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WHERE WE ARE IN CASSAVA BIOTECHNOLOGY

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Introduction

The Cassava Biotechnology Network (CBN) was founded in 1988 as a forum for cassava biotechnologists, collaborating R&D specialists, and cassava research users (farmers and processors or their representatives). CBN's research goals are to improve cassava genetic transformation and processing. Its development goal is to foster interdisciplinary research by national agricultural research programmes in cassava-growing countries, in projects appropriate to improving national food security and contributing to rural income. CBN works towards these goals in three main areas of cassava biotechnology research:

1. Identifying priority objectives.

2. Stimulating complementary, collaborative research on topics of established priority.

3. Fostering exchange of information, including techniques, results, and materials.

The 10th ISTRC Symposium provides an opportunity to concentrate on the third activity: information exchange. Today, CBN's objective is to provide information about the various biotechnologies used in cassava, and information to assist applied R&D workers as they consider how biotechnology can or cannot contribute tools or materials useful to applied research in cassava and other crops. Current cassava biotechnology research results (Thro 1995) are the work of many different laboratories; at the time of writing, more than 120 projects in cassava biotechnology operate in at least 35 countries, and at two international agricultural research centres with a mandate in cassava: the International Institute of Tropical Agriculture (IITA), based in Nigeria, and the Centro Internacional de Agricultura Tropical (CIAT), based in Colombia.

Identifying Priorities for Cassava Biotechnology

Current priorities for cassava biotechnology research are divided according to applications and tools (Table 1). The process of arriving at these priorities involves interdisciplinary debate among cassava scientists, as well as cassava users farmers, processors, traders, and consumers through participatory research and integrated production, processing, and

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marketing projects. Cassava biotechnology priorities have been dynamic, changing in accordance with economic situations, increased knowledge of cassava users' needs, and advances in cassava science.

Recently, the CBN has intensified its contact with users through case studies in sample regions of China and Africa. It is beginning a 5-year project to integrate this micro-level data with international socio-economic data and develop a logical framework for cassava research priority setting, including in biotechnology.

Biotechnologies Used in Cassava Research

Four major biotechnologies are currently used in cassava: tissue culture, molecular markers and mapping, genetic transformation with gene cloning, and microbial biotechnologies.

Tissue culture

Tissue culture has special value for heterozygous, vegetatively propagated crops such as cassava and other root and tuber crops. In these crops, elite cultivars must be vegetatively propagated, leading to a build-up of systemic pathogens in the planting material. Tissue culture provides a means for safe conservation and exchange of healthy germ plasm. Many of its numerous applications in cassava were developed at CIAT.

Tissue culture is used in both international and national cassava germ plasm collections. It permits safe international exchange of cassava germ plasm so that materials can be widely used. The duplication of the world core collection of cassava germ plasm (currently conserved only at CIAT) is now being considered for other sites, including Brazil and Thailand. The objective is to enhance the security and use of the collection. Tissue culture, along with thermo-therapy and virus indexing, will make it possible to ship and store pathogen-free duplicates of the core collection.

Tissue culture can permit rapid initial multiplication of planting material of new cassava cultivars when integrated into varietal development programmes. It can act either as an intermediate stage, delivering plantlets to stake production farms or as a final stage, delivering plantlets directly to farmers. That *in vitro* plantlets can be distributed directly to farmers was demonstrated by the South China Institute of Botany several years ago (Liu et al. 1990). Their innovation was a simple hardening step, involving plain water, which adapted the plantlets to the new conditions.

Innovations to adapt tissue culture to local conditions and local input availability that is, methods for *in vitro* culture at sites where electricity is costly or erratic would greatly

extend the value of this technology for cassava research and cultivar distribution.

Cryopreservation of cassava meristem tips is a further development of tissue culture that can offer significant cost savings to germ plasm collections with the responsibility for long-term conservation of a full range of genetic diversity. Initial research on cryopreservation at CIAT and the Institut français de recherche scientifique pour le développement en coopération (ORSTOM) has been extended to a wider range of genotypes at CIAT. The research demonstrated that direct immersion in liquid nitrogen permits plant recovery rates as high as with the more expensive programmed cooling. The next step will be to study the effect of long-term cryopreservation on genetic stability of the conserved material.

Genetic transformation: a tool for introducing new traits

Genetic transformation can be defined as the insertion, into a cell, of genetic material (DNA) from another source and the subsequent expression of the transgenes in the targeted cell. It is used to introduce traits not found within a species or to alter the level of expression of an existing trait beyond the range achievable by sexual recombination and selection. Genetic transformation is used for genetically simple traits.

Genetic transformation of cassava has been difficult. Interaction among various laboratories, using several different approaches, has been critical to the progress achieved so far. Repeatable transformation of cassava cells was achieved some years ago by several laboratories, including CIAT, the International Laboratory for Tropical Agricultural Biotechnology (ILTAB) with the Scripps Research Institute (USA/ORSTOM), and the Agricultural University at Wageningen.

In cassava, however, the surface layer cells most susceptible to transformation have a lower frequency of regeneration than cells in subsurface layers. Somatic embryogenesis is the only system currently effective for plantlet regeneration in cassava. Somatic embryos arise from multicellular buds, which, if they contain transformed cells, also contain many untransformed ones. Chimeric (sectorially transformed) somatic embryos have been obtained from such buds, and plantlets have been regenerated from them. By subculturing small sectors of chimeric embryos, fully transgenic plants should be obtainable. CIAT has obtained regenerated plants from repeated subculture of chimeric embryos; further tests are being conducted to determine if they are uniformly transformed.

If these plants prove to be uniform transformants, a standard protocol could be developed. For practical crop improvement work, however, a protocol for the routine transformation of cassava is needed. This challenge is being addressed by some laboratories via improvements in transformation and by others via improvements in regeneration.

Advances in transformation methods per se include modified strains of *Agrobacterium* developed at Purdue University to optimize effectiveness for cassava transformation. Its agropine-disarmed Ti plasmid contains mannopine and octopine synthase promoters, octopine-type *vir*G genes, and *Bar* and *uid*A (GUS) markers. A joint project between Rothamstead and the University of Bristol has successfully used electroporation to transform cassava cells. The Eidgenössische Technische Hochschule (ETH) group in Zürich are beginning to direct their meristem micro-targeting methods to cassava.

For regeneration, work on media and culture conditions at ILTAB and Wageningen has dramatically increased the frequency of regeneration from somatic embryos. Most recently, the University of Bath has developed the first true suspension cultures of cassava. The cultures are embryogenic, that is, somatic embryos and plantlets can be obtained from them. Most importantly, very small cell clumps are exposed to the media, thereby providing smaller, more concentrated targets for transformation than were available before. At ILTAB/SCRIPPS, fully transformed embryos have already been obtained from these suspension cultures, using micro-bombardment. The ILTAB group has established the optimal bombardment parameters for use with these cultures and is now working on recovery of transformed plants.

With cassava transformation now appearing very close, the next priorities will be to isolate tissue-specific gene promoters and extend the transformation method to a wider range of agriculturally important cultivars. Work on extending regeneration methods to regionally and locally important cassava genotypes is in progress at IITA (Nigeria) and in Brazil, China, India, and Indonesia. The objective at ETH is to develop a genotype-independent transformation protocol. Work on isolating tissue-specific gene promoters from cassava is just beginning. It may be possible to use appropriate promoters from other crops.

As a spin-off from regeneration research, the new embryogenic suspension cultures broaden the use of tissue culture as a research tool. These embryogenic suspension cultures are already being used at the University of Bath to study cell-level components of cassava resistance to cassava bacterial blight (CBB) (presence or absence of gene products). The new system permits co-cultivation of bacterium and host cells, so the study of biochemistry and cell physiology of disease resistance in cassava becomes possible. As characterized cells or cell groups can be regenerated, making possible the identification of factors that would allow *in vitro* selection for some components of resistance. Artificial seed becomes a technical possibility.

Gene cloning and gene constructs

Gene cloning is the term for the currently used method for isolating and identifying genes for

use in genetic transformation. The isolation method involves cloning in an especially disarmed bacteriophage. Identifying a desired gene, once it is cloned, requires some knowledge of the gene perhaps its biochemical product, its map position, or access to the clone of a similar gene identified in another species. Once a gene is isolated and identified, it is then manipulated into a genetic construct with appropriate gene promoter sequences, for use in transformation.

Cassava genes cloned to date include genes for cyanogenic metabolism and starch biosynthesis. Two key genes in cyanogen breakdown, linamarase and α-hydroxynitrile lyase, have been cloned at the University of Newcastle upon Tyne. As soon as transformation is available, these genes will be used to devise strategies for reducing the hazard of HCN toxicity in cassava products, without compromising any protective or quality-enhancing function of cyanogenesis.

Starch biosynthesis genes cloned at the Agricultural University of Wageningen include ADP glucose pyrophosphorylase B and S, a key enzyme in starch quantity, the branching enzyme involved in amylopectin synthesis, and granule-bound starch synthase (GBSS), involved in amylose synthesis. Cassava GBSS, when used at Wageningen in the antisense or backward configuration to transform potato as a test system, resulted in a potato starch that was almost completely amylose free. Presumably other variations in starch composition would be possible.

Virus coat protein genes of cassava viruses have been cloned by ILTAB/SCRIPPS. In a test system, a viral coat protein gene was effective in protecting the test species (*Nicotiana benthamii)* against cassava common mosaic virus, but not against African cassava mosaic virus (ACMV). Recently, this laboratory has used a dysfunctional viral replicase gene in the same test system; it appeared to be far more effective.

Molecular markers and a molecular map

Molecular markers and maps are recent developments for a classic tool used by breeders and geneticists since Mendel. Plant breeders and selectors have always used morphological markers to follow segregation in hybrid populations. Most agriculturally important traits are not, however, associated with easily observed morphological markers. Molecular marker technology has developed as a better way of following any specific segment of DNA and the traits associated with it. The advantages of molecular markers are that they exist in every genotype; they number in hundreds and thousands in the species so far investigated; and their expression is independent of phenotypic value and of the plant's development stage and external environment.

These attributes give molecular markers several uses. They can be used to identify genotypes (molecular "fingerprints"); for example, the DNA probe M13 has been used at

CIAT to confirm duplication of genotypes similar in their morphological traits and isoenzyme patterns. The DNA probe revealed that some apparently identical clones (e.g., M Per 221 and M Per 242) differ at the DNA level. This information can be used to preserve cassava genetic diversity.

Molecular markers can be used to study genetic variation and genetic relationships within and among cassava and its wild relatives. At present, information on genetic variation within and among breeding populations at the molecular or DNA level is being used, along with other information, to predict crosses likely to have maximum heterozygosis for adaptation to specific ecosystems.

Molecular markers can be used to construct linkage groups for genetic maps, which, in turn, can be used to tag polygenes associated with quantitative traits. The first framework molecular map was developed through teamwork by CIAT and the University of Georgia. Crosses are now being made to use with the map to tag genes associated with ACMV resistance (IITA project), whitefly resistance, and cyanogen level (CIAT). Once established, mapped molecular gene tags can be used to screen large breeding populations as seedlings or any growth stage in any environment. Molecular markers will increase the efficiency of improvement for quantitative traits, while genetic transformation will have its major impact upon simple traits.

Use of molecular markers for plant breeding requires interdisciplinary research among biotechnologists, plant breeders, and disciplinary specialists in cassava production and use. Collaborative laboratory and field research is the only way to establish associations (correlations or linkages) of specific molecular markers with traits of agronomic or market interest.

Microbial biotechnologies

Microbial biotechnologies are those in which microbes (biotic organisms) are used to alter a substrate to improve it for a human purpose. A wide variety of microbial biotechnologies are used in cassava processing. Traditional small-scale cassava processors use microbes to preserve cassava in forms such as *gari*, *foufou*, and *udaga* in Africa, and to provide the self-rising capacity in cassava 'sour starch' in South America. More recently, research is being conducted to enhance the traditional processes via selection of superior microbe strains and definition of fermentation conditions for better or more consistent flavour, nutritional value, and safety.

New applications of microbial biotechnology are being used to develop experimental convenience foods and protein-enriched animal feed, as well as for commercial production of intermediate products for the food-processing industry such as food colours, citric acid, and

lactic acid. Microbial production of enzymes for cassava starch modification is a possibility for the future. Microbial biotechnologies will have an important role in managing cassava-processing wastes, for reducing pollution via cyanide removal and converting residual starch to microbial protein, with reduction of biological oxygen demand and potential economic value of the product.

References

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Table 1. Current priorities for cassava biotechnology research.

Biotechnology applications

(1) New or enhanced processes for desired texture, taste, and nutritional value.

(2) New products for realizing new opportunities.

(3) Starch quantity and quality (pre-harvest modified starch).

(4) Integrated pest management (IPM).

(5) Virus resistance.

(6) Adaptation to stress environments.

(7) Delayed post-harvest deterioration.

(8) Management of cyanogen biochemistry.

Biotechnological tools

(1) Adaptation of *in vitro* micropropagation techniques for use in local conditions and cultivar multiplication programmes.

(2) Genetic transformation protocol for a wide range of cassava genotypes.

(3) Isolation of useful genes and gene promoters.

(4) Establishment of a molecular genetic map of cassava.

(5) Molecular markers for key cassava traits.

(6) Molecular characterization of genomes of cassava and relatives.

(7) Techniques for regulating cassava reproductive biology.