REPORT ON THE FOUNDING WORKSHOP FOR THE AVANCED CASSAVA RESEARCH NETWORK

Held at
Centro Internacional de Agricultura Tropical CIAT

September 6 - 9, 1988
Cali - Colombia

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C O N T E N T S

I. PREFACE

The principal role of improvements in cassava technology in the development process in the tropics is to further the wellbeing of the rural poor and to increase the availability of low cost food to the overall population. In order to ensure that cassava fulfills this role, research at the national and international levels is concentrated in the areas of production, processing, and utilization.

Basic research on cassava problems was however, until recently, very much neglected at both these levels. Modern techniques that might have brought about dramatic results in research have not been applied to cassava, as work of this nature was viewed as being too costly and slow in producing results, to be used on this crop. Research on cassava that might radically change the crop has therefore been given orphan status, despite increasing evidence that exciting possibilities for improvement and development of this important tropical food crop are at hand.

With this in mind, the Centro Internacional de Agricultura Tropical (CIAT) and the International Institute of Tropical Agriculture (IITA) informally exchanged ideas toward creating an advanced research network to focus on cassava. As a first step, both centers set out to identify key constraints in cassava production and processing considered unhandleable through traditional research approaches, but manageable through a multidisciplinary, interrelated network approach.

Scientists in the IARCs are particularly interested in strengthening the link between basic research in advanced research institutions and more applied research carried out by both the international centers and national programs in developing countries. It is clear that a network approach to researching interrelated topics can most efficiently use the limited resources normally associated with this crop, and accelerate advances in cassava research.

The workshop organized to discuss this network, and described in this report, focused on the mechanisms by which a series of high priority collaborative research projects with advanced research institutions could be developed. Priorities were set and potential projects were discussed, in the context of linking these projects in such a way that each member of the network would benefit.

The long-term objective of this network is to improve the technology available to cassava farmers. The major constraints to cassava production, processing and utilization will be identified, and solutions to these problems will be sought through basic research, and use of new technological procedures strategically applied.

We acknowledge with gratitude the enthusiastic collaboration of all participants and especially those who took time to prepare papers for presentation at the workshop. We are pleased to have had the support of CIAT in organizing the workshop.

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II. WORKSHOP PARTICIPANTS

WORKSHOP ON ADVANCED CASSAVA RESEARCH NETWORK
CIAT, Sept. 6 - 9, 1988

Left to right, **Standing:** R. Artunduaga, Colombia; J. Jaynes, USA; L. Bernal, Colombia; J. Jaramillo, Colombia; L. Calvert, CIAT; K. Kartha, Canadá; C. Niblet, USA; R. Bertram, USA; R. Baldwin, USA; R. Beachy, USA; C. Fauquet, France; B. Foroughi-Wehr, FRG; C. Hershey, CIAT; J. M.V. Blanshard, U.K; A.C. Velazco, CIAT; C. Wheatley, CIAT; R. Cooke, U.K.; S.Y. Ng, IITA; J. Shepard, USA; M. Hughes, U.K.; Mroginski, Argentina; A.I. Robertson, IITA, G.G. Henshaw, U.K.; W.M. Roca, CIAT; L. López, Colombia; M. Peferoen, Belgium; L. Withers, IBPGR; B. Nolt, CIAT; M.L. Marín, CIAT; R. Chávez, CIAT; A. Naranjo, CIAT; K. Chun-Yen, China; B. Pineda, CIAT; M. El-Sharkawy, CIAT; **Seated:** R. Reyes, CIAT; T. Ishige, Japan; R. Sayre, USA; C.C. Black, USA; J. Vargas, CIAT; H. Ramirez, CIAT; D.I. Arias, CIAT; M. Carneiro, Brazil; C. Ryan, USA; N.H. Chua, USA; M. Cataño, CIAT; L.M. Tello, CIAT; G. Mafla, CIAT; Y. López, Colombia.

Leading researchers from developed and developing countries who have shown an interest in cassava research and/or those who might have something to contribute to a future network, were invited to attend the workshop. These persons were joined by CIAT and IITA staff in discussions. Representatives of one private company and three potential donor and support agencies also attended.

The following list names the 27 people from 13 countries, in addition to CIAT and IITA cassava researchers, who attended the workshop.

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III. EXECUTIVE SUMMARY

FOUNDING WORKSHOP FOR THE ADVANCED CASSAVA RESEARCH NETWORK

The workshop drew together participants from CIAT, IITA, IBPGR, ORSTOM, IFAR, AID, the Rockefeller Foundation, and from advanced research laboratories in North America, in Africa, Asia, and Latin America. The underlying objective of the workshop was to discuss the creation of a multidisciplinary advanced research network to focus on the resolution of priority constraints in cassava through mobilization of international cooperative research to exploit, wherever possible, modern biological techniques leading to economically identifiable goals within a reasonable time frame.

The workshop recognized the critical importance of cassava in development strategies for the less privileged and less fertile areas of the tropical developing world while at the same time highlighting the relatively low level of advanced research being conducted on this crop, which is among the five most important food crops in the tropics.

The identification of key constraints to productivity and the effective utilization of cassava as a food and foodstock or animal feed was followed by an evaluation of specific research strategies which could conceivably be implemented to resolve those constraints.

The workshop specifically identified as its highest priority a series of interrelated areas of research to which immediate attention should be given:

A. Research integrating several topics in plant physiology, haploid/ di-haploid induction, virology and plant breeding, leading to the identification and resolution of constraints to the use of true cassava seed as a commercial propagation system.

B. Research on the biochemistry and genetics of cyanogenesis which would lead the way to the application of biotechnology techniques with the goal of developing cassava plants with very low, or no, cyanide in the edible parts.

C. Research on developing resistance to economically important cassava virus diseases through the application of genetic transformation techniques.

D. Research designed to better define the factors controlling cassava starch characteristics with a view to improving quality of a wide range of cassava products.

E. Research on a range of interrelated topics in molecular and cell biology which will lead to: a) the genetic transformation of cassava and the regeneration of plants from transformed tissues and b) the understanding of cassava genome structure and variability, gene expression and regulation.

It was felt that the priority research areas identified above would allow researchers at the more applied end of the spectrum, to develop new cassava germplasm with a more desirable combination of characteristics for any particular situation, building on the present international applied research network on cassava. A time frame objective was set for the decade between 2000 and 2010 at which time the earlier developments would be expected to have impact.

The structure, function and modus operandi of the proposed network were discussed and the participants agreed on a common approach to be implemented under the guidance of an elected Steering Committee of seven scientists. The group placed emphasis on a cooperative network structure involving an active and free interchange of research materials and results, training opportunities, and the provision of assistance to the members in identifying funding sources for the priority research areas.

It was agreed that the first scientific meeting should take place eighteen months after the network is formally initiated or as soon as funds become available.

IV. WORKSHOP MODUS OPERANDI

A. PROGRAM

The workshop opened with overviews on cassava research issues in Latin America, Asia and Africa. This was followed by:

a. A series of short presentations covering the state of the art in areas related to the research constraints previously identified by CIAT and IITA. Presentations were organized into four sessions: (i). cassava quality factors; (ii). cassava biotic stresses; (iii). cassava propagation problems and photosynthetic capacity under stress; and (iv). cellular and molecular approaches (See Appendix for the summaries of presentations).

b. Specialized working group discussions on each of the four main sessions referred to above were then organized. The working group discussions focused on a ranking of priorities for the agreed constraints, general research strategies, and proposed cooperative efforts to undertake particular research projects and the appropriate structure and functioning of the cassava network.

c. The proposals of each working group were then discussed by all workshop participants in plenary session, and the research strategies, as well as the role of basic research, and potential of new technological applications were identified and prioritized for each of the four areas of concern. Proposals on the structure and functioning of the network were examined and specific recommendations outlined.

B. RESEARCH PROBLEMS

CIAT and IITA had, prior to the workshop, separately identified the following research problems in cassava production, processing and utilization, considered to have relevance for advanced research approaches. These constraints represent areas where traditional research approaches have shown limited promise and where there is reason to believe that basic research and emerging cellular and molecular research techniques could aid in solving the problem.

CIAT		IITA	
*	1. Cyanide toxicity	*	1 Cyanide toxicity
**	2. Post harvest root deterioration		2. Shy flowering
	3. Propagation related problems		3. Mealybug and green mites
	4. Viral diseases	****	4. Nutritional quality
	5. Cassava hornworm	***	5. Starch quality under stress
***	6. Starch quality under stress		6. Nematodes
	7. Photosynthetic capacity under stress	**	7. Post-harvest root deterioration
****	8. Nutritional quality		8. Mycorrhiza

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* Indicate coincidence between CIAT and IITA.

Out of the original constraints, the IITA representatives finally selected three top priority areas, namely: cyanide toxicity, viral diseases, and nutritional quality (mainly protein). All of the above constraints were included in the working group discussions (see Appendix, summary by Dr. A.I. Robertson on constraints identified by IITA and African national researchers).

V. WORKSHOP DISCUSSIONS AND RECOMENDATIONS

The workshop participants in working groups and in the final plenary session identified priority research areas that can be tackled through the new technologies as well as those that require more basic research before the possibilities for biotechnological approaches can be properly evaluated. The participants also agreed to the establishment and the operational structure of a network of cooperative advanced research for cassava.

A. HIGHEST PRIORITY RESEARCH AREAS

The following highest priority research areas were identified by the workshop participants. A summary of each area is given below.

1. Priority 1: Cassava True Seed Propagation

a. Objective: To develop true (or sexual) seed propagation technology as a commercial possibility.

b. Justification: Vegetative propagation of cassava is associated with important constraints, i.e. virus accumulation, storage problems with planting material, low propagation rates, and plant architecture. Improvement is constrained by the need to provide planting material from lignified stems.

c. Research strategy: In the short term, open pollinated varieties could be developed, while in the longer term development of parental inbred lines through haploid/di-haploid induction and associated studies on seed physiology, flower biology, plant physiology and virology will all be required within a highly focused effort. Another possibility is to incorporate genes for apomixis, or develop techniques which induce apomixis, to produce true-breeding types from heterozygous parents.

d. Time frame: Long-term (+10 years).

e. Linkages with applied research: Plant breeding for parental selection will be required and, in the longer term, testing of combining ability of inbred lines. Cultural practices, seed production and management, and phytosanitary aspects need to be defined. Socioeconomic analysis of appropriate technology design to make adoption more likely would need to be integrated into the project at the outset.

f. Current projects: CIAT and IITA have initiated research on flower biology and haploid induction. IITA has initiated studies on the apomictic-like inheritance found in some of its cassava germplasm.

2. Priority 1: CYANIDE TOXICITY

a. Objective: To eliminate or greatly reduce HCN from the cassava plant organ systems depending on end uses in each region where human and animal toxicity problems exist.

b. Justification: HCN can cause chronic human toxicity problems in regions of Africa where casssava consumption is high and the population is in a poor nutritional state. It has been shown that there are no cassava accessions in the various world germplasm collections without HCN. The residual cyanide content of processed cassava can cause chronic toxicity particularly where processing technologies are not well developed, or not properly carried out.

c. Research strategy: Biochemical and genetic background knowledge on cyanogenesis would open the possibility for a biotechnological approach to the problem. Possible research approaches include: a. screening for variability in cyanide levels, in tissues and plants regenerated from immature pollen, and after mutagenic treatment of haploid tissue; b. removal of cyanogenic glucoside biosynthesis by means of insertional mutagenesis or anti-sense RNA inhibition; c. selective control of cyanide production by means of increasing the level of degradative enzymes in the roots or using non-cyanogenic control systems from other species.

d. Time frame: Long-term (+10 years).

e. Area of impact: Primarily Africa, secondarily Latin America and Asia.

f. Linkages with applied research: Effect of climate and soils on HCN levels, role of HCN in plant metabolism under stress, and study of the role in arthropod resistance. Incorporation of acyanogenic lines into breeding programs and other field research will be linked to the advanced research activities.

g. Current efforts: A proposed project at the University of Newcastle Upon Tyne on the use of molecular techniques (Dr. M.Hughes) requires funding, as does a project at Ohio State University (Dr. R.Sayre). Another project, at the University of Calgary, Canada has been proposed for support by the Rockefeller Foundation. A project has been initiated at CIAT on microspore culture and research by ODNRI (Dr. R. Cooke) as part of a CIAT-IITA agroeconomic study in Africa. This should provide information on processing effects and target regions.

3. Priority 1: VIRAL DISEASES

a. Objective: To first transform cassava for resistance to economically important viruses affecting cassava production in

Africa (ACMV) and Latin America (CCMV), and to concentrate on other important viruses later.

b. Justification: ACMV is the most devastating cassava disease in the African continent while in Latin America the CCMV can be found in most growing areas,causing yield reductions as high as 60%. Breeding for virus resistance is time consuming, and costly.

c. Research strategy: Development of transgenic plants expressing the coat protein gene of these viruses is currently thought to be the most feasible approach.

d. Time frame: Medium term (5 years).

e. Area of impact: Africa, Asia, and Latin America.

f. Linkages with applied research: Development of highly sensitive techniques for virus detection using molecular probes and/or antibody technology, and evaluation of resistance under field conditions followed by the incorporation of resistant clones into breeding programs.

g. Current projects: The Cassava Trans project has been initiated at Washington Univ., St. Louis, Missouri (Dr. R. Beachy) with the collaboration of ORSTOM (Dr. C.Fauquet), CIAT and IITA. This project will become part of the larger advanced cassava research network and has been partially funded. CIAT and IITA will continue virology research to identify and characterize other virus organisms constraining cassava productivity.

4. Priority 1: CASSAVA TRANSFORMATION AND GENOME ANALYSIS

a. Objectives: To develop techniques to allow efficient and consistent transformation and the regeneration of transformed plants in cassava, and to use molecular and cytogenetical tools to develop a basic understanding of the cassava genome structure and variability.

b. Justification: 1. Development of cellular (*in vitro* culture) and molecular techniques will provide the essential tools for achieving the goals of the other highest priority recommendations, as well as opening the way for application to other and longer-term priority research areas identified in the workshop. Such techniques will provide new somatic breeding methods and help improve conventional methods of crop improvement. Initial research in this field has shown the feasibility of transformation in cassava but plant regeneration of non-transformed tissues has been achieved only through somatic embryogenesis.

2. Concerted use of available molecular genetic markers (isozymes, RFLPs), cytogenetical techniques, and morphological genetic markers in cassava can generate powerful tools, for the study of the organization of the cassava genome. This information will be useful to conventional breeding and prepare the ground for non-conventional genetic manipulation. Associated with gene transfer techniques, molecular biology tools will be used for studies on gene expression and regulation regarding the major traits of interest for genetic transformation.

c. Research strategy: 1. Research on somatic embryogenesis conducive to transformation should receive high priority, as well as anther and microspore cultures for use in transformation, selection and conventional breeding through the rapid production of di-haploids. Regeneration from callus and cell cultures should receive early emphasis with the view of facilitating transformation. Research on transformation through biological and physical approaches will need to be tied to the regeneration work and a range of cassava genotypes will need to be explored. Because of the nature of this research, regeneration/transformation studies should be encouraged in as many labs as possible. Preferred transformation systems and associated culture systems will emerge with progress in the subject. In vitro storage of cassava clones and transformed lines by cryopreservation will be emphazided at the outset.

2. Parallel to the efforts on genetic transformation and regeneration, priority attention will be given to the construction of gene libraries, and selection of cDNA clones for implementing RFLP analysis of cassava genomes. Use of morphological, biochemical and RFLP markers will be associated with the development of a cytogenetical understanding of cassava, with particular emphasis on the priority research constraints identified in this workshop. Such tools will be also utilized in the characterization of cassava genotypes and will lead to tagging techniques for important traits. The development of pathogen detection and screening for particular characteristics through molecular probes or antibody techniques will be a valuable spin-off from these studies. The expression and regulation of selected genes is another high priority research area to receive attention.

d. Time frame: Information on cassava genome structure and variability will emerge rather rapidly, short term for transformation and regeneration (5 years), and longer-term for gene characterization/regulation.

e. Current efforts: Limited work took place on cassava ransformation at Vrijes Univ., Belgium but with low level funding. There is specific interest in cassava transformation, gene expression and regulation studies at the Rockefeller

University, New York (Dr. Nam-Hai Chua) and in relation to specific traits at Washington Univ., St. Louis (Dr. R.Beachy), Univ. of Newcastle-Upon-Tyne, U.K. (Dr. M.Hughes); and at Louisiana State Univ., Baton Rouge (Dr. J.Jaynes). There is also a wide interest in associated plant regeneration studies. Work in this area is underway, though with limited resources, at CIAT (also in transformation), IITA, Univ. of Bath; and in various institutions in developing countries, i.e. Zimbabwe. Peru, Brazil (CENARGEN, also in transformation) and in Argentina. Currently, research on cryopreservation of cassava tissues and organs is underway at CIAT in collaboration with IBPGR.

5. Priority 1: CASSAVA STARCH QUALITY

a. Objective: To guarantee acceptable and stable consumer quality characteristics of fresh cassava and processed products, by improving cassava starch quality.

b. Justification: Pre-harvest environmental and genetic factors affect the quality of fresh and processed products, especially starch quality which may be associated with texture and perhaps flavor changes. There is need for quality stability over a range of harvest ages and there is a need of basic knowledge on starch properties to help product development.

c. Research strategy: A series of interrelated short term research projects need to be conducted on effects of genotype, physiological stress, and processing, on starch quality. Possibilities for biotechnology solutions will need to be assessed after the short-term research has been done.

d. Time frame: Short-term (2-3 years) for basic studies and long-term for biotechnology approaches.

e. Current efforts: ODNRI (Dr. R.Cooke) and at the Univ. of Nottingham (Prof. J.M.V. Blanshard) are working in cooperation with CIAT and IITA; work is also underway on starch quality and other nutritional aspects of cassava.

B. OTHER PRIORITY RESEARCH AREAS

1. Priority 2: THE CASSAVA HORNWORM

a. Objective: To develop cassava cultivars resistant to the cassava hornworm (Erinnys ello).

b. Justification: Among the foliage insects, the cassava hornworm is one of the most desvastating, causing yield reductions as high as 50%. The pest is present in all countries of Latin America and the Caribbean and there is no identified

ource of resistance. The pest is susceptible to applications of Bacillus thuringensis suspensions; incorporation of intrinsic resistance to the hornworm is therefore feasible.

c. Research strategy: Development of transgenic plants expressing the B.t. toxin genes. Toxin constructs could be made available through arrangements with private firms, i.e. PGS in Belgium or Monsanto Co; USA. Alternatively, development of transgenic plants expressing proteinase inhibitor genes will be considered.

d. Time frame: Medium term (5 years).

e. Area of impact: Latin America

f. Linkages with applied research: Implications of conferred resistance mechanisms on hornworm biology, including possible acquisition of resistance by the pest and side effects of conferred resistance on agronomic traits, on humans and animals will all be required in support of the basic research.

g. Current efforts: Limited efforts at Vrijes Univ., Belgium (A.Calderon) without special funding.

2. Priority 2: CASSAVA NUTRITIONAL QUALITY

a. Objective: To enhance protein quantity and quality of cassava-based diets within the framework of using cassava as a famine reserve crop for Africa.

b. Justification: Cassava is the principal food source in many areas of Africa. Increasing protein in cassava roots would contribute to resolving nutritional deficiencies. Improving quality in terms of the sulphur containing amino acids would also help decrease effects of HCN toxicity. Improving vitamin A content in cassava would provide additional nutritional benefits and this could be studied in parallel.

c. Research strategy: Several possible routes exist for improving quality protein intake in highly cassava-dependent regions, e.g., diet diversification, fermentation, use of cassava leaves, cassava as animal feed and biotechnological approaches. All will be evaluated and the appropriate approach followed for particular areas of Africa. Biotechnological approaches may include enhanced expression of existing genes or new genetic material. This approach requires precise control of target cells and knowledge on levels of increase of protein necessary to improve the diet.

d. Time frame: Long-term (+10 years).

e. Area of impact: Regions in Africa where cassava is the principal food source.

f. Linkage with applied research: Studies on the implications of increased protein quantity on cassava tolerance to abiotic stresses and effect on consumer acceptability will be required.

g. Current efforts: Basic work is underway at Louisiana State University (Dr. J. Jaynes) and there is possibly interest at Washington State University (Dr. C. Ryan). Currently, work is underway at CENARGEN, Brasil (Dr. M.Carneiro) to develop root gene promoters for cassava for future use in improving nutritional quality.

3. Priority 2: PHOTOSYNTHETIC CAPACITY UNDER STRESS

a. Objective: To generate fundamental knowledge on cassava photosynthesis and respiration with the aim of improving photosynthetic capacity under stress conditions.

b. Justification: It appears that cassava's high productivity under favorable conditions and its tolerance to stress environments are related to unique physiological/biochemical aspects of photosynthesis which can be further exploited in crop improvement.

c. Research strategy: More basic information is needed to characterize cassava's physiological/biochemical responses under stress conditions while work on C4 metabolism and photorespiration and on anatomical aspects of enzymic location need to be carried out.

d. Time frame: Medium term (5 years).

e. Current efforts: Research on the subject is underway at CIAT, and there is strong interest at the University of Georgia, Athens (Dr. C.C.Black) but without special funding.

4. Priority 2: POST-HARVEST PHYSIOLOGICAL ROOT DETERIORATION

a. Objective: To increase the shelf life of cassava roots through significant reduction or delay in physiological deterioration.

b. Justification: The short shelf-life of cassava roots greatly limits the commercial (marketing) potential of the crop. Current improved storage technology exists for certain sectors of the Latin American market but work is required in other areas.

c. Research strategy: Basic biochemical information is required to better understand physiological deterioration before possibilities of new technologies can be assessed.

d. Time frame: Long-term (+10 years).

e. Impact: Throughout the world but especially in Latin America since most cassava is processed in Africa and Asia.

f. Current efforts: None at the present time.

5. Priority 2: BIOMASS PARTITIONING INTO ROOTS

a. Objective: To study sink strength in cassava and elucidate its relationship to biomass partitioning.

b. Justification: Genotypic capacity to partition biomass to roots can be identified and screened through biochemical means. Harvest index appears to be related to total biomass and thus yield, particularly under stress.

c. Research strategy: Basic biochemical mechanisms controlling sink strength in cassava need elucidation followed by screening techniques for variation in sink strength among contrasting cassava clones.

d. Time frame: Medium term (5 years).

e. Impact: This basic work would have impact throughout the developing world.

f. Current efforts: There is interest on the subject at the University of Georgia, Athens (Dr. C.C.Black).

C. ESTABLISHMENT AND ORGANIZATION OF THE WORK

The participants agreed to establish a network for research in the priority areas identified and recommended by the workshop. The guiding principles of the network are in accord with the guidelines provided by Dr. R.Baldwin from IFAR (see Appendix 1), namely: (i) several inter-related, relevant projects were identified with the objective of resolving critical and traditionally recalcitrant constraints to cassava production, processing and utilization by the decade 2000-2010; (ii) the research will be shared according to particular interests and capabilities in several institutions, public and private, and will be inter-disciplinary and multi-disciplinary in nature;

iii) the network participants identified their respective projects and source of funding and additional funding requirements; (iv) and research that is not being done, but is essential to the success of the network was identified. Participants agreed that the network should facilitate exchange of information, exchange and transfer of technology and materials, cooperative training activities, mobility of personnel, communications media like a newsletter, electronic mail networks and periodic scientific review meetings.

A network Steering Committee was elected by the participants. The Committee members for the first 18 months of the proposed network are as follows:
- Dr. R.D. Cooke from ODNRI, London, U.K.
- A representative from CIAT, Colombia
- A representative from IITA, Nigeria.
- A representative from ORSTOM, France
- Dr. G.G. Henshaw, Univ. of Bath, U.K.
- Dr. M. Hughes, Univ. of Newcastle Upon Tyne, U.K.
- Dr. C.C. Black, Univ. of Georgia, USA

The Steering Committee at its next meeting will first discuss further the structural features of the network, including a review of potential institutions and scientists to be involved, funding requirements (current and future), consideration of the appointment of a coordinator for the network, and the location and funding needs of the next Steering Committee and network meetings.

The participants agreed that the network will develop and grow in a way that is dictated by funding availability, scientific progress and the degree of cooperation. Periodic review and flexibility will be essential to its continued development and success.

Funding will be sought both centrally through the Steering Committee and by individual members of the network. The support of IFAR will be sought as the initiator of the network and as a catalyst for the gathering of additional funding. IFAR should identify core funding for network communications, personnel contacts and periodic (yearly) network meetings with the first meeting planned in 18 months.

In summary, the advanced research to be pursued with cassava includes the following categories: a. areas where emerging technological solutions are feasible (cassava true seed propagation, and resistance to viral diseases and the cassava hornworm); b. areas where there is a role for biotechnology, but additional basic biochemical knowledge is also needed (cyanide toxicity and nutritional quality); c. areas where basic biochemical research is needed to identify areas for future new technology approaches (starch quality, post-harvest root deterioration, photosynthetic capacity under stress).

VI. APPENDIX

SUMMARIES OF PRESENTATIONS

T A B L E O F C O N T E N T S

CASSAVA RESEARCH FOR DEVELOPMENT

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Cassava is an important small farm crop. The plant is not only able to survive during drought periods but also produces well with very limited supplies of water. In addition, its tolerance of acid soils allows it to produce good yields on marginal soils without excessive use of costly soil amendments. Cassava is an extremely reliable crop to grow. These qualities have endeared cassava to small farmers by whom it is almost exclusively grown. Furthermore, the relatively large labor requirement, the high cost of specialized mechanization, the logistics of handling this very perishable crop, and the high assembly and transport costs for the harvested roots tend to ensure that small farm systems will continue to dominate and maintain their comparative advantage over larger scale production systems. Given that cassava can be a source of increased income, any gains in productivity will be received by the small farmer.

In order to increase small farmer incomes, however, it is necessary not only to produce more cassava but also to ensure that the farmer can sell his produce. Cassava has traditionally been a rural crop and the end products have been suitable for rural needs, and sufficient change has not occurred in cassava processing and marketing to ensure that the products for sale are suitable for the expanding urban markets. Cassava has, however, multiple end uses and if production, processing and marketing are suitably linked and geared to urban markets it provides an opportunity to markedly increase rural income.

However, development of research plans in agriculture in the third world have frequently been based on the premise that increasing productivity is the objective and that this will benefit the population of the developing countries. We believe that this view is far too simplistic and that by careful planning of the research and development agenda it is possible to ensure that the development process be targeted to certain segments of the population, and that it meets specific political objectives that can be predetermined. In the particular case of cassava, where resources for cassava research are very limited, it is important to ensure that they are coordinated and directed to specific goals. We cannot afford the luxury of doing

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research for the sake of research. This does not mean that research will only be in the downstream applied fields: we may well need upstream basic research to resolve intractable problems faced by our target population.

The environment in which we work, a non-profit research based development organization supported by donations from various sources, and our own personal convictions, lead us to fix the social objectives as: 1) to improve small farmer incomes and food supply and 2) whenever possible to simultaneously increase the overall food supply to the urban population. As a research organization without either the authority or the necessary financial support to be directly involved in the development process, we have to concentrate our efforts on providing the information required by others and stimulate these others to cooperate in our raison de etre.

I shall briefly describe with case studies the planning process we have used, then highlight some gaps still needing to be filled that may be of interest to this workshop. This planning process is itself dependent on factors in the agricultural sector that are external to cassava.

AGRICULTURE AND DEVELOPMENT

Development at the national level is almost invariably associated with urbanization and this is normally supported by a strong agricultural sector. When urbanization is accompanied by industrialization, or the development of efficient service industries, there is increased wealth in the urban sector and demand for agricultural products for food or as the raw materials for manufacturing. This demand for agricultural products leads to a buoyant agricultural sector that requires the services and products of an urban society. If the two sectors develop in a balanced fashion, society as a whole benefits.

The process of urbanization is occurring very rapidly in developing countries at present. The export markets for industrial goods are highly competitive and it is often difficult for developing countries to enter them. This is particularly true of the export of goods to the developed world. Thus the basis for urban development has to be a buoyant rural demand for the low technology products that can be produced by a society that is at the same time developing and urbanizing. Not only must there exist the potential for income generation in the rural sector but also, it must be relatively evenly destributed so as to ensure the demand for low technology goods. If only a small number of large producers receive the benefits of increased agricultural production they will tend to purchase more

sophisticated goods from developed countries whereas a large number of smaller producers will tend to purchase such goods as bicycles, radios and refrigerators.

The smaller scale producer often produces low value, own price inelastic goods. Thus as a group they have little scope for increasing their income drastically unless they can markedly reduce their unit production costs or increase their total production. In the latter case, prices drop and the net income increase would be small. The resolution of this dilemma appears to lie in farmers selling new products that are more own price elastic. This can be achieved either by producing novel crops or by processing traditional crops into different forms. The changing pattern of crops or of processing for end uses are a normal part of the development process. Monke has shown that the use of maize changes from that of direct human consumption to indirect uses as countries develop. This changing use pattern requires changes in the post harvest processing of the crop. For example, maize is sold to the final consumer as poultry or pork, not as maize. The example of maize illustrates a new use for an old crop: the dramatic increase of such crops like soybeans in the US and rape in Europe are examples of changes to more own price elastic crops.

Similar changes are already occurring in the developing countries as part of the development process. In most cases the technological basis for these modifications in the utilization of basic food crops has been imported from the developed countries and depends on possessing crops that are also grown in the temperate areas. Those crops that are exclusively grown in the third world have largely been excluded from this process. Unless these crops are included in the research and development process their comparative advantage is likely to be eroded.

Cassava is one of several tropical crops that has until recently been neglected by research and development agencies. In the early part of the decade of the fifties cassava production using traditional technology in the tropics was very efficient compared with maize production. New technology greatly increased the productivity of maize in the succeeding thirty years and cassava lost ground relative to that crop, with maize research eroding the comparative advantage of cassava relative to maize in productivity. However, there are many regions of the tropics where maize cannot be successfully grown due to climatic and soil restrictions, and in these regions, cassava will have to compete with imports often produced in the developed countries at lower cost due to the use of new technology. This situation is aggravated by the fact that in the area of post harvest technology great advances have been made in the temperate crops.

The importation of grains or other agricultural products by the developing countries is not, however, a viable solution to their development process, as in general they do not have the industrial exports to pay for them unless their wages remain at extremely low levels. As a result most developing country investments in agricultural research agencies, tend to concentrate on raising the yield levels, and not on how to develop technology appropriate to meet the needs of rural development. This approach may well lead to the production of low cost food supplies for the urban sector but will not necessarily contribute to the rural development necessary to stimulate urban development through increased demand for industrial goods and services.

CASSAVA: STATUS QUO

Cassava is the fourth most important crop in terms of calories produced for consumption within the tropics. It is generally produced in the more marginal areas without irrigation. In the areas where cassava is grown farmers often have few if any alternative crops due to the harsh climatic and soil conditions. Present production systems tend not to use inputs commonly associated with modern agriculture. In spite of this levels of productivity at about 3-4 t dry matter/ha year are reasonable considering that it is normally grown in areas where only one crop cycle per year can be obtained. Almost all cassava is grown by small farmers and it is an important source of food and income for millions in the tropics.

The cassava is mostly consumed on the farm or is sold in the local rural markets. In these areas it is a traditional staple and demand is generally inelastic. The crop is highly perishable after harvest; it starts to deteriorate within as little as 24 hours after harvest. Urban markets however require non perishable convenient foods. Cassava does not meet these requirements and as societies urbanize the demand for cassava tends to decrease. This situation is aggravated by the fact that due to its perishability cassava, whilst being a cheap rural staple is an expensive urban food unless suitably processed.

The perishability problem has been partially resolved in certain areas by processing the cassava into a variety of flours or dried products such as Farinha in Brazil, Gari in West Africa and Gaplek in Indonesia. These traditional dry products are generally not preferred goods and are own price inelastic. They also face competition from imports of cheap subsidized grains from the USA and Europe.

This rather dismal picture is brightened by the fact that cassava is a multiple use source of carbohydrates. If suitably processed it should be able to enter into newly developing markets and if price competitive should be able

to substitute for various cereal based products. In this latter case elasticity would at least initially be high as its market share would be so small that increases in supply would have negligible effects on the overall market. However cassava products can only enter into these new markets if the raw material is sufficiently low priced to be competitive and if the processing does not add excessively to the final cost of the product. Furthermore as increased rural income is a desired goal the value added should occur in the rural sector. At the same time the product should be of sufficiently high quality that it is readily acceptable in the urban markets.

A PLANNING FRAME FOR CASSAVA BASED DEVELOPMENT

In order to determine the planning frame it is necessary first to define the objectives. In the case of cassava we have, as stated above, defined the primary objective as: to increase rural incomes by producing low cost goods with an elastic urban demand. Whenever possible these goods should be directed at providing final products which are consumed by the poorer segments of the urban population. This latter condition is however secondary to the rural income objective and is considered as a side benefit that is advantageous when it can be simultaneously achieved. We shall see in later sections that these two objectives are not necessarily mutually exclusive.

In the early sections of this paper we established that basic traditional cassava products are inelastic. Thus in order to meet our objectives we must first of all define the demand characteristics of alternative products, based on cassava. Once this has been done those that are more elastic can be further studied to analyze the use of cassava as the raw material for their production.

In developing this methodology a holistic approach is required as cassava cannot be looked at in isolation. For example, it is only possible to determine the potential for cassava in the animal feed market by analyzing the demand for different animal products, the relative price of other energy sources, the availability of alternative sources of protein and how all these are affected by government policies. At present the general methodology follows the pattern of first identifying the markets with the desired demand characteristics. This is then followed by an analysis of the critical constraints to cassava entering these markets. These constraints are highly variable and are certainly not all on the production side. In fact as will be seen in the following sections the supply side does not normally dominate. Furthermore although one aspect or constraint may dominate in the initial phases of development the resolution of this constraint will normally uncover

other constraints, thus an integrated approach that predicts and resolves multiple constraints must be adopted. The development of this type of integrated approach is illustrated in the following case studies which highlight different initial constraints.

CASE STUDIES OF DEVELOPMENT STRATEGIES

Social organization. In the North Coast of Colombia in the last years of the decade of the seventies the Integrated Rural Development program provided credit and technological assistance to increase cassava production in the region. This traditional production oriented approach did indeed lead to increases in cassava production in the region, however local markets were rapidly saturated and prices dropped in such a manner that farmers were not able to recover their costs. To resolve the problem efforts were directed to opening up new markets. Studies on the production costs of cassava indicated that it could enter the rapidly expanding animal feed market at a competitive price and still leave a respectable profit margin for farmers. Two questions remained unanswered. Firstly, why were cassava farmers only willing to produce limited quantities of cassava and secondly, if the process was potentially so profitable why had a cassava drying industry not grown up spontaneously.

Most of the cassava in the area was used for on-farm comsumption or sold to the local fresh markets. The fresh markets had very stringent quality requirements and at least part of the harvest had to be left in the field as waste. They were not willing to take the large risk involved in planting more cassava with more inputs as they had had the bad experience of not being able to sell their cassava at any price on some occasions. This created an illusory price for cassava that did not reflect production costs but rather the risks inherent in marketing the product and the wastage related to the high quality requirements.

The lack of a drying industry that could lead to a stable price floor was also puzzling. The large feed mills that produce most of the balanced diets faced a deficit of energy sources for their rations and at least one major feed company was interested in using cassava. Further investigation indicated that the feed mills required a certain minimum quantity of dried cassava before they would incorporate it. On the other hand without an established market farmers were not willing to plant more cassava and dry it. Furthermore in view of the high and fluctuating prices of fresh cassava entrepreneurs were not interested in establishing drying plants.

The problem was how to break this deadlock. Various solutions were analyzed, with the most promising appearing to be the establishment of farmers' associations to dry the cassava and sell it to the feed mills. This solution was attractive for the following reasons because 1) individual farmers did not have a sufficiently large resource base to establish drying plants whereas associations did, and; 2) if the price of cassava for fresh market was high the farmer producers could sell in to this market and make substantial profits to pay off loans on the drying plants. The low quality cassava not fit for the fresh market could still be sold to the plants which could function at a low level. If the price of fresh cassava dropped below a threshold value than farmers could sell all their cassava to the plants and still make a profit. This would place an effective floor price on cassava thus stimulating farmers to produce more.

In order to test this development model a pilot project was established to look at the viability of the model and the drying technology. This model did indeed prove to be successful and has been used as the basis for the establishment of what is now a highly successful small scale industry bringing benefits to the small farmer producers.

New Technology. The per capita consumption of fresh cassava is less in the urban areas of Latin America than in the rural zones. The rapid urbanization in the period of 1950-1980 led to a decrease in the demand for fresh cassava and farmers faced a declining market. Conventional wisdom indicated that urban consumers considered cassava to be an inferior good. If this were true then the possibilities for maintaining or increasing rural income through expansion of the fresh cassava market appeared dismal. The conventional wisdom was however backed up by very little concrete data and did not seem to fit with comments concerning cassava made by housewives.

In Colombia, a series of surveys and analyses of cross sectional data indicated that fresh cassava was indeed a preferred food and equally as desired as potatoes and rice. However it was risky to buy and often of low quality. Furthermore the marketing margins of such a perishable crop were extremely high: The farmer price often being less than 25% of the final consumer price. The high marketing margins are related to handling a highly perishable product. Thus both the negative response of urban consumers to cassava and the high cost in urban markets are due to the perishable nature of the product. This suggested that by reducing the perishability of cassava, two objectives could be achieved. First, the market could be expanded with a positive impact on farmer incomes and second, that marketing margins could

be reduced thus providing the urban consumer with a lower cost food supply. Improved production technology was certainly not the solution,but research on post harvest technology to reduce the perishability of fresh cassava offered great promise.

Once a viable technology existed it had to be tested under commercial conditions and consumer response to the new product evaluated. A commercial pilot project was established in the department of Santander in Colombia. Farmers found the technology acceptable and consumers evaluated the product favorably.

The expansion from the pilot to the fully commercial phase is only just beginning at the time of writing, nevertheless initial results are promising. They indicate that by analysis of the market constraint and subsequent technology development directed specifically to the major constraint, development objectives can be met. It is also true to say that other constraints are now appearing, such as how to guarantee a continual supply of good quality fresh roots to be used in the process and also how to maintain quality as production increases.

Policy decisions. The North East of Brazil is economically the poorest area of the country. The population is less urbanized than the rest of Brazil with about 50% of the population in the rural sector. The basic staple of the population is a product called farinha da mandioca. The cassava in the region is almost exclusively produced by small farmers on the less fertile lands with less rainfall. The cassava once harvested is processed in small "casas de farinha" or literally flour houses, into a flour or meal called farinha da mandioca.

The North East of Brazil is noted for its extremely variable rainfall patterns. In fact it is probably due to cassava's tolerance of sporadic rainfall that it is the dominant staple in the area. Nevertheless as a result of the climatic fluctuations, although the cassava crop never fails completely, yields do fluctuate widely. As farinha is a typical basic staple in its demand characteristics it is own price inelastic. Hence as the supply of farinha varies, prices show tremendous variability. This is not conducive to giving farmers a stable income nor is it advantageous to the consumers who have to face uncertainty in the price of their basic staple. The farmers are loathe to increase their production of cassava as they fear that prices will be very low in good rainfall years, whilst in the drought years the landless labor and the urban consumer have to pay extremely high prices.

The solution would seem to be in the establishment of a floor price for farinha. One method of doing this would be for the government to establish a floor price for farinha

with storage facilities. This could become extremely expensive. An alternative strategy is to look for a large alternative market into which the cassava could enter in the case of a bumper year and drop out to a certain extent in a drought year.

Economic analysis of production costs showed that cassava was indeed competitive in the annual feed market. However when prices were looked at cassava was not able to compete, as the transport of other sources such as maize were heavily subsidized. The government of Brazil is now reducing these subsidies and as a result it would appear feasible to establish a cassava drying industry to use all excess production over the farinha market. This would effectively place a price floor on the price of cassava in the region.

As a result of the policy changes several of the North Eastern states of Brazil are including small scale cassava drying in development plans. Efforts on research and development are turning to satisfy the needs of this industry and hence to assist the farmers in increasing their income.

Production technology. It may appear surprising that up to the present in these case studies improved production technology, the traditional product of agricultural research agencies, has hardly been mentioned. In general we feel that production technology per se is only the limiting constraint on raising farmers' incomes when the capacity of the market to absorb increased production is ensured. In the case of Indonesia this seems to have occurred with a whole series of outlets for cassava products in a highly structured market in which demand outstrips supply. Farmers are confident that the bottom will not fall out of the market.

Area planted to cassava in the island of Sumatra in the Lampung district has responded to the situation of demand being greater than supply. In the Asian context, cassava is an introduced crop and the germplasm base for development of improved varieties is limited. It would appear a priori that introduction of exotic germplasm from the center of origin of the crop and the development of new high yielding varieties could assist farmers in increasing their incomes. Furthermore these new varieties need to be fitted into the intricate Asian cropping systems, hence work is required in the area of breeding agronomy and farming systems. Research should then be concentrated on these areas.

PRELIMINARY CONCLUSION

In order to fulfill overall national social and political goals the agricultural sector will have to orient itself not only to providing food for the urban sector but also to

increased wealth in the rural sector so as to create demand for locally produced industrial goods and services. This can only be achieved if the increased wealth is relatively evenly distributed.

Greater wealth in the rural sector will not necessarily be achieved merely by increasing rural production. Increases in rural production must be geared to increasing those goods with elastic demand. As countries become more developed these are generally not the basic staples, or at least the basic staples in their traditional form. Furthermore if the wealth created is to be evenly distributed then a large number of small producers is likely to be the most effective model. Hence, in designing an agricultural research plan it is necessary to look for goods that can be produced by the small farmer sector and analyze the demand for such goods. It should be noted that here we are talking not only of primary products such as the fresh roots in the case of cassava, but also the secondary products that result from processing and give the value added to the producer and processor in the rural sector. Those with large market potential should be chosen and efforts should be concentrated on relieving the constraints on their entering the markets. This may involve research on a whole range of fields from policies through processing to production. If this line is followed then it is our belief that research can be directed in such a manner that its targets are well chosen rather than hoping that as a result of increased production there will be some trickle down to the poorer sectors of the population.

LOOKING TO THE FUTURE

The analysis up to the present looks at developing new uses of cassava and/or production technology but not at radical changes in the crop which might be wrought by using modern techniques. Such changes have been seen as so costly and needing such a long time frame to be achieved that they have been given orphan status. However, possibilities exist that need to be explored. An example to illustrate such possibilities might be **cassava from true seed.**

Cassava is biologically very efficient under drought conditions. However, planting material is always a major problem, particularly when drought is prolonged over several years, as this material is too bulky to bring in from other areas and is often diseased and not well adapted. This major constraint for small farmers, could largely be overcome if we produce cassava from true seed. We could be creating a new crop! In order to achieve this we would need 15 to 20 years of concerted effort in the areas of in vitro techniques to produce haploids, a better understanding of cassava genetics, breeding for seed based cassava, agronomy of seed production, agronomy of production of cassava from

seed, etc. Not all this work would be needed for the overall period; for example if haploid plant production techniques were developed rapidly then research on this aspect could be phased out. However the overall project could only be successful if all aspects are included: working on one aspect in isolation will achieve little or nothing. The question is then as to whether it is possible to mount a long term coordinated research proposal with many cooperating agencies with the objective of producing "A viable technology for farmers to produce cassava from true seed by the year 2010".

I believe that it is this type of exciting long term project that could bring major changes to the cassava world. However before embarking on such projects, we should always ask ourselves "How can this help the small farmer?".

BIOTECHNOLOGY AND THE IMPROVEMENT OF CASSAVA, YAMS AND PLANTAIN IN AFRICA - A VIEW FROM IITA -

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INTRODUCTION

The International Institute of Tropical Agriculture (IITA) undertook a strategic study and planning exercise over the past few years, in order to sharpen the focus of its research activities and develop new modes of collaboration in its region of greatest concern, West and Central Africa. The strategic planning study mobilized the best talents in African agriculture and identified priority areas of research which require urgent attention.

In the area of commodity improvement, some of the new plant breeding objectives cannot be easily realized by means of conventional breeding. IITA felt the need to explore the possibilities offered in biotechnology in order to apply non-conventional technologies in its breeding work, particularly in root, tuber and plantain crops. African agricultural research institutions interested in those applications were invited to discuss the prospects at IITA.

The Meeting on the Use of Biotechnology for the Improvement of Cassava, Yams and Plantain in Africa was held on 8 and 9 August 1988 at IITA in Ibadan, Nigeria, with the participation of 25 experts who represented 14 institutes in 7 countries.

The specific objectives of the meeting were to: 1. Review the status of biotechnology research on cassava, yams and plantain in Africa; 2. Identify common constraints in the research and production of those crops; 3. Discuss the prospects for using advanced, non-conventional technologies to address the problems; 4. Exchange ideas and experiences among the research scientists in Africa.

RECOMMENDATIONS

Participants at this meeting made the following recommendations:

General recommendations

1. Collaboration among different research workers on the priorities identified below should be fostered by IITA through a network of laboratories and support for facilities.
2. The network should assemble once a year, considering that the present meeting was enormously helpful in exchange of knowledge, problems, prospects, hopes and ideas.
3. Scientists from selected laboratories might be invited to hold seminars at such network meetings.
4. An informal newsletter should be established for dissemination of news of biotechnology activities in Africa, to be written by root crop and plantain biotechnology researchers and coordinated through IITA.
5. Encouragement should be given to commercialization of rapid multiplication methods as proof that biotechnology has practical and useful applications .

Recommendations in specific areas requiring urgent attention

6. Basic research. In order to facilitate traditional breeding and selection and the application of modern biotechnological methods, certain basic research needs should be speedily fulfilled. They are:

a. Cytogenetic studies to establish chromosome numbers, ploidy levels and chromosomal variations;
b. Production of haploids and di-haploids;
c. Meiotic analysis to evaluate ploidy levels and the degree of heterogeneity of the genome;
d. Sufficient understanding of the flowering process in order to enable manipulation of flower production;
e. Tissue culture protocol to derive plants from callus;
f. Tissue culture protocol to derive plants from protoplasts.

7. Research Targets.

a. first priority
- acyanogenic lines,
- virus resistant lines-for both yam potex virus and cassava gemeni virus,
- protein fortification-by DNA route and fermentation route
- virus diagnostics-for breeding, movement of germplasm and, the definition of pathogenic variations.

b. Second priority
- black Sigatoka disease-screening, <u>in vitro</u> selection for somaclonal variation,
- processing and related deterioration problems (browning, tannic acids-the identification of enzymes involved and the DNA of their genes),

- mycorrhizae-investigations of local isolates, eco-tolerances and compatibility with host plants,
- cassava mealybug and green spider mite-screening for metabolic blockers or anti-feedants and for humoral antibodies.

c. long-term priority
Nematode resistance is highly desirable, but it was recognized that genome mapping in these crops followed by RFLP analysis of transposable element locations is technically not within reach at this time.

Africa is a very diverse continent, with dramatically different ecological zones from deserts to jungles. It has very varied soils and a wide spectrum of economic and political constraints. As a result no single variety of cassava will suit all situations. What biotechnology seems to offer is the opportunity to have traditional breeding activities supplemented by the "genetic improvement" of single trait addition. These "magic bullets" can be added speedily to already good breeding lines or populations.

In this light it is worth pursuing such traits as protein fortification, virus-resistance and a cyanogenesis in the knowledge that national and local programs can then choose from an array of possible additional genes so that they can tailor-make the final genetic package that is offered to the farmers.

The following questions received priority in discussions:

1. **Cyanide toxicity.** Can or should the HCN content be reduced or removed?

NEEDS: Although no one would want all cassava to be free of HCN, it would be highly advantageous to have some "sweet" cassava varieties that have less or no HCN. Some populations traditionally ferment their cassava, some do not. The HCN increases under (drought) stress and that is the very time when the hungy poor reduce processing and cooking time and thus accelerate the slow poisoning of themselves and their families. Cassava is a backyard, roadside, "anywhere" crop, and if it were totally free of HCN, much would be lost to rodents and mammals, especially monkeys and baboons. However, in more formal plantings for market or commercial production, HCN-free lines would be beneficial, particularly those that consumers could identify as such. The participants were unanimous in according the idea very high priority, in that the need could only get worse as population pressure and rising prices pushed more families back into cassava consumption.

POSSIBLE ACTION: Discussion included three possible approaches to answering the question.

a. Mutation breeding based on cobalt-60 irradiation. Experiments are under way in Ghana and Nigeria along these lines in similar crops. The HCN pathway is redundant, as far as we know, and any enzyme along it may succumb to random lethal mutation.

b. "Reverse genetics": identifying key enzymes, i.e. liniamarase, and interfering with the gene that produces it (Lichtenstein et al.).

c. Wide crosses bringing in the absence of HCN from wild relatives. This is not simple, and would involve a clearer understanding of the apomictic-like inheritance (Hahn) pattern in some such crosses already revealed.

2. **Protein improvement.** Should we aim to fortify the cassava tuber content with more protein and better protein?

NEEDS: Discussion focused on rising prices that protein (meat) commanded (i.e. approximately a 15 fold increase in Nigeria over the past ten years) but that more nutritious vegetable (legumes) are being developed. Should cassava assume the burden for total nutritional needs? Surprisingly many felt that it could do nothing but good, as cassava for cultural reasons is sometimes all that children receive. "Kwashiorkor" is a Ghanaian word and the problem is still there. More protein in cassava would help. It was certainly felt that quantity should improve, after which quality could be addressed.

POSSIBLE ACTION: Two routes, high and low technology, were discussed:

a. High technology: the transfer of a protein storage gene from, perhaps, potato or a seed storage protein of legumes. The impetus for a breakthrough would thus focus on tissue regeneration in cassava. The need was expressed for a protocol for developing plants from callus tissues or, better still, plants from protoplasts. Zimbabwe claimed to be approaching this goal. This would allow Ti transformation of already elite cassava lines. Some discussion probed the possible need for regulatory genes and for switching on the protein synthesis. Would one gene be sufficient or is it a family of genes required?

b. Low technology: fermentation route. Both Meyo in Burundi and Senez in Paris have used fungal fermentation (non-sterile) methods in field trials. Possibilities for adaption (culturally) at village level should be explored. (IITA has a cement mixer based on the ubiquitous oil drum that could be adapted for nonsterile fermenters. Protein

fortification has high priority and we would like to be sure it is achievable.

3. **Virus resistance.** Do we need another method of providing for African mosaic virus resistance?

NEEDS: Mosaic-resistance lines have been developed at IITA. The resistance is not complete but lines derived from IITA do not suffer major losses in the field. However, as the lines are planted further afield, local adaptability requires further crossing and resistance can be lost through low infection pressure during selections (i.e., Zimbabwe). If the infection potential is too severe, resistance can be overcome. It is also true that related crops, particularly yam, and sweet potato suffer from a potyvirus. The "gene-in-a-bottle" is an attractive remedy to have when ongoing breeding is envisaged. The importance of fast and reliable virus diagnostic methods and knowledge of pathogenic variations in this continent have also been expressed.

POSSIBLE ACTION: Collaboration with Beachy and others in Europe makes this option implementable, although the tissue culture regeneration requirement may not be fully satisfied as yet. Yam could prove a useful "dry run" on its potyvirus, as regeneration and Ti transformation have been achieved (Osuji in Nigeria). For virus diseases, diagnosis and study of the pathogenic variations are called for. Monoclonal antibody and cDNA technology could be used (Thottappilly and Rossel at IITA).

On review, it is believed that the need for this virus resistance route and reliable diagnostic technologies may be highest for South American frog skin disease.

Finally it may be worth offering a summary of the socio-economic and philosophic background from which these discussions and recommendations originate. The following

premises seem to influence decisions.

1. If we grow enough food to feed our nation we generate dignity. We thus insulate ourselves from world forces that we cannot influence, so we gain in self-respect.
2. If we export more than we import we generate antonomy. Not even the World Bank can tell us what to do. So we aim to generate self-sufficiency.
3. If we choose to help our neighbors when we can, we will generate self-discipline at home and reap in our region both security and friendship.

BIOCHEMISTRY OF CYANIDE TOXICITY AND PHYSIOLOGICAL DETERIORATION IN CASSAVA

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I. Introduction. Key limitations to the crop's potential are a) the roots are bulky and deteriorate rapidly after harvest; b) the roots are sometimes less acceptable than other staples; a contributing reason being that cassava contains cyanogenic glucosides which are hydrolyzed to hydrogen cyanide when the plant structure is damaged. This cyanide can under some limited circumstances lead to toxicity problems associated with cassava utilization.

Cassava is principally produced by subsistance farmers. The extreme root perishability becomes a major problem for cassava intended for distant markets, a situation reinforced by rapid urbanization. The declining acceptability of cassava relative to cereals often reflects the higher urban price and or the poor quality of the perishable roots. These disadvantages outweigh the agricultural advantages of cassava in many market zones (e.g. Latin America). Interest in post-harvest technologies and processing/utilization aspects has increased recently, with the realization of their central importance to the achievement of the agricultural potential of the crop.

II. Cassava Deterioration.

Cassava roots deteriorate rapidly after harvest; this is related to two separate processes: one being physiological and the other microbiological. Collaboration between CIAT and ODNRI has elucidated the relative roles of these two processes. Initial loss of acceptability is due to physiological deterioration; this begins within 3 days, often within 24 hours after harvest. This is a humidity-sensitive wound response which reflects the degree of mechanical damage encountered by the roots. Microbiological or secondary deterioration involving several saprophytes usually occurs only after the commencement of the physiological deterioration.

Physiological deterioration involves changes in oxidative enzyme activities which generate phenols including catechins and leucoanthocyanidins, which in later stages of discoloration polymerize to form condensed tannins. The visual symptoms are blue or brown discolorations, which

usually appear initially in the peripheral vascular bundles and spread to adjacent parenchyma commonly termed vascular streaking. This is often accompanied by the development of unsatisfactory cooking qualities and adverse tastes. Differences exist in the susceptibility of different cultivars to physiological deterioration, but the cultivars which are slower to deteriorate usually have low dry matter content and are of less value. Deterioration rates are also influenced by different edaphoclimatic conditions: factors which reduce dry matter contents and produce defoliation tend to reduce deterioration.

Genetic modification of the oxidative enzymes involved in deterioration would modify plant phenol metabolism with probable implications for wound healing, lignin biosynthesis, flavor and texture. Consequently genetic modification of deterioration is not considered a promising objective in the medium term.

More effective solutions are low cost storage techniques of fresh roots and processing for urban markets. Recent developments will be summarized.

III. Cyanide Toxicity

The quantities of cyanide commonly present in cassava foods are rarely sufficient to cause acute cyanide-intoxication, the concern is over the long-term effects of cyanide ingestion. These chronic effects will be discussed. Work in Zaire has pointed to a correlation between cassava consumption and the development of goiter and related neurological syndromes in those areas where iodine is in short supply. Studies in Mozambique have drawn attention to the association between consumption of inadequately processed cassava at times of famine and spastic paraparesis. This disease has crippled several thousand women and children in cassava dominated areas of Zaire, Mozambique and Tanzania.

Cassava toxicity can be controlled either by attempting to prevent cyanide biosynthesis or by modified processing/ preparation prior to consumption. Screening trial have so far failed to locate acyanogenic cassava and the cyanide content of the principal tissues does not vary much with plant age. The biosynthetic pathway for cyanogenic glucosides is known in principle, but the cassava enzymes involved have not been characterized. Possible strategies will be considered by Monica Hughes. The link between root cyanide content and yield is thought to be questionable, but acyanogenesis may have implications for pest and disease resistance.

Cassava roots are traditionally processed by a wide range of methods to reduce their toxicity, improve their palatability and convert the perishable fresh roots into stable products. This leads to different degrees of cyanide removal in the product and studies to delineate the mechanisms involved in cyanide removal are in progress. In some cases the glucoside breakdown (linamarase) is the slowest step, in others the hydrolysis of the non-volatile cyanohydrin to HCN (hydroxynitrile lyase) is the limiting step in cyanide removal. Traditional cassava processing or simple modifications thereof are unlikely to remove all the cyanide. The scope for genetic engineering of the plant or (in limited circumstances) of the fermentative microorganisms involved in processing is considered.

CASSAVA STARCH- COMPOSITION, STRUCTURE AND BEHAVIOR

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Composition.
Starch is the major organic component of cassava as can be seen from the following analyses of the tuber and derived starch.

%	Cassava tuber	Cassava starch
Moisture	73.4	8.96
Protein	2.07	0.61
Fat	0.04	0.20
Ash	0.62	0.49
Reducing Sugar	0.54	0.84
Starch	18.2	87.84
Starch (moisture free basis)	68.4	96.4

Distinct differences have been observed in the starch content with increasing maturity. A peak value has been observed eight months after planting while the ensuing decrease to 78% at nine months was accompanied by an increase in sugar concentration from 3.5% to 5.7%.

The starch granules are compound granules varying in size from 5-40 m, the size varying with the maturity of the tuber.

The amylose content of the starch has been variously reported between 13.6 - 25%. Similarly there have been substantial differences in the reported values of the M.W. of amylose; e.g. Suzuki et al. state the weight average of a sample of amylose to be 1249,020 (dpw=7710) while Takeda describes a sample with a molecular weight of 430,920. Data is available comparing the structure of the amylose from cassava with those from corn, rice and wheat and a similar comparison has been made of the p-limit dextrins from these amyloses.

The chain length of the extracted amylopectin has been reported to be 21.2 (Suzuki et al), but a more comprehensive characterization of the distribution of these by Hizukuri has given the following results:

Cassava	Whole	A	B1	B2	B3	B4
CL (max)		11	18	38	62	
CL (Av)	26	12	21	42	69	115
Weight (%)	100	38.5	32.5	23.0	5.1	0.9
Moli (%)	100	47.0	41.9	9.4	1.5	0.2

Structure.
The cassava starches have been reported to exhibit variably "A" and or "C" X-ray diffraction patterns. High resolution (270 MH_z) NMR and IR (pellet) spectra cassava starches show little differences between varieties.

Gelatinization Behavior.
Typical gelatinization temperatures determined by d.s.c. for cassava starch, compared with potato and wheat starches are as follows

	Cassava	Wheat	Potato
$T_{initial}$ °C	54-58	46-53	49-58
T_{peak} °C	58-62	52-58	54-62
T_{final} °C	64-69	58-64	61-68

The gelatinization temperature of cassava starch obtained by light microscopy is 58.75 - 70.0 C, potato 56-66 C, wheat 52-63 C, maize 62-72 C, rice 66-77 C). The pasting viscosity curves with a Brookfield Synchro-Lectric Viscometer model RVT gave the following results:

Sample	Initial pasting temp(C)	Max Viscosity @ 90 C (poise)	Final Viscosity @ 90 C (poise)	Initial Viscosity @ 50 C (poise)	Final Viscosity @ 50 C (poise)
Cassava 4%	62	11	3	3	2
Maize 4%	82	4	5	8	10
Potato 1%	55	31	5	5	5

The values for pasting temperature, peak viscosity range and peak viscosity average with a Brabender viscomylograph of 5% cassava starch concentration are 65-70 C (lower than maize and wheat starch), 500-1500 and 1000 (higher than maize, wheat and waxymaize starch, but lower than potato starch), respectively.

A viscometric study of the dispersion of whole and gelatinized cassava starch suggests that the instability of cassava starch paste and its poor gelling characteristics (compared with maize or wheat starch pastes) is due to the weak intermolecular forces withing and between the starch granules.

The syneresis of 5% pastes, expressed as a percentage of water released from the gel is as follows when stored at room temperature:

% Weight Loss of Water (Syneresis) by Gels on Storage

Storage (days)	Cassava (% loss)	Corn (% loss)
6	-	41.6
10	2.4	41.1
17	5.6	40.8

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GENETIC MANIPULATION OF CYANIDE TOXICITY IN CASSAVA

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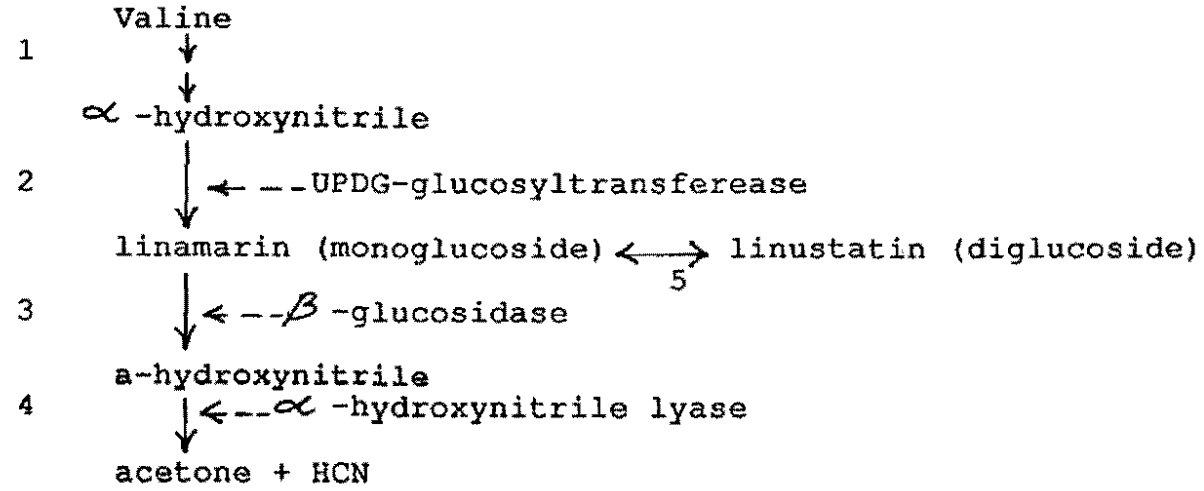
A successful strategy for the genetic manipulation of cyananogenesis in cassava depends upon knowledge of:

a) the biochemical and physiological basis of HCN production in different tissues
b) the genetic control of cyanogenesis
c) interaction of (a) and (b) with the environment.

In general, very little is known about these areas in cassava itself, but since the biosynthesis of cyanoglucosides and their enzymic degradation has been shown to be very similar in different species, some predictions about the mechanisms involved may be made by analogy with other species. Studies on species, such as Trifolium repens (white clover), Linum usitatissimum (flax) and Hevea brasiliensis (rubber) which produce the same cyanoglucosides as cassava are particularly useful. In addition, homology between a white clover gene for the cyanogenic-glucosidase and cassava genes has recently been demonstrated, and this indicates that genetic tools developed in another species will be of future use in cassava studies.

Cassava is an outbreeding species with 2n=36 chromosomes and is usually considered an amphidiploid or sequential allopolyploid. Its putative tetraploid origin does not necessarily mean that four copies of the genes controlling cyanogenesis are present in the genome. However, the fact that it is outbreeding means that, even given the development of an efficient selective screening system, a straightforward mutational breeding program (based on either a conventional or molecular protocol) would not be reliable as a method of eliminating HCN from the crop.

Cyanogenesis in cassava can be summarized as follows:



The physiology, biochemistry and genetic control of steps 1-5 will be discussed in relation to the development of screening and breeding strategies for cassava. The possible use of molecular and cell culture techniques in manipulating cassava cyanogenesis will also be discussed within the framework of these steps. Areas where more fundamental information is required will be outlined.

A vital prerequisite for successful conventional breeding program is the existence of variation within the crop. It is essential that more information is obtained about variation in cyanogenesis in cassava (and possible the related species Manihot pringlei). There are well established techniques available to measure the independently variable biochemical components of HCN production. In addition molecular probes can be developed as useful screening tools.

The extent to which molecular and cellular techniques can contribute to a realistic strategy for HCN elimination will be discussed in relation to the limitations presented by the system under the following headings:

i) screening for potential 'breeding' material
ii) exploitation of somaclonal variation
iii) in vitro genetic manipulation

ISOLATION OF CASSAVA LINAMARASE: EVIDENCE THAT ENDOGENOUS LEVELS ARE INSUFFICIENT FOR EFFECTIVE HYDROLYSIS OF LINAMARIN

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We have purified cassava tuber linamarase to homogeneity by extraction with phosphate buffer, DEAE Sephadex ion exchange chromatography, heat treatment and FPLC chromatofocusing. Similar to linamarases isolated from other species, the cassava enzyme has a molecular weight of 65 Kd and a pI of 3.4. The pH optimum using pNPG as a substrate is 7.5 and the temperature optimum is 55 C, with 55% of optimal activity at 30 C. As previously reported by T. Wood, Tris buffer is inhibitory. Kinetic analyses at optimal temperatures and pH for linamarin indicated that the Vmax and Km were 29.4 (mmol/mg protein/hr) and 1.9 (mMol) respectively.

Numerous investigators have demonstrated that different processing techniques for cassava yield different levels of "bound cyanide" or linamarin in the food product. In general, processing techniques which are less than optimal for linamarase activity yield the highest levels of residual linamarin. We infiltrated crude linamarase (Cooke et al. 1978, Phytochem, 17:381) into 3 mm thick cassava chips and determined levels of linamarin remaining in the tissue after 4 days drying by derivatization with ASTFA and quantification by gas chromatography. In triplicate experiments buffer infiltrated chips has average linamarin contents of 1.02 umol/gdw whereas chips infiltrated with linamarase (activities equivalent to that in the tissue) in buffer had a linamarin content of 0.59 umol/gdw or 40% less. Infiltration with higher levels of linamarase led to further reductions (2X linamarase = 70% reduction). These results suggest that endogenous levels of linamarase are insufficient for hydrolysis of linamarase to safe levels (0.28 umol/gdw) recommended for human consumption.

These observations indicate that a strategy for lowering the cyanide toxicity of cassava food products based upon elevation of celular levels of linamarase is feasible. We are presently cloning the gene which encodes linamarase and in collaboration with Dr. David Bisaro at the Biotechnology Center are investigating the feasibility of using gemini viruses (TGMV) as transformation and expression vectores in cassava. This strategy will be discussed.

GENETIC ENGINEERING OF CROP PLANTS FOR IMPROVED NUTRITIONAL VALUE

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The biosynthesis of amino acids from simpler precursors is a process vital to all forms of life, as these amino acids are the building blocks of proteins. Organisms differ markedly with respect to their ability to synthesize amino acids. In fact, virtually all members of the animal kingdom are incapable of manufacturing some amino acids. There are twenty common amino acids which are utilized in the fabrication of proteins and essential amino acids are those protein building blocks which cannot be synthesized by the animal. It is generally agreed that humans require eight of the twenty common amino acids in their diet. Protein malnutrition can usually be ascribed to a diet which is deficient in one or more of the essential amino acids. A nutritionally adequate diet must include a minimum daily consumption of these amino acids.

When diets are high in carbohydrates and low in protein, over a protracted period, essential amino acid deficiencies result. The name given to this undernourished condition is "Kwashiorkor" which is an African word meaning "deposed child" (deposed from the mother's breast by a newborn sibling). This debilitating and malnourished state, characterized by a bloated stomach and reddish-orange discolored hair, is more often found in children than adults because of their great need for essential amino acids during growth and development. In order for normal physical and mental maturation to occur, the above mentioned daily source of essential amino acids is a requisite. Essential amino acid content, or protein quality, is as important a feature of the diet as total protein quantity or total calorie intake.

Some foods, such as milk, eggs, and meat, have very high nutritional values because they contain a disproportionately high level of essential amino acids. On the other hand, most foodstuffs obtained from plants have lower nutritional value because of their relatively low content of some (or in a few cases, all) of the essential amino acids. Generally, the essential amino acids which are found to be most limited in plants are isoleucine, lysine, methionine, threonine, and tryptophan.

In the diet of people inhabiting a typical developed country, few amino acid deficiency problems arise. Primarily, because their diet is composed of a mixture of a wide range of animal and plant proteins. That situation is, however, not true in many developing countries. In a number of cases, the total food intake, perhaps 80-90%, is highly dependent on a single crop.

Rice, for example, is the major staple in Asia while cassava is the staple in much of South America and Africa. Once there is heavy dependence on plant protein from a single source, its essential amino acid composition becomes of critical importance. In those situations, where the food source is a single plant, it would be highly beneficial to "engineer" the plant to produce proteins with a balanced essential amino acid content. There are several potential methods which exist to achieve this objective. First, utilization of conventional plant breeding techniques to improve protein quality, second, manipulation of the existing storage protein genes to modify them for increased levels of the essential amino acids, and third, construct synthetic genes which encode proteins enriched in essential amino acids.

Although the first method has been applied with some results in the case of maize (i.e. the Opaque 2 mutant, so-called high lysine mutant) the screening techniques and effort involved make this an ineffective method. Besides, plant proteins are deficient in more that one of the essential amino acids which compounds the level of difficulty to achieve a nutritionally complete maize utilizing this method. The second method relies heavily on the isolation and purification of endogenous storage protein genes which is not always feasible and to optimize these existing genes for essential amino acid content is not an easy task. Let us, therefore, look in detail at the third method: the use of synthetic genes to produce proteins rich in essential amino acids for overall enhancement of the quality of total plant protein.

The innovative methods of genetic engineering offer a novel approach to modifying the essential amino acid composition of plant proteins and can thus increase their nutritive value. Our approach is not to try and modify the existing plant proteins, but rather, to supplement these with new synthetic proteins which have a high content of essential amino acids. To produce a synthetic protein of known amino acid composition, we constructed a DNA fragment with the appropriate codon sequence (the three base linear array of the genetic code). These DNA fragments can be produced using modern chemical techniques and can be introduced into various microorganisms via gene cloning or recombinant DNA technology. The cloning and expression of synthetic DNAs, containing repeated codons for a single amino acid, could be

used as a means to supplement the essential amino acid content of plant protein. However, as we have stated previously, plant proteins are deficient in more than a single essential amino acid, so, in the design of a useful synthetic protein, the total amino acid composition of plant proteins must be taken into consideration.

We have constructed, cloned, and obtained expression of genes in bacteria which code for proteins with a high content of the essential amino acids found to be most deficient in plant-derived proteins. This was done in bacteria first in order to facilitate the analysis of the new genes and their protein products. The sequences for several of these synthetic gene fragments (we call this the HEAAE-gene for "high essential amino acid encoding" gene) have been deduced and the particular encoded protein sequences were obtained by inspection of the genetic code. These gene fragments were constructed symmetrically so that a protein, containing a high content of essential amino acids, would be produced no matter which strand of the synthetic DNA was ultimately read by the cell's protein synthesis machinery. The production of these synthetic proteins in the edible portions of these plants can improve their nutritive value and thus increase their importance as basic food crops.

This method of gene synthesis is flexible enough to produce proteins possessing any particular amino acid composition. Therefore, proteins could be specifically designed to supplement any desired animal feed or human food. It should be pointed out thay the insertion of lysine at frequent intervals in these synthetic proteins provides numerous sites for proteolytic attack by trypsin (one of the main protein-degrading enzymes found in the digestive tract). This feature is important as it increases the bioavailability of the supplemental protein. Bioavailability refers to the amount of amino acids actually absorbed from a particular dietary protein and used by the organism to make its own protein.

USAID funded projects between the International Potato Center (CIP) in Lima, Peru, the International Center for Tropical Agriculture, (CIAT) in Cali, Colombia, and the Department of Biochemistry, Louisiana State University, USA, has led to the successful development of routine methods for the insertion of synthetic nucleic acid fragments into the potato and we are working towards the development of the same techniques for sweet potato, cassava, and rice.

MOLECULAR BIOLOGY AS A TOOL FOR THE PROTEIN ENRICHMENT OF TUBEROUS CROPS

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The tropical root crops like Manihot esculenta (cassava), Ipomoea batatas (sweet potato), Colocasia esculenta (taro) and Xantosoma atrovirens (tannia) are mainly cultivated in Latin America, West Africa, Caribbean and Pacific countries. They represent one of the most important source of food in the developing countries located in those areas[1].

The world consumption per person of tropical root crops is 57kg/year and about half of this amount is related to cassava[1]. Root crops are mainly used for human and animal food. The international trade in cassava has been increasing since the early 1970s. Countries like Brazil, Thailand, and Angola, among others, are important exporters of cassava and cassava products to West Germany, U.S.A., France, Japan and Benelux Countriesr[1,2].

Cassava has the following agronomic advantages over other food crops:

1. It can be planted any time of the year in the tropics;
2. It produces more calories per ha per day compared with rice, wheat or maize and at lower cost[3];
3. It is highly tolerant to pests[4], depleted soils[5]. high or low pH and can grow with as little as 51 cm of annual rainfall[1].

All those advantages make cassava an important subsistence crop, but cassava is essentially a starchy food and has to be used in a diet that is adequate in proteins from other sources. Unfortunately, in the poor areas of Brazil and West Africa, people are forced to survive on diets where cassava is the primary food source.Since cassava, as well as the other tropical root crops, is poor in some essential amino acids, like methionine, these people suffer serious protein deficiency problems. Genetic improvement of the protein content has resulted in a plant with a high content of cyanidric acid, low resistence to viral diseases and slow growth in depleted soils[7,8]. Moreover an increase of protein content is only significant when paralleled by an increase in essential amino acids. Therefore genetic crosses may not be the right approach to improvement of this crops' food value.

The advent of recombinant DNA technology and cell culture procedures made feasible the direct modification of plant genomes by introducing specific genes. The success of such

modification is highly dependent on controlling the sequences that dictate how frequently a gene has to be described. The characterization of root-specific promoters would be a major step towards the transfer and successful expression of a foreign gene into root crops.

We are studying the organ-specific expression of plant genes using tropical roots crops as models. Root crops like sweet potato and taro express a few abundant proteins that appear to be root-specific[9]. As storage proteins are mainly being transcriptionally regulated[10], the production of those organ specific proteins might be under control of transcription.

In contrast to the above mentioned crops, cassava expresses a complex electrophoretic pattern of proteins at very low levels[9]. Therefore it is tempting to speculate that the absence of strong promoters contributes significantly to the low amount of proteins in cassava roots. In order to test this hypothesis, strong promoters isolated from sweet potato and taro will be used to express a reporter gene in cassava and other tropical root crops. The effective strong promoters are going to be linked to the coding sequence of the methionine rich protein, that contains 21% of methionine[10,11]. By using suitable vectors, the modified genes will be introduced in the tropical root crop cells, and transgenic plants with improved nutritional qualities may be regenerated.

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ENGINEERING PROTEINASE INHIBITOR GENES TO IMPROVE CROP PROTECTION AND PRODUCTION

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Proteinase inhibitor proteins are part of the complex array of defensive chemicals of plants directed against herbivores and pathogens. The inhibitors comprise at least six non-homologous gene families in the plant kingdom where their expression is developmentally and/or environmentally regulated. Members of the inhibitor I and inhibitor II families from Solanaceae, and members of the Bowman-Birk family from Fabaceae, are expressed in leaves in response to herbivore attacks. The response to wounding is systemic, with inhibitors being synthesized in distal leaves within a few hours following wounding. Oligouronide fragments of the plant'scell wall as small as the dimer and trimer appear to be part of the early signalling mechanism. The systemic induction appears be regulated by an additional second messenger system that is mediated by a phosphorylation of the plasma membrane.

Proteinase inhibitor genes, or chimeric genes containing their 5' and 3' regions fused with the chloramphenicol acetyl transferase open reading frame, have been used to transform tobacco and nightshade plants with foreign wound-inducible genes. Regions of the genes responsible for wound induction are currently being analyzed. The transacting factors responsible for wound-regulation are being sought to further understand the biochemistry and molecular biology of the signalling mechanism. Tobacco, nightshade, tomato, potato and alfalfa are being transformed with foreign proteinase inhibitor genes that code for a spectrum of proteinase inhibitor specificities in order to assess the potential usefulness of this approach to improve natural plant defensive systems.

Proteinase inhibitors comprise about 10-15% of the soluble proteins of potato tubers. The inhibitors are rich in essential amino acids such as lysine and the sulfur-containing amino acids. The levels of inhibitors are generally proportional to the total protein contents of tuber varieties. Levels of inhibitors determined by a rapid immunological assay have been employed to select potato lines having high protein levels in tubers, and tomato species with high protein levels in fruit. Thus, selecting for high levels of proteinase inhibitors or transforming plants with foreign inhibitor genes under tissue specific regulation, could have potential usefulness in increasing plant protection and production as well as improving food quality. (Supported in part by the USDA Competitive Grants Program, National Science Foundation and EniChem Americas Inc.)

NOVEL STRATEGIES FOR VIRUS RESISTANCE IN PLANTS

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We have developed a novel strategy for producing plants that resist virus infection which should be useful for controlling virus diseases in a wide variety of crop plants. The method involves producing genetically transformed plants (i.e., transgenic plants) that express a chimeric nuclear gene comprised of a strong transcriptional promoter, a DNA copy of the viral capsid protein gene, and DNA sequences that control the polyadenylation of the gene transcript (Beachy et al., 1987). In our work we used the coat protein gene from tobacco mosaic virus (TMV) and a transcriptional promoter from cauliflower mosaic virus. This chimeric gene was transferred to a disarmed strain of Agrobacterium tumefaciens. The modified A.tumefaciens was then used to transform tobacco and tomato leaf cells. Transformed cells were then regenerated to produce whole plants that expressed the coat protein (CP) gene, and accumulated between 0.01% and 0.1% (w/w) of the leaf protein as viral capsid protein. The gene was in all cases examined, inherited in progeny as a dominant Mendelian trait (Powel Abel et al., 1986).

Seedlings that expressed the CP gene (CP+) were resistant to virus inoculum concentrations over 4 orders of magnitude. Resistance was exhibited by (1) causing a reduction in the number of sites of infection on the inoculated leaves (Nelson et al., 1987), and (2) reducing the rate of spread of virus in the event of infection. Thus, CP (+) plants either escaped systemic infection, or displayed a delay in development of disease symptoms.

We have expanded considerable effort to characterize the cellular and molecular mechanisms that are responsible for this resistance which we refer to as "coat protein protection". It is known that resistance is expressed in

protoplasts as well as in whole plants, indicating that at least one facet of resistance is an intracellular event (Register and Beachy, 1988). Furthermore, we have found that resistance to systemic spread involves preventing virus from moving from the leaf mesophyll cells into and/or through the vascular system of the CP (+) plants (Wisniewski and Beachy, submitted). We are continuing our investigations into these aspects of coat protein protection.

Since our description of coat protein protection against TMV, other research laboratories have used similar strategies to develop resistance against alfalfa mosaic virus (Tumer et al., 1989; Loesch-Fries et al., 1987; van Dun et al., 1987), cucumber mosaic virus (Guozzo et al., 1988), potato virus X (Hemenway et al., 1988), tobacco rattle virus (van Dum et al., 1988). These and other unpublished reports from several other laboratories (in Europe, Asia, and the U.S.A.) lead us to conclude that this approach will be useful in the control of many different types of plant viruses.

To date, a single report has been published which indicates that transgenic CP (+) tomato plants can control virus diseases under field situations (Nelson et Opal., 1988). In these trials transgenic tomato plants that express the TMV-CP gene were planted in the field, and were later subjected to inoculation with TMV. While all of the CP (-) plants developed systemic infections and disease symptoms in the expected period of time, not more than 10% of the plants that were CP (+) became systemically infected. Furthermore, and to our surprise, these CP (+) tomato plants had a fairly high level of protection against tomato mosaic virus, a virus somewhat related to tobacco mosaic virus. We concluded from these and other experiments that coat protein protection is able to provide a broad range of protection to the virus from which the coat protein gene was taken, as well as to other related strains of the virus. Although many other similar experiments are needed, we are optimistic that genetic transformation can be used to produce field levels of resistance in a rapid and convenient manner readily adaptable to many types of plants, including cassava and other tropical crop plants.

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NOVEL MECHANISMS OF INSECT RESISTANCE IN PLANTS

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In recent years, several methods have been developed to introduce foreign DNA in plant cells. The most successful procedure is the Ti-plasmid mediated gene transfer by Agrobacterium. One of the most interesting applications of this technology is the engineering of insect resistant plants through expression of insecticidal proteins. At the moment, the preferential source of such insecticidal proteins is the bacterium Bacillus thuringiensis (B.t.).

From all different B.t. insecticidal proteins, three major pathotypes have been described: strains toxic to Lepidoplera, Diptera and Coleoptera. The three pathotypes have each a typical crystal protein pattern, as can be demonstrated by SDS-PAG electrophoresis. B.t strains active against Lepidoptera (B.t. kurstaki, B.t. aizawai, etc.) contain crystal proteins in the range of 130 Kd, which are proteolitically processed in the insect midgut to 60 kd toxins. The crystals of the Diptera pathotype, exemplified by B.t. israelensis, contain at least 4 different proteins, ranging from 135 kd to 28 kd. In crystals of B.t. tenebrionis, a Coleoptera specific pathotype, there is one major protein of 66 kd. Even within the group of Lepidoptera toxins, different types can be discriminated by electrophoresis and by their immunological reaction with a panel of monoclonal antibodies. There is a good correlation between a certain crystal protein type and its toxicity for some Lepidoptera species. It is therefore essential to select the B.t. crystal protein with the appropriate toxicity, in order to engineer resistance in a particular crop against its major insect pests.

The idea to engineer insect resistance in plants by transformation with a B.t. gene was first tested with tobacco. We selected a B.t. toxin, Bt2, which is active against Lepidoptera such as the tobacco hornworm (Manduca sexta) and the tobacco budworm (Heliothis virescens), both pests on tobacco. Bt2 is a protoxin and is proteolitically activated in the insect gut into a 60 kd protein. Using deletion clones, the gene fragment corresponding to this toxic polypeptide was accurately mapped. This allows us to eliminate non-essential sequences from the Bt2 gene and to use truncated versions of the gene.

The expression of foreign genes in eukaryotic cells depends upon the site of insertion in the genome, even when they are hooked up to strong expression signals. When using a translational fusion between the gene of interest and the neomycin phosphotransferase gene (neo), a selectable marker gene, one can select for high expressions of the fusion product in transformed cells by selecting for resistance against kanamycin. Chimaeric genes were constructed with the neo gene fused to the entire Bt2 coding sequence or fused to truncated Bt2 genes. These chimaeric genes were transferred to tobacco plants by leaf disk infection with the Agrobacterium tumefaciens vector system and shoots were selected with kanamycin.

Efficient transcription of the genes and expression of the active protein was detected in leaves of the transgenic tobacco plants. Plants transformed with the truncated Bt2 genes expressed higher levels of Bt2 toxin and proved to be highly toxic to tobacco hornworm larvae. The insecticidal trait is stably inherited and several rounds of field trials showed protection of transformed tobacco against feeding damage by both tobacco hornworm and tobacco budworm larvae. The transformation experiments with tobacco have been repeated with tomato and potato plants, resulting in tomato and potato plants resistant to both Lepidoptera.

These results clearly exemplify the feasibility of using genetic engineering techniques to generate plants resistant to certain insect pests. Transfer of different Bacillus thuringiensis genes into a whole range of crops and vegetables may provide agriculture with a new and evironmentally superior method of controlling destructive insect pests.

Cassava is one of the most important sources of food energy in the tropics. Because there are no high-yielding varieties resistant to diseases and pests, average yields of cassava are low. In the Americas the cassava hornworm (Erinnyis ello) is considered as a major pest which can rapidly defoliate plants, resulting in significant loss of yield. Since cassava hornworm outbreaks can be controlled by spraying Bacillus thuringiensis spores, it appears that the hornworm is indeed sensitive to some of the known B.t. toxins. Recent advances in transformation of cassava indicate that it may be possible to engineer insect resistance by transferring Bacillus thuringiensis toxin genes in cassava.

GENETIC ENGINEERING OF CROP PLANTS FOR IMPROVED NUTRITIONAL VALUE

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At the outset, most of our studies have concentrated on the insertion, into plants, of synthetic genes coding for proteins high in essential amino acids. Efficient production of this protein would increase the overall nutritional value of crop plants. Our attention is now extending to the possibility of using both synthetic and purified genes as a way of conferring new forms of resistance to a wide range of plant diseases and pests. The introduction of genes into plants encoding potent antimicrobial proteins, derived from insects, may significantly augment the level of their resistance to bacterial and fungal diseases. Using modern techniques, these genes could be introduced into plant tissue and this tissue could then be manipulated to produce viable plants.

When confronted with a disease problem, the plant breeder has relied on the fact that somewhere, among plants of the same or closely related species, an individual can be found which retains resistance to that particular disease pathogen. This single plant can then be incorporated into the breeding program by attempting to introduce its genes into already well accepted, traditional varieties, through the use of sexual crosses. It is assumed that this will eventually produce a hybrid plant which retains all of the desirable traits including the new one-- resistance to the disease. This has been the hallmark of breeding plants for disease resistance.

One of the main weakness of plant breeding is its dependency upon sexual crosses which are limited to the genes that exist in only one relatively small group of organisms-the species. Recombinant DNA, the manipulation of genes in the test tube, allows the transcendence of inter-species barriers and makes novel genetic combinations possible. Plant genetic engineering offers the possibility of introducing a single trait, without altering other agronomically important characters, into a well accepted or traditional plant variety, providing for a method of "fine-tuning" specific cultivars. This technology will allow the incorporation of desirable and inheritable traits into plants, with some ease, rapidity, and with a high probability of success when allied with modern plant tissue culture techniques. The use of these methods, however, is not a goal in themselves, and should be coupled to conventional breeding in order to achieve plant modification. When used together, the scope is greatly enlarged for the possibility of plant improvement.

The hemolymph of an infected pupa of the Giant Silk Moth, Hyalophora cecropia, contains at least three groups of antimicrobial proteins. Lysozyme, the antibacterial protein found in egg white and human tears, and two other classes of antimicrobial peptides: cecropins and attacins. These antimicrobial proteins have a rather broad spectrum activity and are very effective in killing many different types of plant pathogenic bacteria and fungi. With this array of antimicrobial proteins, a genetically engineered plant would possess a rather potent arsenal composed of three different proteins which appear to work in different ways to actively destroy the pathogen. This multilevel defense system would present a formidable challenge to the invading pathogen, one which would be very difficult to evolve a means to circumvent. The probability that a pathogen would become naturally resistant to all three toxins at once is rather remote (about 1 in 10^{-18}). Also, it has been found that when used together, there is a synergistic effect exerted which will make it even more difficult for the bacterial pathogen to compete. In essence, it was demonstrated that attacin enhanced the activity of cecropin and lysozyme when tested together on E.coli. Primary results obtained in our laboratory seem to indicate that, at least for the plant pathogens and for some animal pathogens, there is a measurable synergism between cecropin and chicken egg-white lysozyme.

Therefore, the genes encoding proteins derived from the humoral immune response in H.cecropia are an attractive genetic system to incorporate into the genome of plants to protect them from diseases induced by bacteria and fungi. Natural resistance to disease and pest organisms, in plants, is a very complicated process. At present, it is an extremely difficult task to identify, isolate, and characterize the genes responsible for pathogen resistance. We believe, that with the introduction of the genes encoding antimicrobial proteins into the plant genome, a crop plants natural resistance to pathogens can be significantly augmented.

The world is a vast repository of potentially valuable genetic material locked within organisms by the natural barriers which isolate species. Recombinant DNA technology and plant genetic engineering allows the Earth's genetic resources to be exploited by providing a means of overcoming these barriers. Indeed, the potential for a new horizon in plant breeding is here and it may be possible, sometime in the near future, to endow plants with a permanent resistance to a variety of pathogens.

FIELD AND LABORATORY RESEARCH WORK ON CASSAVA IN ZIMBABWE

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We started tissue culture work in Zimbabwe in 1978 when I managed to assemble the basic equipment, a flow cabinet, and autoclave, some chemicals and a constant temperature room. We worked first, battling against incredible infection rates, to set up virus-elimination procedures -meristemming- for strawberry and potato. These are now effective and operating and contributing to Zimbabwe's high quality in these crops.

In 1982, we felt ready to tackle a tougher crop -cassava. We collected all local land races and imported both seed and 10 selected lines from IITA. When grown out, and put into single-row variety trials, the IITA lines yielded around 30 t/ha which was a big improvement on the local lines which were 10 to 20 t/ha.

Since 1982 we have embarked on what are now five cycles of crossing and selection, seeking adaptation to our specific conditions. We have been selecting for tuber shape, canopy shape, resistance to African mosaic virus and so on. Recently, many promising lines have been rejected on the basis of a lower tuber-to-total-biomass ratio, that is, harvest index. We are also looking for high dry-matter content in the tuber.

We have half a dozen promising lines which are now at advanced variety trial status on two different locations. We are confident these will yield over 40 t/ha/year in Zimbabwean rainfed conditions.

We are aiming to provide a "food security" crop for our farmers, a crop which will yield every season in proportion to whatever rains it receives. The reason is that many our farmers plant hybrid maize in areas where statistics show that they will get nothing at all every third year because of low or poorly-timed rainfall.

When we were just about ready to launch the best of these lines, we hit two problems. Firstly, there was a build-up of systemic fungus, Colletotrichum, causing severe losses. Secondly, this year and last year, we suddenly had serious African mosaic virus symptoms -again with major losses. Either the heavy virus infection has overcome the genetic resistance or, we suspect, the resistance may have been lost in our selection procedure since the disease does not always

show itself under Zimbabwean conditions. Thus, although the infection potential was regularly present in our susceptible end rows, the disease is not seriously manifested due, we thought, to our rather lower temperature. This has been a serious setback to our program and means we need an indexing system other than field observation. We are working on a tissue culture solution to this, but would welcome a diagnostic kit.

Nevertheless, the yields are still over 40 t/ha/yr and we will begin demonstrating their potential to government, rural farmers and commercial interests this next rainy season. Incidentally, the crop does best in Zimbabwe if it is harvested after two rainy seasons.

Biotechnology in cassava

Our hope is to carry these promising developments further and we look to modern methods of biotechnology to do this. This is part of a dream of building a critical mass of DNA and tissue culture scientists, so that we will participate in the coming gene revolution.

The biotechnology aspect of cassava development is for genetic improvement through: a. Somaclonal selection and b. Gene transfer of desirable traits from other species.

To do this, we need to crack certain technical barriers: 1. As far as I know, no one in the world can produce a plant from a cassava callus. 2. Similarly, no one can produce a protoplast of cassava that will divide and grow into an embryo and on to a plant. 3. Though CIAT was attempting to infect young <u>in vitro</u> leaves with Ti-doctored plasmids to provide a route for gene transfer, there is no sign of success yet. We would all welcome protocols for these three procedures.

In our laboratory in the last months, we have made some progress in both areas, somatic embryogenesis from <u>in vitro</u> leaves and embryogenesis from protoplast culture. It would be exciting if an African laboratory led the way to improving what is such a vital crop to so many African farmers.

Meanwhile, in the belief that the job needs to be done rather than on any guarantee of support, I have set up a small factory for micropropagation in my backyard. It will be run by one of our graduating students as from January 1989 and will provide virus-eliminating strawberries, potatoes and cassava for our farmers. The capital was borrowed from two farming businesses and I shall repay in potatoes and in mushroom spawn.

RESEARCH STRATEGIES TO OVERCOME PROPAGATION-RELATED CONSTRAINTS IN CASSAVA

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As cassava (Manihot esculenta Crantz) is grown under a wide diversity of physical and biological environmental conditions in the tropics and subtropics, it is exposed to a broad range of production constraints. One of the most commonly recognized sets of constraints is related to the vegetative propagation of the crop.

Alternative methods are proposed which would alleviate many of the problems resulting from traditional propagation by lignified stem cuttings. Emphasis is given to the possibility of true seed commercial production. Other possible options could be "artificial seed" from encapsulated somatic embryos, or transplanting of in vitro rapid-propagated plantlets.

Cassava, like many vegetatively propagated crops, appears to suffer from accumulated effects of viruses passed from one generation to the next. While the description of cassava viruses is far from complete, techniques of thermo- or chemo-therapy of meristem tip cultures are being successfully applied to eliminate viruses from vegetative material. Indications to date are that none of the viruses are passed through true seed.

Physical stake quality depends on an array of environmental and management factors. Among the most important of these are effects of stem storage, a common practice throughout the cassava-growing world. Nutrient loss, dessication and pathogen invasion are common during storage, resulting in loss of germination, vigor, yield and quality. On the other hand, true seeds may be stored with no loss of viability for many years under controlled conditions.

Multiplication rate in cassava normally varies between 1:10 and 1:20, and this low rate often limits the expansion of new varieties as rapidly as desired. Through true seed or artificial seed propagation, multiplication rates several fold higher could be attained.

In a seed-propagated cassava plant, the plant architecture would not be constrained by the need for lignified stems for planting material. A completely herbaceous plant might even be imagined, or some other alternative form to upright, late-branching types presently selected for.

A true seed technology for cassava presents many attractive potential advantages, but to make such an option viable will require a concerted research effort integrating several disciplines in both basic and applied fields. The principal areas envisaged requiring contributions in tissue culture or molecular biology are: virus identification and detection techniques, dihaploid production for developing parental inbred lines, physiological studies to model alternative plant architecture for a seed-propagated cassava plant, studies on artificial flower induction, and studies on seed physiology to overcome the erratic and delayed germination under lower than optimum conditions.

PHOTOSYNTHETIC PRODUCTION CAPACITY OF CASSAVA UNDER STRESS

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Cassava is the only known crop plant with photosynthetic characteristics of both C3 photosynthesis and C4 photosynthesis. Thus cassava seems to be evolving toward a C4 type plant from a C3 origin. In some unknown fashion cassava has incorporated C4 traits with C3 photosynthesis while maintaining high yields plus the vigorous ability to withstand environmental stresses such as drought and high temperatures. In sharp contrast in breeding work with other plant species, when C3 plants were crossed with C4 the F1 and other generations were inferior to either the C3 or C4 parent! This early breeding work was with Atriplex and Panicum species.

A number of research questions arise from this knowledge. In seeking answers to these fundamental queries the overall objectives are: i) to increase the yield of cassava: and ii) to enhance its ability to survive and yield well in a wider range of stress environments. The following is a brief listing of research questions which will be elaborated upon in the presentation along with examples of how basic cassava research can be used to facilitate applied production cassava research.

A. What are beneficial C4 traits in cassava? A testable hypothesis would be, that cassava photosynthesis is insensitive to low CO2 concentrations. Can the beneficial traits be transferred to other Manihots without a loss in yield?

B. What are the maximum yield capabilities within cassava? In studies with wheat or other species, yields are much higher than agricultural practices and show characteristic responses to photosynthetic photo flux density. Is this the same for cassava?

C. One explananation for a combination of C3 and C4 traits being beneficial is to increase the CO2 to rubisco through glycine decarboxylase. Is glycine decarboxylase a key enzyme in cassava? Antibodies to this enzyme could be prepared to screen the cassava germplasma for the enzyme and to use in breeding work.

D. How has harvest index changed in developing the cassava plant as a crop? Is there an increase in harvest index accompanied by dry matter increases? Two types of plant yield improvement changes have occurred in other crops; has either or both occurred in cassava?

E. Can the sink strength of cassava be measured biochemically? We have such a measurement for other crops, e.g. potato, and suggest that this new development in plant biochemistry be applied to screen cassava root sink strength.

F. One easily identified and heritable C4 trait is the carbon isotope fractionation associated with the initial CO2 fixation enzymes. This character can be assayed throughout the cassava germplasm and could be used in plant breeding work.

TISSUE CULTURE ACTIVITY AT THE INTERNATIONAL INSTITUTE OF TROPICAL AGRICULTURE (IITA)

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The tissue culture facility at IITA was established in 1979. Its objectives are to evaluate and develop culture media that are suitable for meristems and nodal culture of cassava, sweet potato, yams and cocoyams, to distribute virus-free clonal materials and to develop media for germplasm conservation of these crops. In 1981, when we began to distribute virus-free clonal (plantlet) materials to national programs, we faced problems of low survival rate after transplanting. A training component was therefore added. A three-week tissue culture training course was conducted every year with an average of ten participants per year. Since last year however, we have concentrated instead on individual training.

Currently, we are dealing mainly with cassava, yams, plantain and banana. The tissue culture activities on these crops can broadly be divided into four categories:

1. Disease elimination;
2. Rapid propagation and international distribution of virus-free materials;
3. Germplasm conservation, and
4. Evaluation and development of other tissue culture systems to assist breeders in obtaining desirable cassava and plantain varieties.

1. Disease elimination: meristem culture coupled with reliable virus indexing method has been effective in disease elimination, particularly viruses which are difficult to detect. Media for meristem culture of cassava, yams, sweet potato, plantain, banana and cocoyams were developed and used routinely in our laboratory. In cassava and yam, the efficacy in disease elimination is higher with a combination of heat treatment of mother plants followed by meristem culture. Over a hundred varieties of cassava, four hundred yams, more that one thousand sweet potatoes, two hundred and fifty plantain and banana and one hundred varieties of cocoyams were regenerated from meristem culture.

2. Rapid multiplication and international distribution: media for rapid multiplication using nodal cuttings (apical and axillary buds) are well established in these crops. It is possible to regenerate yam plantlets from tuber discs of freshly harvested yam tubers. The *in vitro* tuberization in yams was encouraged when the sucrose concentration in the media was at 5% level. Aerial tuber formation was also obtained under *in vitro* cultures. This provides opportunity

for distribution of virus-free yam clones using microtubers produced from virus-free plantlets.

Virus-free cassava clones have been distributed to 39 countries in Africa and to CIAT in Colombia.

3. Germplasm conservation: Conservation of root crops and plantain germplasm under field conditions has faced several problems. The most severe ones are the losses due to diseases and pest attack, drought and storage loss. In vitro reduced-growth storage methods have been developed for these root crops. These methods include lower incubation temperature, reduced-growth culture media and a combination of both. We have currently at IITA about one hundred clones of cassava (including ten Manihot spp.), 1,500 clones of sweet potato, 400 clones of yams and 150 clones of cocoyams maintained under in vitro reduced-growth conditions. The plantlets can be maintained for 1 to 2 years depending on the varieties and crop species. We have also 250 clones of plantain germplasm that are maintained under normal growth conditions and we are evaluating the possibility of using reduced-growth storage to maintain this crop.

4. Other tissue culture systems: somaclonal variations and gene transfer are very attractive approaches in developing new crop varieties with desirable traits. However, it has not been possible to regenerate plants from callus cultures of cassava. This is the major drawback to the application of biotechnology in cassava improvement.

We have been able to regenerate roots from callus cultures of cassava. Callus were also obtained from anther/microspore culture, however plant regeneration was not obtained. Somatic embryos of cassava were obtained from leaf (in vitro) explants. We are now testing this system with a wide range of cassava genotypes. Protoplasts were isolated and purified from in vitro leaf of cassava. Micro calli were obtained. Plantlets were also obtained from the culture of embryos of cassava from true seeds.

Embryo culture methodology has also been developed in plantain. It has been applied routinely to germinate hybrid seed of plantain and diploid banana which is resistant to black sigatoka disease. Somaclonal variations were obtained in plantain during the process of multiplication and were found to be genotype specific. One of the important findings in the somaclonal variation was the reversion of false horn type (lack of male flower) to french type (with complete inflorescence) which enabled us to use such materials in the breeding program. Proembryogenic masses were obtained from culturing slices of meristematic tissue of plantain with liquid media. Roots were obtained from the proembryogenic tissue upon transfer to culture media containing NAA and BAP or BAP alne.

In collaboration with scientists at the U.S. Department of Agriculture Laboratory in Beltsville, Maryland, IITA is aiming to use monoclonal antibodies and cDNA for virus indexing and diagnostic.

IN VITRO PLANT REGENERATION FROM SOMATIC TISSUES IN CASSAVA

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It was quickly established in the first in vitro studies with cassava that, whereas techniques involving shoot meristem cultures present few technical problems, those involving more disorganized cell or tissue cultures were likely to be constrained by difficulties associated with plant regeneration. Although progress has now been made with this problem, cassava must still be included in that group of important crop species with which the full range of in vitro techniques cannot be readily employed.

Our own studies, in collaboration with CIAT, fully confirmed the plant regeneration problems with callus derived from most parts of the cassava plant and from a range of genotypes. This lack of success led to more systematic investigations with various juvenile tissues and eventually it was shown that the membranous cotyledons from mature seeds would rapidly produce highly embryogenic tissues in response to media containing 2,4-D. These tissues produced large numbers of somatic embryos when transferred to a second medium containing a cytokinin in combination with a reduced concentration of 2,4-D.

The most obvious feature of the embryogenic tissue produced in this manner is its highly organized structure compared with that of the non-competent friable callus tissue which is produced only too readily from virtually any part of the juvenile or adult plant in response to a wide range of media. It is probably significant that the embryogenic tissue is composed of small densely-cytoplasmic cells which are tightly packed and associated with a distinct epidermal layer. These studies seemed to indicate that the somatic embryos arise by a budding process rather that from single cells, although the latter possibility could not be ruled out entirely.

Subsequent studies have shown that somatic embryos can be produced in a similar manner from immature leaf lobes, with the result that the technique is now applicable to clonal plants. It has also been shown that the embryogenic tissue can be induced to proliferate so that there is now the potential for producing large numbers of somatic embryos from a specific genotype.

Despite these successes, and the occasional reports of plant regeneration from cassava protoplasts, it has to be emphasized that, at present, regeneration can only be induced routinely with the embryogenic tissue, as described above. The competence to produce this type of tissue seems to be confined to explants from the more juvenile regions of the plant and attempts to re-establish it from the friable tissue produced from more mature regions and in rapidly growing cell cultures have so far been unsuccessful. This means that techniques requiring plant regeneration, following manipulations with single cells or protoplasts, cannot yet be used freely with cassava.

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IN VITRO MICROSPORE CULTURE FOR CROP IMPROVEMENT

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In a great number of crop plants haploids can be induced partheno- or androgenetically. Their use in breeding programs is theoretically accepted, and with increasing numbers of such haploids produced from a wide range of genotypes it is also accepted by practical breeders. The main advantage of induction of haploids and their chromosome doubling is the rapid production of complete homozygosity in plant species, and the chance to combine qualitative as well as quantitative inherited characters rapidly. In crosspollinating plant species such as Hevea providing double haploid lines obtained via anther culture is the basis for producing hybrids exhibiting heterosis. During the last few years, much progress has been made in improving the anther culture technique in crop plants, so much so that dicots like rape seed and potato, as well as monocots like rice, wheat, barley and maize lines descending from microspores, are now being checked in official yield trials by seed boards for licensing (Wenzel and Foroughi-Wehr, 1984). In barley, the method has been used successfully for the transfer of barley yellow mosaic virus resistance to commercial winter barley varieties (Foroughi-Wehr and Friedt, 1984).

The procedure of microspore culture involves the in vitro development of immature pollen (microspores with or without any surrounding somatic floral tissue). The success in anther- and microspore culture is predominantly dependent on the genotypes. There is evidence that genotypic differences are heritable and can therefore be transferred to poorly responding genotypes by combination breeding (Wenzel and Foroughi-Wehr, 1984). The physiological status of the plants at the time of anther excision also strongly influences the sporophytic potential of the microspores. The response in culture is predominantly influenced by the different growth conditions (light intensity and temperature) during various seasons (Foroughi-Wehr and Mix, 1979).

In monocots there is always a strong tendency for albino plantlet production from microspores; in average the proportion of albino to green plants was 4:1. Recently, however, the yield of green plants from anther culture in wheat and barley could be improved by using a liquid Ficoll-medium (Olsen, 1987; Chu and Hill, 1988; Bolik et al 1988).

Further progress is envisaged by culturing microspores in isolation from the anther, preventing cross reactions with the somatic tissue or within the densely packed spores. Thus, this offers a more efficient system to regenerate a random sample from the microspore population than does anther culture. This is particularly important when the desired trait is linked with low responsiveness, resulting normally in rather small regeneration rates. Additionally a fraction of vigorous types of pollen can be enriched in the culture. A procedure starting from single microspores passing through embryogenesis and developing at a high frequency into green plants is most advantageous and desirable, because early selection in microspore populations becomes possible. The isolotated microspore culture offers the possibility of combining single cell selection procedures with the advantages of a haploid system; this means direct expression of all genes independent of their recessive or dominant nature. As the presence of such characters can be influenced by parent selection, also simple selection systems will also work, being equivalent to one step of a possibly complex reaction. Microspores are either mechanically isolated from the anthers (in Nicotiana or Brassica; Lichter, 1982) or shed initially into liquid culture media (in cereals; Kohler and Wenzel, 1985). As this regeneration procedure additionally avoids callus formation, the normally undesired somaclonal (gametoclonal) variation will be reduced, resulting in a higher rate of normal green plants.

Furthermore, gene transfer to plant cells is increasingly becoming an important tool for applied plant breeding. The present lack of an appropriate vector system for gene transfer in monocots and the still limited success in regenerating protoplasts of some plant species, make microspores a promising system for gene transfer and subsequent regeneration of transformed plants.

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CRYOPRESERVATION OF CASSAVA MERISTEMS

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Cassava (Manihot esculenta Crantz), a vegetatively-propagated annual root crop, is extensively grown as a source of human food in the humid tropics. Techniques to regenerate plants from shoot apical meristems of cassava have been developed in this laboratory (Kartha et al. 1974) and the technique has been successfully applied to the production of mosaic disease-free plants (Kartha and Gamborg, 1975). The meristem culture technique has been successfully extended to short-term preservation of cassava collections at CIAT, Cali, Colombia (Roca, 1980), but alternative approaches have to be sought to warrant germplasm preservation for any extended periods of time. In recent years, cryogenic techniques have been developed for the preservation of meristems of a number of crop species (for details cf. Kartha, 1985). It would be highly desirable to similarly cryopreserve cassava meristems since the cryogenic storage temperature (-196 C) is believed to arrest all the metabolic activities of the specimens leading to the preservation of genetic fidelity which is vital to any strategy aimed at germplasm preservation.

Cassava, being a tropical species, is sensitive to low temperature injury. After several years of unsuccessful attempts, we were finally able to cryopreserve cassava meristems through the development of a novel droplet-freezing technique (Kartha et al. 1982). The meristems (0.5 mm in length) isolated from fresh sprouts of cassava nodal cuttings were treated with an optimal concentration of 15% DMSO (dimethylsulfoxide) + 3% sucrose solution for 15 min and frozen over an 18 m aluminum foil in 2-3 1 droplets of the cryoprotectants in plastic petri dishes at a cooling rate of 0.5 C min-1 to various sub-zero temperatures (-20; -25; -30; and -40 C) and stored in liquid nitrogen (-196 C). The meristems retrieved from liquid nitrogen storage, upon thawing (37 C) and return to in vitro culture on plant regeneration medium, exhibited various morphogenetic responses such as differentiation of callus and leaves and whole plantlets. The regeneration of plantlets occurred at a very low frequency. In subsequent studies (supported by the International Board for Plant Genetic Resources), the plant regeneration frequency could be increased to 30% by preculturing the meristems, prior to freezing, in liquid nutrient medium containing 9% sucrose and gradually increasing the sorbitol concentration from 0 to 1.0 M over a 24 h period.

Over 250 plants produced from cryopreserved meristems of 4 cassava cultivars were field tested, following further micropropagation, at the CIAT experimental farm by Dr. W.M.Roca. These plants exhibited full stability for all the 5 morphological and 6 agronomic characters examined. Similarly, analysis of esterase isoenzyme activity by electrophoresis showed no variation in the cryopreserved material. These results taken together are indicative of the fact that cryopreservation is a safe and reliable technique for the preservation of cassava germplasm. However, in order for the technique to be routinely employed for cassava germplasm preservation, more research is warranted to increase the recovery of plants post-cryopreservation.

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MULTIPLE REGULATORY ELEMENTS FOR SELECTIVE GENE EXPRESSION IN TRANSGENIC PLANTS

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The development of higher plants requires an orderly expression of selective genes at the right time and in the correct cell type. As a first step toward elucidating the mechanisms that govern differential gene expression during development, we have chosen representative examples of photosynthetic and floral-specific genes and investigated 1) DNA sequence elements for light-responsiveness and cell-type specificity and 2) Nuclear proteins that interact with such cis regulatory elements.

a) Photosynthetic Genes. The two major photosynthetic genes in higher plants are rbcS and Cab which encode the small subunit of ribulose-1, 5-bisphosphate carboxylase and the chlorophyll a/b-binding protein, respectively. Both genes are expressed in chloroplast-containing cells in response to light.

We have used transgenic tobacco as an expression system to analyze in detail cis-acting elements for regulated expression of a pea rbcS-3A gene that contains 410 bp of 5' upstream sequence. Initial experiments indicated that a 280 bp 5' fragment (-330 to -50) functions as an enhancer to confer light-responsiveness and organ-specific expression on a heterologous promoter. Further analyses revealed that genetic information for the two regulated functions is present both upstream and downstream of -170. By site-specific mutagenesis we have identified two conserved sequences, designated boxes II and III (-112 to -150) that are both needed for light-responsive transcription of rbcS-3A. The sequence of box II is GTGTGGTTAATATG and that of box III, ATCATTTTCACT, and together they constitute a light-responsive element (LRE). Sequence motifs similar to those of boxes II and III are repeated at least two times between -410 and -170. The presence of these reiterated LREs explains why sequences both 5' and 3' of -170 can direct light-regulated and organ-specific expression. The effect of mutations in boxes II and III can only be measured when the reiterated LREs upstream of -170 are removed. Thus, in a construct containing only 170 bp of 5' upstream sequence, i.e. only one set of LRE, a GG (146, 147) --> CC transversion is sufficient to abolish transcription.

However, the sequences upstream of -170 are dispensable only in the mature leaves of a green plant. In contrast, in the young, expanding leaves at the top of a green plant, as well as in seedlings, the distal elements are required for high-level expression. Therefore, the functional redundancy is not absolute, and the requirements for rbcS-3A expression change during plant development.

In a separate analysis, we found that boxes II and III can act as a negative LRE when they are interposed between the 35S enhancer and the 35S TATA box. These results imply that there is a sequence overlap between the positive and negative elements. Experiments are in progress to see whether these two functions can be resolved by point mutations.

Analysis of the Nicotiana plumbaginifolia rbcS-8B 5' upstream region reveals results similar to those of pea rbcS-3A. We found a proximal element located between -312 and -102 that confers organ-specific and light inducible expression upon a heterologous promoter. In addition, we uncovered a novel genetic element located between -1038 and -589. This distal element can confer on a heterologous promoter the organ-specificity of a typical rbcS gene but the enhanced transcription in leaves is insensitive to light.

To investigate protein factors that specifically interact with regulatory DNA sequences upstream of rbcS-3A we prepared pea nuclear extracts for gel retardation assays and DNaseI foot-printing experiments. We have identified in the extracts a factor, GT-1, which binds to boxes II and III, as well as to the redundant LREs upstream of -170. Using a series of 2 bp substitution mutations we have defined a core of 6 residues (GGTTAA) within box II (GTGTGGTTAATATG) that are critical for binding. The most detrimental mutation for binding, which changes the double Gs to Cs, is sufficient to eliminate detectable expression in vivo when only 170 bp of 5' flanking sequences are present. The simplest interpretation of these data is that GT-1 is an activator of rbcS-3A transcription. Footprinting experiments show that GT-1 is present in extracts from both light-grown and dark-adapted plants. Thus if GT-1 binds differentially in vivo, it must be post-translationally modified or serologically blocked from binding by another factor in response to light.

b) Floral-specific Gene. 5-Enolpyruvylshikimate-3-phosphate (EPSP) synthase catalyzes a key step in the shikimate biosynthetic pathway. The Molecular Biology Group at Monsanto has reported that the gene for petunia EPSP synthase is expressed 25-fold higher in flower petals as compared to other organs. We have constructed chimeric genes comprised of the EPSP synthase upstream region fused to a CaMV 35S TATA sequence with CAT, the reporter gene. Three

constructs (-1800 to -285; -1800 to -130; and -1800 to -30) have been analyzed in transgenic petunia. The expression pattern of all three constructs resembles that of the endogenous gene, i.e. high levels in the petals, undetectable in the leaves or roots, with low and variable levels of expression in stems. Moreover, the petal expression level is developmentally regulated. There is little CAT enzyme in unopened floral buds, but the level increases dramatically on the second and third days after the bud opens.

To analyze cell-specific expression of the EPSP gene we fused the upstream region (-1800 to -285) to a 35S (-90 to +8) segment and used GUS as the reporter gene. Histochemical analyses of petal sections revealed that the gene is expressed in the upper and lower epidermis of the corolla, whilst in the floral tube, expression is limited to the upper epidermis. Newly opened buds show only very light staining in the epidermis of the corolla while mature buds show intense staining. Thus, the developmental regulation of this gene involves increased expression in the epidermal layers of the petals.

FOCUSED LARGE SCALE PROGRAM FOR A CASSAVA RESEARCH NETWORK

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A mechanism is suggested for tackling agricultural research opportunities that are too large, too complex, too orphaned, or are considered impossible to achieve with the resources and procedures presently available to individual research institutions. The proposed mechanism and institutional arrangement provides for focus, planning, coordination, and supplemental funding needed for "Manhattan" type research projects related to agriculture.

The basic concept is that the suggested approach provides a specific focus on objectives and within a time frame.

Participants should logically include appropriate research cooperators in the public and private sectors, basic and applied, domestic and international. A chosen project would of necessity be a very broad interdisciplinary, inter-institutional research program.

In the past there has apparently been a lack of "convenors" or "initiators" of such broad scale complex projects. A newly formed International Fund of Agricultural Research (IFAR) could be that "catalyst". Implications of such prospects are profound and exciting.

IFAR was established in the USA about a year ago to stimulate interests and support for international agricultural research. It is a non-profit organization with seed money from USAID, the Ford Foundation and the Rockefeller Brothers Fund. Similar support organizations are being formed in other countries. IFAR does not expect to be a "doer" of research, but because of its neutrality and relationship to the international agricultural research community it can serve as a catalyst to stimulate cooperation.

The concept described here to provide focus, planning, coordination and funding to tackle large complex projects would call for: a. identification of a suitable worth-the-effort project with an objective and time focus, b. assembling a group of peers who are currently interested in working on bits and pieces of such a project, c. identifying the "state-of-the-art" including who is doing what where, d. the peer group to outline a program of what is essential in order to achieve the time-focused objective, e. establishing good communication among those working

on relevant parts of the project, f. identification of essential work that is not being done, and g. securing supplemental funding so that all parts come together to achieve the desired results.

Many potential projects could fit this concept. A specific Cassava Cooperative Research Networking project is suggested:

"Hybrid (F_1) True Seed Cassava with Virus Resistance and No Significant Cyanide Toxicity Problems by the Year 2000"

Following the modus operandi outlined above as it relates to such a project could be as follows:

1. Identify this as a project or choose another but make it a. have very important world wide implications especially for small farmers and as an assured supply of low cost food for consumers, b. large scale, worth-the-effort, c. involve several disciplines and institutions, d. have identifiable focussed objectives within a specified time frame.

2. Have a working group composed of interested participants to identify a. requirements for the project to be successful, b. who is already doing what that is relevant to the project as a whole, c. current resources available to potential cooperators, d. a draft protocol to help guide how the network would operate e. results of this conference as a "state-of-the-art" report or commission supplemental work, and, f. invite other relevant participants to join the network.

Since this would be a voluntary cooperation, participants could drop out or new ones be added at any time.

3. The group as a whole would choose a leader, or chairperson to act as secretariat, coordinator and convenor.

4. Network participants would each identify his or her part of the project and the current source of funding. It would not be expected that additional funding would be used to replace current funding.

5. Participants would identify work that is not being done but is essential to the success of the project and also identify who could work on the missing links. Thereafter they can recommend the need for additional funding. IFAR would try to identify sources of such funding and/or help secure the funding.

6. The project "secretariat" would provide communications by: a. personal contacts b. periodic conferences (at least yearly) c. some form of newsletter. Funding for these activities could be identified by or raised by IFAR.

7. The periodic conferences would provide a means of: a. keeping all participants up-to-date, b. providing critiques, advice, peer reviews, c. guiding the program toward its goals, d. identifying additional essential research and recommending it for funding, e. providing a general cohesiveness and 'esprit des corps', and f. encouraging the spin-off of results.

8. Private sector participants should be invited to be an important part of the project from the beginning. They could provide valuable advice, counsel,and experience as well as appropriate basic and/or applied research with the objective of moving the process toward field trials and distribution as soon as practical.

9. If distribution of the project results is to be via the public sector, such as the international centers, and/or national agricultural institutions, they would probably provide the applications and adaptation research, field testing, selection, and release or distribution of the hybrid seed as expeditiously as possible.

10. Marketing and distribution of the seed by either the public or private sector to producers and their successful use of it for improved production of improved quality cassava is really the key objective of the program.

11. Independent publication of research results as the project progresses should disseminate basic information and spin-off ideas to scientists around the world.

Possible Role of IFAR

1. Encourage you to develop a large scale, focused, long range program to make major improvements in cassava.

2. Arrange for funding to initiate the process and support for core services to keep it going, such as: a. the secretariat b. communications and data base handling c. periodic conferences.

3. Try to identify and/or secure funding for essential recommended research that is not otherwise funded, i.e. help "package" the program in such a way that it is attractive to funding groups that like focussed, results-oriented research. Indications are that this is a very "marketable" or "saleable" approach.

4. IFAR will try to help you make such a network program a "go". But you, the scientists, will have to provide the science, and the cooperation and enthusiasm to make it a success. IFAR is anxious to encourage and help you to that end.