feed and starch industries. About 20% of world production is fed to animals and about 6% goes into starch for industrial processes.

Cassava is a perennial woody shrub, with underground storage roots. It is a highly heterozygous, polyploid species (2n=4x=36); both cross- and self-pollination occurs naturally and is highly susceptible to inbreeding depression. The crop is traditionally propagated by stem cuttings. Sexual propagation is important for breeding purposes; production of variability through hybridization is relatively easy in cassava and once a superior genotype has been identified, it can be indefinitely maintained by means of vegetative propagation.

Cassava genetic resources are very large; nearly 100 wild Manihot species have been identified, Manihot esculenta being the only cultivated species of the genus.

Germplasm collections exist in international and national institutes. The cassava collection maintained at CIAT comprises over 5,000 cultivated accessions and nearly 30 wild species.

CASSAVA GERMPLASM DEVELOPMENT RESEARCH AT CIAT

Cassava research at CIAT forms part of a global cassava research and development system. Together with its sister research program at the International Institute of Tropical agriculture (IITA), the CIAT program is dedicated to exploiting cassava as an important traditional rural and urban staple and to developing new forms of utilization to satisfy diversified markers. The CIAT cassava research program was formed at the time of the Green Revolution of rice and wheat. In contrast to these two crops, the scientific base for cassava improvement was rudimentary. By the end of the seventies, a great deal had been achieved in improving the basic understanding of the plant. In the early eighties, research on cassava utilization was initiated involving integrated production, processing and marketing projects. Currently, as CIAT moves from applied to strategic research, the most important objectives of cassava germplasm research include (CIAT, 1992): (i) developing components of production technology that form the basis of cassava based sustainable cropping systems, (ii) developing genetic based and management technology that allows cassava to be grown on presently under exploited lands; (iii) developing processing technology that makes cassava a low-cost, high quality, convenient food; (iv) assisting in the development of novel uses of cassava that increase the overall demand for the crop; (v) increase the capacity of national programs to carry out cassava research and development projects.

BIOTECHNOLOGY RESEARCH AT CIAT

The objective of biotechnology research at CIAT is to increase the efficiency and cost effectiveness of CIAT strategic germplasm research with cassava (Manihot esculenta), common beans (Phaseolus vulgaris), rice (Oryza sativa) for Latin America, and tropical forage species like Brachiaria spp, Arachis spp and Stylosanthes spp. In order to achieve these objectives CIAT has adopted the following strategies:

(i) Establishment of the Biotechnology Research Unit (BRU). Organized in 1985, the BRU is designed to perform as a scientific bridge for developing biotechnologies to address priority production and utilization constraints in CIAT crops.

(ii) Integrating biotechnology with strategic research. Relevant constraints are first identified in CIAT germplasm research; the BRU monitors basic research developments worldwide and brings to CIAT new information and methodologies. Cooperation with

CIAT program scientists to utilize biotechnology tools, is essential to implement this strategy;

(iii) Networking. The BRU cooperates with CIAT programs to bring prioritized research to the attention of the world scientific community. This leads to the establishment of international cooperative efforts and research linkages between basic research institutions with the strategic research conducted at CIAT;

(iv) Institution development. Biotechnological information transfer to CIAT partners in developing countries is basic to CIAT's bridging role in biotechnology research. Advanced training as post-graduated research is the main modality of information transfer. Critical issues in biotechnology research, such as biosafety, socio-economic impact, and issues relating to intellectual property protection are included in CIAT's institution building activities.

RESEARCH PRIORITIES FOR CASSAVA BIOTECHNOLOGY

Modern biological technologies have not been fully applied to cassava because major advances in the new biology have mostly taken place in developed countries, where cassava is not grown nor used as a food.

The identification of constraints to cassava production and utilization is the first step in the development of advanced biotechnological research relating to cassava. In 1988, CIAT, IITA and leading researchers from developed and developing countries and donor organizations founded the Cassava Biotechnology Network (CBN) (CIAT, 1989), and identified a series of priority research topics for biotechnological and more basic research approaches. Constraints include those related to the crop vegetative propagation such as insect pests and viral diseases, cassava true seed propagation, and to quality such as cyanide toxicity, starch and protein quality, and root perishability after harvest. Under the auspices of the Dutch Ministry of Foreign Affairs, additional priorities were identified for cassava biotechnology (DGIS, 1991), including soil acidity and drought, biological pest control, and cassava processing constraints.

As with all agricultural research the ultimate goal is to generate an appropriate, efficient and effective technology that can be adopted by as large a number of users as possible. In our case, the target group is the small-scale farmer and/or processor. Table 1 demonstrates that constraints which limit cassava productivity, e.g. viral diseases and insect pests share similar potential in Africa and Latin America, being less important in Asia; cassava quality constraints have similar potential for biotechnological approaches in the three continents, except for a lower priority of cyanogenesis in Latin America.

Table 1 also attempts to capture how cassava production and/or marketing is influenced by biotechnology innovations. Since yield reductions from a range of viral

diseases and insect pests can be as high as 70-80%, especially in Africa and Latin America, a biotechnological solution to these constraints would results in a very high increase in cassava yields, but with low direct advantage of the crop in the market. On the contrary, the relative impact of biotechnological innovations on cassava quality constraints would be only slight in terms of yield increase, but would provide a large market advantage to cassava products. Other technologies will impact only on the marketing side of cassava.

In addition to yield increase or marketing advantage, sustainable management of the natural resource base in another important issue, especially for resource-limited small-scale cassava farmers, who generally grow their crops on marginal lands under difficult climatic conditions. Any new technology should also optimize the conservation of the natural resource base.

Another set of cassava research constraints includes the techniques of cellular and molecular biology whose full development is necessary to approach the production and utilization challenges refered to above. Plant regeneration from somatic and reproductive cells, genetic fingerprinting and molecular mapping of the cassava genome, genetic transformation, cryopreservation, and advanced pathogen diagnostic techniques, are some of the most important.

Biotechnology research is not different from more traditional research regarding its integration with other disciplines (i.e. socio-economics, agronomy, etc.) in developing appropriate innovations. One may even argue that the integration with other disciplines becomes even more important with biotechnology research since particular innovative technologies like true cassava seed (TCS), require a very extensive and intensive farmer-participatory research and development approach. Moreover, as is the case of TCS, in-depth knowledge of seed markets and marketing becomes fundamental for successful acceptance, adoption and impact.

EARLY APPLICATIONS OF BIOTECHNOLOGY TO CASSAVA AT CIAT

Enhancing vegetative propagation and production of clean planting material.

Cassava vegetative propagation is not only slow (10-20 stem cuttings can be obtained from one mature plant per year) but often exposes the crop to pests and diseases which are transmitted through successive generations. Viral diseases, in particular, may lead to a decline in crop yield and quality over a period of time. We have developed virus elimination techniques followed by micro-propagation of disease-free cassava stocks. In vitro multiplication of cassava is needed for (i) increasing the number of meristem tip-derived plants for virus testing; (ii) increasing pathogen-tested clones for international exchange and germplasm storage; (iii) obtaining a sufficient number of plants

for the production of basic 'seed' and (iv) achieving massive propagation of elite clones for direct field planting.

There are two possible routes for in vitro propagation of cassava: (i) through enhancement of pre-existing meristems, i.e. by means of single node cuttings and multiple shoot cultures (Roca, 1984), and (ii) through de novo formation of somatic embryos from immature leaves or meristem tips (Szabados et al., 1987). The former is the most common route for cassava micropropagation. In vitro techniques largely surpass traditional methods in propagation potential. Single node culture and multiple shoot culture can produce $60x10^3$ and $60x10^4$ stem cuttings per mother plant per year, respectively. The multiplication potential of somatic embryogenesis has not been tested.

Local cassava cultivars show significant yield gains when the planting material is generated and multiplied by micropropagation techniques (Table 2). Micropropagation of disease-free stocks has been carried out in the national programes of Brazil, China, Cuba, Panama, Paraguay, Peru and Venezuela. In China, for example 30-70% yield increase over local varieties was obtained by micropropagation of the CIAT clone CM 321-188, and over 30.000 hectares have been planted with this and other genotypes in Southern China in 1988.

In vitro conservation and international exchange of cassava genetic resources.

Conventional maintenance of cassava germplasm collections is carried out by continuous vegetative cultivation in the field; besides being a costly operation, field maintenance often exposes the valuable material to insect and disease attack and soil or climatic problems.

An in vitro active gene bank has been developed at CIAT, which maintains over 4,800 cassava clones, collected from 23 tropical countries (Table 3). The in vitro collection represents over 95% of the world cassava germplasm collection held at CIAT. The estimated running cost of the bank is 30,000 U.S.$ per year which represents one half of the cost of maintaining the collection in the field.

The international exchange of cassava germplasm has been greatly facilitated by in vitro techniques. In the last ten years, over 900 cassava elite clones have been cleaned from viral diseases; out of these, 850 clones haven been distributed from CIAT to national research institutions in 35 countries of Latin America, South-East Asia and Africa. On the other hand, some 2800 cassava cultigens, collected from 14 tropical countries, have been introduced to CIAT as in vitro cultures (Table 3).

Thus, in vitro culture techniques have contributed to cassava improvement by increasing the availability of germplasm resources for genetic improvement, and the

transfer of elite clones to national research programs for evaluation and varietal development.

ADVANCED CASSAVA BIOLOGICAL RESEARCH

As refered to earlier, the Cassava Biotechnology Network (CBN) constitutes an effective mechanism for accessing basic information in molecular and cellular biology. The CBN has received wide acceptance by the scientific community. Since its foundation, the number of projects has increased steadily; currently over 25 projects are underway in developed and developing countries, dealing with critical research challenges in cassava (Table 4). Virus and insect resistance and photosynthetic behavior under stress, plant regeneration from cell cultures, genetic transformation, cassava genome characterization and cryopreservation of genetic resources are the most important topics receiving attention by researchers. Perishability of cassava roots after harvest, starch quality, nutritional quality of roots including protein and carotene content, have been proposed as new initiatives for immediate research (Bertram, 1990; DGIS, 1991).

CIAT's approach to cassava biotechnology is constraint-driven. However there are critical techniques whose full development is necessary for tackling the research constraints refered to above. With this in mind, CIAT has given special attention to research on technology development in the last few years.

DEVELOPMENT OF MODERN CASSAVA BIOTECHNOLOGIES AT CIAT

Characterization and identification of useful genetics variability.

Genetic fingerprinting. Electrophoretic fingerprinting of cassava by α, ß-Esterase (EST), Diaphorase (DIA), Acid Phosphatase (AcP), Peroxidase (PrX), and Glutamate Oxalo Acetate (GOT) isozymes have been developed at CIAT. We have studied the genetics of the EST-1 locus using 300 progenies from 11 crosses. Five alleles (Ao, A1, A2, A3, A4) with 11 phenotypes, and 5 non-observable null alleles, were demonstrated. This locus behaves as a monomer and its inheritance pattern is compatible with a diploid model.

Because many EST alleles per loci are polymorphic in cassava this system has been used at CIAT to analyze over 4300 cassava accessions. A large portion of the accessions showed unique patterns, implying genotypic differences; but other accessions had similar banding patterns suggesting possible duplication.

The development of DNA-based techniques for the analysis of genetic diversity of Manihot is advanced. Techniques for DNA extraction and the construction of genomic libraries have been carried out. Polymorphisms were detected with several clones.

Other probes have been tested for detecting polymorphism in cassava. The human minisatellite probe resulted ineffective in detecting polymorphism; however, the phage M13 probe and several random amplified polymorphic DNA markers (RAPD) did yield sufficiently variable band patterns to differentiate cassava varieties. We have confirmed the higher power of DNA fingerprinting over EST isozymes for discriminating cassava genotypes.

Molecular mapping. A collaborative research project to construct the molecular and physical maps of cassava using random genomic, cDNA and YAC libraries was initiated at CIAT in 1992. The goal of the project is to tag agronomically important traits, simply and quantitatively inherited, draw genetic relationship between cassava and its wild relatives, and as a step towards the isolation and cloning of cassava genes.

A PstI random genomic library was generated using the var. MCol 22. Some 200 single copy clones, of a total of 500, have been characterized, insert size ranges from 0.2 to 3 kb. Cassava DNA digested with EcoRI, EcoRV, XbaI and HindIII provided probes with highest polymorphisms. Polymorphism between the cv. MCol 22 and its wild relative M. aesculifolia was dramatically higher than the polymorphism found between two cultivated genotypes (Table 5).

Plant regeneration and genetic transformation.

The development of cassava transformation requires a plant regeneration technique, a genetic construct containing appropriate selectable and/or reporter markers, a house keeping or tissue specific promoter; the technology to monitor the transcriptional and post-translational products of gene expression and the inheritance of the introduced gene.

Cassava somatic embryogenesis. Plant regeneration can be achieved through somatic embryogenesis on immature leaves or apical meristems. We have demonstrated the usefulness of the technique with a range of cassava genotypes with 4-8 mg/l 2,4-D. Embryos are induced directly on an embryogenic tissue, and plantlets are regenerated from primary and secondary embryos in the presence of benzyl amino purine and gibberellic acid (Szabados et al., 1987).

Agrobacterium tumefaciens-mediated transformation. We have screened a number of cassava varieties with 25 A. tumefaciens strains. Two cassava varieties showed very high sensitivity to both the wild and the disarmed strain.

We used the plasmid construction pGV 1040 (provided by PGS, Belgium) containing uid A, bar and nptII genes. Expression of opine synthesis genes of a wild Agrobacterium strain, isolated from cassava tumors, was obtained after transformation of immature leaves.

Sensitivity levels of cassava tissues to the commercial herbicide Basta, used as selection agent, were determined. Minimum lethal doses of the herbicide are 1 mg/l for immature leaves and 18 mg/l for somatic embryos. Currently, work is underway using the wild Agrobacterium strain carrying the GV 1040 plasmid.

Particle bombardment-mediated transformation. Globular stage somatic embryos were bombarded using the pGV 1040 plasmid construct. GUS expression after 3 days of bombardment has been observed, with an average rate of 20 GUS spots per 0.5 cm^2 of embryogenic tissue. Recently, GUS expression was detected in expanded tissue areas of somatic embryos after one month of bombardment, suggesting that multiplication of cells which expressed the GUS gene initially took place.

Cryopreservation

Currently, ex-situ conservation of cassava germplasm at CIAT is carried out both as a field crop and in the laboratory as shoot tip cultures. The in vitro conservation constitutes an active collection and clones are sub-cultured and re-cycled every 12-18 months depending on the genotype. The availability of a long-term storage technology for cassava, in reduced space, free of genetic change, and at a low cost can be achieved by cryopreservation.

A research project on cassava cryopreservation began at CIAT in 1988; the project comprises three phases: (i) The first phase was carried out in cooperation with IBPGR (1988-91) and resulted in the recovery of plants from frozen shoot tips in liquid nitrogen. With the cv. MCol 22, recovery rates ranged from 30 to 50%. However, several cassava cvs. had a low response or did not respond at all. (ii) The second phase of the research (1991-present), was designed to improved the previous protocol for increasing the recovery rate of plants and minimizing genotypic differences. Lower temperature and higher illumination of donor cultures increased recovery from liquid nitrogen with consistent rates around 60%. Use of high concentration of sucrose in lieu of sorbitol and DMSO in the pre-culture stage, allowed up 80% plant recovery from frozen shoot tips. Rapid freezing, i.e. direct immersion of shoot tips into liquid nitrogen, resulted in similar or higher recovery rates than slow freezing, i.e. 0.5°C/min. On the other hand, recovery rate of otherwise unresponsive genotypes has significantly increased with the improved technique. This work has paved the way to the development of a long-term, base gene bank of cassava clones using liquid nitrogen.

CONCLUSIONS

Table 6 summarizes the research activities carried out at CIAT in the last 12 years aimed at developing biotechnology capabilities with cassava. Constraints associated with cassava clonal propagation and germplasm management were first tackled by means of tissue culture micropropagation and related techniques. These applications are now

under use at CIAT and several developing countries. Later on, the need for characterizing and making more efficient cassava germplasm collections stimulated our work on genetic fingerprinting. Likewise, these technologies have been incorporated into on-going cassava research at CIAT. More recently, CIAT efforts have focused on developing advanced biotechnology tools to allow cassava genetic mapping and tagging, and manipulation at the cellular and molecular levels; the goal is to bring cassava science and technology to the level of other important food crops.

CIAT efforts in cassava biotechnology have been effectively supported by the development of the Cassava Biotechnology Network. This cooperative international initiative facilitates the tackling of priority constraints and the exchange of information, and links research projects with the needs of small-scale cassava farmers.

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Table 1. Relative importance of cassava biotechnology research topics by continent and impact of biotechnological innovations on small-scale cassava farmers and marketing of cassava products.

Biotechnology research topics	Importance			Impact of innovations	
	Africa	Latin America	Asia	Yield increase	Market advantage
Viral diseases	+++	+++	+	+++	+
Insect pests	+++	+++	+	+++	+
Cyanide toxicity	+++	+	++	0	++
Starch quality	++	++	+++	0	+++
Post harvest root deterioration	++	+++	+++	0	+++

+++ large; ++ medium; + small; 0 no change.

Table 2. Yield of three local cassava cultivars following pathogen elimination and micropropagation by shoot tip culture.

Cassava cultivar	Growth cycle (year) (a)	Yield (ton/ha)	
		Fresh roots	Starch
'Secundina'	I	30.2	8.2
	II	25.9	7.7
	III	20.7	6.1
	IV	17.6	5.2
	Control(b)	17.0	5.3
'Quilcace'	I	17.4	5.3
	II	12.9	3.9
	Control(b)	7.5	2.1
'MCol 113'	I	16.5	4.3
	II	7.2	1.9
	Control(b)	4.6	1.2

(a) Planting material (stem cuttings) for each cycle was obtained from mature plants of preceding cycle.

(b) Planting material obtained from farmers fields, i.e. without micropropagation.

Table 3. Tissue culture for the production of disease free clones, international exchange and conservation of cassava germplasm (Work carried out at CIAT between 1980-91).

	Virus elimination*	Introductions to CIAT	Distributions from CIAT	*In vitro* gene bank
N° Accessions	950	2800	850	4850
N° Countries	-	14	35	23

* Cassava common mosaic, virus X, frog-skin disease and latent viruses.

Table 4. Major research projects underway in developed and developing countries dealing with critical constraints and development of biotechnological tools for cassava*.

Research topic	Collaborating institutions	Country	Funding source
Cyanogenesis	Univ. of Newcastle Upon Tyne Royal	U.K.	RF/EC/ODA
	Vet.Agric.Univ.	Denmark	RF/EC
	Royal Vet.Agric.Univ.	Denmark	DANIDA
	Mahidol Univ.	Thailand	
	Ohio State Univ. Colombus, Free	USA	USAID
	Univ. Amsterdam	The Netherlands	
Virus resistance	The Scripps Institute, La Jolla	USA	ORSTOM/RF/ USAID/GTZ
	Free Univ. Amsterdam/	The Netherlands	DGIS
	Univ. Zimbabwe	Zimbabwe	
Insect resistance	Washington State Univ., Pullman	USA	RF
Photosynthesis	Australian Nat.Univ. Canberra	Australia	AIDAB
	Univ. of Georgia/CIAT	USA	USAID
Plant regeneration	Univ. Paris, Orsay	France	EC
	Univ. of Bath	U.K.	ODA
	Univ. of Zimbabwe	Zimbabwe	DGIS
	South China Inst. Botany	China	RF
	Univ. Wageningen	The Netherlands	
	CIAT	Colombia	Core
	IITA	Nigeria	Core
Genetic transformation	Univ. Nottingham.	U.K.	RF
	Univ. of Guelph	Canada	RF
	CENARGEN	Brasil	
	IITA	Nigeria	Core
	CIAT	Colombia	Core
DNA fingerprinting and Molecular mapping	Washington Univ., St. Louis	USA	RF
	Univ. of Georgia, Athens	USA	RF
	CIAT	Colombia	RF
Cryopreservation	CIAT	Colombia	Core
CBN activities	CIAT	Colombia	DGIS
Other activities	CIAT	Colombia	Core
	IITA	Nigeria	Core

* As of June, 1992.

Table 5. Degree of polymorphism in cassava detected with at least one restriction enzyme, using five random genomic libraries as source of probes.

Library**	MCol 22 vs. MCol 1505	MCol 1505 vs. M. aesculifolia
PstI	60%	85%
XbaI	60	85
HindIII	55	95
EcoRI	40	60
BamHI	30	45

* Best restriction enzymes, in order of polymorphism, are: EcoRV, XbaI, EcoRI, HindIII.
** Library constructed with cassava cv. MCol 22.

Table 6. Research activities at CIAT for developing cassava biotechnology capabilities: Summary for the period 1980-1992.

Research topics-technologies	Funding source		Status of technology
	Core	Special	
1. Micropropagation/pathogen-free clones	X		In use
2. *In vitro* conservation/germplasm exchange	X	X	In use
3. Genetic fingerprinting			
· Isozyme fingerprinting	X	X	In use
· DNA fingerprinting		X	Advanced
4. Molecular mapping		X	Initiated
5. Genetic transformation	X		Initiated
6. Cryopreservation	X	X	Advanced
7. Gene identification/cloning(a)	X		Initiated
8. Cassava Biotechnology Network	X		Underway
9. Training developing countries	X	X	Underway

(a) Genes involved in photosynthesis under stress conditions: rbcs, mdh, me, ppc.

AgBiotech News and Information 1992 **Vol. 4 No. 10** 303N–308N

#1915 ARTICULO RESUMIDO

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CIAT activities in developing biotechnology applications for cassava over the last decade are described. The strategies used to identify research topics and to implement in-house capabilities and an international cooperative effort in cassava biotechnology are emphasized. Successful integration of selected biotechniques into the research on germplasm conservation, characterization and propagation at CIAT and national programmes in developing countries is highlighted, and progress in developing advanced biotechnology tools for genetic mapping and manipulation at CIAT is presented. CIAT efforts are oriented to link modern biological research with the needs of cassava farmers through the generation of low input, environmentally friendly, germplasm-based technologies.

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Modern biological technologies have not been fully applied to cassava because major advances in the new biology have mostly taken place in developed countries, where cassava is not grown nor used as a food.

The identification of constraints on cassava production and utilization is the first step in the development of advanced biotechnological research relating to it. In 1988, CIAT, IITA and leading researchers from developed and developing countries and donor organizations founded the Cassava Biotechnology Network (CBN) (CIAT, 1989), and identified a series of priority research topics for biotechnological and more basic research approaches. Constraints include those related to vegetative propagation, such as insect pests and viral diseases and cassava true seed propagation, and those related to quality, such as cyanide toxicity, starch and protein quality, and root perishability after harvest. Under the auspices of the Dutch Ministry of Foreign Affairs, additional priorities were identified for cassava biotechnology (DGIS, 1991), including resistance to soil acidity and drought, biological pest control, and processing constraints.

As with all agricultural research the ultimate goal is to generate an appropriate, efficient and effective technology that can be adopted by as large a number of users as possible. In our case, the target group is the small-scale farmer and/or processor. Table 1 demonstrates that constraints which limit cassava productivity, e.g. viral diseases and insect pests share similar potential in Africa and Latin America, being less important in Asia; cassava quality constraints have similar potential for biotechnological approaches in the three continents, except for a lower priority of cyanogenesis in Latin America.

Table 1 also attempts to show how cassava production and/or marketing is influenced by biotechnology innovations. Since yield reductions from a range of viral diseases and insect pests can be as high as 70–80%, especially in Africa and Latin America, a biotechnological solution to these constraints would result in a very high increase in cassava yields, but with little direct advantage of the crop in the market. In contrast, the relative impact of biotechnological innovations on cassava quality constraints would be only slight in terms of yield increase, but would provide a large market advantage to cassava products. Other technologies will impact only on the marketing side of cassava.

In addition to yield increases and marketing advantages, sustainable management of the natural resource base is another important issue, especially for resource-limited small-scale cassava farmers, who generally grow their crops on marginal lands under difficult climatic conditions. Any new technology should also optimize the conservation of the natural resource base.

Another set of cassava research constraints concerns the techniques of cellular and molecular biology whose full development is necessary in order to tackle the production and utilization challenges referred to above. Plant regeneration from somatic and reproductive cells, genetic fingerprinting and molecular mapping of the cassava genome, genetic transformation, cryopreservation and advanced pathogen diagnostic techniques are some of the most important.

Biotechnology research is not different from more traditional research regarding the need for integration with other disciplines (i.e. socioeconomics, agronomy, etc.) when developing appropriate innovations. One may

Table 1. Relative importance of cassava biotechnology research topics by continent and impact of biotechnological innovations on small-scale cassava farmers and marketing of cassava products.

Biotechnology research topics	Importance			Impact of innovations	
	Africa	Latin America	Asia	Yield increase	Market advantage
Viral diseases	+ + +	+ + +	+	+ + +	+
Insect pests	+ + +	+ + +	+	+ + +	+
Cyanide toxicity	+ + +	+	+ +	0	+ +
Starch quality	+ +	+ +	+ + +	0	+ + +
Postharvest root deterioration	+ +	+ + +	+ + +	0	+ + +

+ + + large; + + medium; + small; 0 no change.

even argue that integration with other disciplines becomes even more important with biotechnology research since particular innovative technologies, such as true cassava seed (TCS), require a very extensive and intensive farmer-participatory research and development approach. Moreover, as is the case with TCS, in-depth knowledge of seed markets and marketing becomes fundamental for successful acceptance, adoption and impact.

EARLY APPLICATIONS OF BIOTECHNOLOGY TO CASSAVA AT CIAT

Enhancing vegetative propagation and production of clean planting material

Cassava vegetative propagation is not only slow (10–20 stem cuttings can be obtained from one mature plant per year) but often exposes the crop to pests and diseases which are transmitted through successive generations. Viral diseases, in particular, may lead to a decline in crop yield and quality over a period of time. We have developed virus elimination techniques followed by micropropagation of disease-free cassava stocks. *In vitro* multiplication of cassava is needed for (i) increasing the number of meristem tip-derived plants for virus testing; (ii) increasing pathogen-tested clones for international exchange and germplasm storage; (iii) obtaining a sufficient number of plants for the production of basic 'seed'; and (iv) achieving massive propagation of elite clones for direct field planting.

There are two possible routes for *in vitro* propagation of cassava: (i) through enhancement of pre-existing meristems, i.e. by means of single node cuttings and multiple shoot cultures (Roca, 1984), and (ii) through *de novo* formation of somatic embryos from immature leaves or meristem tips (Szabados *et al.*, 1987). The former is the most common route for cassava micropropagation. *In vitro* techniques largely surpass traditional methods in propagation potential. Single node culture and multiple shoot culture can produce 6.0×10^3 and 6.0×10^4 stem cuttings per mother plant per year, respectively. The multiplication potential of somatic embryogenesis has not been tested.

Local cassava cultivars show significant yield gains when the planting material is generated and multiplied by micropropagation techniques (Table 2). Micropropagation of disease-free stocks has been carried out in the national programmes of Brazil, China, Cuba, Panama, Paraguay, Peru and Venezuela. In China, for example, a 30–70% yield increase over local varieties was obtained by micropropagation of the CIAT clone CM 321–188, and over 30,000 hectares were planted with this and other genotypes in Southern China in 1988.

In vitro conservation and international exchange of cassava genetic resources

Conventional maintenance of cassava germplasm collections is carried out by continuous vegetative cultivation in the field; besides being a costly operation, field maintenance often exposes the valuable material to insect and disease attack and soil or climatic problems.

An *in vitro* active gene bank has been developed at CIAT, which maintains over 4,800 cassava clones, collected from 23 tropical countries (Table 3). The *in vitro*

Table 2. Yield of three local cassava cultivars following pathogen elimination and micropropagation by shoot tip culture.

Cassava cultivar	Growth cycle (year)[a]	Yield (ton/ha)	
		Fresh roots	Starch
"Secundina"	I	30.2	8.2
	II	25.9	7.7
	III	20.7	6.1
	IV	17.6	5.2
	Control[b]	17.0	5.3
"Quilcace"	I	17.4	5.3
	II	12.9	3.9
	Control	7.5	2.1
"MCol 113"	I	16.5	4.3
	II	7.2	1.9
	Control	4.6	1.2

(a) Planting material (stem cuttings) for each cycle was obtained from mature plant of preceding cycle.
(b) Planting material obtained from farmers' fields, i.e. without micropropagation.

Table 3. Tissue culture for the production of disease free clones, international exchange and conservation of cassava germplasm (work carried out at CIAT between 1980–91).

	Virus elimination*	Introductions to CIAT	Distributions from CIAT	*In vitro* genebank
N° Accessions	950	2800	850	4850
N° Countries	-	14	35	23

*Cassava common mosaic, virus X, frog-skin disease and latent viruses.

The international exchange of cassava germplasm has been greatly facilitated by *in vitro* techniques. In the last ten years, over 900 cassava elite clones have been cleared of viral diseases; of these, 850 clones have been distributed from CIAT to national research institutions in 35 countries in Latin America, South-East Asia and Africa. In addition, some 2800 cassava cultigens, collected from 14 tropical countries, have been introduced to CIAT as *in vitro* cultures (Table 3).

Thus, *in vitro* culture techniques have contributed to cassava improvement by increasing the availability of germplasm resources for genetic improvement, and by facilitating the transfer of elite clones to national research programmes for evaluation and varietal development.

ADVANCED CASSAVA BIOLOGICAL RESEARCH

As referred to earlier, the Cassava Biotechnology Network (CBN) constitutes an effective mechanism for accessing basic information in molecular and cellular biology. The CBN has received wide acceptance by the scientific community. Since its foundation, the number of projects has increased steadily; currently over 25 projects are underway in developed and developing countries, dealing with critical research challenges in cassava (Table 4). Virus and insect resistance and photosynthetic behaviour under stress, plant

Table 4. Major research projects underway in developed and developing countries dealing with critical constraints and development of biotechnological tools for cassava*.

Research topic	Collaborating institutions	Country	Funding source
Cyanogenesis	Univ. of Newcastle upon Tyne	UK	RF/EC/ODA
	Royal Vet. Agric. Univ.	Denmark	FR/EC
	Royal Vet. Agric. Univ.	Denmark	DANIDA
	Mahidol Univ.	Thailand	
	Ohio State Univ. Colombus,	USA	USAID
	Free Univ. Amsterdam	Netherlands	
Virus resistance	The Scripps Institute, La Jolla	USA	ORSTOM/RF/ USAID/GTZ
	Free Univ. Amsterdam /	Netherlands	DGIS
	Univ. of Zimbabwe	Zimbabwe	
Insect resistance	Washington State Univ., Pullman	USA	RF
Photosynthesis	Australian Nat. Univ., Canberra	Australia	AIDAB
	Univ. of Georgia/CIAT	USA	USAID
Plant regeneration	Univ. Paris, Orsay	France	EC
	Univ. of Bath	UK	ODA
	Univ. of Zimbabwe	Zimbabwe	DGIS
	South China Inst. Botany	China	RF
	Univ. Wageningen	Netherlands	
	CIAT	Colombia	Core
	IITA	Nigeria	Core
Genetic transformation	Univ. Nottingham	UK	RF
	Univ. of Guelph	Canada	RF
	CENARGEN	Brazil	
	IITA	Nigeria	Core
	CIAT	Colombia	Core
DNA fingerprinting and molecular mapping	Washington Univ., St. Louis	USA	RF
	Univ. of Georgia, Athens	USA	RF
	CIAT	Colombia	RF
Cryopreservation	CIAT	Colombia	Core
CBN activities	CIAT	Colombia	DGIS
Other activities	CIAT	Colombia	Core
	IITA	Nigeria	Core

*As of June, 1992

roots including protein and carotene content, have been proposed as new initiatives for immediate research (Bertram, 1990; DGIS, 1991).

CIAT's approach to cassava biotechnology is constraint-driven. However, there are critical techniques whose full development is necessary for tackling the research constraints referred to above. With this in mind, CIAT has given special attention to research on technology development in the last few years.

DEVELOPMENT OF MODERN CASSAVA BIOTECHNOLOGIES AT CIAT

Characterization and identification of useful genetic variability

Genetic fingerprinting

Electrophoretic fingerprinting of cassava using α, β-esterase (EST), diaphorase (DIA), acid phosphatase (AcP), peroxidase (PrX), and glutamate oxaloacetate transaminase (GOT) [aspartate aminotransferase] isozymes has been developed at CIAT. We have studied the genetics of the EST – 1 locus using 300 progenies from 11 crosses. Five alleles (*A0, A1, A2, A3, A4*) with 11 phenotypes, and 5 non-observable null alleles, were identified. This locus behaves as a monomer and its inheritance pattern is compatible with a diploid model.

Because many EST alleles per loci are polymorphic in cassava this system has been used at CIAT to analyse over 4300 cassava accessions. A large portion of the accessions showed unique patterns, implying genotypic differences, but other accessions had similar banding patterns, suggesting possible duplication.

The development of DNA-based techniques for the analysis of genetic diversity of *Manihot* is now at an advanced stage. DNA extraction and the construction of genomic libraries has been carried out. Polymorphisms were detected with probes based on several cassava DNA clones.

Other probes have been tested for detecting polymorphism in cassava. The human minisatellite probe was ineffective in detecting polymorphism; however, the phage M13 probe and several random amplified polymorphic DNA (RAPD) markers did yield sufficiently variable band patterns to differentiate cassava varieties. DNA fingerprinting was shown to be superior to EST isozymes for discriminating between cassava genotypes.

Molecular mapping

A collaborative research project to construct the molecular and physical maps of cassava using random genomic, cDNA and YAC libraries was initiated at CIAT in 1992. The goal of the project is to tag agronomically important traits (both simply and quantitatively inherited) in order to characterize the genetic relationship between cassava and its wild relatives, and as a step towards the isolation and cloning of cassava genes.

A *Pst*I random genomic library was generated using the cv. MCol 22. Some 200 single copy clones, of a total of 500, have been characterized, with insert size ranging from 0.2 to 3 kb. Cassava DNA digested with *Eco*RI

Table 5. Degree of polymorphism in cassava detected with at least one restriction enzyme, using five random genomic libraries as source of probes.

Library**	MCol 22 vs MCol 1505	MCol 1505 vs *M. aesculifolia*
*Pst*I	60%	85%
*Xba*I	60	85
*Hin*dIII	55	95
*Eco*RI	40	60
*Bam*HI	30	45

*The best restriction enzymes, in order of polymorphism, are: *Eco*RV, *Xba*I, *Eco*RI, *Hin*dIII.
**Library constructed with cassava cv. MCol 22.

*Eco*RV, *Xba*I and *Hin*dIII provided probes with highest polymorphisms. Polymorphism between MCol 1505 and its wild relative *M. aesculifolia* was markedly higher than the polymorphism found between two cultivated genotypes (Table 5).

Plant regeneration and genetic transformation

The development of cassava transformation requires a plant regeneration technique, a genetic construct containing appropriate selectable and/or reporter markers, a housekeeping or tissue specific promoter and the technology to monitor the transcriptional and post-translational products of gene expression and the inheritance of the introduced gene.

Cassava somatic embryogenesis

Plant regeneration can be achieved through somatic embryogenesis on immature leaves or apical meristems. We have demonstrated the usefulness of the technique with a range of cassava genotypes with 4–8 mg 2,4-D/litre. Embryos are induced directly on an embryogenic tissue, and plantlets are regenerated from primary and secondary embryos in the presence of benzyl amino purine [benzyladenine] and gibberellic acid (Szabados *et al.*, 1987).

Agrobacterium tumefaciens-mediated transformation

We have screened a number of cassava varieties with 25 *A. tumefaciens* strains. Two cassava varieties showed very high sensitivity to both wild and disarmed strains. To monitor transformation experiments we used the plasmid construct pGV 1040 (provided by Plant Genetic Systems, Belgium) containing *uidA*, *bar* and *nptII* genes (encoding GUS, glufosinate resistance and neomycin phosphotransferase, respectively). Expression of opine synthesis genes of a wild *Agrobacterium* strain, isolated from cassava tumours, was obtained after transformation of immature leaves.

Sensitivity levels of cassava tissues to the commercial herbicide Basta [glufosinate], used as a selection agent, were determined. Minimum lethal doses of the herbicide are 1 mg/l for immature leaves and 18 mg/l for somatic embryos. Currently, work is underway using a wild *Agrobacterium* strain carrying the GV 1040 plasmid.

Particle bombardment-mediated transformation

Globular stage somatic embryos were bombarded using the pGV 1040 plasmid construct. GUS expression after 3 days of bombardment has been observed, with an average level of 20 GUS spots per 0.5 cm^2 of embryogenic tissue. Recently, GUS expression was detected in expanded tissue areas of somatic embryos one month after bombardment, suggesting that multiplication of cells which initially expressed the GUS gene took place.

Cryopreservation

Currently, *ex situ* conservation of cassava germplasm at CIAT is carried out both in the field crop and in the laboratory using shoot tip cultures. The *in vitro* conservation constitutes an active collection and clones are subcultured and recycled every 12–18 months depending on the genotype. Long-term storage of cassava, in a reduced space, free of genetic change, and at a low cost can be achieved by cryopreservation.

A research project on cassava cryopreservation was commenced at CIAT in 1988. The first phase was carried out in cooperation with IBPGR (1988–91) and resulted in the recovery of plants from frozen shoot tips in liquid nitrogen. With MCol 22, recovery rates ranged from 30 to 50%. However, several cassava cultivars had a low response or did not respond at all. The second phase of the research (1991-present), was designed to improve the previous protocol for increasing the recovery rate of plants and minimizing genotypic differences. Lower temperature and higher illumination of donor cultures increased recovery from liquid nitrogen with consistent rates around 60%. Use of a high concentration of sucrose in lieu of sorbitol and DMSO in the pre-culture stage allowed up 80% plant recovery from frozen shoot tips. Rapid freezing, i.e. direct immersion of shoot tips into liquid nitrogen, resulted in similar or higher recovery rates than slow freezing, i.e. 0.5°C/min. Additionally, the recovery rate of otherwise unresponsive genotypes has significantly increased with the improved technique. This work has paved the way to the development of a long-term, base gene bank of cassava clones using liquid nitrogen.

Table 6. Research activities at CIAT for developing cassava biotechnology capabilities: Summary for the period 1980–1992.

Research topics/technologies	Funding source: Core	Funding source: Special	Status of technology
1. Micropropagation pathogen-free clones	X		In use
2. *In vitro* conservation: germplasm exchange	X	X	In use
3. Genetic fingerprinting: Isozyme fingerprinting	X	X	In use
DNA fingerprinting		X	Advanced
4. Molecular mapping		X	Initiated
5. Genetic transformation	X		Initiated
6. Cryopreservation	X	X	Advanced
7. Gene identification cloning[a]	X		Initiated
8. Cassava Biotechnology Network	X		Underway
9. Training developing countries	X	X	Underway

(a) Genes involved in photosynthesis under stress conditions: *rbcs, mdh, me, ppc.*

CONCLUSION

Table 6 summarizes the research activities carried out at CIAT in the last 12 years aimed at developing biotechnology capabilities with cassava. Constraints associated with cassava clonal propagation and germplasm management were first tackled by means of tissue culture micropropagation and related techniques. These techniques are now in use at CIAT and several developing countries. Later on, the need to characterize and make more efficient cassava germplasm collections stimulated our work on genetic fingerprinting. Likewise, these technologies have been incorporated into ongoing cassava research at CIAT. More recently, CIAT efforts have focused on developing advanced biotechnology tools to allow cassava genetic mapping and tagging, and manipulation at the cellular and molecular levels; the goal is to bring cassava science and technology to the level of other important food crops.

CIAT efforts in cassava biotechnology have been effectively supported by the development of the Cassava Biotechnology Network. This cooperative international initiative facilitates the tackling of priority constraints and the exchange of information, and links research projects with the needs of small-scale cassava farmers.

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