

IN VITRO SWEET POTATO PLANTLETS - TRANSPORT & TRANSFER

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.

1. TRANSPORT OF TISSUE CULTURE MATERIAL

- **Packing.** The material is packed in polystyrene in a cardboard container. Each package contains small glass test-tube, each with one well-developed plantlet. Extra agar media is added to prevent damage from movement during shipment

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

- **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.
- **Handling after arrival.** The cultures should be cleared from customs as quickly as possible. When 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.

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

- **TRANSFER TO PLANTING MIX**

Materials:

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| • Peat moss | • Distilled water |
| • Fine sand (1mm diameter) | • 1% calcium-hypochlorite solution |
| • Aluminum foil | • 70% alcohol solution |
| • Pots (8 to 10 cm diameter) | • Strong soap |
| • Larger pots (20 cm diameter) | |
| • Fertilizer with high content of phosphorous (5-50-17) or (12-12-12) | |

- Mix peat moss and sand (1:2 by volume)
- In an autoclave if available, fill the pots with peat moss/sand mix, cover them with aluminum foil, and sterilize for 1 hour. If an autoclave is not available wash the 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.)
- Take the pots and the in vitro culture to a clean bench that is protected from air currents, dust, dirt, insects, or other contaminants.
- Wash your hands with strong soap and a 1% calcium-hypochlorite solution. Then rinse hands in 70% alcohol.
- Irrigate the pots with a small amount of water
- Prepare the pot to receive the plantlet by making a hole in the center of the peat moss/sand mix with a clean stick or pencil.
- 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.
- With clean hands remove the parafilm and the plastic cover from the test tube. Work with one tube at a time.
- Gently pull the plantlets with the agar out of the test tube using sterilized forceps (flamed to red heat and cooled).
- Wash the agar from the roots by gently immersing them several times in sterilized water, trying not to wet the rest of the plantlet.
- Plant each plant individually in the holes in the potting mix with the roots plus one or two nodes below the surface.
- Place sterilized sand around the plantlet and press lightly to keep the plantlet straight in the pot.
- Place the plant into a humid chamber during 48 h. Remove the humid chamber and wait until the roots are established (about 10 days)
- Keep the pots in a clean location, at 25 to 27C with 14-16 hours' illumination.
- 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.
- 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.
- Gradually expose the plants to the normal atmosphere by removing the beakers for short periods each day.
- 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 5g N:P:K at 12-12-12 per liter water. Apply 50-100 cm³ per 20 cm diameter pot. Again, do not overwater.