

1. Tissue Culture Laboratory

1.1 MICROPROPAGATION UNIT

A micropropagation unit includes a tissue culture laboratory and a propagation greenhouse. When planning a micropropagation unit we have to consider the following factors: available space, environment, financing, type of work to be developed, and required production capacity. According to the production capacity and the available space, we may consider three types of micropropagation units:

a) Small scale. The facilities for in vitro work can be adapted for a house setting, using the available equipment and materials to carry out the basic micropropagation activities. This method could be used to micropropagate plants for interested people, or mother plants for greenhouses but with great care to avoid contamination problems.

b) Medium scale. It is necessary to design, implement and/or prepare specific working areas, and to acquire equipment and materials to increase the efficiency and uniformity of the results.

c) Large scale. The facilities and the equipment must be designed to actually perform the work and to maintain an optimum production flow.

Basic Processes

The basic processes normally carried out in a tissue culture laboratory are:

- a) Glassware washing
- b) Culture media preparation

- c) Media and equipment sterilization
- d) Ex-plants preparation and aseptic transference of cultivated materials
- e) Incubation and growth of cultivated materials up to maturity
- f) The rooted-plantlets transplantation, is accomplished, in part, with the help of laboratory personnel.

Basic Organization

The laboratory for plant tissue culture requires a basic organization that comprises three areas:

- a) General laboratory (or media preparation area) provided with spaces for common or independent work. Some equipment and materials can be used by several workers at the same time.
- b) Area for the aseptic manipulation of plant material (or transference area).
- c) Culture maintenance area (or culture rooms) with controlled conditions for light, temperature and humidity.

There should be two separate rooms at least. One for washing, sterilization, storage and culture media preparation; and another one for culture maintenance (culture room).

The transference chamber can be located in the general laboratory or in an area specifically designed as a transference room, according to available conditions.

Washing and Media Preparation Area

The area for washing should have a big washbasin (of stainless steel, and be acid and alkali resistant), tap water, tables that allow stand-up work and shelves to dry and keep the washed materials. The media preparation area must be equipped with a refrigerator to keep the chemicals and solutions used in the media culture, scales, a potentiometer, a kitchen, a media mixer, a water distiller, and an autoclave or pressure pot. The last two must be located as close as possible to the washbasin. The stove may be used to dry the materials.

Culture Area

This is the culture incubation area, where optimum media conditions change according to the species in culture. So, temperature variations, light intensity and quality, relative humidity and photoperiod should be taken into consideration. Temperature is controlled with air-conditioning equipment or heaters. According to the cultivar, the average temperature of an incubation room should be $25^{\circ}\text{C} \pm 1-200$. For higher or lower temperatures, air-conditioning equipment should be

used to reach the appropriate temperature. It is recommended to use thermostats which prevent temperature variations in the room from exceeding the culture requirements.

The airflow must be uniform within the culture room to maintain the same temperature in the whole environment.

The air-conditioning equipment indirectly controls the relative humidity. If the relative humidity drops below 50%, there will be a water loss in the culture media, and an increase in the mineral salts concentration, which can damage the cultures. With a high relative humidity (80-100%) contaminants could enter the culture containers. The optimum average is between 50 and 70%. The light source is provided by fluorescent lamps and the photoperiod is controlled by an hourly timer. The fluorescent lamps have an advantage over the incandescent lamps because they have better light quality, distribute the light uniformly and produce less heat. However, some cultivars grow better with a combination of both types of light.

Most of the cultivars require an illumination that varies between 500 and 3,000 lux. Some of them need more than 5,000 lux, and others just need darkness as in the case of in vitro tube induction. When using fluorescent lamps we should consider that the ballast generates heat which affects the culture room temperature. That is why they must be installed outside the room.

The arrangement and number of the shelves, where magenta vessels and tubes with the cultures are placed, will vary according to the room's dimensions. Shelves can be metallic or wooden, and should be painted white.

The shelves' dimensions may vary. However, it is recommended to have an incubation platform of 0.45 x 0.90 m, with a height of 0.30 m among the shelves because it allows good illumination, access to, and control of the incubated materials. The space between the soil and the first platform must be 0.15 m to facilitate soil cleaning. A distance of 0.05 to 0.10 m must be kept between the wall and the shelves to allow the free circulation of air.

For the laboratory walls antifungal-epoxy-paint (used in cool temperature chambers) is recommended as a preventive measure.

It is recommended to put a tray on the floor, with a rug containing an acaricide and a fungicide to impregnate the shoes of those who enter the culture room.

The culture room must be isolated from the external environment to maintain the appropriate temperature and the relative humidity and to avoid the entrance of contaminants. Just in case, the windows should also be sealed. Access to the culture room will be allowed only to the people who work there.

A tissue culture laboratory can be located in any geographical area. The internal controlled environmental conditions allow isolation with a minimum of external influence.

To decide where to build the tissue culture laboratory, the following factors must be taken into consideration:

Environmental growing conditions for the species that will be cultivated

Availability of electricity

Availability of water and drainage

Good all-round communication

Production Process

The production process involves:

- The in vitro culture establishment stage
- The production stage

In vitro culture establishment stage

It consists of taking plants for the test-tube from the field: for this, a clean lot is selected (free from pathogens) that guarantees the quality, uniformity, and strength of the material to be placed in the market when the production stage is finished.

The selected plants will be those with optimum growth, development, and phytosanitary conditions. These plants can go through the process of thermotherapy and meristem culture. The plantlets will be used as a source of explants for the production process. If pathogen cleaning is not necessary, entire buds are taken and placed in a temporary culture medium where they will be observed for one or two weeks and, if a bacterial infection is noticed, they are treated with antibiotics until the infection disappears.

Production stage

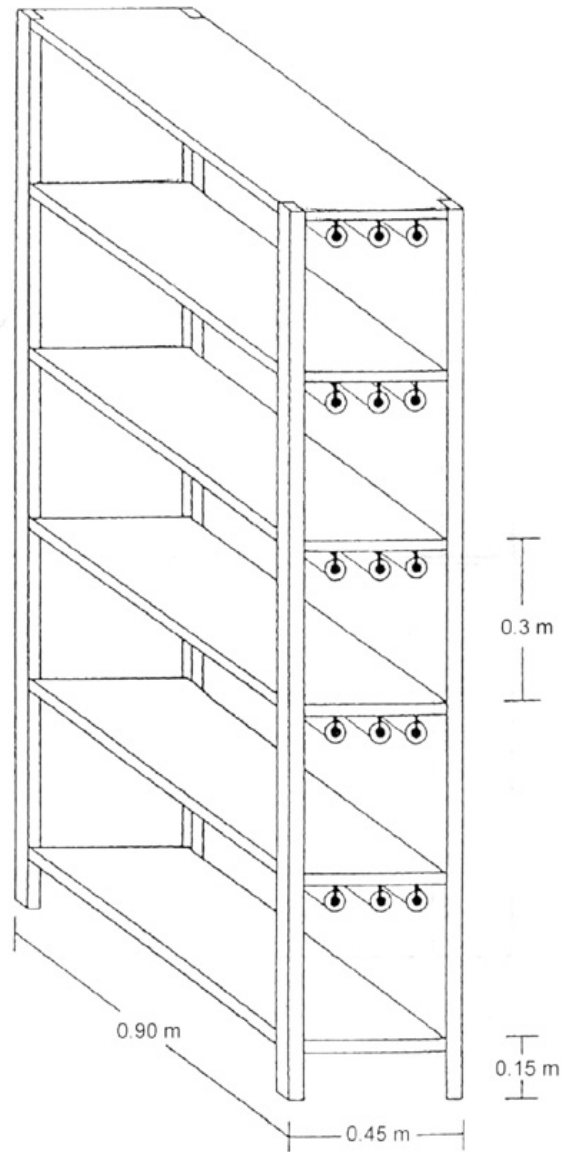
It consists of the massive propagation of explants and plantlets.

The propagation range depends on the species: the ranges commonly present in most of the micropropagated crops have been taken as a reference. In the same crop, the propagation range may vary according to the phytohormones in the culture medium.

The time of each propagation cycle depends on the species behavior, the culture medium, and the environmental conditions to which it is subjected: the average is between three or four weeks for each step.

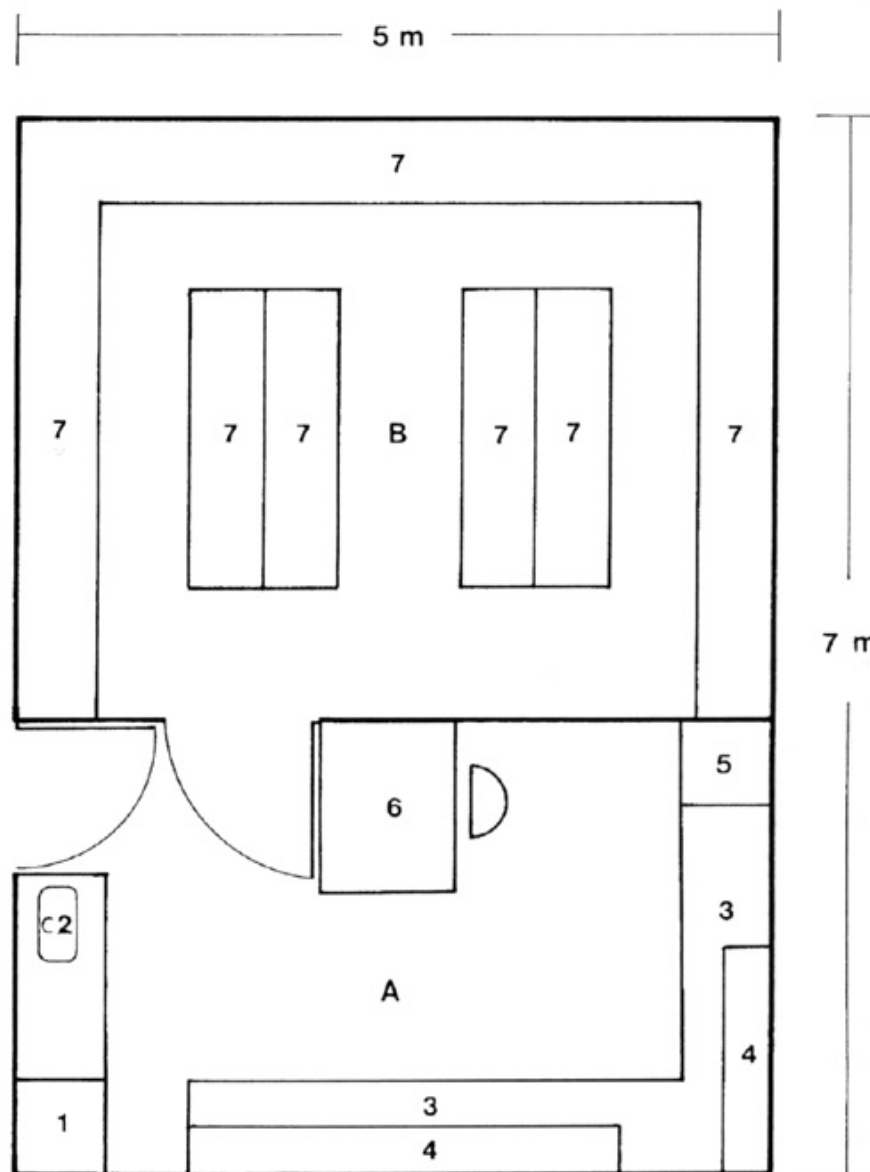
The Magenta plastic containers have been taken as standard, but any others can be prepared for this purpose. It is considered that 20 explants per container make a good plantlet development possible.

The graphics below show the design of the incubation shelves and of the micropropagation laboratory.



Nelson Espinoza R.

Figure 1 Incubation shelf



SCALE 1:50

A Laboratory

B Incubation room

1 Autoclave

2 Washbasin

3 Working tables

4 Wall cabinet

5 Refrigerator

6 Transference chamber

7 Incubation shelves

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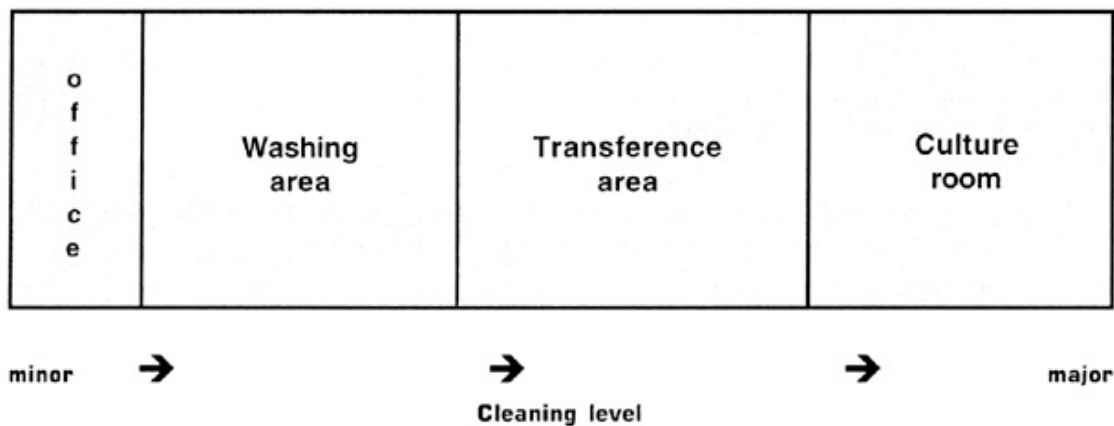
Figure 2 Basic Small - Scale Micropropagation Laboratory

Aseptic Conditions in the Laboratory

Asepsis in the tissue culture laboratory is one of the most important requirements. A good asepsis guarantees the plantlets' growth and the good use of the culture media.

Asepsis in the Facilities

The tissue culture laboratory must have four basic environments: an office, a washing and media preparation room, a transference room, and a culture room, which require a minor to major grade of cleaning, as well as a minor to major restriction on personnel access.



The main purpose of asepsis is to maintain the rooms isolated from the external environment. Cleaning is carried out with more diligence on floors, furniture, and shelves by using disinfectants, and avoiding direct entrance from greenhouses or fields.

The entrance doors must have rugs continuously sprayed with acaricide powder

When entering, it is necessary to use lab coats to avoid dispersing dust from clothes.

Asepsis in the Washing and Media Preparation Room

The wash room must be provided with the necessary materials for cleaning the used tubes, on covered shelves to avoid dust. Tables and shelves must be continuously cleaned.

All rejected material (tubes with old plants or tubes with used medium) must be immediately sterilized to avoid environmental contamination.

The media preparation area is the room where material is sterilized, so it requires a good cleaning to avoid media contamination before it is used. This area must be maintained in isolation to protect it from dust and other air-borne contaminants.

Asepsis in the Transference Room

The transference room, where the laminar flow chambers are, is a place which must be kept very clean, since it is next to the culture room.

In this room a washstand is necessary where the personnel can wash their hands before they start propagation.

In this area, great care must be taken in the laminar flow chamber; the internal walls must be cleaned with alcohol 70⁰/o].

The filters and pre-filters (Hepa) within the chambers must be continuously revised to avoid contamination.

The tools within the chamber must be disinfected over a burner flame.

During propagation, hands and table surface will be continuously cleaned with a piece of cotton soaked in alcohol.

Only the material necessary for propagation will be permitted within the chamber. Other appliances (radios, books, etc.] are not permitted.

Never open a contaminated tube (or magenta] within the chamber; it must be sterilized immediately.

Asepsis in the Culture Room

The disinfection of floors and shelves must be continuous. The tube racks must be cleaned with an acaricide/fungicide solution before they are put into the culture room for the first time.

The inside doors and walls must be frequently examined to avoid the preserve of fungi.

The air-conditioner must be regularly checked.

1.2 PREBASIC SEED PRODUCTION PLAN

In seed production programs, propagation systems must be developed to make possible the maximum use of the places allocated for this purpose, Thus, greenhouses must be used economically for in vitro plantlets.

The diagram included here shows an example of a production plan developed in the INIA station at Huancayo, where three campaigns per year are carried out, and material is propagated for six greenhouses by using 3,850 plantlets per greenhouse.

During the first season, the in vitro plantlet propagation starts in August; they grow under the continuous observation of laboratory personnel. On November 2, the plantlets are taken to the greenhouses.

At the same time, soil-disinfecting activities and bed preparation will be carried out in the greenhouse to have it ready for the reception of material on November 2.

It is suggested that laboratory personnel take the plantlets to the greenhouse and participate in their transference to the beds, as well as in the washing of the magentas, before taking them back to the laboratory.

On March 3, (according to the diagram] plants will be ready to harvest and then the greenhouse is prepared for the following campaign. Note that harvesting and cleaning take one week (fumigation, renewal of beds, etc.)

The subsequent campaigns are similar Modifications may be carried out according to the availability of personnel and facilities.

PRE-BASIC SEED PRODUCTION PLAN

Variety: Perricholi

Place: INIA - Santa Ana Station, Huancayo

Conditions:

Two people in micropropagation

Three seasons per year

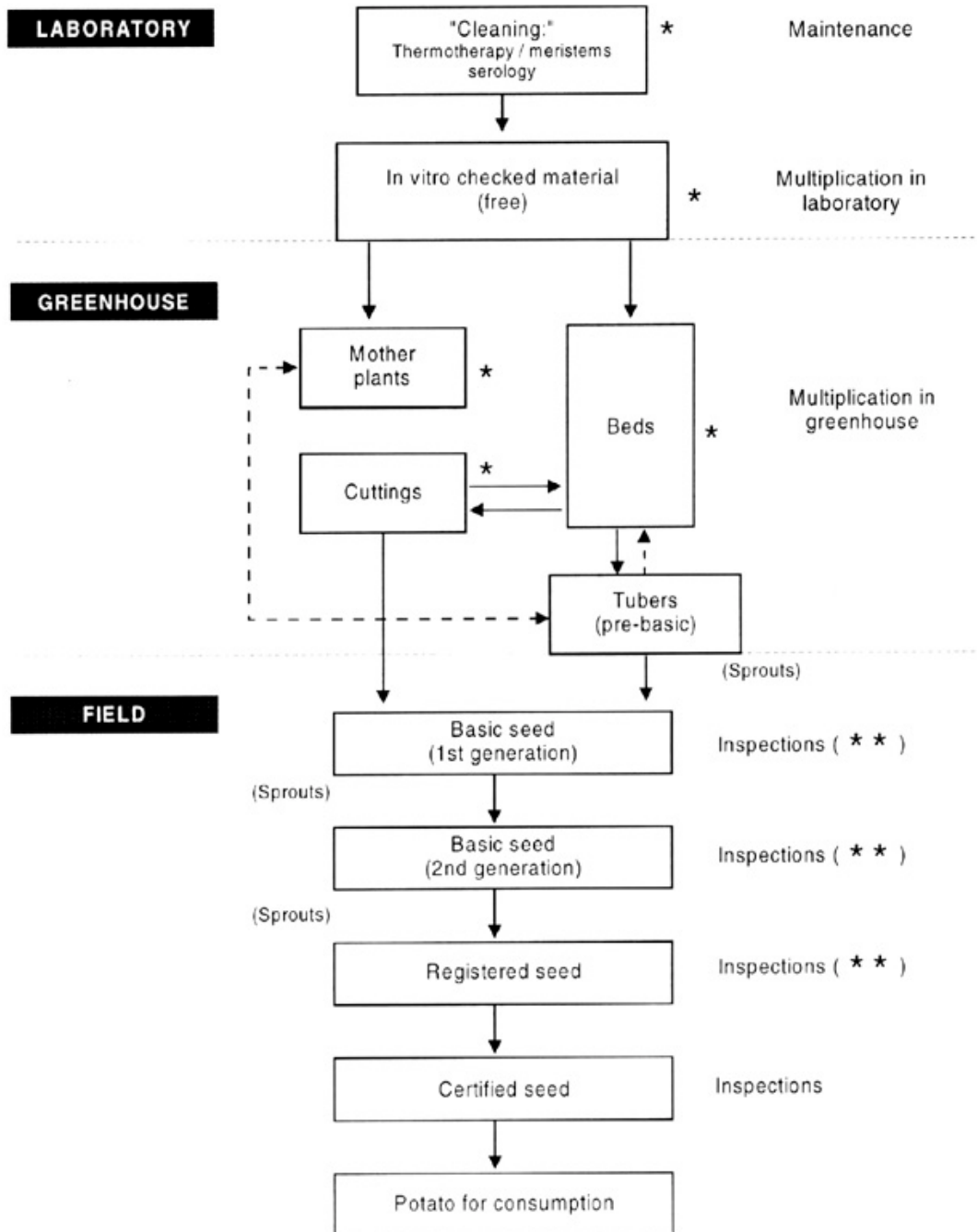
Six greenhouses

	Aug Sep Oct Nov Dec Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov
Tissue culture lab personnel Activities	<p>X →→→ 2</p> <p>X →→→ 7</p> <p>X →→→ 12</p>
Greenhouse personnel activities.	<p>2 → → → 3</p> <p>7 → → → 7</p> <p>12 → → → 13</p>

This program indicates a specific case in the coordination of in vitro propagation activities and the transference of plantlets to greenhouses.

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GENERAL SCHEME OF POTATO TUBER-SEEDS PRODUCTION



- * Quality control with ELISA
- ** ELISA is optional

Source: Dr. Oscar Hidalgo

1.3 MULTIPLICATION PLAN FOR IN VITRO PLANTLETS FOR GREENHOUSES

The infrastructure of the tissue culture laboratories makes possible the propagation of large quantities of plantlets to provide material for one or several greenhouses.

The multiplication of 3,600 to 4,000 plantlets must be carried out under a defined program according to laboratory conditions.

The growing of the in vitro propagated plantlets will depend on the medium, the environmental conditions of the incubation room, and the variety. So, it is necessary to evaluate the plantlets in growing conditions in relation to time to establish an efficient system later, according to our requirements. Next, three propagation procedures, followed in different laboratories (different environmental conditions), are presented.

Procedure 1 (Tissue culture laboratory, INIA-Huancayo)

- Propagate nodes in 8 tubes (4 nodes per tube) to obtain 32 plantlets with 5 nodes each, in 2 1/2 weeks: the plantlets are cut, apical buds and roots are separated, and stems are planted in a liquid medium (5 stems of 4 nodes per erlenmeyer flask).
- Plant the apical buds (32 buds) in magentas (25 buds per magenta).
- Leave the stems growing in a liquid medium during 2 1/2 weeks to obtain 128 plantlets: bring them together with the plantlets growing in the magenta (32 plantlets) to obtain a total of 160 plantlets.
- Out the plantlets again (apicals and roots), and transfer stems only to a liquid medium (5 stems of 4 nodes per vial) for 2 1/2 weeks.
- Plant the apical buds (160 apicals in total) in magentas (25 buds per magenta). The 160 stems planted in a liquid medium produce 640 plantlets: if we add the 160 plantlets that are growing in magentas, a total of 800 plantlets with 5 nodes is obtained. That is 4,000 explants (nodes).
- Plant the nodes in magentas: after 3 1/2 weeks 4,000 plantlets will be ready for the greenhouse.

This procedure is carried out in 11 weeks under a solid-liquid-liquid-solid system: a total of 6,079 ml of culture medium is used.

Procedure 2 (Private laboratory, Huancayo)

- Put 5 stems in a herlemeyer flask (the apical bud and the root are removed). They will produce 30 plantlets in three weeks (5 nodes per plantlet, 150 explants).

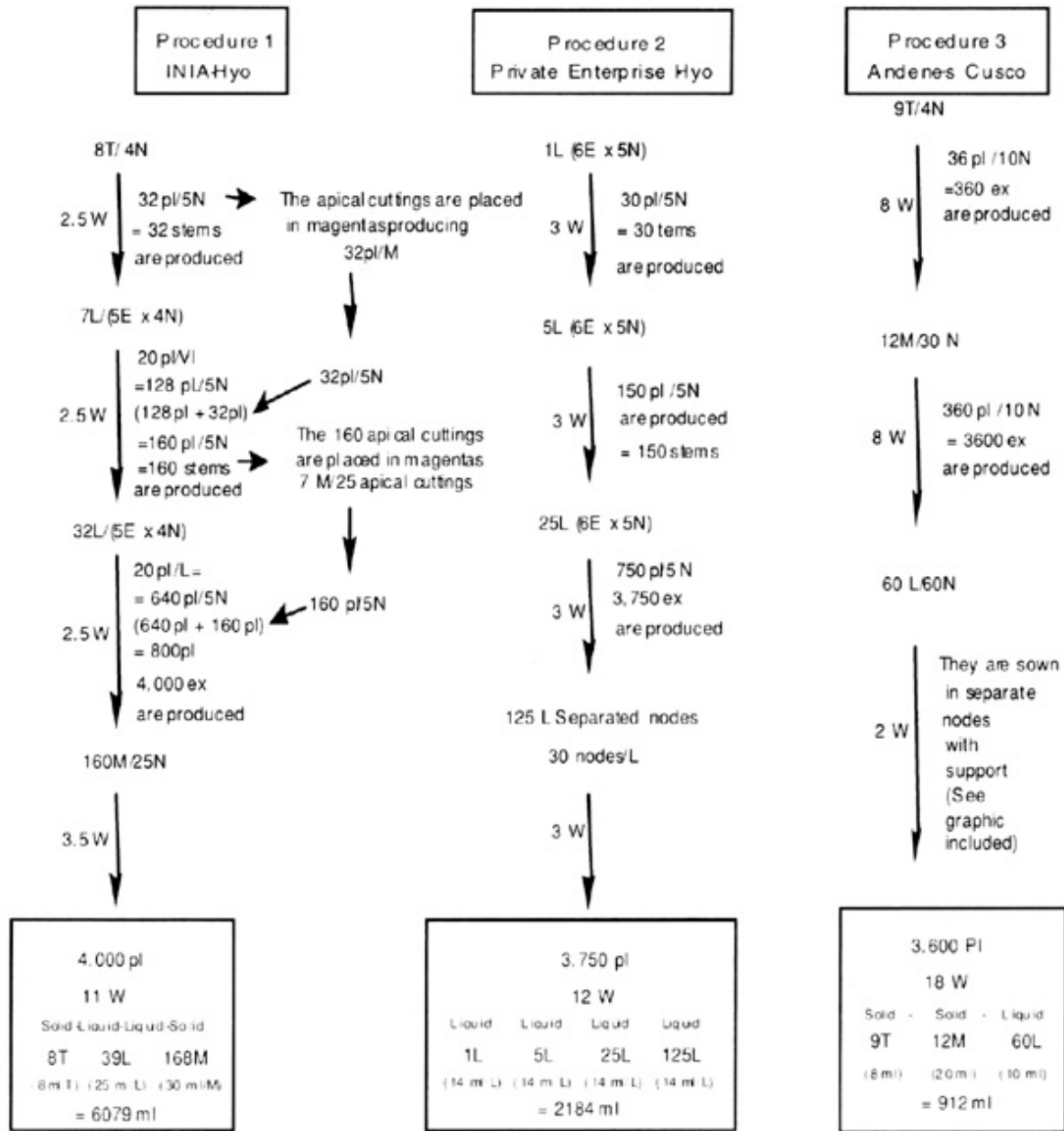
- Propagate the plantlets in 5 erlenmeyer flasks containing a liquid medium (6 stems of 5 nodes per flask]; after three weeks 150 plantlets are obtained (5 nodes each, 750 explants).
- Propagate the plantlets in 25 flasks with a liquid medium (6 stems of 5 nodes per vial]; after three weeks growth 3,750 nodes are obtained (5 nodes per plant] that will be propagated, as separated nodes, in flasks containing a liquid medium.

This procedure is carried out in 12 weeks under 4 steps of liquid media using 1.4 ml/each.

Procedure 3 (Laboratory Andenes, Cuzco)

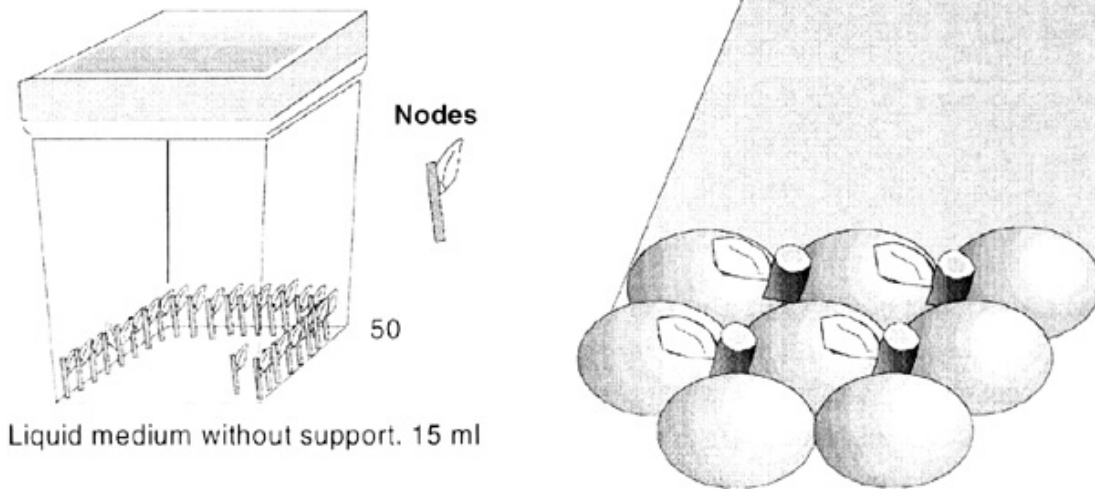
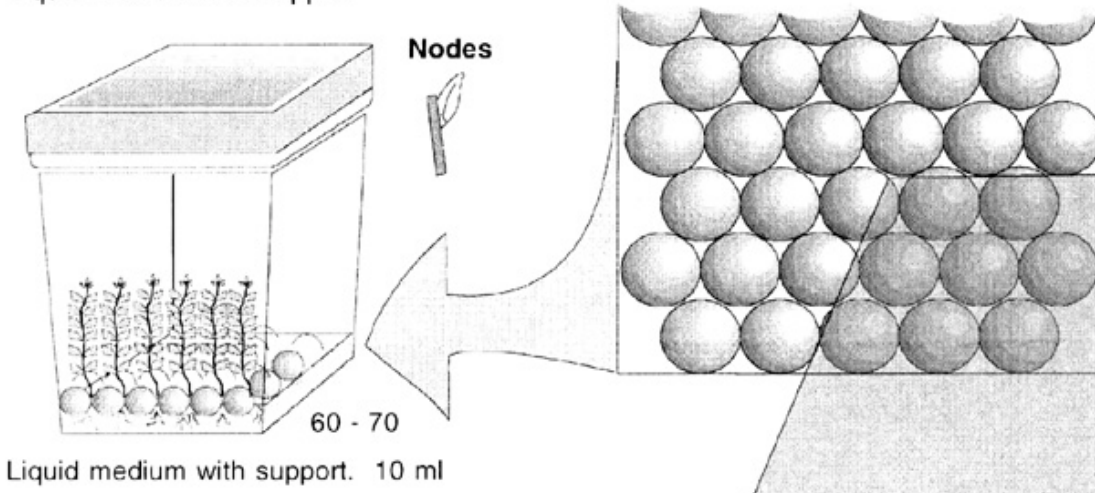
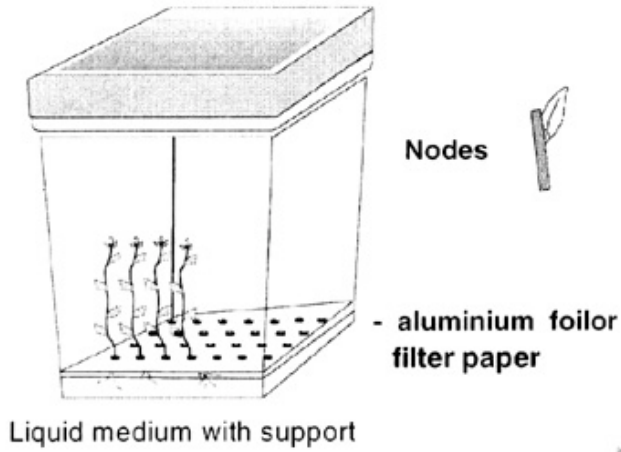
- Propagate plantlets in 9 tubes with 4 nodes each, and let them grow during 8 weeks until 10 nodes per plantlet (total: 380 nodes] are obtained.
- Propagate the plantlets in magentas (30 nodes per magenta], and 10 nodes per plant are obtained after 8 weeks' growth. This procedure produces 3800 explants which are propagated in 80 flasks containing a liquid medium (60 nodes per flask] (see graphic below]. After two weeks 3,600 plantlets will be ready to be transferred to the greenhouse.

This procedure is carried out in 18 weeks using a solid-solid-liquid system (9 tubes, 12 magentas and 60 liquid media: 912 ml of culture medium were used (the tubes contain 8 ml of medium, the magentas 20 ml and magentas with liquid media contain 10 ml).



T=tubes N=nodes L=erlenmeyer Flask C=cuttings W=weeks pl=plantlets M=magentas ex=explants

USE OF LIQUID MEDIUM IN PLANT PROPAGATION IN PROCEDURE 3



2. Stock Solutions

SOLUTION PREPARATION

INTRODUCTION

The growth of the in vitro plantlets depends mainly on the media utilized and, consequently, on the prepared solutions. The Murashige & Skoog basal medium L19B2~ is used a lot in potato plantlet production laboratories; its concentration of salt and vitamins are adequate for the normal growth of plantlets in in vitro conditions. At the present time, the basal media are easily obtained in their commercial presentation; however, the use of «stock solutions» makes possible the availability of culture media for many years, and reduces the plantlet production costs. Additionally, the research in tissue culture requires the utilization of growth regulators, which must be appropriately prepared and preserved for their maximum effect.

On the other hand, in spite of sterile conditions and good management, the presence of bacteria in the medium is possible; the use of antibiotics could be helpful in the temporary maintenance of the plantlets. It is necessary to establish proper disinfecting procedures, to avoid the abuse of antibiotics, which damage the genetic stability of the plant, and alter the resistance levels of the bacteria.

Afterwards, procedures to prepare salt, hormones, antibiotics, and other stock solutions used to prepare culture media will be developed.

SUMMARY FOR LAB USE

MURASHIGE & SKOOG BASAL MEDIUM

STOCK A (SALTS)

NH_4NO_3	35.0 g
KNO_3	40.0 g
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	10.3 g
KH_2PO_4	3.5 g
H_3BO_3	0.1 g
$\text{MnSO}_4 \cdot \text{H}_2\text{O}$	0.4 g
$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	0.2 g
KI	0.02 g
NaMoO_4	0.004 g

Dissolve in 200 ml of distilled water

$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	5 mg
$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	5 mg

Dissolve in 10 ml with distilled water

Use 1 ml of the solution and bring to 200 ml with distilled water

STOCK B

$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	3.7 g
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Dissolve in 100 ml of distilled water

STOCK C

Na_2EDTA	0.75 g
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Dissolve while hot with 20 ml of distilled water

$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	0.55 g
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Dissolve in 20 ml of distilled water

Mix both solutions while cold and bring to 100 ml with distilled water.

STOCK D

Thyamine-HCl	20 mg
Glycine	100 mg
Nicotinic acid	25 mg
Piridoxine-HCl	25 mg

Dissolve them in 500 ml of distilled water. Mix well.

Distribute in 20 ml vials and keep them at 0°C.

**BASAL MEDIUM: 100 ml STOCK A + 10 ml STOCK B + 5 ml STOCK C +
10 ml STOCK D + 100 mg Inositol. BRING TO 1 L.**

2.1 PREPARATION OF THE MURASHIGE & SK000 BASAL MEDIUM (MS)

PREPARATION OF STOCK SOLUTIONS

1. STOCK A: SALTS

1. Weigh the following reagents:

NH_4NO_2	35.0 g
KNO_3	40.0 g
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	10.3 g
KH_2PO_4	3.5g
H_3BO_3	0.1g
$\text{MnSO}_4 \cdot \text{H}_2\text{O}$	0.4 g
$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	0.2 g
KI	0.02g
NaMoO_4	0.004 g

Dissolve them in 200 ml of distilled water

Keep the solution in a conveniently labeled vial at 4°C.

2. Weigh 5 mg of the following reagents:

$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$
 $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$

Dissolve them in 10 ml of distilled water. To 1 ml of the previous solution add 200 ml of distilled water.

Keep the solution in a conveniently labeled vial at 4°C.

2. STOCK B: MgSO_4

Weigh 3.7 g of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ in 100 ml of distilled water.

Keep the solution in a conveniently labeled vial at 4°C.

3. STOCK C:

1. Weigh 0.75g of Na_2EDTA .

Dissolve while hot in 20 ml of distilled water. Let the solution cool.

2. Weigh 0.55 g of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$

Dissolve it in 20 ml of distilled water

3. Mix both solutions and fill up to 100 ml by adding distilled water Keep the solution in a dark, conveniently labeled vial at 4°C.

4. STOCK 0: Vitamins

1. Weigh the following reagents:

Thyamine HCl	20mg
Glycine	100mg
Nicotinic acid	25 mg
Pyridoxine HCl	25 mg

Dissolve them in 500 ml of distilled water Stir well.
Dispense the solution in 20 ml vials and keep at 0CC.

MS BASAL SOLUTION*

For 1 liter of basal medium mix:

- 100 ml of Stock Solution A
- 10 ml of Stock Solution B
- 5 ml of Stock Solution C
- 10 ml of Stock Solution D
- 100 mg of Inositol

Make up to 1 liter with distilled water

* Stocks A+B+C+D

2.2 PREPARATION OF SOLUTION VGA* (VITAMINS AND GIBBERELIC ACID)

For 500 ml of VGA mix:

Thyamine HCl	10mg
Glycine	200mg
Nicotinic acid	50mg
Pyridoxine HCl	50mg
Gibberellic acid	
(Stock 1 000 ppm)	10ml

Make this solution up to 500 ml with distilled water

Distribute this solution in 20 ml. vials.

Use 5 ml/l

* This solution was previously called MSA.

2.3 PREPARATION OF VITAMIN SOLUTION

For 500 ml of solution mix:

Thyamine	HCl10mg
Glycine	200mg
Nicotinic	acid50mg
Piridoxine	HOI50mg

Make it up to 500 ml with distilled water

Distribute this solution in 20 ml vials.

Use 5 ml/l.

2.4 PREPARATION OF HORMONE STOCK SOLUTION

GIBBERELIC ACID (GA₃)

Stock solution of gibberellic acid: 1,000 ppm

1. Weigh 0.2 g of gibberellic acid and dissolve well with some alcohol drops.
Add 200 ml of distilled water
2. Keep in a conveniently labeled vial at 0°C.

The gibberellic acid may be sterilized together with the culture medium: however, the loss of some activity is also possible.

One ml of concentrate solution (1,000 ppm) contains 1 mg of gibberellic acid.

NAFTALENACETIC ACID (NAA)

1 Stock solution of NAA 1,000 ppm

Weigh 0.2 g of NAA and dissolve well with some NaOH 1N drops.

2. Add 200 ml of distilled water.

Keep it in a conveniently labeled vial at 0~5°C.

One ml of stock solution (1,000 ppm) contains 1 mg of NAA.

BENCYLAMINOPURINE (BAP)

Stock solution of BAP: 1,000 ppm

1. Weigh 0.2 g BAP and dissolve well with some drops of NaOH 1N.

Add 200 ml distilled water.

2. Keep in a conveniently labeled vial at 0°C.

BAP may be sterilized together with the culture medium; however, the loss of some activity is also possible.

One ml of stock solution (1,000 ppm) contains 1 mg of BAP

INDOLEACETIC ACID (IAA)

Stock solution of IAA: 1,000 ppm

1. Weigh 0.2 mg of IAA and dissolve well with some alcohol drops.

Add 200 ml of distilled water.

2. Keep it in a conveniently labeled vial at 0°C.

Sterilization by filtration is recommended.

One ml stock solution (1,000 ppm) contains 1 mg of IAA.

KINETINE (KIN)

Stock solution of KIN: 1,000 ppm

1. Weigh 0.2 g KIN and dissolve well with some drops of NaOH 1N. Add 200 ml of distilled water.

2. Keep in a conveniently labeled vial at 0°C.

KIN may be sterilized together with the culture medium; however, the loss of its activity is also possible.

One ml of the stock solution (1,000 ppm) contains 1 mg of KIN.

2, 4-D

Stock solution of 2,4-D: 1,000 ppm

1. Weigh 0.2 g of 2,4-D and dissolve well with some alcohol drops. Add 200 ml of distilled water.

2. Keep in a vial conveniently labeled at 0°C.

2,4-D may be sterilized together with the culture medium; however, a loss of its activity is also possible.

One ml of the stock solution (1 000 ppm) contains 1 mg of 2,4-D.

2.5 Preparation of antibiotics

Rifampicin (Rimactan 300)

1. Cut small squares of filter paper (10 mm x 10 mm).
2. Place them in a petri dish and sterilize them.
3. (In a flow chamber) Place the squares carefully on sterilized petri dishes, slightly separated one from the other.
4. Dissolve a capsule of Rimactan (300 mg) in 150 ml of distilled water. Sterilize with filters of 0.22 µm.
5. Place 3 drops of the antibiotic solution, approximately 0.09 ml, on each square.
6. Let the antibiotic dry in the flow chamber. Keep all the squares in petri dishes, covered and sealed with parafilm.
7. Keep the temperature at 4°C, until the petri dishes are ready to be used.

When ready to use:

with forceps, take a square containing antibiotic by one side, and introduce it in a tube. Press over the medium close to the place where the node will be planted. The antibiotic will diffuse and cover the planted area including the node.

SODIC CEFOTAXIM (CLAFORAN)

1. Cut small squares of filter paper (5 mm x 5 mm).
2. Place them in a petri dish and sterilize them.
3. (In a flow chamber) Place the square very carefully with a forceps, on the surface of sterilized petri dishes, slightly separated from each other.
4. Prepare an antibiotic solution, dissolving 1 g of Claforan in 25 ml of sterile distilled water. Sterilize with 0.22 µm filters.
5. (In a flow chamber) Place a drop of approximately 0.03 ml on each square.
6. Let the antibiotic dry in the flow chamber. Keep all the squares in petri dishes, covered and sealed with parafilm.
7. Keep the temperature at 4°C, until the petri dishes are ready to be used.

When ready to use:

with forceps, take a square with antibiotic by one side and put it into a tube. Press over the medium close to the place where the node will be planted. The antibiotic will diffuse and cover the planted area including the node.

2.6 PREPARATION OF CALCIUM HYPOCHLORITE

1. Weigh 50 g of calcium hypochlorite. Dissolve it in 1.000 ml of distilled water (50/0).
2. Shake it for 3 to 4 hours and let it rest for 8 hours, or over night.

3. Filtrate the solution by using a filter paper and maintain it hermetically closed in a flask in a safe place.
4. Use 50 ml of solution and add 50 ml of distilled water

2.7 PREPARATION OF AN ACARICIDE SOLUTION

1. Weigh 5 g of an acaricide and dissolve it in 1,000 ml of distilled water Stir well.
2. Use the solution while fresh. Do not keep it.

2.8 PREPARATION OF SOLUTIONS FOR pH ADJUSTMENT

Solution to bring down the pH - Hydrochloric acid (HCl) I N

1. Pour 91.4 ml of distilled water into a beaker (Use a mask and gloves to protect yourself from the acid vapors).
- 2 With a pipette take out 8.8 ml of hydrochloric acid (commercial concentrate, 36. 5-38.0⁰/o].

WARNING: Do not breathe when taking out the acid. Use a rubber-bulb pipette.

3. Homogenize and keep in a broad-mouth vial, closed and at room temperature.

Solution to bring up the pH Potassium hydroxide (KOH) I N

1. Place 50 ml of distilled water in a beaker
2. Add 5.6 g of KUH and dissolve well.
3. Bring to 1 00 ml with distilled water Keep it in a closed broad-mouth vial at room temperature.

Uses

According to pH of the medium, add the solutions drop by drop until the required pH is reached.

3. Culture Media

CULTURE MEDIA IN POTATO PROPAGATION

The growth of the in vitro plantlets depends on nutritional and environmental factors which interact to produce a plantlet with similar characteristics to those grown in the field. The nutritional factors are based on the Murashige & Skoog medium (1962) composed of organic salts, vitamins, aminoacids, carbohydrates, growth regulators. and organic supplements. In the case of the potato, the compounds used for the culture media preparation contain the basal medium, vitamins and other substances, according to the explant to be used. Thus, for the in vitro introduction one of the most important components is gibberellic acid, which breaks bud dormancy and accelerates explant growth.

In the conservation medium, Sorbitol is used to induce osmotic stress, which retards nutrient absorption and, therefore, growth.

The tuberization medium contains growth regulators which induce the micro-tubers' production through stress.

Other substances added to the culture media are:

Vitamins, which participate in cell enzymatic functions, stimulating the growth of the explants. Polyamines, nitrogenous compounds derived from the aminoacids, that stimulate growth through cell division.

Activated carbon absorbs the eliminated inhibitory substances through the explants' sap in the medium, and stimulates root morphogenesis and growth.

3.1 IN VITRO INTRODUCTION MEDIUM

1. Dissolve a packet of MS [Murashige & Skoog basal medium) in 600 ml of distilled water
2. Add 25 g of sucrose and 5 ml of vitamin solution. Stir
3. Add '1 ml of gibberellic acid.
4. Bring the volume up to 1,000 ml with distilled water.
5. Measure the pH and adjust it to 5.6 [bring it up with KOH 1N, or down with HCl 1 N).
6. Add 3.5 g of Phytigel.
7. Dissolve the gelling agent with heat [microwave oven: 1000/0 intensity, 1-2 minutes). Stir well until it dissolves, preventing it from boiling.
8. Distribute 2 ml in each 13 x 100 mm tube.
9. Sterilize at 121°C and 15 pounds pressure, for 20 minutes.
10. Take the culture tubes off the autoclave and keep it at 4°C until planting.

3.2 POTATO PROPAGATION MEDIUM

1. Dissolve a packet of MS [basal medium Murashige & Skoog) in 600 ml of distilled water
2. Add 25 g of sucrose and 5 ml of VGA solution. Stir
3. Fill up to 1000 ml with distilled water
4. Measure the pH and adjust it to 5.6 [bring it up with KOH 1N or down with HCl 1 N).
5. Add 3.5 g of Phytigel.
6. Dissolve the gelling agent with heat [microwave oven: 1000/0 intensity, 12 minutes). Stir well until the gelling agent is dissolved, preventing it from boiling.
7. Distribute '1-2 ml in 25 x 150 mm tubes.
8. Sterilize at 121 (sf3 and 15 pounds pressure for 20 minutes.
9. Take the culture tubes off the autoclave and keep it at 4°C until planting.

In the conservation medium, Sorbitol is used to induce osmotic stress, which retards nutrient absorption and, therefore, growth.

The tuberization medium contains growth regulators which induce the micro-tubers' production through stress.

Other substances added to the culture media are:

Vitamins, which participate in cell enzymatic functions, stimulating the growth of the explants.
Polyamines, nitrogenous compounds derived from the aminoacids, that stimulate growth through

cell division.

Activated carbon absorbs the eliminated inhibitory substances through the explants' sap in the medium, and stimulates root morphogenesis and growth.

3.1 IN VITRO INTRODUCTION MEDIUM

1. Dissolve a packet of MS [Murashige & Skoog basal medium) in 600 ml of distilled water
2. Add 25 g of sucrose and 5 ml of vitamin solution. Stir
3. Add '1 ml of gibberellic acid.
4. Bring the volume up to 1,000 ml with distilled water.
5. Measure the pH and adjust it to 5.6 [bring it up with KOH 1N, or down with HCl 1N).
6. Add 3.5 g of Phytigel.
7. Dissolve the gelling agent with heat [microwave oven: 100% intensity, 1-2 minutes). Stir well until it dissolves, preventing it from boiling.
8. Distribute 2 ml in each 1.3 x 100 mm tube.
9. Sterilize at 121°C and 15 pounds pressure, for 20 minutes.
10. Take the culture tubes off the autoclave and keep it at 4°C until planting.

3.2 POTATO PROPAGATION MEDIUM

1. Dissolve a packet of MS [basal medium Murashige & Skoog) in 600 ml of distilled water
2. Add 25 g of sucrose and 5 ml of VGA solution. Stir
3. Fill up to 1,000 ml with distilled water
4. Measure the pH and adjust it to 5.6 [bring it up with KOH 1N or down with HCl 1N).
5. Add 3.5 g of Phytigel.
6. Dissolve the gelling agent with heat [microwave oven: 100% intensity, 12 minutes). Stir well until the gelling agent is dissolved, preventing it from boiling.
7. Distribute '1.2 ml in 25 x 1.50 mm tubes.
8. Sterilize at 121°C and 15 pounds pressure for 20 minutes.
9. Take the culture tubes off the autoclave and keep it at 4°C until planting.

3.3 POTATO MERISTEMS MEDIUM

1. Dissolve a packet of MS (Murashige & Skoog basal medium) in 600 ml of distilled water.
2. Add 25 g of sucrose and 5 ml of vitamin stock solution.
3. Add 2 ml of putrescine (stock solution: 10,000 ppm). and 0.5 ml of gibberellic acid
4. Fill it up to 1,000 ml with distilled water.
5. Measure the pH and adjust it to 5.6 (bring up with KOH 1N or down with HCl 1N).
6. Add 6 g of agar.

7. Dissolve the gelling agent with heat (microwave oven: 100% intensity, 1 2 minutes). Stir well until the gelling agent is dissolved, preventing it from boiling.
8. Distribute 2 ml in 1 3 x 100 mm tubes.
9. Sterilize at 121⁰C and 15 pounds pressure for 20 minutes.
10. Take the culture tubes off the autoclave and keep it at 4⁰C until planting.

3.4 POTATO CONSERVATION MEDIUM

1. Dissolve a packet of MS (Murashige & Skoog basal medium) in 600 ml of distilled water
2. Add 20 g of Sucrose, 40 g of Sorbitol and 5 ml of vitamin solution. Stir.
3. Fill it up to 1,000 ml with distilled water
4. Measure the pH, adjust it to 5.6 (bring it up with KOH iN or down with HCl 1N).
5. Add 7. 5 g of agar.
6. Dissolve the gelling agent whit heat (microwave oven: 100% intensity, 12 minutes). Stir until the gelling agent is dissolved, preventing it from boiling
7. Distribute 12 ml of medium in 25 x 125 mm tubes.
8. Sterilize at 121⁰C and 15 pounds pressure for 20 minutes.
9. Take the culture tubes off the autoclave and keep it at 4⁰C until planting.

3.5 POTATO TUBERIZATION MEDIUM

1. Dissolve a packet of Murashige & Skoog basal medium in 600 ml of distilled water. Stir well.
2. Add 80 g of Sucrose, 5 ml of BAR and 500 mg CCC. Stir.
3. Fill it up to 1,000 ml with distilled water
4. Measure the pH and adjust it to 5.6 (bring it up with KOH 1N or down with HCl 1N).
5. Distribute in bottles.
6. Sterilize at 121⁰C and 15 pounds pressure, for 20 minutes.
7. Take the bottles off the autoclave and keep them at 4⁰C until induction.

3.6 PREPARATION OF THE PROPAGATION MEDIUM WITH ACTIVATED CARBON

1. Dissolve a packet of MS (Murashige & Skoog basal medium) in 800 ml of distilled water.
2. Add 25 g of sucrose and 5 ml of VGA solution. Stir
3. Fill it up to 1,000 ml with distilled water
4. Measure the pH and adjust it to 5.8 (bring it up with KOH 1 N or down
5. Add 4 g of Phytigel.
6. Dissolve the gelling agent with heat (microwave oven: 100% intensity, until the gelling agent is dissolved, preventing it from boiling.
7. Add 10 g of activated carbon, using a mask.
8. Distribute 12 ml of medium in 25 x 150 mm tubes.

9. Sterilize at 121°C and 15 pounds pressure for 20 minutes.
10. Take off the culture tubes from the autoclave and keep them at 4°C until planting.

PREPARATION OF POTATO CULTURE MEDIA (1 LITER)
POTATO CULTURE MEDIA FORMULATION

Reagents	Introduction	Propagation	Meristem	Conservation	Tuberization	Activ. Carbon
MS basal salts	1 - 1 l	1 - 1 l	1 - 1 l	1 - 1 l	1 - 1 l	1 - 1 l
VGA solution		5 ml				5 ml
Vitamin stock	5 ml		5 ml	5 ml		
AG ₃ (1,000 ppm)	1 ml		0.5 ml			
Putrescine (10,000 ppm)			2 ml			
BAP(1,000 ppm)					5 ml	
CCC					500 mg	
Sucrose	25 g	25 g	25 g	20 g	80 g	25 g
Sorbitol				40 g		
Activated carbon						10 g
Phytigel	3.5 g	3.5 g				4 g
Agar			6.0 g	7.5 g		

4. Tissue Culture Techniques

TISSUE CULTURE TECHNIQUES

Introduction into in vitro conditions

Fungi and bacteria grow on the plant surface and contaminate the culture media when they are not adequately eliminated. The process of explant introduction into in vitro conditions depends mainly on the disinfecting phase.

Problems in handling produce contamination of the in vitro plant and so, a similar disinfection process to that of the in vitro introduction technique is recommended. However, it is possible to use antibiotics included in the medium, but just temporarily, while the plantlets are growing. Among the bacterial contaminants are *Bacillus* sp., *Erwinia* sp., *Pseudomonas* sp., etc.

In the case of systemic infections, the use of disinfectants is not effective, because the pathogens are located in the vascular system. In this case, it is recommended to use specific antibiotics and meristems or bud cuttings.

On the other hand, the process of in vitro introduction involves the use of explants in different physiological stages, as dormant buds, which require growth regulators for example, Gibberellic acid) to stimulate and accelerate bud growth.

During the process of in vitro introduction there is a close connection between the greenhouse and the laboratory; that is why the necessary precautions should be taken to avoid the entrance of contaminants into the laboratory.

A) In the greenhouse

1. Take cuttings from the mother plant, or buds from greenhouse tuber sprouts.

B) In the laboratory

1. Immerse the cuttings for 10 minutes in a beaker conveniently labeled with the accession name, and containing a solution of 0.5% acaricide with three drops/l of Tween 20.
2. Throw away the acaricide solution and rinse the nodes with tap water
3. Prepare the laminar flow chamber.
4. Immerse the cuttings in 70% alcohol for 30 seconds and take the beakers immediately to the laminar flow chamber

C) In the laminar flow chamber

1. Eliminate the alcohol and replace it with a 2.5% solution of calcium hypochlorite. Keep the cuttings immersed during 15 minutes.
2. Throw away the hypochlorite, and rinse the cuttings three times with sterile water
3. Keep the nodes immersed in sterile water until bud extraction.
4. Dissect the buds on a sterile plate and place them in the culture medium.

4.1 MICROPROPAGATION

In vitro plantlets, which are free of pathogens, are used as initial material for potato and sweetpotato seed programs. The methods used in these micropropagation programs mainly depend on their production volume and the available infrastructure. In the case of potato micropropagation, the basic methods have been already described (Dodds, 1985; Espinoza et al., 1992). They have been verified in many institutions and they are based on the rapid growth of individual node cuttings, or stems with multiple node cuttings. Afterwards, the basic micropropagation methods are described.

A. Node micropropagation

This method 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 axillary bud, resulting in a new in vitro plantlet.

This type of propagation promotes the development of a pre-existent morphological structure. The nutritional and hormonal condition of the medium breaks the dormancy of the axillary bud and promotes its rapid development (Lizárraga et al. 1989).

Greenhouse

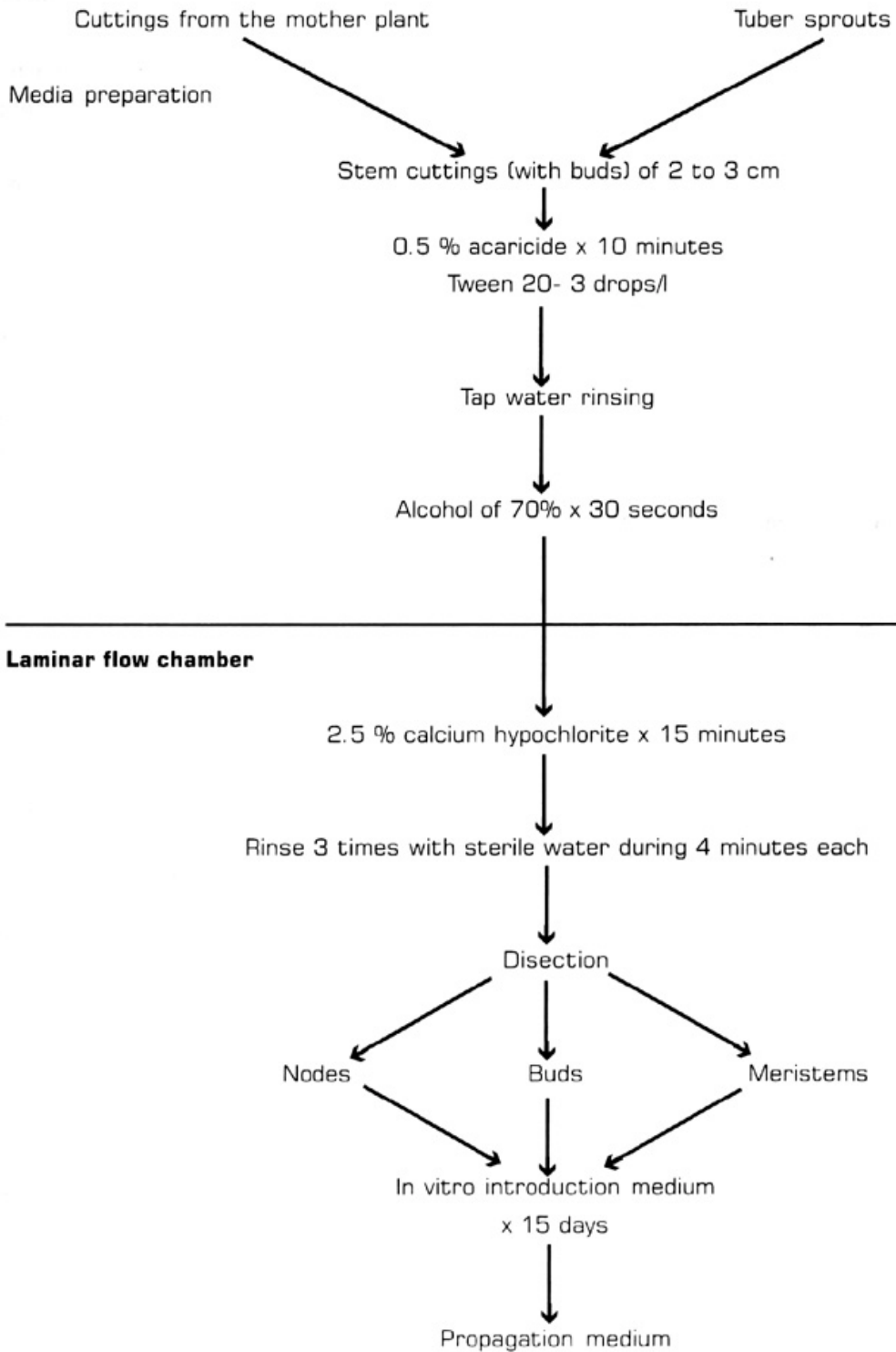


Figure 1 Procedure for in vitro introduction



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

Under room-controlled conditions micropropagation is fast. Each node planted in a propagation medium will produce a plantlet which will occupy the full length of the test tube, after approximately four weeks for potato, and six weeks for sweetpotato. The resultant in vitro plantlets may be transplanted to in vitro conditions in small pots in the greenhouse.

B. Micropropagation by node cuttings in a liquid medium

This technique is applied both with potato and sweetpotato to produce a large number of nodes rapidly. Stem cuttings with 5 to 8 nodes are prepared by removing both the apex and the root of the in vitro plant to be propagated. The stems are placed in the corresponding propagation liquid medium (Espinoza et al., 1992; Lizárraga et al. 1989). It is also possible to use isolated nodes: the nodes will sprout and new plantlets will develop over a period of 3 to 4 weeks.

Micropropagation procedure

1. Sterilize petri dishes (placed in paper bags or comets) and prepare the laminar flow chamber by disinfecting the internal surfaces with alcohol.

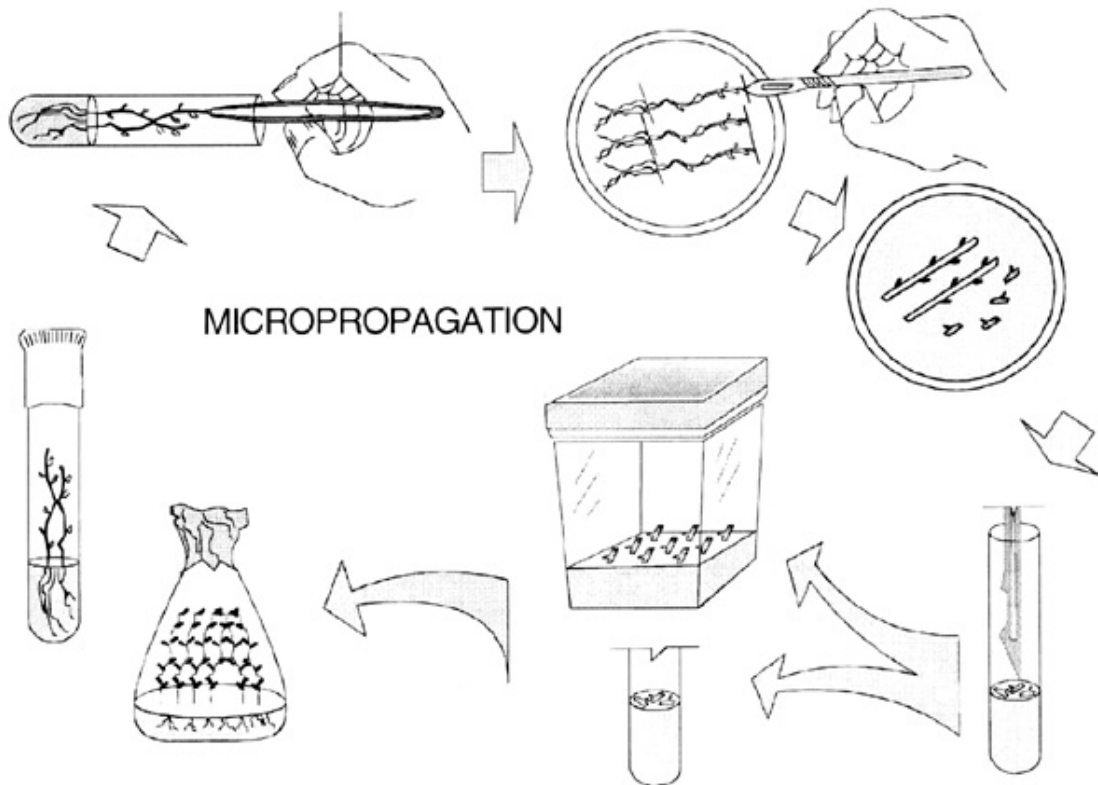


Figure 2 Potato micropropagation process scheme

Sterilize the tools with an instrument sterilizer and place them on a sterile dish.

2. Open the tube, take off the plantlet and place it on a petri dish with the help of forceps.
3. Remove the leaves and cut the nodes.
4. Open a tube containing fresh sterile medium and place a node inside, trying to plunge it slightly into the medium with the bud up.

Close the tube.

5. Seal the tube with a gas-permeable plastic tape (parafilm or saram wrap) and label it correctly.

It is recommended to place two explants in 16 x 125 mm tubes, three in 18 x 150 mm tubes, five in 25 x 150 mm tubes, and 20-30 in magenta vessels.

C. Common problems in micropropagation

Some problems may appear in tissue culture according to the crop or variety. To solve them, it is necessary to apply one or several preventing/solving methods such as:

Phenolization

The explants frequently become brown or blackish shortly after isolation. When this occurs, growth is inhibited and the tissue generally dies. The young tissues are less susceptible to darkening than the more mature ones.

Prevention.

The tissue darkening— mainly that of the recently isolated explants and that of the medium—may be generally prevented by:

1. Removing the phenolic compounds produced by dispersion. Absorption by means of activated carbon.

Absorption by polyvinilpyrrolidone (PVP).

2. Modifying the redox potential.

Reducing agents: ascorbic acid, citric acid, L-cisteine HCL, ditriotreitol, glutation and mercaptoethanol

Less availability of oxygen: stationary liquid media.

3. Inactivating the phenolase enzymes.

Chelating agents: NaFeEDTA, EDTA, diethyldithiocarbamate, dimethyl-dithiocarbamate

4. Reducing the phenolasic activity and the availability of substrate.

Low pH

Darkness

Absence of rooting

The explants can naturally form roots during propagation, without an additional rooting stage, as with the potato. However, some wild potato species may show root production deficiency. Rooting may be induced by incorporating auxins, such as IAA, NAA, and IBA, or activated carbon to the culture medium.

4.2 POTATO IN VITRO TUBERIZATION

Most of the potato microtubers are used in seed programs in Europe, where large amounts (hundred of thousands) of pre-basic seed of a few varieties are produced. By means of this technique, microtubers are produced and stored, and it is possible to store thousands of them in a small area (in humid containers at 4°C) for long periods of time.

The microtuber induction is produced through a stress effect by the CCC (chlorocholine chloride), BAP (B-benzylaminopurine) and Sucrose, which under darkness will produce from 3 to 4 microtubers per plant, according to the variety.

In the beginning, microtubers were used as an alternative for germplasm distribution and in vitro conservation.

Curing the plantlet in vitro multiplication process, usually there is a multiplication of higher amounts to those required in the greenhouse and so, after the transference, some magentas are left over. These plantlets may be used for microtuber induction. In accordance with the indicated procedures, the induction medium is added to the magentas and then they are placed in the dark room. After three months the microtubers will be produced. These may be harvested and transferred to sterile containers (at 4°C), where they can be maintained up to 10 months, or used in the next campaign instead of in vitro plantlets.

Tuberization in vitro

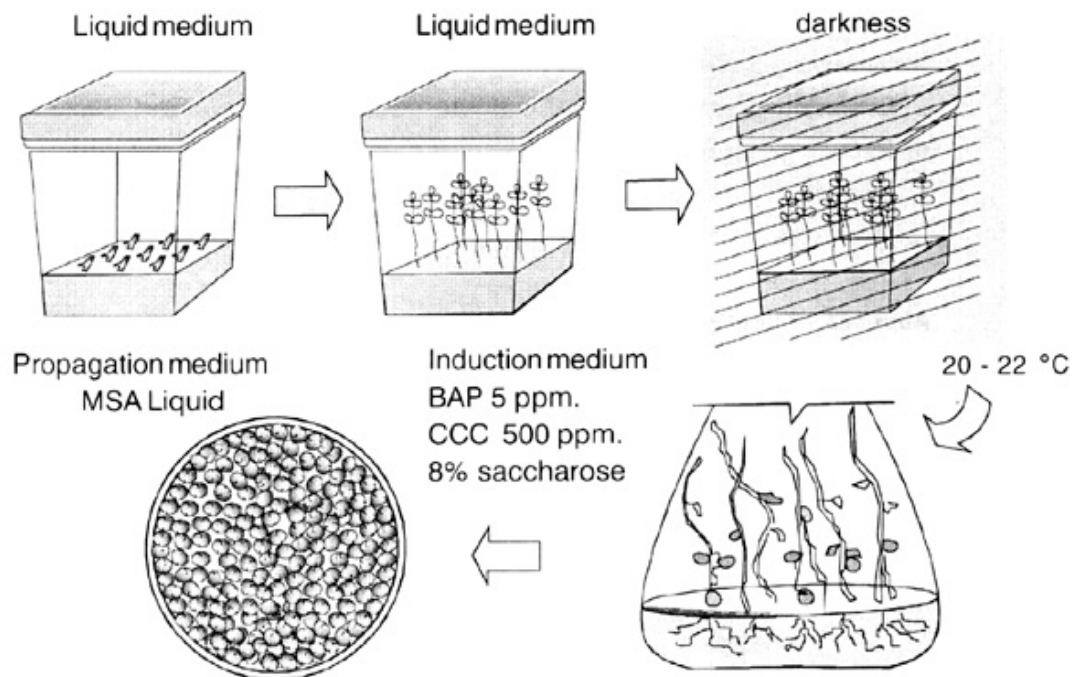


Figure 3 Potato in vitro tuberization process scheme

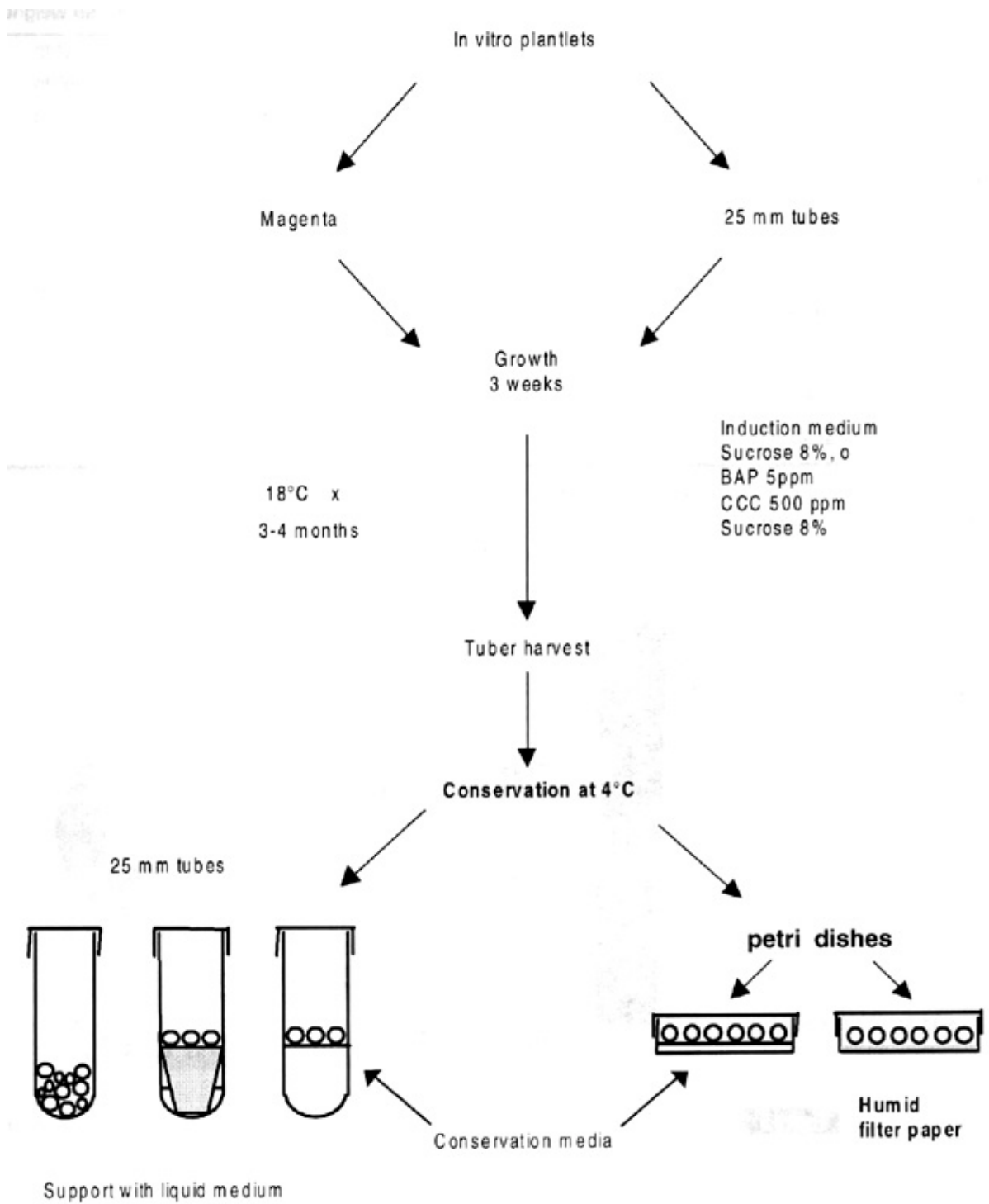


Figure 4 Potato in vitro tuberization process scheme

Number of microtubers per flask and fresh weight of tubers*.

Accession	No. microtubers/flask	Fresh weight/tuber
Mariva	9.9	163.3
LT-2	12.1	230.0
DTO-2	9.7	108.3
LT-7	19.4	64.5
DTO-33	8.2	80.9
LT-1	15.3	54.9
Piñaza	20.8	39.0
Atacama	8.9	59.0
702867	13.4	93.1
Atzimba	9.8	77.7

Source: R. Estrada, P. Tovar, J. Dodds. 1986. International Potato Center.

* 30 nodes per vial were used

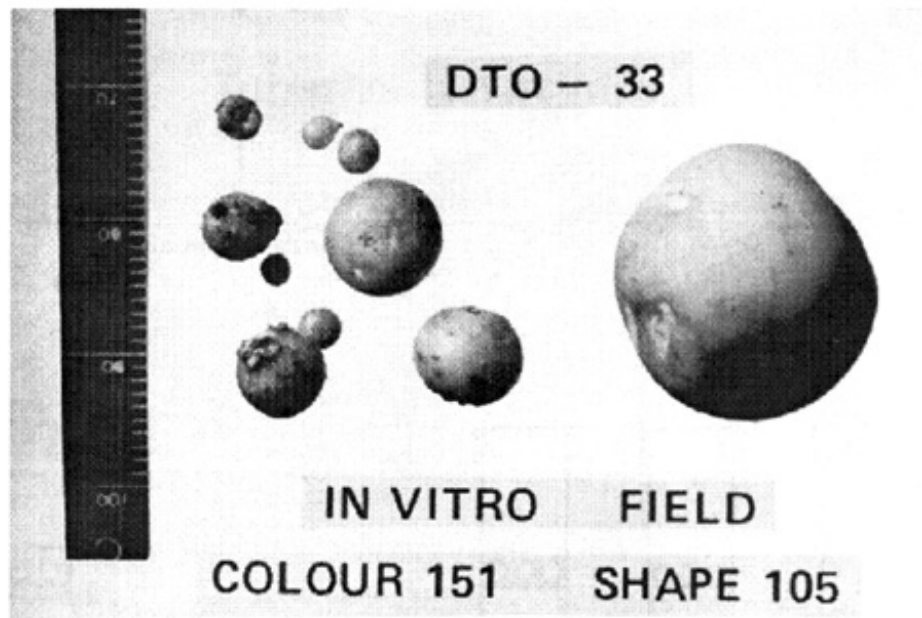


Figure 5 Comparison, in size and shape, of in vitro microtubers with a normal one from the field

These microtubers can also be used as a reserve, in case in vitro material is contaminated or dies, because of temperature or handling effects.

Both the table and Figure 5 show the considerable amount of produced tubers in comparison with the prebasic tubers produced in the greenhouse (Estrada R, et al, 1986). The high number and bigger size of the microtubers increases the success rates for the transfer to the greenhouse.

4.3 MEDIUM TERM GERmplasm MAINTENANCE

The in vitro germplasm maintenance under normal growth conditions requires a series of transfers of the plantlets into a fresh medium. This leads to a consumption of time, increases the possibility of losses because of material contamination during successive sub-cultures, causes a loss of material by human error or failure of some equipment, and demands more labor. A way to avoid these problems is through limitation, restriction or inhibition of growth. This approach consists of growth speed reduction by modifying the physical or chemical conditions of the culture, and it is effective for a short or medium term period.

In vitro conservation methods

These consist in maintaining the cultures (buds, plantlets derived from nodes or directly from meristems) under physical (environmental factors) or chemical (culture medium composition) stressed conditions that make it possible to extend, as much as possible, the interval of transference into the fresh media, without affecting the viability of the cultures.

The methods to reduce the in vitro cultivated plant growth include the reduction of temperature and light during storage, the incorporation of growth retardants in the medium, and the induction of osmotic stress in the medium, or a combination of all these.

Temperature

Temperature reduction has been the most used way to curb culture growth. Most of the in vitro cultures are maintained at temperatures between 12 and 20°C: at lower temperatures, the growth rate decreases but the reduction depends on the species.

Nutrients concentration

The reduction of the carbohydrate concentration and the nitrogenous components of the nutritive medium may affect the growth rates. In addition, the continuous absorption of these nutrients during plantlet growth will bring a nutritional deficiency, which could produce a premature death of the plants.

Use of growth regulators

The abscisic acid (ABA), phosphon-D, maleic hydrazide, and succinic acid are some of the most

frequently used growth regulators.

Osmotic concentration

Growth limitation caused by osmotic concentration is due to the reduction of the water and nutrients absorbed from the medium. For example, at high concentrations, sucrose acts osmotically and it is highly metabolized. Non-metabolized osmotics, such as Mannitol and Sorbitol, are possibly more effective than sucrose in culture growth limitation.

Evaluation of in vitro preserved material

To evaluate material under these conditions, some important facts about in vitro maintenance such as genetic viability and stability should be considered.

The viability evaluation of the in vitro cultures must be systematic. In slow growth conditions, when the sub-culture or transfer period extends during months or years, the frequency of the culture evaluation increases. The most important characteristics to be evaluated in the low-growth cultivars storage of apical buds are: contamination, leaf senescence, the number of green sprouts, the number of viable nodes in relation to the stem length, the presence or not of roots, and callus formation.

Maintenance of accessions in seed program

In a seed program, a group of accessions free of virus is maintained for pre-basic seed production: however, most of them are not propagated in the greenhouse so they are maintained in vitro to be used in the future. The continuous propagation of the in vitro plantlets damages the material, mainly if we consider that environmental conditions are not adequate (temperature and light):

the alternative is to maintain the accessions in conservation media. Each accession must be maintained in test tubes with five replications to avoid possible losses.

The maintenance conditions have been tested in CIP's potato collection that consists of more than 5,000 accessions, by means of which it is possible to assure the normal recovery of the material after using stress producers.

The plantlets used in each campaign must come from the maintenance phase. Then, they will be propagated in normal media where their growth will be reestablished, and the elected procedures for plantlet multiplication in the greenhouse will continue. Each season must be initiated with this material to start off with strong plants. In addition, the plantlets taken for multiplication must be replaced, trying to maintain 5 tubes per accession in the conservation media.

The sub-cultures in the conservation medium are carried out approximately every one or two years. The conservation medium renovation must pass through a previous sub-culture in the

propagation media to rejuvenate the explants.

4.4 VIRUS ERADICATION THROUGH MERISTEMS CULTURE AND THERMOTHERAPY

If a healthy plant is sown in the field, it is exposed to infections caused by pathogens as nematodes, fungi, bacteria, phytoplasmas, virus and viroids, which have a negative effect on yield, and in some cases may kill the plants. However, not all the plant cells may become infected. A group of cells, which are in a continuous non-differentiated multiplication, are virus-free: the meristem.

The in vitro meristems culture, together with growth at high temperatures produces potato plantlets free from viruses in more than 90% of planted meristems. This routine method was established in the International Potato Center to obtain virus-free plantlets for national and international distribution.

The in vitro maintenance of virus-free plants provides the possibility to maintain all the time a bank of healthy and more vigorous plants, and with a more accelerated growth, than the infected ones.

In a seed program, it is essential to initiate this work with virus-free plantlets since this will affect the seed quality and the yield as well. The virus cleaning procedure is too long and expensive: that is why CIP, through its germplasm distribution program, has provided a list of the principal virus-free potato varieties to all users.

PROCEDURE

1. Approximately 18 to 20 plantlets (virus-infected) are propagated in magentas.
2. After a growth period of 20 to 25 days, or when the plantlets are 4-5 cm high, they are placed in the thermotherapy chamber. The growth conditions are:

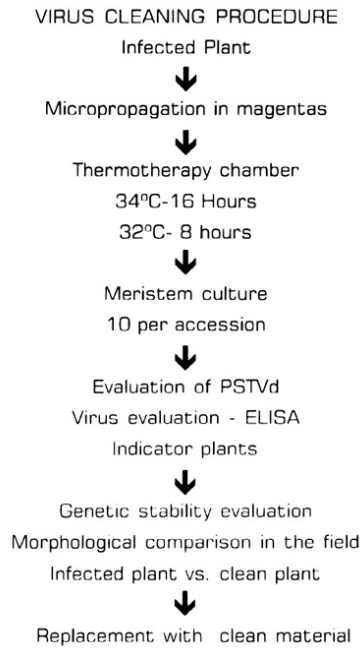
16 hours of light 34°C; 8 hours of darkness 32°C

3. The magentas are maintained in the thermotherapy chamber for one month.
4. Afterwards, the magentas are taken out of the chamber, and the outside is cleaned with 98⁰/_o alcohol. Then they are introduced to the culture room.
5. Meristems are obtained as follows:
Cut the apical portion and remove the leaves that cover the meristem (approximately 3 to 4 leaves); the meristem is observed with a prominent leaf primordium.
Remove the meristem with part of the leaf primordium; cut only the translucent portion.
Use a new knife.
Place the meristem in the culture medium. Be sure that the meristem is in the tube.
6. Evaluate meristem growth and transfer them to fresh media if necessary.
7. Each meristem that originates a plant is called «line», which will be labeled according to the

accession it belongs to. For example, Yellow Line 1, Yellow Line 2, Yellow Line 3, etc.

8. Five tubes with several plants are propagated to evaluate the potato virus PSTVd, PVT, to determine the host range, the morphologic evaluation, and the in vitro maintenance.

9. The results of each evaluation are obtained, and the infected material is replaced with clean material.



Temperature

The growth of the plantlets at temperatures higher than 20°C is accelerated (Do not use temperatures higher than 30°C).

2. Materials and Equipment for the Tissue Culture Laboratory

Table 1 Materials for the Tissue Culture Laboratory

Description	Catalogue	No Cat	Units
Stainless-Steel Utility trays	FISHER	13-361d	ea
Beaker glass x 1000 ml	FISHER	02-540P	6/pkg
Beaker glass x 2000 ml	FISHER	02-540R	4/pkg
Beaker glass x 4000 ml	FISHER	02-540T	ea
Beaker glass x 600 ml	FISHER	02-540M	6/pkg
Beaker plastic x 100 ml	FISHER	05-591-10B	12/pkg
Reusable plastic Beakers-1000 ml	FISHER	02-591-10G	3/pkg
Reusable plastic Beakers-2000 ml	FISHER	02-591-10H	ea
Reusable plastic Beakers-4000 ml	FISHER	02-591-10J	ea
Reusable plastic Beakers-600 ml	FISHER	02-591-10F	4/pkg
Polypropylene with spigot	FISHER	02-963-2B	ea
Large diameter Pyrex-desiccator	FISHER	08-631B	ea
Fisherbrand-Sterilizer bos	FISHER	03-460	ea
Pipette pump II	SIGMA	02-963-2B	ea
Erlenmeyer flasks without stoppers, 1000 ml	FISHER	10-040K	6/pkg
Erlenmeyer flasks without stoppers, 125 ml	FISHER	10-040D	12/pkg
Erlenmeyer flasks without stoppers, 2000 ml	FISHER	10-040M	8/pkg
Erlenmeyer flasks without stoppers, 250 ml	FISHER	10-040F	12/pkg
Erlenmeyer flasks without stoppers, 4000 ml	FISHER	10-040P	4/pkg
Spatula with V shaped spoon	FISHER	21-401-25B	ea
Fisherbrand spoonulet-lab spoon	FISHER	14-3756-20	ea
Plastic racks, 13 mm	FISHER	14-809-22	ea
Plastic racks, 16 mm	FISHER	14-809-24	ea
Plastic racks, 18 mm	FISHER	14-809-26	ea
Magenta 7-Way tray	SIGMA	T8654	10/pkg
Dissecting knife blade # 11	FISHER	08-916-5B	100/pkg
Dissecting knife blade # 15	FISHER	08-916-5D	100/pkg
Pipette washers/rinsers	FISHER	15-530-95A	ea
Magenta GA-7 Vessel	SIGMA	V8505	25/pkg
Glass burner	Local		
Parafilm paper 4" x 250 ft	SIGMA	S-8501	ea
Parafilm M 4" x 250"	SIGMA	P-7668	ea
Delicate thumb forceps, cushioning	FISHER	08-953C	ea
Pipette glass serological 1 ml	FISHER	13-650-4	6/pkg
Pipette glass serological 10 ml	FISHER	13-650-4C	6/pkg
Pipette glass serological 5 ml	FISHER	13-650-4B	6/pkg
Pipette serological disposable 1 ml	FISHER	13-675-15C	200/pkg
Pipette serological disposable 10 ml	FISHER	13-675-20	200/pkg
Pipette serological disposable 5 ml	FISHER	13-675-22	200/pkg
Wash Bottle x 250 ml	FISHER	03-409-22B	6/pkg

Description	Catalogue	No Cat	Units
Wash Bottle x 500 ml	FISHER	03-409-22C	6/pkg
Petri dish glass 150x20mm cover-bottom	FISHER	08-747F	12/pkg
Petri dish culture 100 x15 mm cover-bottom	FISHER	08-747C	12/pkg
Sigmaware lab markers,broad tip,black	SIGMA	S5894	10/pkg
Sigmaware lab markers, fine tip, black	SIGMA	S5769	10/pkg
Cylinder glass 1000 ml	FISHER	05-548G	2/pkg
Cylinder glass 2000 ml	FISHER	08-562-5G	2/pkg
Cylinder glass 250 ml	FISHER	08-548E	2/pkg
Cylinder glass 500 ml	FISHER	08-548F	2/pkg
Magenta GA-7/GA-7 Vessel Cover	SIGMA	C0542	25/pkg
Bacticapall polypropylene 13 mm	FISHER	14-127-28A	1000/pkg
Bacticapall polypropylene 16 mm	FISHER	14-127-28B	1000/pkg
Bacticapall polypropylene 18 mm	FISHER	14-127-28C	1000/pkg
Closures pyrex rimless culture tubes	FISHER	14-957-85E	100/pkg
Closure disposable Sigmaware for 25 mm			
Culture tubes, natural color	SIGMA	C7591	500/pkg
Max./Min. Thermometers	FISHER	15-093	ea
Culture test tube 13 x 100 mm	FISHER	14-957C	720/pkg
Culture test tube 16 x 125 mm	FISHER	14-957F	576/pkg
Culture test tube 18 x 150 mm	FISHER	14-457H	576/pkg
Culture test tube 25 x 150 mm	FISHER	14-957M	288/pkg
Scissors 115 mm	Local		ea
Wool	Local		ea
Comercial cotton	Local		ea
Lab coat	Local		ea
Scissors 7"	Local		ea
Small light-Duty cart	FISHER	11-926-50	ea
Kik-Step Stool	FISHER	11-931	ea
Stainless-Steel Basket	FISHER	14-799-4	ea
Scienceware Polypropylene support	FISHER	13-712-10	ea
Scienceware electric glassware dryer	FISHER	11-387	ea
Stainless-Steel Drain/Dy	FISHER	91-162	ea

Table 2 Chemical Reagents

Description	Catalogue	No Cat	Units
Murashige and Skoog Basal Salt Mixture	GIBCO	23118-037	10 x 1l
Sucrose Grade II 5 kg.	SIGMA	S5391	5 kg
alcohol rectified	Local		
Phytigel 5 kg.	SIGMA	P8169	5 kg
Agar Powder	SIGMA	A1296	1 kg
Thyamine hydrochloride crystalline	SIGMA	T3902	100 g
Glycine free base	SIGMA	G6143	100 g
Nicotinic acid free acid	SIGMA	N0765	100 g
Pyridoxine hydrochloride crystalline	SIGMA	P8666	100 g
Gibberellic acid x 10 gr	SIGMA	G7645	10 g
Charcoal activated neutralized	SIGMA	C3790	2.5 kg
Spermidine trihydrochloride	SIGMA	S2501	5 g
Tween 20 x 500 ml	MERCK	822184	500 ml

Description	Catalogue	No Cat	Units
Indole-3 acetic acid	SIGMA	I2886	25 g
Naphthalene acetic acid x 100 g	SIGMA	N0640	100 g
Myo-inositol	SIGMA	I3011	100 g
D-Mannitol	SIGMA	M1902	500 g
D-Sorbitol	SIGMA	S8143	1 kg
Putrescine	SIGMA	P3178	25 g
D-Pantothenic acid	SIGMA	P6045	100 g
D-(+)-Glucose	SIGMA	G7520	5 kg
L-Ascorbic acid (Vitamin C)	SIGMA	A2174	100 g
Chlorochoine Chloride	SIGMA	C4049	25 g
8-Benzylaminopurine	SIGMA	B3408	5 g

Table 3 Equipments

Description	Catalogue	No Cat
pHmeter	SIGMA	P-7449
pHep Stick meter	FISHER	13-300-73
Water distiller wheaton 5 L	WHEATON	09-124-100
6' Horizontal Laminar Flow Clean Bench	BAKER	EG-6252
2' Horizontal Laminar Flow Clean Bench	BAKER	IV-22
Hepa replacement filter 30 x 48 x 5-7/8 for TT4830 (cameras Envirco)	Envirco Corporation	69302-p
Hepa replacement filter 24 x 72 x 5-7/8 for TT7224 (cameras Envirco)	Envirco Corporation	69212-p
Fisher Porcelain-top stirring hotplates	FISHER	11-499-7SH
Ohaus Portable model 200	SIGMA	B-4658
Ohaus Model A5605	SIGMA	B-8785
Sterilmatic - sterilizers Chamber 26L x 16"H	FISHER	14-460
Lightweight Sterilizer	FISHER	14-461-5
Microscope Nikon Mod. SM-S-D	Geoimpex	NMAG1aI
Haker	THOMAS	8290-f26
PC Gateway Proffessional System P5-100	Gateway	P5-100
Sterilizer, Infrared, 120V	SIGMA	S3398
Unispense UP W/ standard 220V	WHEATON	374302
3 mm rub tub assembly	WHEATON	374311
Biotronette Mark III Environmental Chamber	CMS	258-521
Bottle top dispenser Brinkmann	SIGMA	D5174
Air conditioner "Carrier" 60,000 BTU	COLD IMPORT	
Maxi Mix II, 240 V	Thermolyne	M37610-26