RESEARCH GUIDE
GUIA DE INVESTIGACION
GUIDE DE RECHERCHE

CIP

CIP Research Guide 38

TRANSPORT, RECEIPT, AND PROPAGATION OF IN VITRO SWEET POTATO PLANTLETS

1991

John H. Dodds, Ana Panta, and James E. Bryan

INTERNATIONAL POTATO CENTER (CIP)
CENTRO INTERNACIONAL DE LA PAPA (CIP)
CENTRE INTERNATIONAL DE LA POMME DE TERRE (CIP)

CIP Research Guide 38

TRANSPORT, RECEIPT, AND PROPAGATION OF IN VITRO SWEET POTATO PLANTLETS

1991

John H. Dodds, Ana Panta, and James E. Bryan

CIP
P.O. Box 5969
Lima, Peru

Location
Av. La Universidad s/n
La Molina, Lima

Fax 351570
Tel. 366920
Telex 25672 PE

CIP's Research Guides (CRGs)

Describe technologies that have been developed and used by CIP and National Programs in order to promote the research and the exchange of information among scientists. They are regularly updated according to the scientific advance.

Dodds, J. H.; Panta, A.; Bryan, J. E. 1991. Transport, receipt, and propagation of in vitro sweet potato plantlets. CIP Research Guide 38. International Potato Center, Lima, Peru. 17 pp.

1 TRANSPORT OF TISSUE CULTURE MATERIAL

1.1 **Packing.** The material is packed in polystyrene in a cardboard container. Each package contains several small glass test-tubes, each with three well-developed plantlets. Extra agar media is added to prevent damage from movement during shipment (Figure 1).

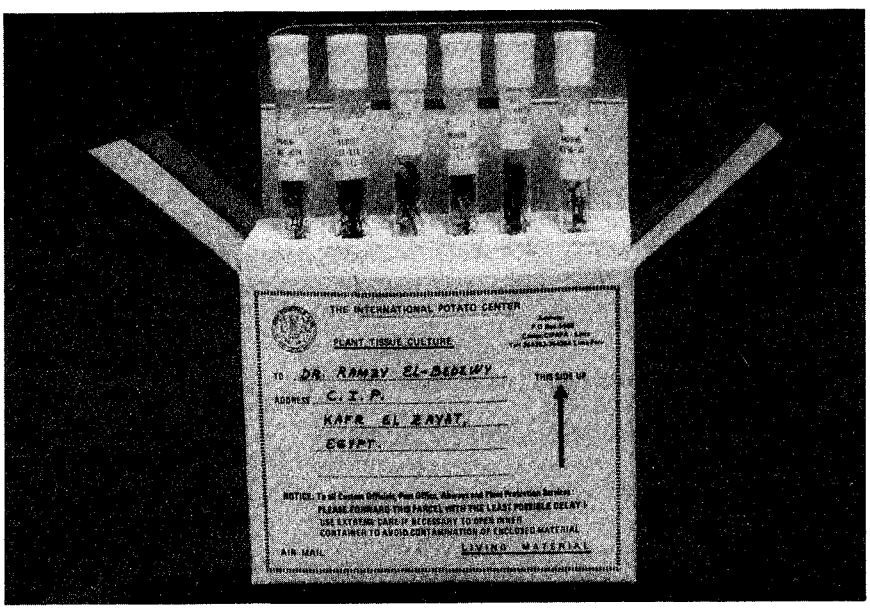

Figure 1

The test tubes are capped with plastic covers and sealed with parafilm to prevent entry of contaminants into the cultures and loss of water from the medium.

1.2 **Shipment.** Whenever possible, tissue cultures are hand-carried to ensure rapid transport. When this is not feasible, the fastest possible method is usually airfreight. In vitro plantlets can survive two to three weeks without light.

TRANSPORT, RECEIPT, AND PROPAGATION OF IN VITRO SWEET POTATO PLANTLETS

1 **Transport of tissue culture material**
2 **Transfer to planting mix**
3 **Micropropagation**
4 **Murashige-Skoog medium (modified)**
5 **Medium to sweet potato micropropagation**
6 **Bibliography**

Tissue culture materials consist of small, aseptic plantlets growing on a synthetic nutrient medium. The aseptic nature of this material makes it an ideal method for international exchange of germplasm as it minimizes the risk of transmitting fungal and bacterial diseases.

This document contains information for the recipient of in vitro plantlets on the handling procedures to be followed for further micropropagation or transfer to non-sterile growing conditions.

1.3 **Handling after arrival.** The cultures should be cleared from customs as quickly as possible. When advance notice of the shipment is known, alert the customs officials of its expected arrival. Carefully remove the test tubes from the package in a laboratory or clean room. Do not open the tubes. Do not remove the plantlets.

If the plantlets have become yellow, place the test tubes under diffused light for about 1 week in a clean room.

In vitro shoot cultures are free from diseases. Work under clean conditions according to the following description (steps 2.4 and 2.5) to prevent contamination during and after unpacking.

1.4 **Use of tissue culture material.** The plantlets can be used in two different ways:

- transfer to planting mix (Section 2),
- micropropagated (Section 3).

2 TRANSFER TO PLANTING MIX

2.1 Materials:

- peat moss
- fine sand (1 mm diameter)
- aluminium foil
- clay pots (8 to 10 cm diameter) or jiffy pots
- larger pots (20 cm diameter)
- distilled water
- 1% calcium-hypochlorite solution
- 70% alcohol solution
- strong soap
- fertilizer with high content of phosphorous (5-50-17) or (12-12-12)

2.2 Mix peat moss and sand (1:2 by volume).

2.3 If an autoclave is available, fill the pots with peat moss/sand mix, cover them with aluminium foil, and sterilize for 1 hour. If an autoclave is not available wash the clay pots (jiffy pots are already sterile) with detergent, rinse them well with running water, and sterilize the planting mix and some additional sand separately by any other means (heat, steam, fungicides, etc.).

2.4 Take the pots and the in vitro culture to a clean bench that is protected from air currents, dust, dirt, insects, or other contaminants.

2.5 Wash your hands with strong soap and a 1% calcium-hypochlorite solution. Then rinse hands in 70% alcohol.

2.6 Irrigate the pots with a small amount of water.

2.7 Prepare the pot that is to receive the plantlet by making a hole in the center of the peat moss/sand mix with a clean stick or pencil.

2.8 Before removing the plantlets, disinfect the outside of the test tube using a piece of cotton or cloth moistened with 70% alcohol to reduce the risk of contamination.

2.9 Using clean fingers, remove the parafilm and the plastic cover from the test tube. Work with one tube at a time.

2.10 Gently pull the plantlets with the agar out of the test tube using sterilized forceps (flamed to red heat and cooled) (Figure 2).

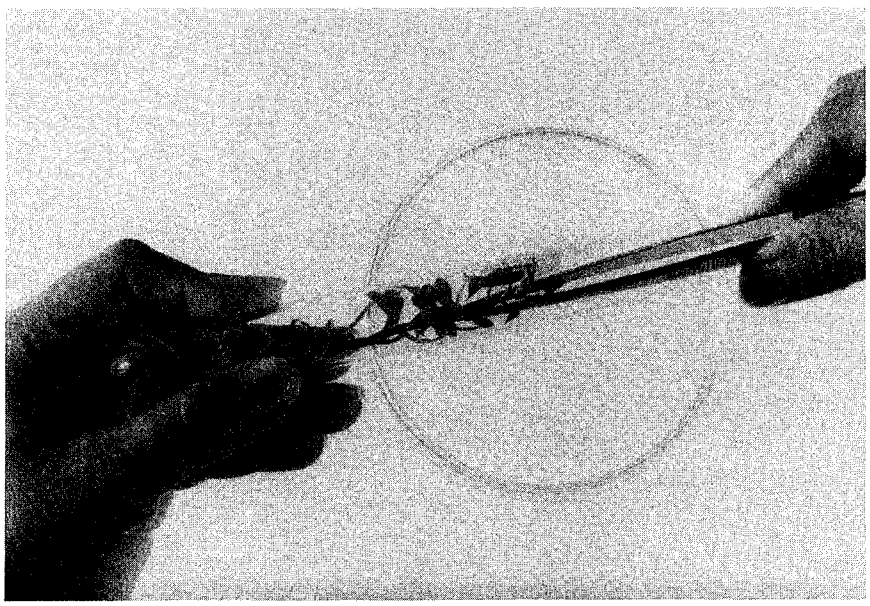

Figure 2

2.11 Wash the agar from the roots by gently immersing them several times in sterilized water, trying not to wet the rest of the plantlet.

2.12 Plant each plant individually in the holes in the potting mix with the roots plus one or two nodes below the surface (Figure 3).

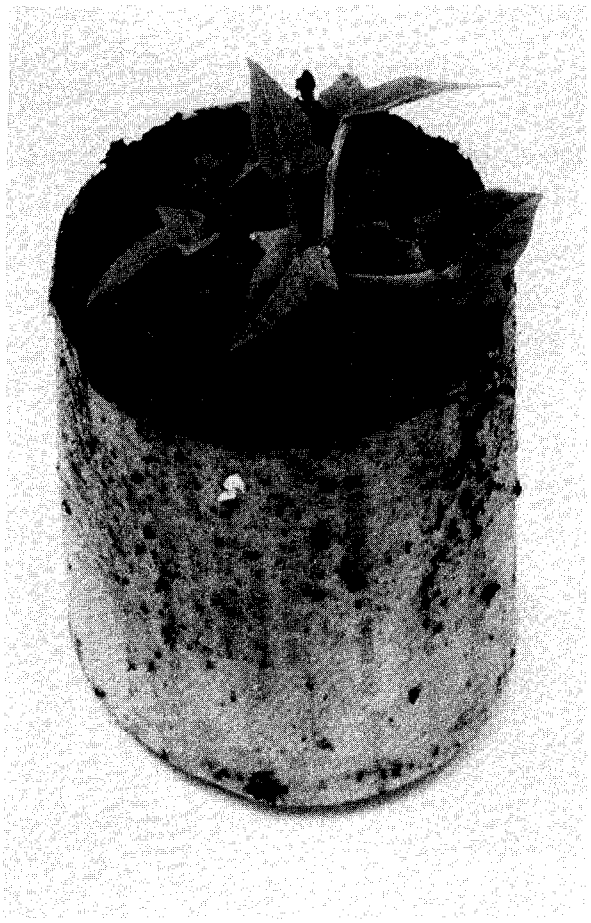

Figure 3

2.13 Place sterilized sand around the plantlet and press lightly to keep the plantlet straight in the pot.

2.14 Place the plant into a humid chamber during 48 h. Remove the humid chamber and wait until the roots are established (about 10 days).

2.15 Keep the pots in a clean location, at 25 to 27°C with 14-16 hours illumination.

2.16 Until the plants are well rooted irrigate lightly with tap water if it has a low salt content; otherwise use demineralized or rain water. Do not overwater.

2.17 When roots are established, you may dissolve supplementary nutrient in the irrigation water. Commercial peat moss often contains fertilizer, thus less additional nutrient may be required.

2.18 Gradually expose the plants to the normal atmosphere by removing the beakers for short periods each day.

2.19 Once the plants are established, transfer to larger (e.g. 20 cm diameter) pots. Be careful not to break the roots. When the plants are well rooted, normal fertilizer can be dissolved in the irrigation water. At CIP we use 5 g N:P:K at 12-12-12 per liter water. Apply 50-100 cm^3 per 20 cm diameter pot. Again, do not overwater.

3 MICROPROPAGATION

3.1 Materials and equipment

- Culture medium (see Section 4 and 5)
- test-tubes
- plastic caps or cotton wool
- forceps
- scalpel
- parafilm
- alcohol lamp
- alcohol 70%
- autoclave
- sterile work area ("microvoid")

3.2 Prepare the nutritive growth medium according to the procedure given in Section 5.

3.3 Dispense 4 cm^3 of the medium in each test tube. Cap the tubes with plastic caps or cotton wool plugs and autoclave them for 15 minutes. Keep the test-tubes vertical while the agar sets.

3.4 Working under sterile conditions (sterile area or "microvoid") follow steps 2.8 to 2.10.

3.5 Transfer the plantlets from the test tube to a sterile petri dish and make nodal cuttings using sterile scalpel and forceps. Each nodal cutting consists of a 0.2-0.5 cm stem segment with an axillary bud (Figure 4).

3.6 Place each nodal cutting in a test tube. Ensure that each cutting lies on the agar surface with its axillary bud pointing upwards (Figure 5). Place 1 or 2 cuttings per test tube.

3.7 Close the test tubes, seal with parafilm, label and place in a clean area where the room temperature is 25-27°C. Give 45 $\mu E/m^2/seg^2$ or 3,000 lux illumination for 14-16 hours each day.

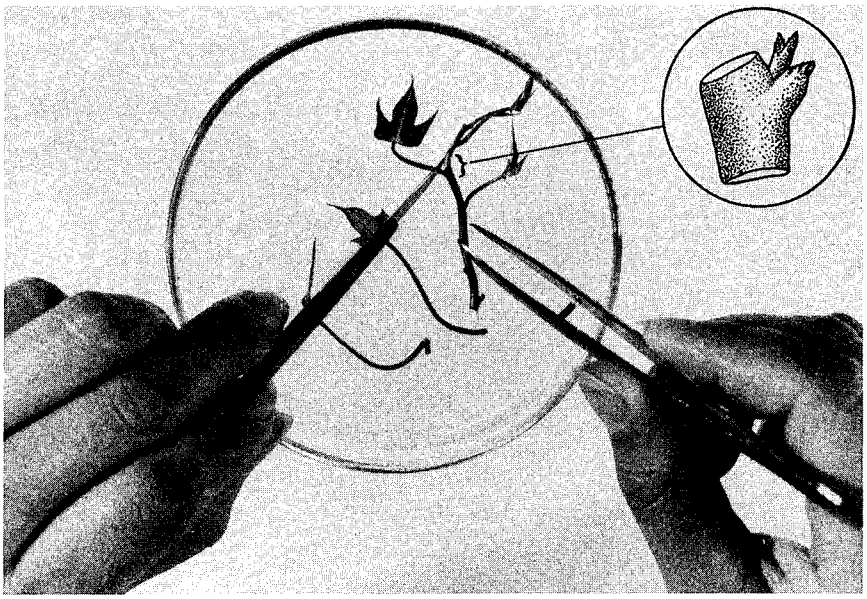

Figure 4

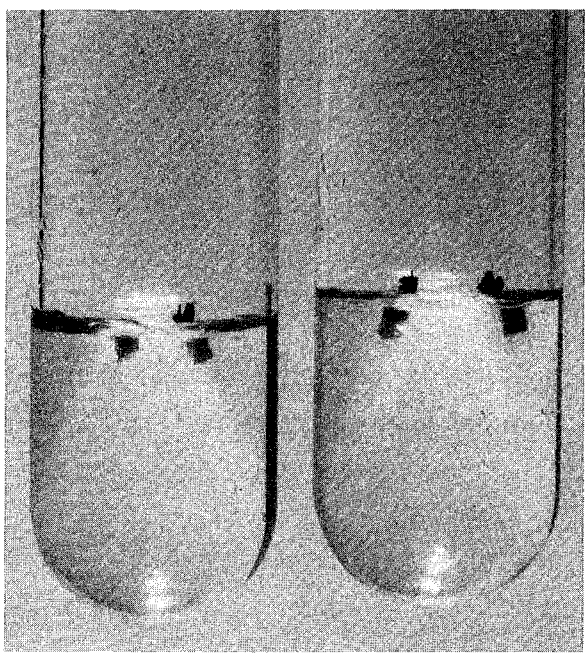

Figure 5

3.8 The axillary bud of each nodal cutting grows into a new plantlet within 2-4 weeks and is ready for transplanting to pots as previously described, or for further micropropagation (Figure 6).

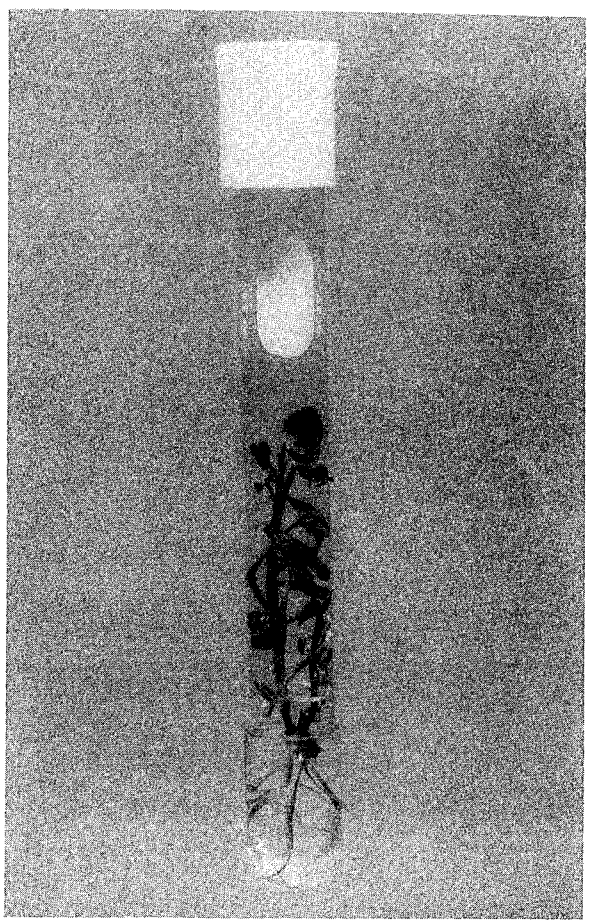

Figure 6

4 MURASHIGE-SKOOG MEDIUM (MODIFIED)

Prepare the stock solutions in four separate parts:

- Salts
- MgSO4
- Iron
- Vitamin

Salts stock solution: Dissolve each in 200 cm^3 glass distilled water.

-	NH_4NO_3	35.0 g
-	KNO_3	40.0 g
-	$CaCl_2.2H_2O$	9.0 g
-	KH_2PO_4	3.5 g
-	H_3BO_3	0.1 g
-	$MnSO_4.4H_2O$	0.5 g
	($MnSO_4.H_2O$	0.4 g)
-	$ZnSO_4.7H_2O$	0.2 g
	($ZnSO_4.H_2O$	0.1 g)
-	KI	0.02 g
-	$Na_2MoO_4.2H_2O$	0.005 g

Dissolve 5 mg (0.005 g) of the following salts together in 10 cm^3 of water; add 1 cm^3 of this solution to 200 cm^3 water for the stock solution

$CuSO_4.5H_2O$ y $CoCl_2.6H_2O$

Mix the ten individual salt solutions together to make 2,000 cm^3 of the salt stock solution.

$MgSO_4$ stock solution

- $MgSO_4.7H_2O$	3.7 g in 100 cm^3 distilled water

Iron stock solution

- Na_2EDTA	0.75 g
- $FeSO_4.7H_2O$	0.55 g

Dissolve $FeSO_4.7H_2O$ in 20 cm^3 distilled water; Na_2EDTA in 20 cm^3 distilled water heating it up. Mix the solutions, cool, and make up to 100 cm^3 with distilled water.

Vitamin stock solution

- Thiamine HCl	40 mg in 100 cm^3 distilled water

Medium preparation. Prepare 1 liter of the Murashige-Skoog basic medium by mixing the stock solutions with additional materials in the following proportions:

- Salts	100 cm^3
- $MgSO_4$	10 cm^3
- Iron	5 cm^3
- Vitamin	1 cm^3
- Inositol	100 mg
- Gibberellic acid	0.25 ppm
- Calcium pantothenic acid	2.0 ppm
- Sucrose	3.0 %
- Agar	0.8 %

Autoclave for 15 minutes.

5 MEDIUM TO SWEET POTATO MICROPROPAGATION

The medium used for this work is based on the salts Murashige-Skoog (1962). Prepare 1 liter of Murashige-Skoog medium (Section 4) with additional nutrients in the following proportions:

-	Calcium pantothenate	2 ppm
-	Gibberelic acid	20 ppm
-	Ascorbic acid	100 ppm
-	Calcium nitrate	100 ppm
-	Putrescine HCl	20 ppm
-	L-Arginine	100 ppm
-	Coconut milk	1%
-	Sucrose	5%
-	Agar or	0.7%
	Phytagel/Gelrite	0.25%

Autoclave for 15 minutes.

7 BIBLIOGRAPHY

CONGER, B. V. 1981. Cloning agricultural plants via in vitro techniques. CRC Press, Boca Ratón, USA.

ESPINOZA, N.; ESTRADA, R.; TOVAR, P.; BRYAN, J.; DODDS, J. H. 1984. Tissue culture micropropagation, conservation, and export of potato germplasm. Specialized Technology Document 1. International Potato Center, Lima, Peru. 20 pp.

HENSHAW, G. 1975. Technical aspect of tissue culture storage for genetic conservation. *In* Frankel, O.H., Hawkes, J.G. (eds.). Crop Genetic Resources for Today and Tomorrow. Cambridge University Press, Cambridge. pp. 349-358.

HU, C. Y.; WANG, P. J. 1983. Meristem, shoot tip, and bud cultures. *In* Evans, D.A.; Sharp, W.R.; Ammirato, P.V.; Yamada, Y. (eds.). Handbook of plant cell culture I. MacMillan, New York. pp. 177-227.

HUSSEY, G. 1983. In vitro propagation of horticultural and agricultural crops. *In* Smith, H. (ed.). Plant Biotechnology. Cambridge University Press, Cambridge. pp. 111-138.

MOREL, G. 1975. Meristem culture techniques for the long-term storage of cultivated plants. *In* Frankel, O.H.; Hawkes, J.G. (eds.). Crops Genetic Resources for Today and Tomorrow. Cambridge University Press, Cambridge. pp. 327-332.

MOREL, G.; MARTIN, G. 1952. Guérison de dahlias atteintes d'une maladie 'a virus. Comp Renel 235:1324-1325.

MURASHIGE, T. 1974. Plant propagation through tissue culture. Annual Reviews Plant Physiology 25:135-166.

MURASHIGE, T.; SKOOG, F. 1962. A revised medium for rapid growth and biossays of tobacco tissue cultures. Physiological Plantarum 15:473-497.

ROCA, W. M.; BRYAN, J. E.; ROCA, M. R. 1979. Tissue culture for the international transfer of potato genetic resources. Amer. Potato J. 56:1-11.

ROCA, W. M.; ESPINOZA, N. O.; ROCA, M. R.; BRYAN, J. F. 1978. A tissue culture method for rapid propagation of potatoes. Amer. Potato J. 59:691-701.

SCHILDE-RENTSCHLER, L.; ESPINOZA, N. O.; ESTRADA, R.; LIZARRAGA, R. 1982. In vitro storage and distribution of potato germplasm. Proc. 5th International Plant Tissue Culture Congress, Tokio. pp. 781-782.

WILKINS, C. P.; DODDS, J. H. 1982. The application of tissue culture techniques to plant genetic conservation. Science Progress 68:281-307.

WITHERS, L. A. 1980. Tissue culture storage for genetic conservation. Technical Report. IBPGR, Rome. 22 pp.

Processed by Training Department, CIP, Lima, Peru, December 1991

Copies printed: 1000 XIX-TR-E-15-O-1000

INTERNATIONAL POTATO CENTER (CIP)
CENTRO INTERNACIONAL DE LA PAPA (CIP)
CENTRE INTERNATIONAL DE LA POMME DE TERRE (CIP)

The International Potato Center (CIP) is a scientific, autonomous, and non-profit institution dedicated to develop and disseminate knowledge for greater use of the potato and other tuber and root crops as basic foods in the developing world. CIP was established by agreement with the Government of Peru and is supported by the Consultative Group on International Agricultural Research (CGIAR) whose members provide funding for international agricultural development.

El Centro Internacional de la Papa (CIP) es una institución científica, autónoma y sin fines de lucro, dedicada a desarrollar y diseminar conocimientos sobre la papa y otros cultivos de tubérculos y raíces, para lograr su mayor utilización como alimentos básicos en los países en desarrollo. El CIP fue establecido mediante convenio con el Gobierno del Perú y es apoyado por el Grupo Consultivo sobre Investigaciones Agronómicas Internacionales (GCIAI) cuyos miembros proveen fondos para el desarrollo agrícola internacional.

Le Centre International de la Pomme de Terre (CIP) est une institution scientifique, autonome et sans but lucratif, dont l'objectif est de développer et diffuser des connaissances pour mieux tirer profit de la pomme de terre et autres tubercules et racines comme aliments de base dans les pays en voie de développement. Le CIP a été établi avec l'accord du Gouvernement du Pérou et a l'appui du Groupe Consultatif pour la Recherche Agricole Internationale (GCRAI) dont les membres fournissent des fonds pour le développement agricole international.