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TISSUE CULTURE MICROPROPAGATION,  
CONSERVATION, AND  
EXPORT OF POTATO GERMPLASM

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TISSUE CULTURE MICROPROPAGATION,  
CONSERVATION, AND  
EXPORT OF POTATO GERMPLASM

- 1 Advantages of tissue culture techniques
- 2 Meristem isolation
- 3 Micropropagation
- 4 Maintenance and long-term storage
- 5 In vitro shipment
- 6 Media
- 7 Bibliography

Tissue culture allows the rapid clonal propagation of large numbers of plantlets in a short period and the conservation of potato germplasm under controlled conditions requiring reduced space and labor. The pathogen-free status of many in vitro cultures greatly facilitates international germplasm exchange.

This document describes the advantages, methodology, and materials of tissue culture techniques applied at the International Potato Center (CIP) and discusses the techniques of meristem isolation, micropropagation, long-term storage, and in vitro export of germplasm.

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## 1    ADVANTAGES OF TISSUE CULTURE TECHNIQUES

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CIP maintains a potato germplasm collection with some 6000 clones which serve as a rich source of genetic diversity for use by breeders in potato improvement programs. The maintenance of this collection in the field is expensive, and the collection is susceptible to a wide range of risks such as disease infection or adverse weather conditions. Maintenance of the collection in vitro holds a number of advantages over growing the collection each year in the field:

- reduction of labor costs
- avoidance of field infections
- avoidance of environmental hazards, e.g. hail, frost
- easy availability of material for micropropagation
- easy availability of material for pathogen elimination
- availability of material for propagation and export all year round

The transfer of the potato germplasm collection to in vitro (tissue culture) conditions is currently in progress and will take several years to complete.

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In this document we will follow the path of a potato clone through meristem culture, micropropagation, storage, and eventual export as shown in Figure 1.

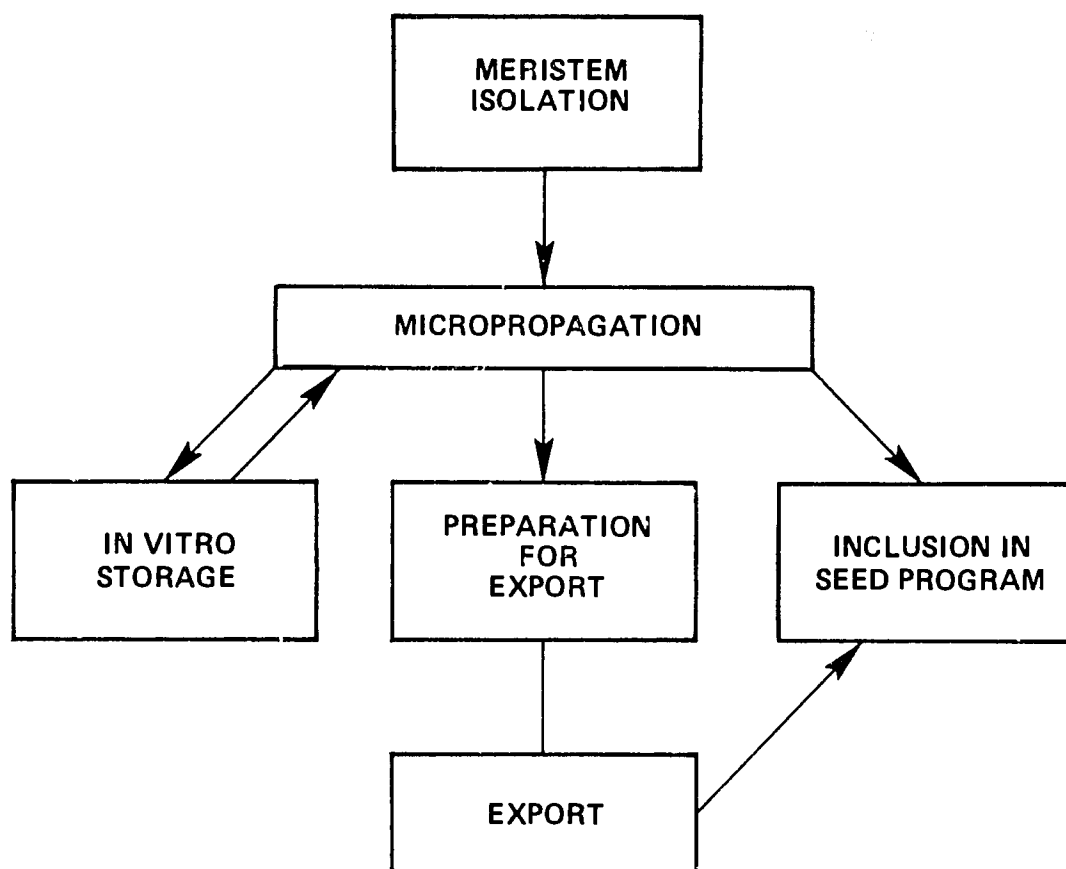


Fig. 1. Flow diagram showing the stages that plants pass through before eventual in vitro export.

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## 2 MERISTEM ISOLATION

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The active growing point of the plant shoot is the meristem. This is a small organ composed of rapidly dividing (meristematic) cells. For propagating potato shoot cultures, it is the ideal starting material as it has two favorable characteristics:

- The isolated meristem develops in culture in a genetically stable form. This is not the case, for example, in disorganized callus cultures that show major genetic irregularities.
  
- The isolation of meristems reduces the level of viral infection in the tissue and, under appropriate conditions, can be used for complete pathogen eradication.

The dome of a shoot apical meristem contains the truly meristematic cells and is surrounded by leaf primordia and primary leaves. Since the more differentiated vascular tissues occur away from the meristem (towards to older tissues of the stem), the vascular elements of the leaf primordia are still very incipient and have not yet made contact with the main strand of the vascular system in the stem. Therefore, virus particles, which may be present in the vascular system, can reach the meristematic region of the apex only through movement from cell to cell. This is one of the main reasons why in a virus-infected plant, virus concentration decreases acropetally toward the meristem of the apical as well as the axillary buds. This situation is, in principle, the same for all crops, the only difference being the shape of the meristem and the leaf primordia.

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Whether other factors, such as the production of virus inhibitory substances by the meristematic cells or the effect of hormones in the culture medium, play a role in the elimination of virus by meristem culture has not been proven.

Isolation of the apical part, called the meristem tip, under aseptic conditions and its culture on an adequate aseptic medium leads to the development of plantlets. This development, in principle, follows a pattern similar to that in the entire plant; cells of the meristem divide and differentiation of tissues continues. The nutrition of the excised portion of the plant is supplied by the artificial medium. This technique, called meristem culture, was first applied by Morel and Martin (1952) on dahlia, and can be used to produce pathogen-free plants.

The aseptic dissection of the meristem is a delicate process and requires many hours of practice. The sequence of dissection is shown photographically (Figure 2) and is carried out as follows: Cut stems into segments each containing one node with its axillary bud. Carefully remove the leaves. Disinfect pieces for 30 seconds in 70% alcohol, followed by 2.5% calcium hypochlorite for 15 minutes. Wash four times with sterile distilled water to remove excess hypochlorite.

Under a binocular dissecting microscope, cut and remove the leaflets surrounding the growing point until only the apical dome and a few leaf primordia remain. Cut off the dome and two leaf primordia and transfer to Medium A (Section 6). Transfer weekly to fresh medium. After 6-8 weeks, transfer the small plantlets to larger tubes for further growth. When the plants are 4 cm high, micropropagation can begin.

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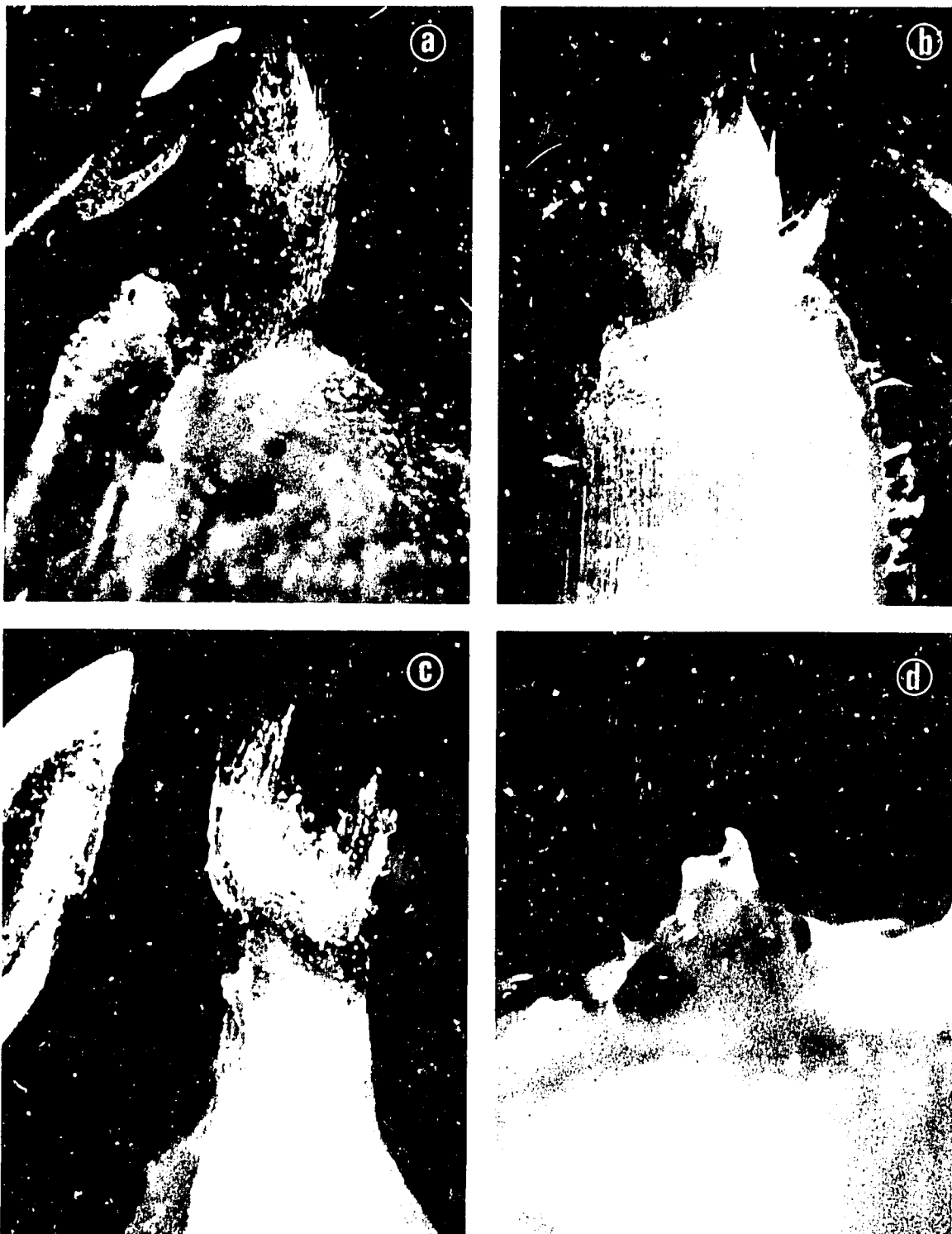


Fig. 2. Photographic sequence of meristem dissection:  
a - The isolated surface-sterilized bud.  
b - and c - Various stages in the dissection where primary  
leaves are removed.  
d - The fully dissected meristem with two leaf primordia.

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### 3 MICROPROPAGATION

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The objective of micropropagation is to obtain large numbers of clonal plants in a short period. At CIP, micropropagation is carried out by two methods:

- nodal cuttings, and
- shaker cultures.

Nodal cuttings. Single nodes with leaves are excised from small in vitro plantlets and the large leaves are carefully removed. Each node is then inoculated onto the surface of agar-solidified Medium B. The axillary bud quickly grows out (Figure 3), and in 3-4 weeks six or seven more nodes will be available for transfer.

Shaker cultures. In vitro plantlets are cut in pieces with three to four nodes; the large leaves are removed. Each nodal piece is placed in 15 ml of liquid Medium F and the flask is shaken (80 rpm). After 2-3 weeks of rapid growth each flask contains 60 or 70 nodes (Figure 4).

Once a suitable number of small plantlets have been produced, they need to be rooted and transferred to non-sterile conditions.

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Rooting and transfer to non-sterile conditions. After removal of the large leaves, 16 - 25 excised single nodes of in vitro plantlets are placed on agar-solidified Medium B in a 6 x 6 cm plastic container. When the plantlets are 3 - 5 cm high and have developed a good root system (Figure 5), they are ready for transplanting into pots or beds containing a suitable compost mixture (Figure 6). Take care not to damage the roots, and ensure good contact between roots and planting media. Keep the plants in an environment with high relative humidity for the first few days.



- Fig. 3. Growth of single node cuttings; tubes from left to right show a sequence of growth.
- Fig. 4. Shaken culture for rapid propagation. The flask shown is now ready to be subcultured.
- Fig. 5. Box containing 25 individual rooted plantlets ready for transfer from the sterile environment to compost.
- Fig. 6. Plantlet a few days after transfer to compost.

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#### 4 MAINTENANCE AND LONG-TERM STORAGE

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In vitro material can be kept in culture indefinitely, provided sufficient care is taken to avoid contamination and transfers to fresh media are made at appropriate intervals. In this way a pathogen-tested stock can be kept as a reserve for future use.

For short-term maintenance, plantlets are grown on Medium B or D in tubes sealed with autoclavable plastic caps. Cotton wool plugs are easily penetrated by fungi and, although suitable for short-term storage, are not recommended for medium- or long-term storage.

The growth rate of the plantlets depends on incubation temperature, medium composition, and cultivar. It follows, therefore, that for long-term storage, modifications to the media and incubation temperature are required.

Both Media C and D exert an osmotic stress and can be used for storage at 25°C; the stress reduces the growth rate and produces short internodes. Many nodes are thus available when propagation of the stored material is required. Under these conditions material only needs to be transferred annually. However, when using Medium C the temperature can be lowered to 8°C, thereby significantly reducing the growth rate of the plantlets. At this low temperature transfers are only required once every 2 or 3 years.

It is possible, therefore, to maintain a germplasm collection under the storage conditions outlined above. When a request is received for a particular genotype, it can be removed from storage and micropropagated.

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## 5 IN VITRO SHIPMENT

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Material from the pathogen-tested collection can be exported to other countries as in vitro plantlets. Single node segments can be obtained from micropropagated material and transferred to tubes containing Medium E. When the in vitro plantlets are 2 cm high and have a well-developed root system, the tubes with plantlets are carefully packed (Figure 7) for air shipment. The consignee is informed of the flight and airway bill number by telex or telegram, so that the shipment can be quickly released from customs. Attached to every in vitro shipment is a leaflet explaining the handling of material after receipt, a phytosanitary certificate, and a card to return to CIP giving details about the state of arrival.

The in vitro plantlets received in this way can be transferred directly to compost in plant pots or further micropropagated (Section 3).

At the present time all in vitro shipments are in the form of 2 cm high plantlets; however, investigations are under way to complement or substitute this method with export of in vitro tubers (Figure 8). These are very small, aseptic, pathogen-free tubers that can be induced in culture. They are easier to transport and handle than in vitro cultures.

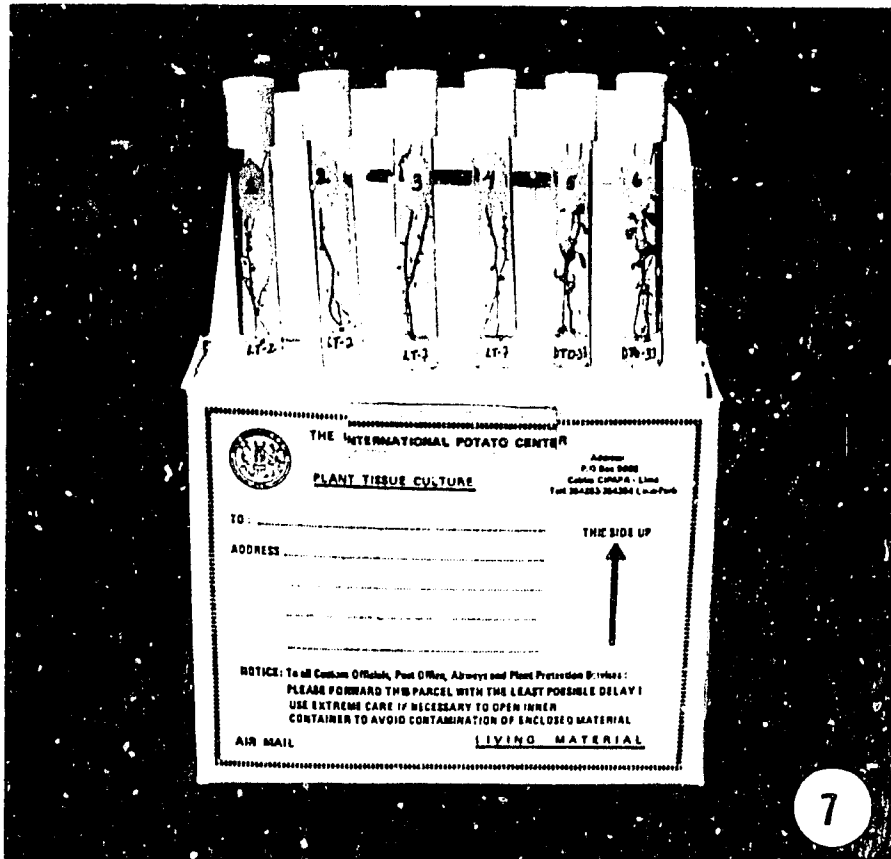


Fig. 7. In vitro plantlet export package.

Fig. 8. In vitro tubers sprouted after storage. The 9 cm petri dish gives an idea of average tuber size.

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6 MEDIA

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All media used for this work are based on the salts of Murashige and Skoog (1962). The salt stock solutions are normally prepared in four separate parts:

- Salts
- $MgSO_4$
- Iron
- Vitamins

Salts stock solution: dissolve each in 200 ml glass distilled water

- $NH_4NO_3$	35	g
- $KNO_3$	40	g
- $CaCl_2 \cdot 2H_2O$	9	g
- $KH_2PO_4$	3.5	g
- $H_3BO_3$	0.1	g
- $MnSO_4 \cdot 4H_2O$	0.5	g
( $MnSO_4 \cdot H_2O$ )	0.4	g)
- $ZnSO_4 \cdot 7H_2O$	0.2	g
( $ZnSO_4 \cdot H_2O$ )	0.1	g)
- KI	0.02	g
- $Na_2MoO_4 \cdot 2H_2O$	0.005	g

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

- $CuSO_4 \cdot 5H_2O$  and  $CoCl_2 \cdot 6H_2O$

Mix the ten individual salt solutions together to make 2000 ml of the salt stock solution.

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MgSO<sub>4</sub> stock solution

- MgSO<sub>4</sub>.7H<sub>2</sub>O                      3.7 g in 100 ml distilled water

Iron stock solution

- Na<sub>2</sub>EDTA                              0.75 g
- FeSO<sub>4</sub>.7H<sub>2</sub>O                      0.55 g

Dissolve FeSO<sub>4</sub>.7H<sub>2</sub>O in 20 ml distilled water; Na<sub>2</sub>EDTA in 20 ml distilled water heating it up. Mix the solutions, cool, and make up to 100 ml with water.

Vitamin stock solution

- Thiamine HCl                      40 mg in 100 ml distilled water



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

Salts	100 ml
MgSO <sub>4</sub>	10 ml
Iron	5 ml
Vitamin	1 ml
Inositol	100 mg

Add the relevant hormones and sucrose (see below), and autoclave the medium with or without agar for 15 minutes.

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Additions to the basic MS medium for preparation of specific media types:

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Medium	MS media + the following additions		
A	0.25	ppm	Gibberellic acid
	2.00	ppm	Ca Pantothenic acid
	3.0	%	Sucrose
	0.6	%	Agar
B	0.25	ppm	Gibberellic acid
	2.00	ppm	Ca Pantothenic acid
	3.0	%	Sucrose
	0.8	%	Agar
C	4.0	%	Mannitol
	3.0	%	Sucrose
	0.8	%	Agar
D	4.0	%	Mannitol
	0.5	%	Sucrose
	0.8	%	Agar
E	0.25	ppm	Gibberellic acid
	2.00	ppm	Ca Pantothenic acid
	3.0	%	Sucrose
	1.0	%	Agar
F	0.40	ppm	Gibberellic acid
	0.50	ppm	Benzylamino purine
	0.01	ppm	Napthalene acetic acid
	2.00	ppm	Ca Pantothenic acid
	2.0	%	Sucrose

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7 BIBLIOGRAPHY

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- Conger, B.V. 1981. Cloning agricultural plants via in vitro techniques. CRC Press, Boca Ratón, USA.
- 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 à virus. *Comp Rend* 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 bioassays 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., Lizárraga, R. 1982. In vitro storage and distribution of potato germplasm. *Proc. 5th International Plant Tissue Culture Congress, Tokio* pp. 781-782.
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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.

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