The Cassava Biotechnology Network: [illegible]es and accomplishments in biotechnology for a small farmer crop

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Introduction

Cassava has two roles in tropical agriculture. Cassava is a an irreplaceable food security crop for some of the most hard-pressed populations in tropical countries. Cassava is tolerant of seasonal drought and poor soils, and has an unequalled ability to recover after damage to aerial parts by pests, diseases, or war. Cassava also provides a raw material for agroindustrial development. Because it cassava is a highly reliable crop even on relatively poor soils, it can serve these roles even in areas that are otherwise poor in resources.

The Cassava Biotechnology Network (CBN) is one of the products of CIAT's response to the situation of a decade ago, when the importance of cassava was seldom mentioned internationally and was not well known outside the tropics. Powerful new biotechnological tools for agricultural research were developing rapidly, but chiefly in countries where cassava was not grown. Consequently, little was being done to apply these new tools to cassava. Yet biotechnology is an important tool for enhancing cassava as a traditional staple and for developing new forms of utilization to satisfy diversified markets.

CBN was founded in 1988 to provide a forum for cassava biotechnology issues and to foster cassava biotechnology research on priority subjects. At about the same time, the Special Programme for Biotechnology and Development Cooperation of the Directorate General for International Cooperation (DGIS) of the Netherlands began taking an increasingly active role in directing the attention of biotechnology research to the needs of small farmers. The interests of DGIS and CBN converged because cassava is primarily a small-farmer crop. In 1992 DGIS joined the CBN Steering Committee and funded a full-time CBN Coordinator. Since that time, CBN has been able to be increasingly active in three general areas:

Identification of priorities for cassava biotechnology

Stimulation of complementary, collaborative biotechnology research on topics of established priority

Fostering of free exchange of information on cassava biotechnology research, including techniques, results, and materials

Priority-setting for cassava biotechnology research

Priorities for cassava biotechnology research were originally derived by debate and consensus in interdisciplinary meetings of national and international experts. An axiom underlying these discussions was that cassava production, postharvest processing, marketing, and consumer aspects must be integrated, both in research and needs assessment, in order for research to be targeted correctly to the desired impact. CBN has emphasized its role in providing biotechnologists with information on the needs of developing country cassava users, including small-scale producers, processors, and marketers of cassava, and cassava consumers.

Recently, CBN has sought methods of structuring the ways that it involves cassava users in priority setting. In Latin America, CBN has had access to farmer contact through CIAT's involvement in participatory research with members of farmer-processor cooperatives organized around small-scale local processing facilities. In search of direct farmer contact in other regions, CBN recently chose to conduct a first case study in Africa, a region where cassava is critical not only to economic prosperity but to survival. A second case study will be conducted in South China, a very different region, later in 1994.

The CBN case studies explore the potential for obtaining the perspectives and opinions of cassava users directly, informally, and quickly, in a way that can be repeated at intervals or in different regions by any program like CBN whose main investment must be in research but which requires for its direction a practical method of current interaction with end-users.

To be useful for international cassava biotechnology priority setting, the information obtained in the case studies must be combined with a mosaic of other micro-studies, and with macro-scale data from international studies, national agricultural statistics, and other sources, to permit an assessment of what research needs (or opportunities) are shared by the greatest number of cassava users within Africa, Asia, or Latin America

CBN, CIAT, and DGIS are collaborating with the Rockefeller Foundation and IITA in a study to develop a framework for combining and evaluating micro and macro data for cassava research priority setting, with special reference to choice of research approach, including biotechnology.

Current CBN priorities

Research priorities for cassava biotechnology are divided into high-priority target applications, and the biotech tools necessary to permit these applications. Priority applications include starch quality; texture, taste, and nutritional value; and new product development, for realizing new opportunities for the crop. Problem-solving priorities include integrated pest management, virus resistance, management of cyanogen biochemistry, and others.

Priority tools include the development of a robust, genotype independent genetic transformation protocol for cassava, cloning of useful genes and gene promoters, molecular markers for key traits and a molecular map of cassava, molecular characterization of cassava and its relatives, and techniques for the regulation of cassava reproductive biology.

Until the new, collaborative priority-setting framework method is ready, findings from the case studies will be incorporated into the debate-and-consensus process for continual update of current CBN priorities. One result of CBN's first case study has already been confirmation to CBN of the importance of microbial biotechnologies to African cassava users.

Cassava biotechnology research

Cassava Tissue Culture

Cassava tissue culture is a mature technology used by national programs in more than a dozen developing countries. Tissue culture is particularly valuable for cassava, because cassava varieties, unlike rice varieties or maize inbreds, do not reproduce true to type from seed. Previously, the only way to conserve or exchange cassava varieties was by using stakes, which transmit diseases.

CIAT has been the leader in development of practical in vitro protocols for cassava that have permitted its wide use in international and national cassava genebanks and international exchange of cassava germplasm. Cryopreservation, a development from tissue culture, has recently been achieved for a range of cassava genotypes at CIAT and ORSTOM. The next step will be tests of long-term viability and genetic stability of cryopreserved samples.

Several European universities have begun using tissue culture techniques to study cassava physiology and pathology at the cellular level and for cellular-level screening for drought tolerance and disease resistance.

Cassava molecular markers

A framework genetic map of cassava based on molecular markers is now under construction, via collaborative interchange agreements at CIAT, the University of Georgia, and Washington University/St. Louis (both in the USA). Several types of molecular markers are being used in the initial mapping work, including RFLPs from both total genomic DNA and cDNA and also RAPD primers.

CIAT and ORSTOM are using molecular markers to study and manage their cassava germplasm collections. Washington University, CIAT, and CENARGEN are also collaborating to study phylogenetic relationships of cassava and its wild *Manihot* relatives using molecular markers and highly conserved chloroplast DNA sequences.

ORSTOM and IITA, soon to be joined by CIAT, are using molecular markers to study the genetics of the host-plant-environment interaction in the internationally-important disease cassava bacterial blight. Other molecular marker-assisted studies are in the planning or exploratory stages for cassava photosynthesis and postharvest deterioration, among other traits.

Transgenic cassava

Cassava gene constructs

Current research on gene constructs for cassava involves genes of highest importance for the food security value of cassava and for development of market opportunities. These genes are being cloned concurrently with cassava transformation research in order to permit minimum delay when a transformation protocol is ready.

The University of Wageningen has cloned the genes involved in cassava starch metabolism, which, in different expression constructions, will permit the development of cassava varieties with specific starch qualities. Such varieties are expected to expand the market and price competitiveness for cassava starch, providing an industrial export crop to regions such as northern Southeast Asia where soils are too poor for rice.

Several groups, including the University of Newcastle upon Tyne, the Royal Agricultural and Veterinary University of Copenhagen, Mahidol University in Bangkok, and Ohio State University, have cloned genes for key rate-limiting enzymes in cassava cyanogen metabolism. The cyanogenic potential of cassava has long been a source of controversy. High-cyanogen cassava is toxic if not properly processed, yet in some regions, farmers who have access to both high- and low-cyanogen varieties traditionally choose to grow mostly high-cyanogen varieties, stating that they are more productive, more pest-resistant, and have better processing quality. The ability to produce acyanogenic plants and/or plants acyanogenic in specific plant parts, will at last permit unambiguous comparative experiments on the association of cassava cyanogenesis with the food security value of cassava, and to begin to design plant types that will optimize nutritional safety and production stability.

The International Laboratory of Tropical Agricultural Biotechnology (ILTAB) is well advanced in development of gene constructs for resistance to cassava viral diseases. Recently, a major improvement was made in the effectiveness of some of these constructs against the serious gemini virus ACMV (African Cassava Mosaic Virus) in the virology model species *Nicotiana benthamiana.*

Other gene cloning research in cassava includes work on protein genes at Long Ashton, UK; and work on the starch deposition process in cassava roots at CENARGEN/EMBRAPA.

Cassava regeneration

Cassava has proven recalcitrant to regeneration from callus or single cells; embryogenesis from somatic tissue is the only currently successful system. Somatic embryogenesis of cassava was first achieved at the University of Bath, followed by CIAT. It was further developed at the Agricultural University of Wageningen into a cyclic system permitting the production of much larger numbers of regenerable secondary somatic embryos.

The somatic embryogenesis regeneration system has, however, some serious limitations as a system for genetic transformation. New somatic embryos usually originate from several cells instead of a single cell. If some of the cells are transformed and others not, the embryo and resulting plantlet will be chimeric. This can be overcome by subculturing segments of chimeric embryos. However, though cells near the surface of somatic embryos are most susceptible to genetic transformation, new embryos tend to arise from cells deeper within the tissue. Transformed (sub)epidermal cells divide but rarely form embryos.

The University of Bath has recently achieved true suspension cultures of cassava cells which regenerate via the formation of globular embryos. Small cell clusters in these cultures are currently being used in a new generation of transformation experiments, and results are awaited with interest.

Cassava genetic transformation

Cassava cells are susceptible to transformation from both *Agrobacterium* and microprojectile bombardment. Transformation using both methods appears to achieve stable integration into the genome, since tissues express the GUS reporter gene 90 days after transformation and longer.

Using subculturing of plantlets obtained using a specific combination of native Colombian *Agrobacterium* strain and a cassava accession from the international collection held at CIAT, CIAT has achieved regeneration of plantlets that appear to be uniformly transformed with marker genes for resistance to phosphinothricin (the <u>Bar</u> gene) and kanamycin, and which carry the GUS reporter gene <u>uid</u>A. PCR tests of these plants using primers for <u>Bar</u> and <u>uid</u>A were positive. The plants are growing normally in a containment facility and Southern blot tests for GUS and phosphinothricin are being conducted now to confirm transformation.

Purdue University has developed an *Agrobacterium* strain optimized for cassava transformation. Its agropine-disarmed Ti plasmid contains mannopine and octopine synthase promotors, octopine-type virG genes, and <u>Bar</u> and <u>uidA</u> (GUS) markers. ILTAB, Wageningen, Rothamstead, and several other laboratories are actively involved in research on efficient transformation systems for cassava.

CBN estimates that with about two to four years additional work, robust cassava transformation protocols may be available for use as a new tool in crop improvement research. Advanced programs, working with marker genes and readily-regenerable genotypes, may be producing transformed plants in small numbers well before then.

Microbial biotechnologies

Microbial biotechnologies are so-called because they use microbes (biotic organisms) for deliberate alteration of a substrate to improve it for some human purpose. More recently, genetic improvement biotechnologies such as molecular markers and genetic transformation are being used to improve the microbes themselves.

A wide variety of traditional microbial biotechnologies are found across cassava-growing regions. These include preservation of the crop, which is highly perishable once harvested; removal of toxic cyanogens; cassava beer brewing; and achievement of desired qualities such as higher protein content, improved baking quality, or preferred taste.

Selection of superior microbes and development of improved conditions for faster processing and better quality products is being conducted at many institutions including CIRAD and ORSTOM of France, the Natural Resources Institute of the UK, Wageningen, and national programs, especially in Nigeria, Tanzania, India, Brazil, Indonesia, Vietnam, Thailand, and the Philippines.

Research on microbial and plant biotechnologies for plant-soil relationships is being conducted in very few institutions and CBN hopes that this research area will increase in importance.

CBN small grants program to stimulate cassava biotech research

CBN has just announced its first small grants program, designed to stimulate research in areas identified as high priority for cassava biotechnology. The small grants program for 1994 has a budget of approximately US $ 100,000. It is intended to provide funds for international collaborative project development, bridging funds or start-up funds for critical projects on highest priority objectives, and specific short-term training. The response of international and bilateral donors to projects developed with the support of CBN small grants will be key to ensuring that cassava biotechnologies, in most cases still in the basic or strategic research stage, can be developed to the point of usefulness to national programs and their clients, cassava users in developing countries.

Information exchange

CBN publishes a newsletter and a directory and coordinates an e-mail network. In addition, CBN has organized two international scientific meetings, both in Colombia: the founding workshop attended by 35 participants in 1988, and the first full-fledged CBN meeting attended by 135 participants in 1992. These meetings have provided opportunities for national program and advanced lab researchers to meet, learn about each other's work, and develop collaborative research projects. They have also served as discussion fora for priority setting.

The next CBN meeting is scheduled for this coming August in Bogor, Indonesia. The latest results in cassava biotechnologies will be presented, including new developments in regeneration and transformation and a report on the molecular mapping project, and research priorities will be reviewed in light of progress, changing economic and climatic situations, and new information from cassava users. This year's meeting will be followed in 1996 by a CBN meeting in Africa.

CASSAVA BIOTECHNOLOGY RESEARCH PRIORITIES

CBN

Priority level	
	Biotechnology applications: For realizing cassava opportunities
H	Starch quantity and quality for diverse end uses
M	Fermentation, biochemistry, and molecular genetics for: (1) new product development and (2) desired texture, taste, and nutritional value
M	Plant nutrient cycling efficiency
M	Extended range and increased productivity in suboptimal agroecological zones by research on photosynthesis under stress and enhanced mycorrhizal interactions, bio-fertilizers
	Biotechnology applications: For solving cassava problems
H	Integrated pest management, including host/pathogen and host/pest interactions
H	Resistance to important viral diseases
H	Modified cyanogen biochemistry for optimal cassava production and use
M	Enhanced fermentation systems for: (1) cyanogen reduction and (2) waste management
M	Delayed postharvest deterioration
M	Development of true seed for cassava production
	Biotechnological tools: For genetically improving cassava
H	Molecular and cytological characterization of *Manihot* species genomes
H	Framework genetic map and international database of genomic data
H	Useful genes and gene promoters, characterized and cloned
H	Improvement of plant regeneration systems
H	Improvement of genetic transformation techniques
M	Techniques for regulation of reproductive biology (flowering, pollen conservation, haploid production, apomixis)
	Biotechnological tools: For conserving and exchanging Manihot genetic diversity
H	Diagnostic methods for clean germplasm transfer
M	Cryopreservation for long-term conservation of genetic resources
M	Tissue culture for germplasm conservation and micropropagation

Setting priorities for cassava biotechnology research

CBN places *high priority* on ***socioeconomic studies***, which provide knowledge of the perspectives of small-scale cassava farmers and processors and of cassava problems and opportunities, and on ***interdisciplinary studies*** to understand complex issues such as cyanogenesis, rapid postharvest deterioration of cassava, and the use of true seed instead of vegetative propagation.

Priority level H, high; M, medium (low priority research areas are omitted)