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CASSAVA BIOTECHNOLOGY: International collaboration current situation and perspectives

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ABSTRACT

The cassava crop is important both for food security in areas of climatic, economic, and political stress, and as a source of high-quality starch as a raw material for economic development. Biotechnology can improve the availability or value of cassava in both of its roles. The Cassava Biotechnology Network (CBN) was founded in 1988 to foster the application of biotechnology to cassava research priorities. Among the objectives of the network are to collaborate with cassava researchers across disciplines to continually evaluate research priorities using biological and socioeconomic information, and to link many organizations and countries for exchange of information, materials, and training. Progress in cassava biotechnology since 1988 has been significant in the areas of methodologies for germplasm conservation and use, *Manihot* genome studies, regeneration and transformation, molecular markers for identification of genotypes and framework mapping, and quality factors. Identification and cloning of useful genes and molecular mapping of quantitative trait loci are two of the areas that are expected to emerge as biotechnological tools for cassava are improved.

THE CASSAVA CROP AND BIOTECHNOLOGY

Cassava (*Manihot esculenta*) is a staple for as many as 500 million people. Its starchy roots produce more calories per unit of land than any other crop, except sugarcane. Cassava leaves provide vitamins and protein when eaten as a vegetable, a common practice in Africa.

Cassava can play two roles in tropical agriculture: it is a reliable crop for food security, and it provides a raw material for economic development. Because it tolerates drought and low fertility, cassava is primarily grown by small-scale farmers in areas with poor soils or unfavorable climates. It can be processed into different forms for a wide variety of end uses, and much of this processing can be carried out locally, providing jobs and income in rural areas. In many cultures, cassava production, processing, and marketing are women's activities.

Until recently, there was little international awareness of cassava's importance to millions of the world's poorest people. Powerful biotechnological tools for agricultural research were developing rapidly, but little was being done to apply these new tools to cassava. Because biotechnology is one of the set of

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research and development tools for exploiting cassava as an important traditional rural and urban staple and for developing new forms of utilization to satisfy diversified markets, the Cassava Biotechnology Network (CBN) was formed in response to the need for a forum on cassava biotechnology issues and to foster use of biotechnology in priority areas of cassava research.

THE CASSAVA BIOTECHNOLOGY NETWORK: A FORUM FOR COLLABORATION

CBN draws together the experience and efforts of many different organizations and countries; encourages them to collaborate in research and to share techniques, results, genetic materials, and training efforts; and, through broad-ranging dialogue, defines and continually reexamines priorities for research (see Box). CBN encourages the use of biotechnology where it serves important objectives of cassava germplasm research. Some of these objectives are: (i) components of production technology for cassava-based sustainable cropping systems, both where cassava is currently grown and on presently under-exploited lands; (ii) processing technology that makes cassava a low-cost, high quality, convenient food; (iii) novel uses of cassava that increase the overall demand for the crop (CIAT, 1992).

To implement its objectives, CBN seeks to provide a communications link between entities such as associations of small-scale cassava farmers and processors; nongovernmental organizations; national agricultural research and development systems in developing countries; other networks that focus on cassava, development, or biotechnology; advanced research laboratories around the world; international agricultural research centers; and agencies that fund international development.

Objectives of the Cassava Biotechnology Network

1. **Integrate priorities of small-scale farmers and processors and consumers in cassava biotechnology research planning**

   Involve farmer and small-scale processor groups in setting objectives and priorities for cassava research. Identify those areas of high priority for which a biotechnological approach would offer a comparative advantage in solving problems and opening new opportunities for farmers and national economies.

2. **Stimulate cassava biotechnology research on topics of established priority**

   Coordinate the experience and efforts of a spectrum of organizations in the scientific and donor communities of industrialized and developing countries to address high priority research in cassava biotechnology.

3. **Foster free exchange of information on cassava biotechnology research, including techniques, results, and materials**

Organize biennial scientific meetings, other meetings, and workshops. Publish scientific proceedings, a semiannual newsletter, and other material.

CBN is structured as a research consultation network for cassava biotechnology. Within the CBN, informal working groups have formed around priority cassava biotechnology research themes (Table 3), according to the technical demands of each theme and the comparative advantages of various groups. CBN supports these working groups through facilitating contact and encouraging meetings to clarify progress and coordinate planning.

CBN is advised by a Steering Committee and a Scientific Advisory Committee. Their members represent national programs of cassava-growing regions, international centers, donors, and biological and socioeconomic sciences. A small core budget, provided by the Special Programme for Biotechnology and Development Cooperation, Directorate General for International Cooperation (DGIS), serves as seed money and training funds to enable high priority projects to start. The further funding of a project comes from individual donors or institutions. (CBN is not, however, a donor agency).

SETTING RESEARCH GOALS FOR CASSAVA BIOTECHNOLOGY

The identification of constraints to cassava production and utilization is the first step in the development of advanced biotechnological research relating to cassava. As with all agricultural research the goal is to generate appropriate, efficient and effective technology that can be adopted by as large a number of users as possible. In the case of cassava, the target group is the small-scale farmer and/or processor, and urban consumers.

Table 1 shows researchable constraints that limit cassava productivity, e.g. viral diseases and insect pests, and cassava quality constraints, and their importance by world region (Roca et al., 1992). 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 other hand, 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.

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 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 such as socioeconomics, agronomy, food science, when developing appropriate innovations. Table 2 shows current priorities for cassava biotechnology research, as identified by CBN after consultation with other disciplines. Priority setting for biotechnology, as for all research, is a dynamic and continual process. CBN will increasingly seek to involve organizations of small-scale farmers and processors in its priority setting process.

CASSAVA BIOTECHNOLOGY PROGRESS

Some work in cassava biotechnology, particularly in tissue culture and *in-vitro* conservation, had been started at CIAT as early as 1978. Cassava biotechnology research began in earnest in 1988, when CBN was founded. As was evident at the first International Scientific Meeting of the CBN (Roca and Thro, 1993), greatest progress to date has been in the development of biotechnology tools for cassava. Development of these tools has been emphasized as they are necessary before many biotechnology applications can proceed.

Biotechnology tools for conservation and use of cassava and *Manihot* species genetic diversity are most advanced. Micropropagation and in-vitro conservation of cassava is in use in several countries, including Barbados, Brazil, Cameroon, China, Cuba, Indonesia, Nigeria, Panama, Peru, Samoa, Venezuela, Zaire, and others. Molecular markers are in use to identify duplicates in germplasm collections (e.g., Ocampo et al., 1993). Cryopreservation methods for low-cost, secure conservation of cassava genetic diversity have been developed at CIAT (Escobar et al., 1993) and ORSTOM (Engelmann, pers. comm., 1993) and are now ready for long-term testing.

In the area of *Manihot* genome research, studies have shown that there is chromosomal compatibility among species (Bai et al., 1993), while that molecular polymorphisms exists within and between species (Angel et al., 1993). Molecular phylogeny research indicates that the center of origin of cassava may be northwestern South America and that the closest wild relative may be *M. aesculifolia* (Bertram, pers. comm., 1993).

A molecular map of cassava for gene tagging and efficient breeding is under construction, via collaborative interchange agreements among five groups. Genomic libraries and a mapping population have been generated and a framework map initiated (Angel et al., 1993).

Progress toward genetic transformation of cassava has been substantial. To date, regeneration has been achieved through somatic embryogenesis in a wide range of genotypes (e.g., Cabral et al., 1993; Mroginski and Scocchi, 1993; Raemakers et al, 1993; Sudarmonowati and Henshaw, 1993; Taylor and Henshaw, 1993). The critical bottleneck has been regeneration of uniformly transformed plantlets from single transformed cells. No regeneration from callus or protoplasts has yet been reported. Embryo suspension culture studies are promising at this time, and the possibility of other single-cell based regeneration systems should be investigated.

Transformation of cassava cells has, however, been proven, and chimeric somatic embryos are regularly obtained (e.g., Chavarriaga et al., 1993; Sarria et al., 1993). If funding for current cassava transformation research is maintained, transgenic plants are expected within a reasonable time frame.

Transgenic resistance to cassava viruses using the coat protein method has been shown to be highly effective against CMV in test systems using *Nicotiana*. Effectiveness against ACMV in test systems is still being sought (Fauquet et al., 1993). Direct application will follow when transformation is available.

In the important area of cassava quality, significant progress has been made in understanding the biochemistry of cassava cyanogenesis (e.g., Bokanga et al., 1993; MacMahon and Sayre, 1993; White and Sayre, 1993), root protein (Shewry, 1993), and root starch (Carvalho et al., 1993; Salehuzzaman et al., 1993). A multidisciplinary proposal has been developed to address the production/marketing constraint of rapid post-harvest deterioration of cassava roots, a problem that four years ago was not sufficiently understood to be considered ready for a project. The project will integrate biotechnology and crop improvement via recent advances in molecular genetics.

The gene has been cloned for linamarase, a key enzyme in the cyanogenesis pathway (Hughes et al., 1993). New methods for determination of cyanogen content have been developed that are quicker, less expensive, and less toxic than former methods (O'Brien et al, 1993; Bainbridge et al., 1993; Essers, pers comm., 1993). Understanding of the implications of cyanogens for cassava production and use, crucial for cassava biotechnology research, has increased. There is now more information on the types of crisis circumstances likely to lead to cyanogen toxicity (Rosling et al, 1993), and evidence for a possible association of cyanogenic glucosides with preferred cassava quality and with insect resistance (Bellotti and Arias, 1993).

Cassava photosynthesis has puzzled researchers because it has features of both C3 and C4 species. Data is now available to show that early products of cassava photosynthesis are C3 acids, but gas exchange behavior during cassava photosynthesis resembles CO2 assimilation of C4 species (Black et all, 1993). Cassava has also been found to have a novel biochemical pathway for extremely rapid synthesis of sucrose during photosynthesis.

PERSPECTIVES FOR CASSAVA BIOTECHNOLOGY

Experiences with other crops suggest that a genetic transformation protocol for cassava is not far off. Work on a framework map to be used for gene tagging and locating major genes is now under way and is expected to be completed toward the end of 1994. After these initial tools are developed, the next generation of cassava biotechnology research, research that is in some cases already begun, will move ahead: identification and cloning of useful genes for applications such as starch metabolism, mapping for quantitative trait loci, and biochemical manipulations and novel processes for new products and waste management. Wise national regulations for environmental release of transformed plants, including cassava, will become critical to CBN.

Still somewhat farther off may be biotechnology research to understand and manipulate cassava physiology and plant/environment interactions, such as photosynthesis and soil nutrient cycling. Biotechnology research involving such complex aspects of cassava biology will require improved understanding of cassava agronomy and physiology, as well as biochemistry.

Also important in the future direction of cassava biotechnology will be socioeconomic studies, which provide knowledge of the perspectives of small-scale cassava farmers and processors and of cassava problems and opportunities, and interdisciplinary studies to understand complex issues unique to the cassava crop, such as the possibility of commercial use of true seed instead of vegetative propagation, cyanogenesis, and rapid postharvest deterioration of cassava.

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Table 1. Relative importance of cassava constraints and opportunities for which biotechnology may have a relative research advantage, by region, and anticipated impact of biotechnological innovations on small-scale farmers and market value of cassava.

Biotechnology research topics	Importance by region			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	+++

+++ high; ++ medium; + low; 0 no change.

Ref: Roca, W. M., G. Henry, F. Angel, and R. Sarria, 1992
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Table 2. Cassava biotechnology research priorities

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, biofertilizers
	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	Rapid 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)

Table 3. Cassava biotechnology research projects 1993

RESEARCH AREA	No. of projects	COUNTRIES & INTERNATIONAL CENTERS
Tissue culture, micropropagation	Many	Barbados, Brazil, Cameroon, Cuba, China, Indonesia, Nigeria, Panama, Peru, Samoa, Venezuela, Zaire, and others; CIAT, IITA
Regeneration	9	China, France, Netherlands, UK, USA, Zimbabwe; CIAT, IITA
Transformation	7	Canada, Brazil, UK, USA; CIAT, IITA
Molecular map, markers, fingerprinting	6	France, UK, USA; CIAT, IITA
Virus resistance	3	Netherlands, USA, Zimbabwe
Cyanogenesis	7	Denmark, Netherlands, Thailand, USA; CIAT, IITA
Photosynthesis	2	Australia, USA
Cryopreservation	2	France; CIAT
Processing	Many	Argentina, Brazil, CIAT, Colombia, Congo, France, Ghana, India, Nigeria, South Africa, Tanzania, UK, and others