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CIP Research Guide 32

**TISSUE CULTURE OF *Ipomoea batatas*:  
MICROPROPAGATION AND MAINTENANCE**

1992

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CENTRO INTERNACIONAL DE LA PAPA (CIP)  
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**TISSUE CULTURE OF *Ipomoea batatas*:  
MICROPROPAGATION AND MAINTENANCE**

- 1 Advantages of tissue culture**
- 2 Introduction of in vivo material into in vitro**
- 3 Isolation of meristems**
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- 6 Culture media**
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Tissue culture allows the rapid clonal propagation of a large number of plantlets over a short period, as well as the maintenance of germplasm under controlled conditions in small spaces and with reduced labor requirements.

This document describes the advantages, methodologies and materials used for tissue culture at the International Potato Center (CIP). It analyzes isolation, micropropagation, and long term storage techniques.

Sweetpotato (*Ipomoea batatas* (L.) Lam.) ranks seventh among the world's food crops according to FAO statistics (1978).

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## 1 ADVANTAGES OF TISSUE CULTURE

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CIP maintains a sweetpotato germplasm collection of over 5,000 accessions. Their clonal maintenance on the field is expensive and involves the risk of loss due to infectious diseases or unfavorable climatic conditions. Thus, in vitro maintenance presents the following advantages:

- lower labor costs,
- absence of field infections,
- protection against unfavorable climatic conditions,
- timely access to material under maintenance,
- timely access to material for pathogen clean-up,
- permanent availability of (when pathogen tested) material for propagation and exportation.

To date CIP maintains 2,800 in vitro accessions in its sweetpotato germplasm collection.

The *I. batatas* (L.) Lam. species is variously known in different parts of the world as batata, batata doce, boniato, camote, cumar, and sweetpotato. We shall hereafter refer to *I. batatas* as sweetpotato.

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## 2 INTRODUCTION OF IN VIVO MATERIAL INTO IN VITRO

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The in vivo mother plant must come from the greenhouse. It should be two to three months old, in excellent health conditions, and free of lateral buds that are too sprouted (at any rate, these must not be included). The stems are excised from the mother plant and the leaves removed. Part of the petiole should be left to cover the buds. Stem cuttings 2 to 3 cm long each must include an axillar bud and a portion of the internode behind the bud for ease of manipulation. Before sending them to the in vitro laboratory, the stem cuttings are treated with a wide spectrum acaridicide (that will destroy the acari at any stage of development) such as Morestan-Bayer (Chinometonat) at 0.5% for 10 minutes. The acaricide is next rinsed away by washing the stems in running water. They are then placed in a clean bottle covered with a petri dish until starting surface disinfection.

To start surface disinfection of the stem cuttings, remove the water from the container, add 96% alcohol and let stand for two seconds. Next, remove the alcohol and immediately add a 2.5% solution of calcium hypochlorite (brought to pH 8 by addition of HCl). Sodium hypochlorite or chlorine (bleach) may also be used. If possible, add a few drops of a dispersing-adhering agent such as Tween 20 or 80 (4 drops/l of solution). The bottle is then placed in the laminar flow transfer chamber. After 15 minutes under sterile conditions, the hypochlorite is eliminated by washing three times with sterile water. To reduce phenolization of the explants, after the last rinse they are left in a sterile 100 ppm solution of ascorbic acid before proceeding to excision.

Under these conditions, proceed to excise the buds by eliminating the largest possible number of leaflets and leaf primordia. The excised portions should be as small as possible. The optimum size is 0.6 mm but the explant may be bigger if no adequate excision instruments, such as a stereoscopic microscope, are available.

The buds are planted in an MMB-I culture medium and are kept there for 15 days. They are then transferred to an MMB-II medium where the plantlets will grow over

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a period of 30 to 60 days. Propagation by individual nodes may then be carried out in an MPB medium.

In the first propagation stage, 16 x 125 mm tubes are used. In the second stage, either 18 x 150 mm or 25 x 150 mm tubes may be utilized.

Due to the fact that in these cases meristematic portions larger than 0.6 mm are used, some material may be contaminated mainly by saprophytic bacteria. If so, proceed in either of two ways.

- 1 If a stereoscopic microscope is available, excise meristems between 0.4 and 0.6 mm long, plant them in the introduction medium (MMB-I) and transfer them weekly to the MMB-II medium.
- 2 Eradication of bacteria or yeasts may be attempted by adding antibiotics to the medium. For bacteria it is recommended to use Rifampicine (Rimactan 300 CIBA) at 400 ppm.

A concentrated (12,000 ppm), filter-sterilized solution of Rifampicine is used to soak small (5 x 5 mm) squares of filter paper which are allowed to dry in the laminar flow chamber. Approximately 0.03 cm<sup>3</sup> of concentrate solution is used for every square of filter paper. They are best if used before seven days for they progressively lose their effectiveness. This process must be carried out under aseptic conditions. The paper squares are introduced into the culture medium with the planted bud which must be transferred to a fresh medium with another antibiotic paper every 3 to 5 days. Other antibiotics such as Cefotixine (Mefoxin Merck) in doses of 500 ppm may also be used.

For contamination by yeasts it is advisable to use 0.25 to 0.5 ppm doses of Amphotericine B. The filter paper procedure described above will be used in all instances.

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### 3 MERISTEM ISOLATION AND CULTURE

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The meristem is a tissue made of cells under division and is the active growth point of the bud. The dome of the bud contains the meristematic cells and is surrounded by foliar primordia and primary leaves. The meristematic cells divide and form new tissue. Nutrition of the dissected section is provided by the artificial medium.

The isolation of the meristematic zone under aseptic conditions and its culture in an adequate nutritive medium allow plantlet development with a differentiation pattern similar to that of a normal plant.

The aseptic dissection of the meristem for virus eradication is a delicate process requiring skill. Figure 1 shows a photographic sequence of the dissection procedure. The steps involved are:

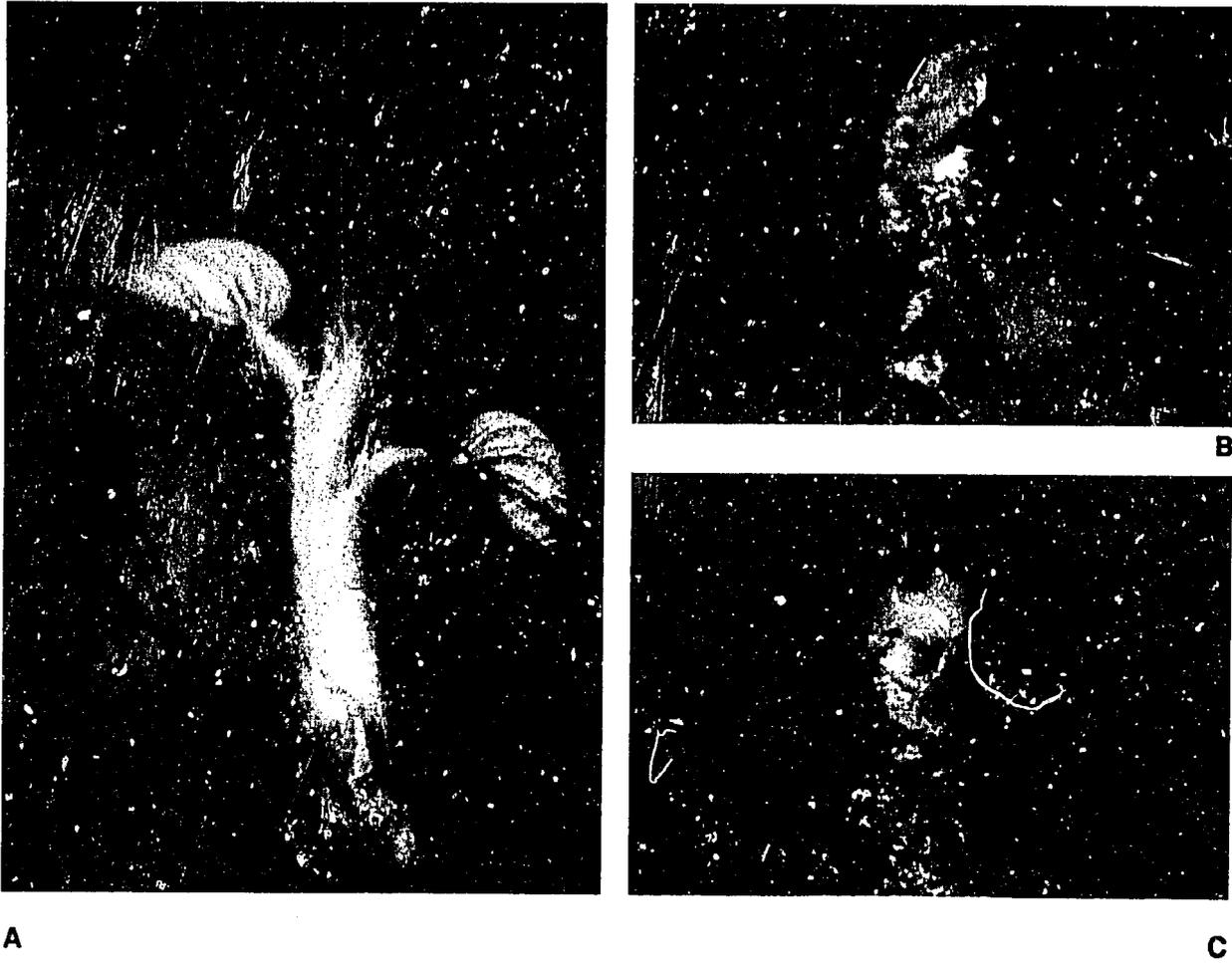
The plant stems are cut in segments including a node and the corresponding axillar bud. The material is disinfected with Morestan (Bayer) and sodium hypochlorite as for the introduction of in vitro material (see page 5).

After rinsing in distilled sterile water, place the material under the dissection microscope and use a needle or surgical knife (bistoury) to remove the leaves around the growth point until only the cupule and two or three foliar primordia are left.

The cupule and foliar primordia are dissected with the bistoury and transferred to culture medium MMB-I. The dissected meristem is transferred weekly to a fresh MMB-II medium. After 6 to 8 weeks the meristem will develop into a plantlet. The plantlets are now ready for subculture in the propagation medium (see section 6: Culture media).

**Thermotherapy:** At CIP before excision of the meristems, the plants undergo one month thermotherapy at 38°C for 16 hours and at 32°C for eight hours under constant light conditions. This high temperature treatment has increased the efficiency of the production process of virus free material. After thermotherapy either axillar or apical meristems may be used indistinctly.

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**Fig. 1. Photographic sequence of meristem dissection:**

- A: Isolated and desinfested apical bud**
- B: Dissection after removal of primary leaves**
- C: Meristem with two foliar primordia**

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## 4 MICROPROPAGATION

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The purpose of micropropagation is to obtain a large number of clonal plants in a short time. The following methods are used at CIP:

**Propagation by nodes.** This is based on the principle that the node of an in vitro plantlet placed in an appropriate culture medium will induce the development of the axillar bud resulting in a new in vitro plantlet. It must be noted that this type of propagation is based on the development of a pre-existing morphological structure. The nutritional-hormonal condition of the medium plays a simple role in breaking the dormancy of the axillar bud and promoting its rapid development. The propagation medium described in section 6 is used.

Callus formation and plant regeneration must be carefully avoided because they tend to affect the genetic stability of the genotype.

The plantlets grow under long day conditions (16 hours of light at  $45 \mu\text{E}/\text{m}^2/\text{seg}^2$  or 3,000 lux) and at temperatures ranging from  $25^\circ\text{C}$  to  $28^\circ\text{C}$ . Under these conditions, micropropagation is fast. Each node will develop into a plantlet occupying the full length of the test tube. The plantlets will be ready for subculture after six weeks (Figures 2 and 3).



**Fig. 2. Plantlet growing from a node.**

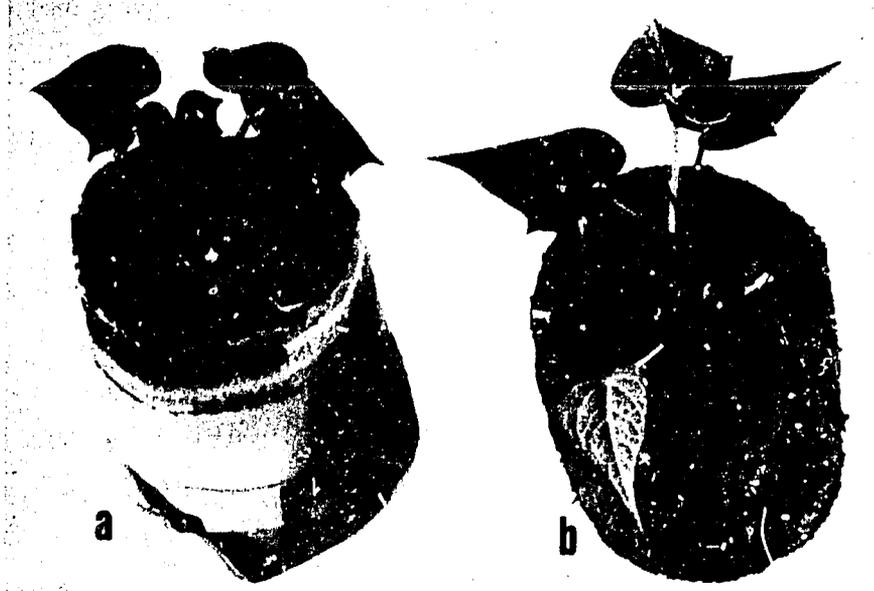


**Fig. 3. Sequence of In vitro development.**

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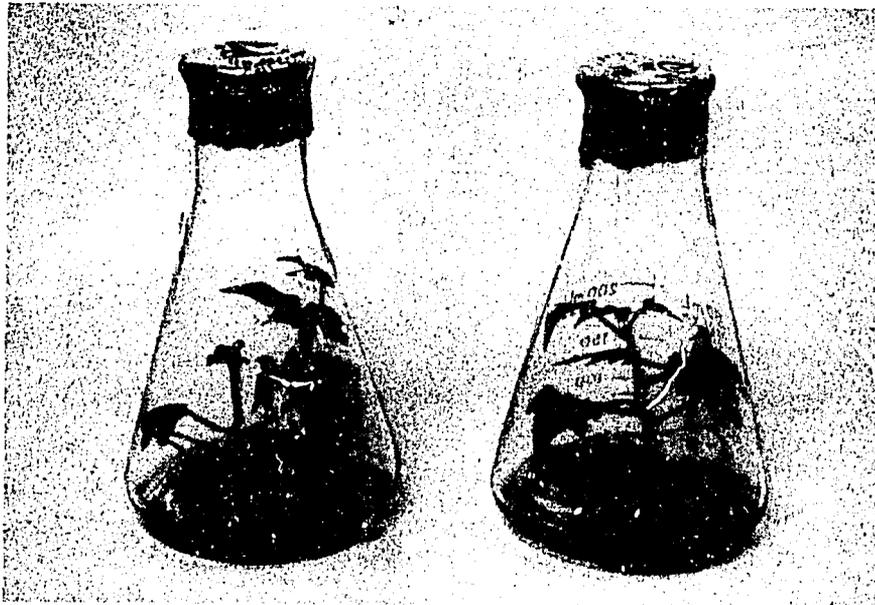
The resulting sweetpotato in vitro plantlets may be easily transplanted to in vivo conditions either in small pots or directly to field beds (Figure 4).

**Propagation by stem cuttings in liquid medium.** As with the potato, it is possible to micropropagate sweetpotato in bottles containing a liquid medium (Figure 5). Stem cuttings with 5 to 8 nodes are prepared by removing both the apex and root of the plantlet to be propagated. The cuttings are placed in a liquid medium containing gibberellic acid to break the dormancy of the stem cutting's axillar buds. The nodes will sprout and new plantlets develop over a period of 3 to 4 weeks. The plantlets may be used as initial material for simple node propagation or once again for propagation with stem cuttings in liquid medium, depending on program needs. Shaking of cultures may accelerate and promote the development of new plantlets. However, this is not essential.



**Fig. 4. Plantlets after transfer to peat-moss pot.**

- a. homemade peat-moss pot (newsprint)**
- b. commercial peat-moss pot (Jiffy 7)**



**Fig. 5. Liquid medium culture for rapid propagation.**

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## 5 LONG TERM MAINTENANCE

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Long term maintenance is important for propagation and conservation itself. For clonally propagated cultures it is important that every propagule be free of even the slightest genetic alterations that may build up from one generation to the next and result in major changes affecting uniformity and production.

For the maintenance of germplasm clones, it is crucial to conduct a detailed analysis of the culture's genetic stability. The clonal storage of germplasm consists on the maintenance of specific gene combinations (genotypes). If a plantlet's genetic combination changes during storage, the validity of the storage techniques must be examined. The capacity to detect genetic changes during propagation and maintenance depends on the methods used.

In many germplasm collections the stored genotypes are routinely evaluated for the morphological characteristics of the plantlets growing under controlled conditions. If the plants show new morphological characteristics (e.g. leaf shape or storage root color) some genetic changes are obvious. However, genetic changes such as virus resistance may not be detected through the observation of morphological changes.

Biochemical methods are now used to study the genetic stability of both potato and sweetpotato. They are the analysis of soluble protein and isoenzyme patterns. Although these methods are highly effective in determining changes in genetic products, they do not allow to directly determine changes in the genes.

New methods such as Restricted Fragment Length Polimorphism (RFLP) are considered to be more sensitive ways of determining genetic changes. It is important that germplasm banks and seed programs use the most sensitive methods in determining the genetic fidelity of their propagation and maintenance systems.

**Growth restriction media.** After many years of research, propagation media for sweetpotato have been developed that optimize rapid in vitro growth. However, maintenance requires limiting growth to a minimum while maintaining culture viability. Use of growth restriction media allows to maximize the interval between

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transfers (subcultures) of in vitro plantlets. At CIP, transfer of most sweetpotato material under maintenance is carried out once a year, and in some cases once every year and a half.

Laboratory experiments aimed at limiting in vitro growth of sweetpotato include the use of hormonal growth retardants such as abscisic acid (ABA), growth inhibitors such as B 995 or chlorocoline chloride (CCC), as well as osmotic regulators with addition of low assimilation sugars such as manitel or sorbitol.

The difficulty involved in this type of study is that under these conditions genotypes react differently. Studies of germplasm collections in vitro maintenance should aim at developing maintenance media broad enough for a large variety of genotypes.

Also, the storage medium should not allow callus induction that may result in genetic alterations.

Many storage methods have been reported for sweetpotato. At present, the method described in section 6 is used at CIP.

**Restriction of storage temperature.** The growth of in vitro plantlets may be restricted by reducing incubation temperature. Adequate in vitro growth of sweetpotato can be obtained with temperatures between 28°C and 30°C. At 8°C, survival time is less than one month. For genotypes studied to date, 15°C seems to be the optimum temperature. However, this has to be confirmed yet.

As with other in vitro cultures such as cassava and potato, low temperature and growth retardants may be used simultaneously. For now, the combined use of osmotic stress and low temperature (15°C) appears as the best and least costly way of maintaining sweetpotato germplasm collections.

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## 6 CULTURE MEDIA

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Culture media used in the work reported here are based on Murashige-Skoog (1962) and Gamborg B-5 (1968) salts.

### Medium for in vitro introduction (MMB-I).

- Calcium panthotenate	2	ppm
- Gibberellic acid	20	ppm
- Ascorbic acid	100	ppm
- Calcium nitrate	100	ppm
- Putrescine HCl	20	pmm
- L-Arginine	100	pmm
- Coconut milk	1	%
- Sucrose	5	%
- Agar or	0.7	%
- Phytigel/Gelrite	0.25	%

### Medium for transfer of meristems or buds (MMB-II)

- Calcium panthotenate	2	ppm
- Gibberellic acid	15	ppm
- Ascorbic acid	100	ppm
- Calcium nitrate	100	ppm
- Putrescine HCl	20	ppm
- L-Arginine	100	ppm
- Saccharose	5	%
- Agar or	0.7	%
- Phytigel/Gelrite	0.25	%

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**Propagation medium (MPB).**

- Calcium panthotenate	2	ppm
- Gibberellic acid	10	
- L-Arginine	100	
- Ascorbic acid	200	
- Putrescine HCl	20	
- Sucrose	3	%
- Agar or	0.8	%
- Phytigel/Gelrite	0.3	%

**Maintenance medium (MCB)**

- Glucose	2	%
- Sorbitol	2	%
- Putrescine HCl	20	ppm
- Phytigel/Gelrite	0.4	%

A pH of 5.8 is used in all media.

**Note:** These culture media were prepared to attain maximum uniformity in a collection including a large number of varieties.

When few varieties are involved, it is advisable to use simple culture media such as Murashige and Skoog salts with the addition of gibberellic acid and sucrose.

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