
Procedure for the Shipment of Cassava *in vitro* Material

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PROCEDURE FOR THE SHIPMENT OF CASSAVA *IN VITRO* MATERIAL

The Genetic Resources Program (GRP) of the International Center for Tropical Agriculture (CIAT) conserves and distributes *in vitro* germplasm of the genus *Manihot*. Quarantine regulations for this genus have been applied since 1980 when it was stipulated that only *in vitro* plants could be used for exchange throughout the world, and technical guidelines for the safe movement of germplasm were developed (Frison & Feliu, 1991).

In vitro techniques have been used for two purposes: 1) to introduce into the *in vitro* germplasm bank of CIAT a large number of materials collected in the main centers of variability and to conserve them *in vitro*, and 2) to distribute selected germplasm from CIAT to national programs and other users.

These activities are performed under the standards established in the International Treaty on Plant Genetic Resources for Food and Agriculture effective since June 29, 2004.

This section describes the procedures for shipment of materials to the Genetic Resources Program of CIAT.

Preliminary Aspects

The GRP introduces materials into its collections under the condition that they are new materials, i.e. accessions that are not already registered with the International Treaty's Multilateral System.

The first filter of selection of materials to be sent to the genebank must be the verification of the passport information. Once the materials are received, a characterization and evaluation of the germplasm is performed to check whether the received materials are duplicates or copies of the ones already conserved in the collection.

Legal Aspects

Once the list of materials to be sent is defined, the acceptance and signature of the Standard Material Transfer Agreement (SMTA) must be done by the supplier (page 8 of SMTA) and recipient of the materials, in this case CIAT (pages 8 and 12 of the SMTA).

With the detailed information about the type of material, country of origin, units, type of packing and quantity per package, the import permit must be obtained from the Instituto Colombiano Agropecuario-ICA, quarantine Colombian institution. This document is sent to the provider of the germplasm by certified airmail, and by email.

Preparation of shipment. The materials are micropropagated using the medium 4E (Roca et al., 1991). See "Handbook of Procedures for the *in vitro* conservation of germplasm of the genus *Manihot*," paragraph 3.4 (Mafla et al., 2010).

The tubes are sealed with extensible tape, marked with permanent ink marker using paper label, indicating in clear and legible letters and numbers the identification of the materials. The label is attached to the tube with extensible tape; the use of adhesive labels is recommended (Fig. 1).



Figure 1. Sealing system and labeling of *in vitro* material suggested for the exchange of germplasm.

Sealed and labeled tubes are placed in growth conditions (temperature 26-28°C, 18.5 lighting $\mu\text{mol.m}^{-2}.\text{s}^{-1}$ and 12 hours photoperiod). After two to three weeks, roots sprout in each test tube; the step of rooted plants is the most appropriate way, from the standpoint of management, for the exchange of germplasm clonal species.

Evaluation, Packing and Shipping of *in vitro* Material

Evaluation. The general condition of the plants, the growing medium, and the sealing and proper labeling of materials are evaluated.

Pack and ship only tubes with plants in good physiological (strong and rooted) and phytosanitary conditions (free of bacterial and/or fungal contamination or without any suspicion of it). There should be no doubts in relation to the identity of the materials.

Packing and Shipping. Upon verification of the correct identification of materials to be sent, proceed with the packing. Three tubes for each material are the minimum number of tubes to send. According to the total number of tubes, different systems can be used for packing the materials. Shipments of more than 60 tubes (20 accessions) are made on racks.

a. Shipment in trays:

- The tubes are placed on styrofoam trays or cardboard (preferably corrugated) and attached to them with adhesive tape.
- The trays are placed one above the other and the whole is secured with adhesive tape.
- In this shipment system it is important to indicate the direction in which the materials must be transported and the fragile condition of these (Fig. 2).

Figure 2 shows the arrangement of the test tubes in styrofoam trays, the location of the tubes and the final labeling of the box.



Figure 2. Packing system of *in vitro* material in trays.

b. Shipment in racks:

The *in vitro* seedlings can also be arranged in styrofoam racks according to the size of tube used. It is important to secure the tubes and racks properly; sheets and structures in styrofoam and/or cardboard are used for this (Fig. 3).



Figure 3. Packing system for *in vitro* seedlings in styrofoam racks: a) Packing system for individual racks, and b) Packing for two racks, in both cases racks are protected by styrofoam structures.

Individual or grouped racks are packed in cardboard boxes. The boxes are labeled with the following information:

- Sender and receiver with their respective contact addresses.
- Packing content (living plant material, *in vitro* plants) and the documentation accompanying the material such as the import permit and phytosanitary certificate.
- Terms of handling and transport. The position to be given to the boxes, as well as the fragility and the optimal conditions for storage is indicated through stickers. The purpose of this is to alert carriers about the conditions for a proper handling during transport and storage (Fig. 4).



Figure 4. Labels used on the boxes containing *in vitro* materials.

Shipments must be accompanied by their respective Import permit, a Phytosanitary Certificate, issued by the competent authority of the donor country, including indications for treatment and testing of disease detection applied to plant material. Also, the list of materials submitted with the passport information must be included.

CIAT has developed a guide with instructions for sending air cargo which must be taken into account when drawing up the relevant documentation and labeling of the boxes in the shipment (Annex 1).

Shipments are done by air in any of the following ways: as accompanied baggage, as cargo or mail. The shipment of the materials must be coordinated in advance, and after the shipment of the materials, the number of the air waybill must be reported, so that custom can be cleared as soon as possible.

During transportation, the materials are exposed to conditions of variable temperature and continuous darkness, which can damage the material. The darkness during transportation induces elongation and chlorosis. Temperature variations for short periods do not affect crops. The materials must be stored at temperatures of 15-25°C.

Germplasm Management after the Reception

Once materials are received the Colombian quarantine authorities should be informed about their arrival; then these materials must be placed in optimal conditions for their growing while the initial evaluation of the physiological and health status of incoming materials is done.

Material with fungal and/or bacteria contamination problems should be destroyed by autoclaving in presence of an ICA officer. Subsequently, the ICA issues the Certificate of Destruction for the materials that have been eliminated because of contamination, and a Phytosanitary Certificate of Nationalization for those that have entered the country in good condition.

Introduced materials are multiplied for their conservation and in order to carry out the certification process against viruses of quarantine importance. Once the process of certification against viruses of quarantine importance is completed, the materials are available to users.

Bibliography

- Frison, E.A., E. Feliu. 1991. FAO/IBP GR technical guidelines for the safe movement of cassava germplasm. Food and Agriculture Organization of the United Nations-FAO and International Board for Plant Genetic Resources, Rome, Italy.
- Roca, W.M., B. Nolt, G. Mafla, J.C. Roa, R. Reyes. 1991. Eliminación de virus y propagación de clones en la yuca (*Manihot esculenta* Crantz). En: Roca, W.M., Mroginski, L.A. (eds.), Cultivo de tejidos en la agricultura: Fundamentos y Aplicaciones, pp. 403-421.
- Mafla, G.M., J.C. Roa, E. Aranzales, D.G. Debouck. 2010. Handbook of Procedures for the *in vitro* conservation of germplasm of the genus *Manihot*. Genetic Resources Program. Centro Internacional de Agricultura Tropical (CIAT), Palmira, Colombia. 56p.

ANNEX 1

CIAT SHIPPING INSTRUCTIONS FOR AIR CARGO

1. The Air waybill –AWB should be consigned to:

**DEPOSITO PRIVADO - CIAT
CENTRO INTERNACIONAL DE AGRICULTURA TROPICAL
DR. RUBEN ECHEVERRÍA – DIRECTOR GENERAL
RECTA CALI-PALMIRA, KM 17. CALI, COLOMBIA**

Also, include a short description of the items.

2. Marks and labels:

**DEPOSITO PRIVADO - CIAT
CENTRO INTERNACIONAL DE AGRICULTURA TROPICAL
DR. RUBEN ECHEVERRÍA - DIRECTOR GENERAL
RECTA CALI-PALMIRA, KM 17. CALI, COLOMBIA**

3. DOCUMENTS: Send original and two (2) copies of the Air waybill, packing list, commercial invoice and, if applicable, insurance certificate to:

**CIAT DEPARTAMENTO DE IMPORTACIONES
Contact: Julian Montoya / Elkin Collazos
Email: jamontoya@cgiar.org / ecollazos@cgiar.org
Tel: 57-2-445-0000 Ext. 3685 / 3252
Centro Internacional de Agricultura Tropical “CIAT”
Recta Cali-Palmira, Km. 17
Cali, Colombia.**

4. INSTRUCTIONS:

- a. Consular documents are not necessary.
- b. The following must appear on all documents:

"ESTA IMPORTACIÓN PERTENECE A UN CONVENIO INTERNACIONAL Y ESTÁ EXENTA DE IMPUESTOS. NO NECESITA REGISTRO DE IMPORTACIÓN DE ACUERDO CON LA LEY 29 DE MARZO 18 DE 1988 Y NO REQUIERE CERTIFICADO DE INSPECCIÓN SEGÚN EL ARTÍCULO 3 DEL DECRETO 861 DE MAYO 26 DE 1995".

- c. The following must appear on the commercial invoice:

"DECLARAMOS BAJO JURAMENTO QUE LOS PRECIOS DE ESTA FACTURA SON LOS MISMOS QUE CARGAMOS AL CLIENTE Y QUE LA MERCANCÍA ES ORIGINARIA DE (Country of Origin). EN FE DE LO EXPUESTO FIRMAMOS ESTA DECLARACIÓN EN (City and date).

- d. Description of items on invoice should preferably be in Spanish.