TISSUE CULTURE propagation of Potato
CIP SLIDE TRAINING SERIES

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Tissue culture (in vitro culture) offers an excellent technique for the rapid propagation of potato plants. The methods used depend on a synthetic growth medium, which stimulates the growth of axillary buds. The objective of this slide set and guidebook is to enable the users to further propagate in vitro potato plants they receive.

CIP continuously distributes in vitro potato germplasm to more than 50 developing countries. To ensure viability upon arrival, CIP sends in vitro material by air on the fastest route possible.

In vitro germplasm is distributed in test tubes. Each tube contains small, rooted plantlets growing in an agar solidified medium. The plantlets originate from meristems and have passed tests to detect known pathogens. For transport, the tubes are packed into a polyethylene box enclosed in a card-
board box. Each in vitro shipment is accompanied by an official Peruvian Phytosanitary Certificate and a phytosanitary statement of CIP.

A. After the export package (containing the in vitro recovered plantlets) has been in transit, sometimes up to three weeks, some of the plantlets can become etiolated.

B. Upon receipt the tubes should be removed carefully from the package and placed in diffused light (approximately 1000 lux) for about one week to recover. Once the plantlets have recovered their green color, the material is ready for further in vitro propagation.

Plantlets can be propagated in vitro by two different methods.

In the first method, single-node cuttings are excised and then grown in tubes on an agar solidified medium.

In the second method, known as shaken liquid cultures, stem segments are transferred into a flask of agitated liquid medium.

Let us first look in detail at propagation by single node cuttings.
PROPAGATION BY SINGLE-NODE CUTTINGS

The equipment needed to propagate potatoes in vitro by single-node cuttings is simple and consists of:

- Culture medium
- Glass tubes and caps
- Scalpel and forceps
- Alcohol lamp
- Petri dish

The operations are carried out under aseptic conditions. The optimal method for achieving this is to work at a laminar-air-flow sterile bench.
Successful results can also be obtained by working in a simply constructed box of approximately 50 x 50 cm: stretch a plastic sheet over a wooden frame, leaving the front open for access. Sterilize the interior of the box by wiping with 70% alcohol and 2.5% calcium hypochlorite before beginning sterile transfer operations.

Working under sterile conditions, quickly flame the mouth of the tube and whilst holding over a petri dish, carefully remove the plantlet(s) using a pair of forceps.

In the petri dish, carefully remove the leaves from the stem and cut the stem into single-node segments. If the plantlet is highly etiolated, remove stem segments to obtain short nodes having intact axillary buds.
This single-node cutting has had the small leaf removed, however, the axillary bud remains undamaged. The average single-node cutting is 2 to 3 mm long.

Using the tip of a scalpel, transfer the isolated nodes onto the surface of a fresh propagation medium prepared as described later in this slide set. Quickly flame the mouth of the tube before capping. Do not damage the single-node cutting with the point of the scalpel. Make sure that the cutting is in good contact with the surface of the medium, but it should not be pushed below the surface.

When beginning this type of work, transfer only one cutting per tube to minimize the risk of infecting the material; with more experience it is possible to transfer three or four cuttings per tube.

Single-node cuttings begin to grow rapidly into new, rooted plantlets. Here we see freshly transferred cuttings (far left) alongside material after two, four, and eight weeks of growth. The material can be subcultured as needed every 7 to 8 weeks.
The tubes are incubated in a growth room to allow plantlets to grow. The ideal growth temperature is 20-22 °C with 16 hours of light supplied by warm-white or gro-lux fluorescent tubes.

It is also possible, however, to grow the plantlets in front of a window as long as they are protected from strong direct sunlight.

LIQUID SHAKEN CULTURES

The second method of propagation is the use of liquid shaken cultures in flasks. This method is more expensive to set up since an electric shaker is required; however, it is much more rapid and produces more plantlets ready for subculture after only 3 weeks.
Remove the plantlet from the tube and place on a petri dish.

Remove the shoot pex, roots, and leaves. This results in a stem segment with axillary buds along its length. Transfer three or four of these stem segments into flasks of liquid medium.

Liquid Murashige and Skoog medium is used. Gibberellic acid has been added to this liquid medium to release the dormancy of all the axillary buds along the length of the stem and to promote rapid and bushy growth.
The flasks containing these stem segments are then incubated at 20 °C in 16 hours of light for 3 weeks. During this time, the flasks are shaken on a horizontal shaker at 80 rpm. When the plantlets are fully grown the flasks will contain material for further production of individual rooted in vitro plantlets.

To produce individual plantlets, prepare single-node cuttings again.

A. Single-node cuttings may be transferred into autoclavable, sterile, plastic boxes, or mermelade jars.

B. After two weeks of growth small plantlets can be seen.

C. After four weeks of growth, strong, individual, rooted in vitro plantlets are ready for a number of uses. The larger containers facilitate movement of large numbers of plantlets and work well for seed multiplication programs.
Uses of In Vitro Plants

- Production of seed tubers
- Production of mother plants
- Induction of in vitro tubers
- Conservation of germplasm
- Distribution of germplasm

In vitro plantlets can be used for several purposes:

- Production of seed tubers
- Production of mother plants
- Induction of in vitro tubers
- Conservation of germplasm
- Distribution of germplasm

High quality seed tubers (pre-basic seed) can be produced by removing the in vitro plantlets from tubes or sterile boxes and planting them into seedbeds. The beds, containing an appropriate planting mix, are located in a greenhouse or screenhouse. High relative humidity is important, especially during the first day following transplanting. Care must be taken to water the young plants with a fine mist.

The in vitro plantlets are normally transplanted at a high density in 1-m² seedbeds. The density of planting will depend on the genotype, but it is normally between 50-150 plants/m².
The plants grow vigorously and after 90 days the tubers are harvested.

The yield and size of tuber per m² depends on the genotype and density. In the example shown, the tubers are separated on the basis of seed tuber weight < 1 g, 1-5 g, 5-10 g, 10-20 g, 20-40 g, > 40 g. Tubers of 5 g and larger can be planted in the field for further multiplication. Those less than 5 g can be replanted in seedbeds at a high density producing more pre-basic seed tubers.

It may be desirable to use individual in vitro plantlets for producing mother plants to use in conventional rapid propagation methods; for example, stem, single-node, or leaf bud cuttings. Cuttings can also be taken from plants in the seedbeds.
It is also possible to take in vitro plants (*flask A*) and by a change in medium composition induce the formation of in vitro potato tubers (*flask B*).

The diameter of these in vitro tubers is around 5 mm. CIP is already using these in vitro tubers experimentally for germplasm distribution. They also grow well when planted at high density in seedbeds.

In vitro plantiets are also used for the conservation of germplasm. By adding 4 % Mannitol to the medium, it is possible to restrict plantlet growth to the extent that subculturing is required only once every two to three years.
In vitro plantlets are being used more and more for the international exchange of germplasm. In vitro potato plantlets are being packaged and distributed worldwide by CIP.

Here we see the various schemes by which the potato can be clonally propagated in vitro.
- Single-node cuttings
- Shaken liquid cultures
- In vitro tubers

For more information contact the International Potato Center (CIP), P.O. Box 5969, Lima, Peru.