ESTABLISHMENT AND OPERATION OF A PILOT IN VITRO ACTIVE GENEBANK OF CASSAVA

To be submitted to: Diversity

By R. Chavez, W. Roca, D. Arias[1], L. Withers and I. Williams[2]

1. Biotecnology Research Unit, CIAT
A.A. 6713 Cali, Colombia

2. IBPGR Rome, Italy

1. INTRODUCTION

Although often vital adjuncts as breeders' collections, many existing tissue culture collections do not necessarily constitute genebanks suitable for long-term conservation of germplasm. In this context, the IBPGR has been laying the theoretical foundations for the development of in vitro techniques for the conservation of certain crop species, and it was necessary to test out the theoretical framework developed. The IBPGR has assigned to CIAT worldwide responsibility for cassava (Manihot esculenta Crantz) germplasm conservation. CIAT's stock, at the present, includes a field collection of 4500 accessions, a in vitro collection of 3500 accessions, and approximately 1100 accessions maintained in the form of sexual seed.

The principle of genetic conservation that material retrieved shoud represent the material accessed has caused concern about the use of in-vitro systems because of the release of somaclonal variation. As a consequence of genetic instability there can be variation chromosome number and structure and in morphological and biochemical traits. It has also been suggested that certain in vitro storage conditions can put germplasm integrity at risk because of directional genetic change in response to selection. This project pays special attention to this issue, by looking at the extent to which this variation can occur during in vitro storage of organized structures, and how this variation can be detected by using morphological, cytological and biochemical criteria.

2. OBJECTIVES

The overall objective of this IBPGR-CIAT Collaborative project is to assess and demonstrate the technical and logistical aspects of establishing and operating an in vitro Active Genebank (P.IVAG) according to standards set by IBPGR and CIAT using cassava (Manihot esculenta) as a model crop.

3. GENERAL WORK PLAN

Using the experience gained at CIAT on *in vitro* conservation of cassava clones and the recommendation of IBPGR on monitoring genetic stability of root crops, the major steps required for the establishing and running of a pilot-IVAG were outlined for a period of three years. The major steps are: a) selection, sampling and initial characterization of clones, b) introduction of clones into *in vitro* culture, subculturing and intensive micropropagation, c) *in vitro* storage under minimal conditions of growth; and d) monitoring of genetic stability. At present the first two steps mentioned above have already been performed.

4. SELECTION OF GENOTYPES FOR THE P-IVAG.

The cassava germplasm being used in the P-IVAG comprises 100 clones selected from a total of 4500 accessions currently maintained at CIAT in the field genebank. Selection was made mainly on the basis of phenotypic variation, so interclonal differences and morphotypes could be easily recognized reducing the number of morphological duplicates. Hopefully the clones should represent a wide range of the genetic variation present in the cassava gene pool. In addition to morphological variation, three other criteria were taken into account for clonal selection: a) adaptation to the main edaphoclimatic (ecosystems) zones of cultivated cassava, b) geographical latitude, longitude, altitude and country of origin; and c) a few elite clones with outstanding yield potential.

5. SAMPLING OF MATERIAL

Five plants per selected clone were labeled in the field genebank. Four stem cuttings (stakes) were taken from each one of the labeled plants, i.e. twenty stakes per clone, from which five stakes were planted in the Associated Field Genebank (AFG) and fifteen were planted in the glasshouse for disease indexing, electrophoretic characterization, thermoterapy and meristem tip culture. Due to the large work load and space required during the initial sampling and processing stages the material was handled as five lots each comprising twenty clones, with a 4-5 weeks interval lots. The lineage identity of clones, plants and stakes are being kept during the all steps of sampling and introduction of material to *in vitro* culture and storage.

6. DISEASE INDEXING

The phytosanitary conditions of mother plants was determined prior to thermotherapy and meristem-tip culture using a combination of symptomatology, graft inoculation, ELISA and dsRNA analysis for the detection of cassava common

mosaic virus (CCMV), cassava X virus (CsXV), the frogskin disease (FSD), caribbean mosaic disease (CMD) and cassava latent disease (CVL). FSD root symptoms were observed in 13 of the 100 clones. Eleven clones were positive by graft inoculation to the susceptible clone "Secundina". CCMV was not detected in any of the clones by the ELISA test. However the ELISA test did detect in CsXV in five clones. Virus-specific dsRNA was detected in 52 clones of the 100 clones tested. All clones testing positive for virus will be retested after thermotherapy and meristem-tip culture, and prior to *in vitro* storage. Should any of the clones remain positive following treatment, microthermotherapy will be carried out *in vitro*.

7. IN VITRO CULTURE AND STORAGE

Thermotherapy of the 500 mother plants was carried out in the growth chamber, exposing plants to high temperatures 35-40 °C at an illumination of 3.000-5.000 lux, for 2-3 weeks. Meristem tips were excised from fast growing shoots and placed in culture medium developed at CIAT. Subculturing of plantlets and subsequent micropropagation were made in medium 17 N until enough replicates were obtained for evaluation and for storage under minimimal conditions of growth, using the medium 8S. Up to the present all clones from the first group have been introduced into *in vitro* storage. A total of 25 individuals will be maintained in test tubes for the P-IVAG (fig.1)
Three markedly different clones, from the genetic view point, each with 25 replicates, are being used as controls for monitoring the effect of subculturing frequency during storage and the effect of meristem origin, either apical or axilar.

8. MORPHOLOGICAL CHARACTERIZATION

Preliminary morphological evaluation was made in the original field genebank from which the material for the P-IVAG was selected. More detailed morphological evaluation was carried out in the Associated Field Genebank (AFG) where growing conditions are more uniform. Results of this evaluation indicated a wide range of genetic variability for most of the internationally recognized cassava descriptors among these 100 clones, representing the spectrum of genetic variation within the crop species, *Manihot esculenta*. This core cassava germplasm also displays an evolutionary pathway of the crop ranging from very primitive cultivars to advanced elite clones.

A total of 62 descriptors were recorded for each genotype, out of which 21 are passport data, and the remaining comprises discrete morphological traits, qualitative and quantitative, disease indexing, electrophoretic patterns and pollen fertility of flowering clones. The information was recorded in field charts

especially designed for use in field and for direct transcription to the computer. Characterization of growing plants both in the field genebank and in the AFG has been done on six months old plants. Leaf shape, size and color was mainly studied on the 11th leaf starting from the top.

9. ELECTROPHORESIS

Isozyme electrophoretic methods have been applied to the characterization and discrimination of the 100 selected genotypes. Isozyme clustering data of 16 identified major bands shows an association of the 100 clones in 32 clustered groups and enabled the identification of six duplicates among the material. Electrophoregrams from the 100 clones were computer analyzed; up to the present 10 alfa and 6 beta esterase bands were identified to differentiate each genotype. Seventeen clones showed consistent and well defined electrophoregrams, from which, the pattern of CV. MBRA309 was taken as a reference to compare with all banding patterns from the core germplasm. Out of this group 3 clones were selected for use in a related experiment to test the effect of subculture frequency on genetic stability. Clustering data of alfa and beta esterases are being supplemented with the data obtained from the new LKB ultrascan XL laser densitometer that overcomes the difficulties of accurately quantifying electrophoretic gels. Beta esterease band, most comon to the core cassava germplasm, was used as a reference diring the scanin of gels. The comparison of ultrascan XL and clustering of electrophoregrams will give us incomparable help for further monitoring of the genetic stability on *in vitro* cultures.

10. ASSOCIATED FIELD GENEBANK (AFG)

The AFG represents, a) the original core cassava germplasm without treatment under any *in vitro* condition, and b) the field plot with plants originate from in-vitro conditions. They are used systematically for clonal evaluation and as a control for evaluating the I-IVAS to compare any possible phenotypic change in *in vitro* stored clones under minimal conditions of growth, mainly on discrete morphological traits. Stake samples from each identified plant growing in the field genebank were taken and planted in 28 x 60 m 2 plot. Five plants per clone were planted in a distance between plants 1 m, between rows 2 m and between lines 3 m. Plants are growing under two conditions and are also displaying contrasting morphological features.

11. DATA BASE

A computerized data base on characterization of morphological traits, disease indexing and biochemical data has already begun. A software package is being developed

for running all aspects of the P-IVAG using the International Data Management System (IDMS). This software will be used with the new hardware to be purchased very soon for the P-IVAG.

12. FACILITIES FOR RUNNING THE P-IVAG

The following CIAT laboratories and other facilities have been used for establishing and operating the P-IVAG.

1- Tissue culture lab.
2- Transference room (in vitro)
3- Propagation room (in vitro)
4- In Vitro storage room
5- Cytology lab.
6- Biochemistry (Electrophoresis) lab.
7- Virology lab.
8- Computer Center
9- Photographic and library facilities
10- Glasshouses
11- Screen Houses
12- Field plots

13. STAFFING

Because the complex nature of the P-IVAG project a lot of interaction is involved and remarkable help, collaboration and advice has been received from several CIAT scientists, assistants, technicians and administrative personnel. It is mentioned only the main staff of the P-IVAG:

a. Principal Research Staff
   Dr. R. Chavez
   Dr. W. Roca
b. Research Assistants
   Miss D. I. Arias (fulltime)
   Miss Cataño (parttime)
c. Technician
   Mr. Guillermo (fulltime)
   Mr. R. Arias (fulltime)
d. Collaborators and advisers
   Dr. C. Hershey (Cassava Program)
   Dr. D. Wood (Genetic Resources Unit)
   Dr. M. Holle (IBPGR)
   Dr. B. Nolt (Cassava Program)
   Dr. L. Chapas (Data Services Unit)
   Dr. L. Withers (IBPGR)

For more detailed information on P-IVAG activity, contact: R. Chavez /W. Roca. Centro Internacional de Agricultura Tropical (CIAT) A.A. 6713 Cali-Colombia.

Fig. 1. Storage of cassava clones under minimal conditions of growth (P.IVAS). Liliana Muñoz, Lab. Technician from the Biotechnology Research Unit-CIAT holds a test tube No. 25 Containing a cassava in-vitro culture.

Margins 1" 1 1/2" Margins 1 1/2" 1"

THEORETICAL AND APPLIED GENETICS (TAG) 1988 (In press)

Transfer of Resistance to PLRV Titer Buildup from *Solanum etuberosum* to a Tuber-bearing Solanum Gene Pool

R. Chavez[1], C. R. Brown*[2], and M. Iwanaga[3]

[1] Biotechnology Research Unit, Centro Internacional de Agricultura Tropical (CIAT) A.A. 6713, Cali, Colombia

[2] USDA-Agricultural Research Service, Irrigated Agriculture Research and Extension Center, P.O. Box 30, Prosser, WA, 99350, USA

[3] International Potato Center, Department of Germplasm Resources, APDO 5969, Lima, Peru