Specialized Technology Document 1

Tissue Culture Micropropagation, Conservation, and Export of Potato Germplasm

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INTERNATIONAL POTATO CENTER (CIP)

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TISSUE CULTURE MICROPROPAGATION, CONSERVATION, AND EXPORT OF POTATO GERMPLASM

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T I S S U E C U L T U R E M I C R O P R O P A G A T I O N ,
C O N S E R V A T I O N , A N D
E X P O R T O F P O T A T O G E R M P L A S M

It is possible to use tissue culture technology to propagate, conserve, and transfer potato germplasm. 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 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.

1 ADVANTAGES OF TISSUE CULTURE TECHNIQUES

The International Potato Center (CIP) maintains a potato germplasm collection with some 6000 clones. This collection serves 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. The maintenance of the germplasm collection in vitro holds a number of advantages over conventional field maintenance -- these include:

- reduction of labor costs,
- avoidance of field infections,
- avoidance of environmental hazards, i.e. hail and 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 more to complete.

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

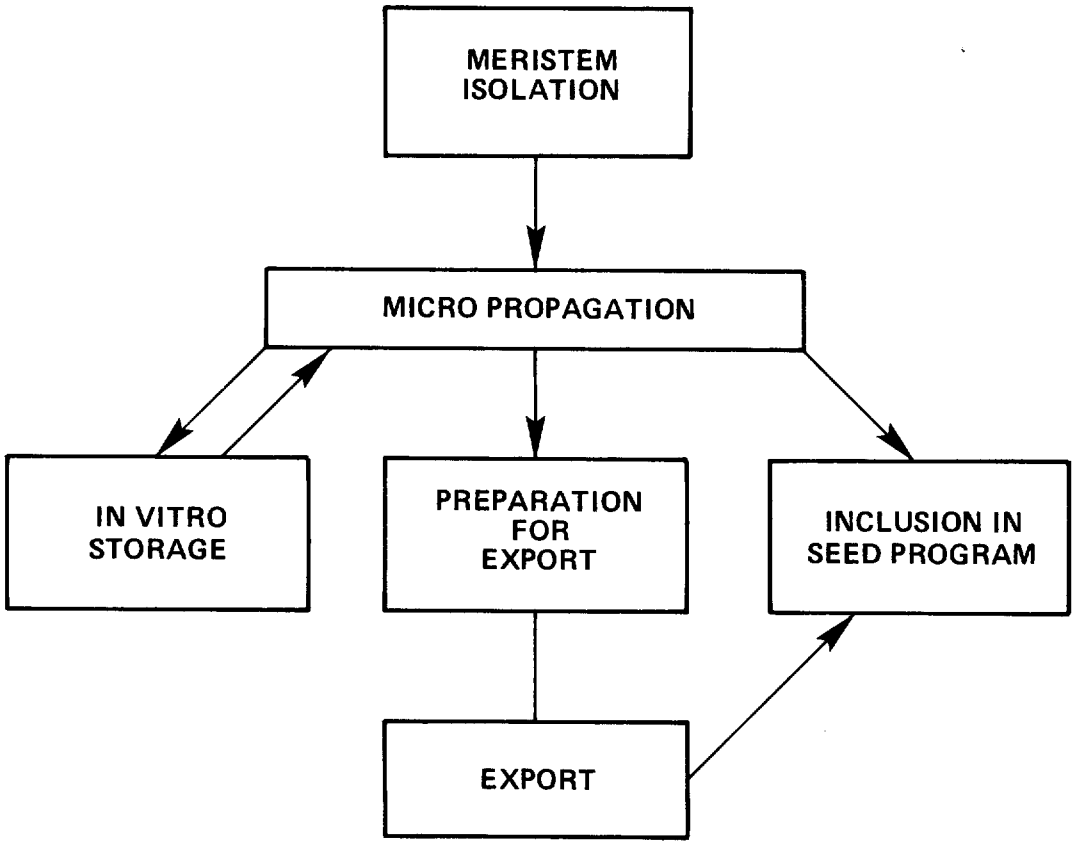


Fig. 1 - Flow diagram showing the stages that plants pass through before eventual in vitro export.

2 MERISTEM ISOLATION

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 (see "Meristem culture and thermotherapy for pathogen elimination" - Specialized Technology Document, in preparation), 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 these elements 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 is in the shape of the meristem and the leaf primordia.

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: the cells of the meristem divide and the differentiation of tissues continues. The nutrition of the excised portion by the plant is supplied by the artificial medium. This technique, called meristem culture, was applied first some 30 years ago by Morel and Martin on Dahlia (1952), and it can lead to 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 % Ca hypochlorite for 15 minutes. Wash four times with sterile distilled water to remove excess hypochlorite.

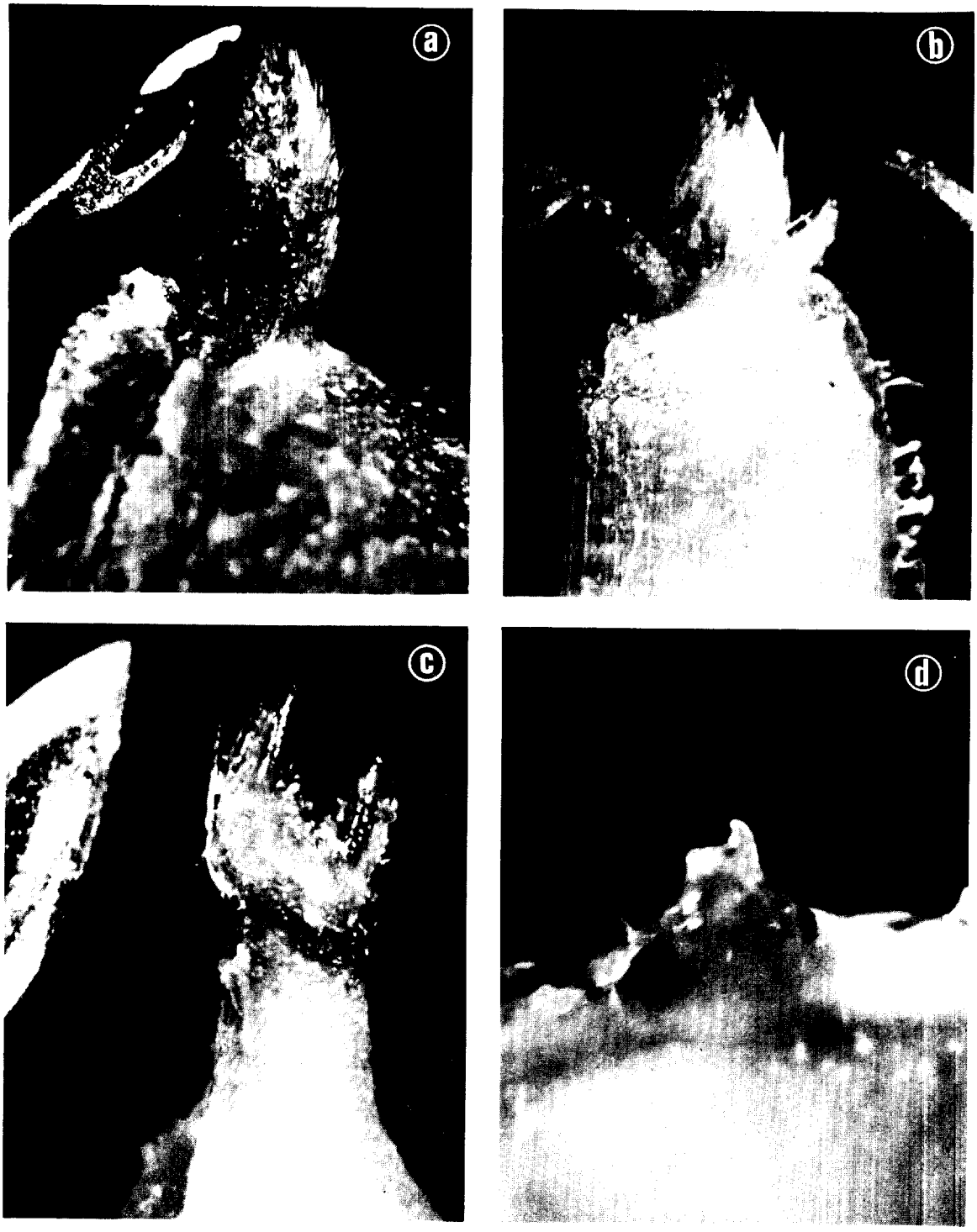


Fig. 2 - Photographic sequence of meristem dissection:
a - The isolated surface sterilized bud.
b - and c - Various stages in the dissection where primary leaves are removed.
d - The fully dissected meristem with two leave primordia.

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.

3 MICROPROPAGATION

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

- nodal cutting and
- shaker cultures.

Nodal cuttings. Single nodes with leaves are excised from small in vitro plantlets. The large leaves are carefully removed. The single node is then inoculated onto the surface of agar solidified Medium B. The axillary bud will quickly grow 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 and large leaves are removed. These nodal segments are then placed in 15 ml of liquid Medium F and the flask is shaken (80 rpm). After 2-3 weeks very rapid growth has taken place (Figure 4) and each flask will contain 60 or 70 nodal sections.

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

Rooting and transfer to non-sterile conditions. From excised single nodes of *in vitro* plantlets, large leaves are removed and the nodes are placed on agar solidified Medium B for rooting. When the plantlets are 3-5 cm high and have developed a good root system, they are ready for transplanting (Figure 5). The rooted plantlets can be transplanted into pots or beds containing a suitable compost mixture (Figure 6). Take care not to damage the roots, and ensure that good contact is made between roots and planting media. Keep the plants in an environment with high relative humidity for the first few days.

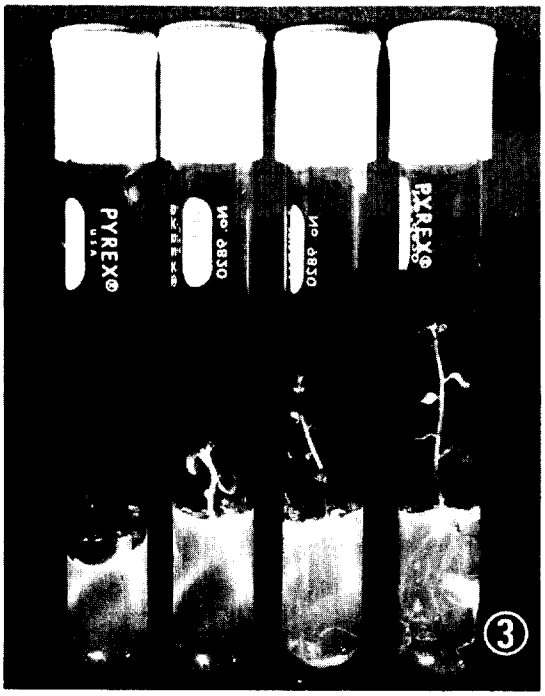


Fig. 3 - Growth of single node cuttings, tubes from left to right show a sequence of growth.

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 removal from the sterile environment to transfer to compost.

Fig. 6 - Small in vitro derived plantlets a few days after transfer to compost.

4 MAINTENANCE AND LONG-TERM STORAGE

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

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

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

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. Storage times under these conditions are such that material only needs to be transferred annually.

However, using Medium C, the temperature can be lowered to 8 °C. This significantly reduces the growth rate of the plantlets and transfers under these conditions are only required once every 2 or 3 years.

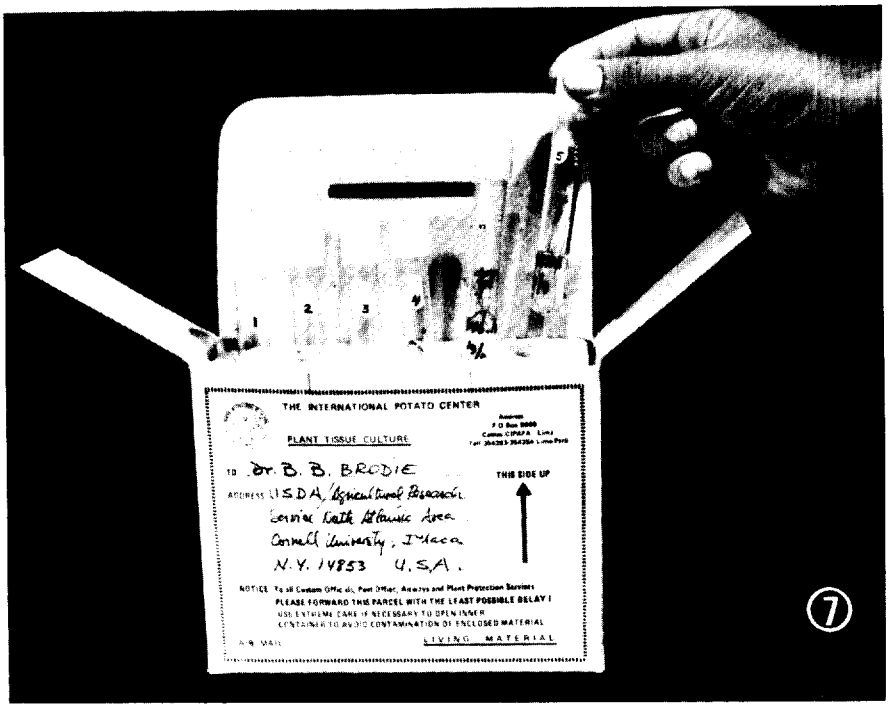
It is possible, therefore, to maintain a germplasm collection under the storage conditions outlined above. When a request is received for export of a particular genotype, it can be removed from storage, and micropropagated.

5 IN VITRO SHIPMENT

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 packed carefully (Figure 7) for air shipment. The receiver is informed of the flight and airway bill numbers by telex or telegram, so shipment can be liberated 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 to compost in 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 more easily handled by the receiving country and are far easier to transport. The induction and value of in vitro tubers is covered in a separate specialized technology document.



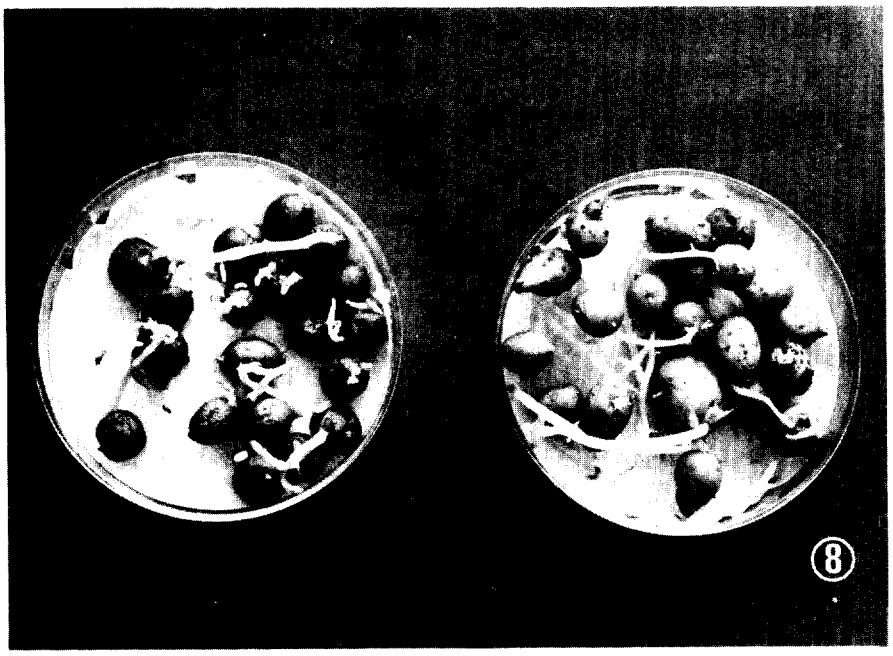


Fig. 7 - An *in vitro* plantlet export package.
Fig. 8 - A sample of *in vitro* tubers after storage showing sprout outgrowth. 9 cm petri dish gives an idea of average tuber size.

6 MEDIA

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 (10 x concentrated stock solution); each one in 200 ml glass distilled water

-	NH_4NO_3	33 g
-	KNO_3	38 g
-	$CaCl_2.2H_2O$	8.8 g
-	KH_2PO_4	3.4 g
-	H_3BO_3	0.124 g
-	$MnSO_4.4H_2O$	0.446 g
	$(MnSO_4.H_2O)$	0.338 g
-	$ZnSO_4.7H_2O$	0.172 g
	$(ZnSO_4.H_2O)$	0.1228 g
-	KI	0.0166 g
-	$Na_2MoO_4.2H_2O$	0.005 g
-	$CuSO_4.5H_2O$	0.0005 g
-	$CuCl_2.6H_2O$	0.0005 g

Total = 2200 ml

$MgSO_4$ (100 x concentrated stock solution)

- $MgSO_4.7H_2O$ 3.7 g in 100 ml distilled water

Iron (100 ml of stock solution)

- Na_2 EDTA 0.745 g
- $FeSO_4.7H_2O$ 0.557 g

Dissolve $FeSO_4.7H_2O$ in 20 ml distilled water; Na_2 EDTA in 20 ml distilled water heating it up. Mix $FeSO_4.7H_2O$ and Na_2 EDTA, cool, and complete to 100 ml with water.

Vitamins (100 ml of stock solution)

- Thiamine HCl 40 mg in 100 ml distilled water

Preparation of media. 1 lt of MS (Murashige-Skoog) basic media is prepared by mixing the stock solutions above in the following proportions:

Salts	100 ml
$MgSO_4$	10 ml
Iron	5 ml
Vitamins	1 ml

The relevant hormones and sucrose (see below) are added, and the media with or without agar is autoclaved for 15 minutes at 15 lb/in^2 at 121 °C.

The additions to the basic MS medium for preparation of specific media types are as follows:

Medium	MS media + the following additions		
A	0.25	ppm	Gibberellic acid
	2.00	ppm	Ca Pantothenic acid
	3	%	Sucrose
	0.6	%	Agar
B	0.25	ppm	Gibberellic acid
	2.00	ppm	Ca Pantothenic acid
	3	%	Sucrose
	0.8	%	Agar
C	4	%	Mannitol
	3	%	Sucrose
	0.8	%	Agar
D	4	%	Mannitol
	0.5	%	Sucrose
	0.8	%	Agar
E	0.25	ppm	Gibberellic acid
	2.00	ppm	Ca Pantothenic acid
	3	%	Sucrose
	1	%	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	%	Sucrose

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