

Brazil**Farmer participatory project for *in vitro* cleaning and multiplication of local and improved cassava varieties**

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Participating Agencies

- Biotechnology Research Unit of EMBRAPA Mandioca e Fruticultura (CNPMF), Cruz das Almas, Bahia
- Participatory Plant Breeding Group of EMBRAPA-CNPMF, Cruz das Almas, Bahia
- Empresa Baiana de Desenvolvimento Agrícola (EBDA), Caetité, southeast Bahia
- Farmer communities in Caetité, southeast Bahia



This project is characterized by synergism between research

(EMBRAPA-CNPMF), delivery (EBDA), and end-use (farming communities). Seven farmer-preferred cassava varieties, made up of two local varieties (Lazam and Aipim Cachorro) and five improved clones (003, 005, BGM 1318, BGM 1393, and BGM 1389S), were selected from farmer participatory breeding trials on the basis of resistance to CBB and drought tolerance. These varieties are to be used for *in vitro* cleaning, rapid multiplication, and redistribution to farmers. Project activities are distributed as follows:

EMBRAPA-CNPMF-Biotechnology Research Unit

- Receive and clean, and conduct *in vitro* indexation and rapid *in vitro* multiplication of planting materials of cassava clones supplied by the Participatory Plant Breeding (PPB) Group. The rapid *in vitro* multiplication protocols are adapted from those previously developed at CIAT.
- The Unit also trains extension agents and farmers in tissue culture protocols. It helped set up the pilot laboratory and protocols in the Maniçau community of Caetité. It ensures continuous supply of *in vitro* materials to the pilot laboratory, helps transfer materials from the laboratory to the greenhouse while ensuring a follow up with the host community. The Unit also participates in on-farm validation of materials arising from *in vitro* methods.

EMBRAPA-CNPMF-Participatory Plant Breeding Group

- The PPB Group interacts with the communities selected as pilot study sites and, in conjunction with EBDA, leads the selection of research materials, communities, farmers, and extension workers. It also, in association with farmers and with EBDA's help, selects, in a participatory manner, the improved and local varieties adapted to the pilot zone.
- The Group also supplies planting stakes from the chosen varieties to the Biotechnology Research Unit, trains farmers and extension agents in two-node rapid multiplication, actively participates in the transfer of *in vitro* plantlets from the laboratory to the greenhouse, and subsequently follows up with the farmers and technicians on the rapid propagation theme.
- Data collection on technology adoption and impact.

Farmers

- The farmers as a group participate in varietal selection, are trained in the *in vitro* and rapid cassava multiplication techniques (about 3 persons), transfer the received training to other group members, and engage in follow up and feedback with EBDA, the Biotechnology Research Unit, and the PPB Group.
- Responsible for the establishment and maintenance of the planting materials reservoir.

Empresa Baiana de Desenvolvimento Agrícola

- In conjunction with the PPB Group, EBDA selects the communities, farmers, and extension workers who will take part in the participatory selection of improved and local cassava varieties adapted to the zone, become trained in *in vitro* and rapid cassava multiplication, and pass their knowledge on to others of their respective groups.
- EBDA follows up and gives feedback on the processes with the different players to EMBRAPA-CNPMP's Biotechnology Research Unit and PPB Group.

Arising from the rapid *in vitro* multiplication of these five selected varieties, 432 plantlets have been produced in EMBRAPA-CNPMP's Biotechnology Laboratory. Once multiplied, these clones will be transferred as plantlets to the pilot laboratory in Caetit , southeast Bahia.

1. Identifying Information

Project title: Application of low-cost *in vitro* propagation techniques to preserve native varieties and produce quality cassava seed in southwestern Colombia

Date: 30-01-03

Reporting period: 30-06-02 to 30-12-02

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2. Achievements and Constraints

During this phase of the project, activities focused on fine-tuning the phases of hardening *in vitro*-produced material and transplanting it to the field. Several native cassava varieties were also collected and identified using morphological descriptors, and will be subsequently analyzed by AFLP technique.

Organizational and gender analyses helped strengthen community organization, according to the objectives agreed upon with L. Sperling and the late C. Gines in December 1991.

Main achievements include the following:

- 2.1 A methodology adjusted for the adaptation of *in vitro* material to the greenhouse phase and its subsequent planting in the field. Plants were placed for 8 days in water and then transferred as such to the definitive planting site, where they were planted in plastic bags containing sterilized soil. This practice eliminates the additional expenses incurred when transporting the material between the laboratory and the farms, while also reducing the possibility of lodging during transportation.

- 2.2 Continued incorporation of new equipment and less expensive reagents into the process. A laminar flow chamber was built with local materials, costing 10 times less than an imported chamber.

Advances were also made in the adjustment of the MS basal media. Cassava seedlings showed best growth when the commercial product Ferrovital–NF was used.

- 2.3 Use of the rapid propagation system based on two-budded cuttings by the group of farmers to complement the *in vitro* system. With this methodology, farmers multiplied nearly 6,000 plants of the variety MCOL 1468 from *in vitro* material certified by the Colombian Agriculture and Livestock Production Institute (ICA, its Spanish acronym)
- 2.4 Establishment of a bank in farmers fields with *in vitro* material from 6 clones of interest to farmers (MCOL 1522, HMC 1, CMC 523-7, CM 6740-7, MBRA 383, and MPER 183) to provide FSD-free material. When these clones are harvested in 2003, they will be multiplied by the rapid propagation system, using two-budded cuttings, and distributed to local farmers.
- 2.5 Collection of 14 native cassava varieties in the municipalities of Caldono, Piendamó, Morales, Santander de Quilichao, and Caloto (Department of Cauca) and their identification using the morphological descriptors applied by CIAT's germplasm bank. A sample of each variety was planted on two farms in the municipalities of Caldono and Piendamó, as well as in the greenhouse at CIAT-Palmira.

These varieties will be evaluated in CIAT's Biotechnology Unit Lab, using AFLP to determine the degree of diversity among materials. To continue this process, a project proposal has been submitted to the Cassava Biotechnology Network (CBN) to complete the identification of these materials during 2003, and subsequently distribute clean seed of these native varieties to farmers.

- 2.6 Systematization of the traditional knowledge of local men and women regarding native varieties, especially their tolerance to different stress factors and their resistance to the most common pests and diseases affecting cassava in the region, uses, starch quality, and use of plant parts (other than roots).
- 2.7 Definition of a participatory work scheme involving men and women to strengthen the formal cassava seed production system using *in vitro* technology and rapid propagation in the rural communities of Bajo Santa Ana, Alto Santa Ana, and Quinamayó.
- 2.8 Easy adoption by the group of farmers of the rapid propagation technique using two-budded cuttings. This method was implemented by the project as complementary to the *in vitro* method and farmers described it as easy and simple, and said it produced immediate results.

3. Outstanding Results

- 3.1 An *in vitro* cassava seed production scheme to be managed by small farmers was defined. The scheme consists of 6 phases: (1) receipt of certified material; (2) *in vitro* multiplication in the rural laboratory; (3) adaptation in the greenhouse of the material multiplied through tissue culture; (4) planting of material in farmer fields with adequate management of irrigation, pest control, and fertilization; (5) multiplication of cuttings obtained in the field by rapid propagation (two-budded cuttings); and (6) production of good quality cuttings through the conventional system.
- 3.2 During both phases of this project, rural laboratories were built with their corresponding work areas, and the use of locally available, low-cost materials in their construction was evaluated. In addition, most of the components of the culture medium, such as salts, hormones, and sugars, were replaced with products obtained in the local market. The multiplication rate (1:3-4) obtained when local inputs were used to prepare the culture media was similar to that obtained when imported raw materials were used.
- 3.3 Several of the native materials collected in central northern Cauca, for example Algodona Amarilla and Algodona Grande, present good starch quality and high percentage of starch. Therefore, an agreement was reached with several farmers and starch producers to increase the area planted to these two materials using *in vitro* propagation. FIDAR assumed production costs and CIAT provided the certified materials. These two cassava clones will hopefully be able to compete better with the varieties imported by starch factories in Ecuador and other regions of Colombia.
- 3.4 The women belonging to the group were able to reach a consensus about their needs and ways to solve them. Priority will be given to the search for income-generating alternatives. The *in vitro* production of cassava seed is one of these alternatives, but because of the time involved, they decided to participate in complementary production activities such as the planting of cassava, pineapple and bean, individually and in association, and the multiplication of commercial cassava varieties using rapid propagation.
- 3.5 The results of group participation reported in this phase are characterized by the empowerment and commitment of farmers, who have showed interest in undertaking other initiatives that benefit the community, for example a program to improve the quality and coverage of basic secondary education and allowing the access of young people and adults, who because of economic problems cannot attend nearby schools. An improved educational level has allowed the group to better understand and analyze the processes of *in vitro* technology.

4. Difficulties Encountered When Executing Work Plan

- 4.1 The cost of an *in vitro* cassava plant produced in the rural laboratory was estimated at US\$0.29 (see final project report 2001). This value is still quite high and not

sufficiently competitive for small farmers to purchase these plants. The project continued working on this aspect during the current reporting phase and was able to integrate *in vitro* production technology with the rapid propagation system using two-budded cuttings. The data needed to determine whether the integration of these two systems reduced costs should be available at the end of 2003.

New systems are being studied to reduce the cost of handling and transporting cassava seed in the greenhouse, its definite adaptation in farmers' fields, and the diversification of use of rural laboratories for tissue culture of banana and fruit trees.

- 4.2 The sharing of a common language and the level of confidence demonstrated by the group of farmers in the facilitating farmer ensured that project participants completed the six phases of the *in vitro* cassava production process in the rural laboratory and the multiplication in the field. However, the lack of academic training prevented several farmers from converting measurements of volume and weight and from making decisions to solve problems of contamination.
- 4.3 Macro and microeconomic factors affected community participation in the project, for example:
- High fluctuations in the exchange rate of the Colombia peso versus the US dollar
 - Low price of cassava roots in Colombia over the last 10 months
 - Legal and illegal importation of sour starch by large companies in the region
 - Increase in the number of agronomic and plant health problems (whitefly and diseases such as frog skin) over the last two years

However, the adoption of several complementary measures (production projects, training in cassava cultivation) has allowed the group to continue. The importance of acting in an organized fashion to achieve project/group objectives in benefit of the community has been recognized.

5. Communication and Dissemination of Information

Project results have been disseminated at the local and national levels through the participation in different forums and workshops on topics related to farmer application of cassava tissue technology.

5.1 Seminars and Workshops

Researchers and technicians working with the project participated in the following seminars and workshops in the capacity of lecturers:

- First Regional Workshop on Rapid Propagation (In Vitro) and Genetic Transformation of Cassava. CIAT, 25 February - 2 March 2002.
- Biotechnology in the Development of Colombia. First Colombian Congress on Biotechnology. Universidad Nacional de Colombia. Bogotá, June 2002.

- Intensive training course in modern cassava production and processing systems. CIAT, June 2002.
- Rapid propagation as a technology to support the multiplication of in vitro cassava materials by farmers. Santa Ana (Cauca), September 2002.
- Cassava seed production workshops held for the farmer associations of El Agrado, the Toez Indigenous Council, and La Arrobleda. August, September, and October 2002.

5.2 Publications

During 2003, two articles summarizing the project's experience will be submitted to the journals *Illeia* and *Scientific American Latinoamérica*

- Tissue culture for farmers: participatory adaptation of low-input cassava propagation by a resource-poor rural community in southern Colombia. R.H. Escobar, C.M. Hernández, G. Ospina, J. Restrepo, L. Muñoz, J. Tohme, and W.M. Roca. 2003.
- Aplicación de la tecnología de propagación in vitro para producir semilla de yuca (*Manihot esculenta* Crantz) por pequeños agricultores: descripción y análisis de la experiencia. J.M. Restrepo, G.I. Ospina, C.M. Hernández, R.H. Escobar, J. Tohme and W.M. Roca. 2003

6. Lessons Learned and Future Work

By implementing and assessing this *in vitro* cassava seed multiplication technology, farmers were able to maintain informal cassava seed production systems that yielded propagation rates of 1:3-4 every 45-60 days. The system was capable of producing 3250 plants per initial explant (plant), reaching an efficiency of 400% compared with the conventional vegetative seed propagation system currently used by regional farmers. Outstanding results were also obtained in the identification of technical parameters to build a low-cost rural laboratory, that could be easily operated by farmers, as well as of equipment and inputs to prepare the culture media, achieving an efficiency similar to that of specialized laboratories.

In addition, the farmer-farmer training methodology implemented by project researchers and technicians proved to be correct because it ensured that participants understood the concepts and acquired the skills needed to operate the rural laboratory. However, more time was needed than that initially planned for the group to understand and self-manage the different processes (prepare the culture media, plant the tissues, hardening the plants, establish plantlets in the field).

Although the simplification of the tested technology significantly reduced the costs of the infrastructure, inputs, and culture media used, the labor costs implied by the different processes continue to be quite high and make it impossible for farmers to assume *in*

vitro seed multiplication. It is therefore important to continue evaluating new systems and diversify the use of the laboratory with other crops to ensure its long-term sustainability. Mechanisms must also be sought to attract the participation and support of different local institutions.

The use of *in vitro* technology by farmers is an alternative that solves the problem of availability of good quality seed, especially in the case of new varieties or when seed is scarce because of climatic and plant health problems.

The rural laboratory can also be used to multiply native cassava varieties of northern Cauca. These are currently being identified and cleaned at CIAT's Biotechnology Unit Laboratory for subsequent redistribution to the communities for their *in situ* conservation and multiplication of seed of those varieties enjoying greatest acceptance by farmers and starch producers.

**Plant Breeding Small Grants Program
System-Wide Program on Participatory Research and
Cassava Biotechnology Network (CBN)**

Small Grants Financial Report (US\$)

Project: Application of low-cost *in vitro* propagation techniques to preserve native varieties and produce quality cassava seed in southwestern Colombia

Institutions: FIDAR and CIAT

Reporting period: 30-01-2002 to 30-06-2002

Income
PRGA: US\$7,000
CBN: US\$13,000

Partner contributions
CIAT-FIDAR: US\$5,900

	Expenses	Expenses
Personnel	6,500	3,500
Services	2,000	
Travel	3,000	
Communications	800	
Workshops	1,000	1,000
Capital	4,000	1,400
Indirect costs	2,700	0
	20,000	5,900
Total	20,000	5,900
Financial Statement	0	0

APPROVED BY:

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Rapid propagation of Cassava Planting Material by Temporary Immersion Bioreactors.

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Introduction

Cassava has been considered the most important alternative crop since it is the only crop that yields acceptably under marginal conditions, with minimum inputs. Demand for cassava planting material is high and conventional methods of propagation don't satisfy the needs (Buitrago 1999).

Recently a temporary immersion system has been described by Teisson and Alvard (1995) for plant propagation. This system, known as RITA®, has been successfully used with embryogenic tissues of banana (Alvard et al 1993), coffee (Berthouly 1991), rubber (Etienne et al 1993) and sugarcane (Lorenzo et al 1998). In collaboration with the Colombian Biotechnology Program, PBA, we are adapting RITA® to produce enough planting material of desired, indexed commercial clones using nodes as initial explants.

Objectives

- Develop and validated operative schemes to produce planting-material for the different cassava growing zones in Colombia
- Scale up the cassava propagation methods as possible solutions for the lack of planting material.
- Adapt and establish a low -cost method for massive propagation.

Methodology

Two cassava clones, Venezolana (MCol 2215) and Verdecita (MCol 1505), were used to develop the technique due to great interest of farmers in the North Coast of Colombia. Initial tissues were propagated by conventional methods, using solid 4E medium (Roca 1984).

We set up the systems with a potential capacity of 8 manifolds with 24 RITA each, to growth nodal explants at different immersion periods. The system was compared with the conventional cassava multiplication system on 4E solid medium. Different combinations of growth regulators (BAP, Kinetin and TDZ) were tested together with application of CO₂.

The evaluation was done for 8 weeks, and expressed in terms of number of new explants/initial single nodal cultured. Currently we had validated this systems with 16 cassava clones.

Results

We increased propagation rates up to 1:6 to 1:10 (Table 1), depending upon the genotype, which was higher if compared with rates (1:3-4) of normal propagation on 4E solid media.

Plants produced with this system were transferred to the screen-house and compared with plants produced on solid media. No morphological differences were observed.

Cassava clone	Common name	Recovered tissue		
		Shoot	Nodes	Propagation rate
CM 3306-4	Ica Negrita	50	51	10.1
CM 4574-7	-	40	25	6.5
CM 523-7	Catumare	46	60	10.6
MBra 383	Brasileña	43	25	6.8
NBra 507	Tucuma	43	35	7.8
MCol 2215	Venezolana	58	30	8.8
MCol 1505	Verdecita o P12	28	25	5.3
MCub 74	Señorita	31	42	7.3
MEcu 72	Injerita	26	42	6.8
MTai 8	Rayon 60	26	35	6.1
SGB 765-2	Carlbeña	37	20	5.7
SBG 765-4	Rojita	27	32	5.9
CM 6740-7	Reina	32	18	5.0
CM 3555-6	-	46	40	8.6
CG 1141-1	Ica Costeña	48	40	8.8
MVen 25	Querepa amarga	40	40	8.0

Carbon dioxide was injected into RITA. Partial results show bud activation but not elongation. More time may be required for shoot elongation.

We are adapting bioreactors at low cost. RITA vessels are being replaced with recyclable soda glasses, and silicone tubing by aquarium flexible pipes. Hydrophobic, 0.22µm filters were substituted by hand-made, cotton filters. We are setting up plans to run a low-cost, pilot bioreactor system, that can be transferred to small-scale farmer systems.

A member of the PBA regional committee (Mr. Eduardo Erazo) discussed with us the importance of including in our propagation schemes some regional varieties. Farmers from the Atlantic Coast gave us stakes of local clones "Ramirana", "Yema de huevo" and "Por encima". A GRU staff isolated meristems and treated them with chemotherapy. We are now running RITAs with these materials.

We are also adapting RITAs to improve somatic embryo induction in *Bracharia* and rice, and to improve rice another culture. We are also using RITA for FEC regeneration to support cassava transformation activities.

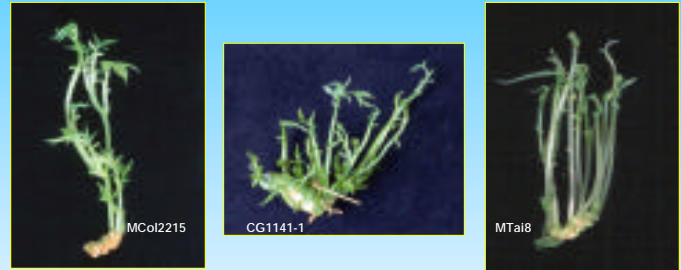


Figure 1: Preliminary results for nodal propagation of cassava commercial varieties using RITAs. The observed rate of propagation was 1:6 to 1:7 for MCol2215, and 1:8 to 1:9 for CG1141-1 and MTai8, which seems high compared to conventional propagation methods (1:3).

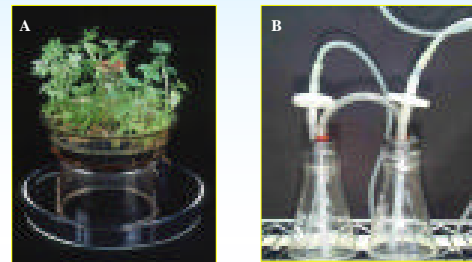


Figure 2: (A) Massive response of nodes to RITA systems. (B) Preliminary assays to adapt the RITA principle to establish low-cost propagation methods.

Conclusions

- Propagation rates using RITA vary from 1:6 to 1:10, which are higher than conventional propagation on 4E solid medium. Rates are genotype dependant.
- Type and concentration of Cytokinin affect the response of tissues. TDZ is better than BAP for propagation. Application of CO₂ seems to activate node response.
- RITA could be adapted to reduce implementation costs to establish low-cost propagation systems.
- RITA could improve somatic embryogenesis and plant regeneration in other crops.

Ongoing Activities

- Fine-tuning conditions to improve propagation rates reducing hiperhydricity.
- Field testing of plants produced in RITA.
- Meeting with farmers, representatives of National Programs and PBA-regional committees to exchange experiences, and transfer this technology.
- Reduce the costs of adopting RITA using locally made bioreactors.

Acknowledgments

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