FROM SUGAR TECH TO BIOTECH

Plant Tissue Culture: MAGIC in a BOTTLE

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Crop Science Cluster, College of Agriculture, UPLB

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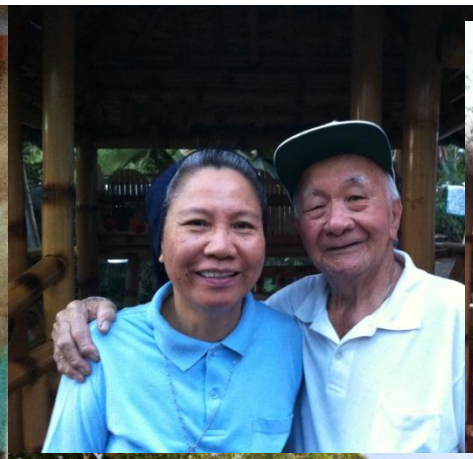
FROM SUGAR TECH TO BIOTECH

Plant Tissue Culture: MAGIC in a BOTTLE

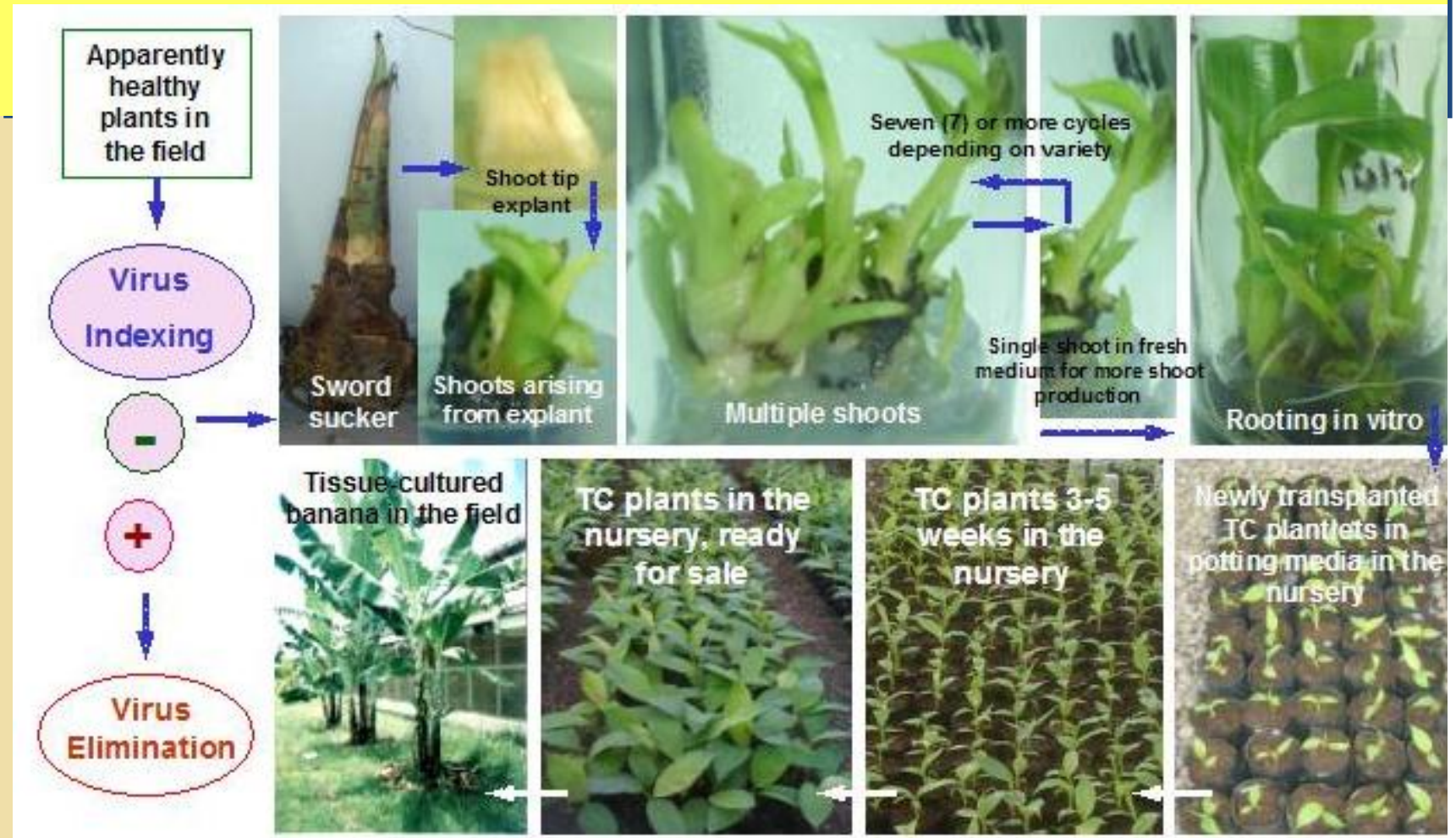


IPB

UNIVERSITY OF THE PHILIPPINES LOS BANOS
College of Agriculture
SUGAR TECHNOLOGY (1970)

Institute of Plant Breeding (IPB)
Plant Cell and Tissue Culture Laboratory
Dec 1975 – Present
(40 yrs – 31 as Researcher, 9 as Faculty)

PCTCL–IPB BANANA TISSUE CULTURE TECHNOLOGY



Pateña and Barba, 2012

Fig. 1. PCTCL-IPB Banana Tissue Culture Technology

Introduction

- **Tissue culture of banana coupled with serology is the only way of producing disease-free planting materials.**
- **It is the accepted practice now in the Philippines and throughout the world.**

IPB –PCTCL Tissue Culture Technology

History:

International Development Research Center (IDRC), Canada

PCARRD

Institute Of Plant Breeding (IPB), CA, UPLB

Objective:

- to develop a system of promoting quick and massive plantings of 'Saba' banana in poor rural communities

In 1982, then Director of IPB, Dr. RM Lantican, submitted an invited proposal on banana, prepared by Dr. RC Barba and LF Pateña, to the International Development Research Center (IDRC), Canada through PCARRD.

In 1983, IDRC awarded IPB the banana grant with RC Barba as Consultant/Adviser and LF Pateña as Project Leader of the tissue culture project.

History:

The project developed micropropagation protocol for more than 50 different banana cultivars with OP Damasco as MS student advisee of RC Barba and MCE Umali as Project Research Assistant.

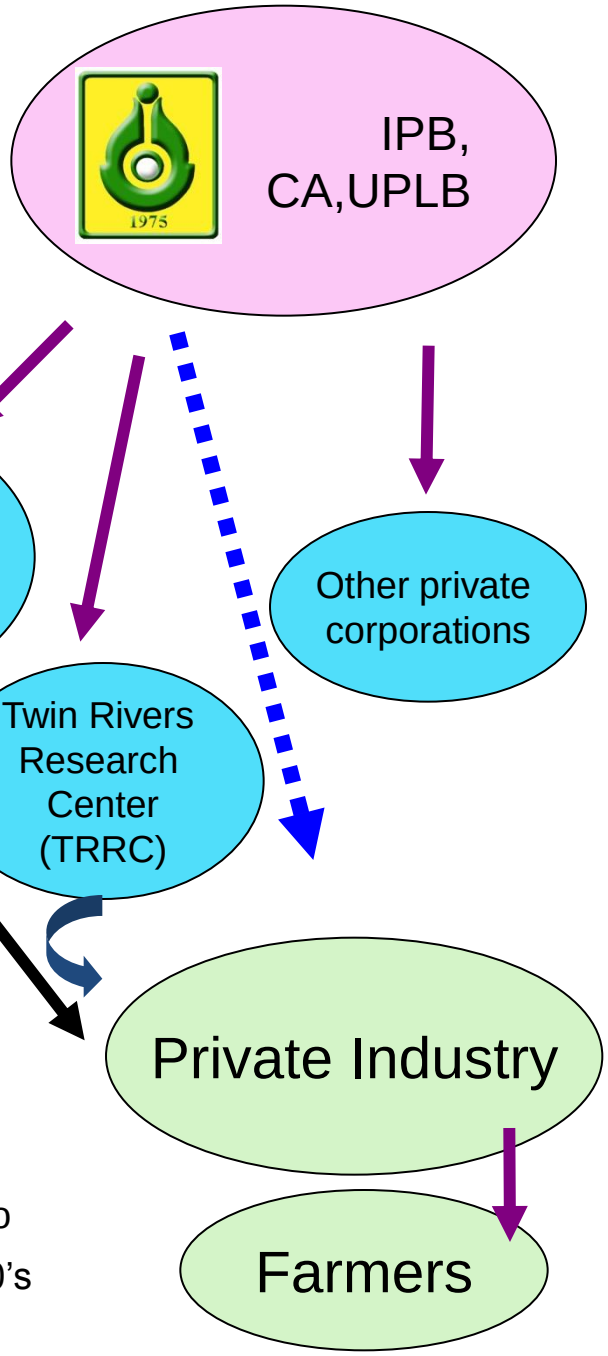
In 1983-1984, LF Pateña prepared a training module on micropropagation of banana.

The IPB Banana Tissue Culture Team, with AB Zamora (vice LF Patena who went on study leave) as Project Leader, conducted the training.

The training facilitated the transfer, for the first time, of the banana micropropagation technology to the different Asian countries, including the Philippines.

In, the Philippines, the 2 trainees (L. Magnaye and A. Jadraque) adapted what they learned in their training at IPB to the benefit of commercial plantations in Mindanao where bananas are grown for export.

At IPB, TC bananas were produced and sold to local clients in the late 80's to the early 90's and then stopped, then revived in 2006.



IPB –PCTCL Tissue Culture Technology

Virus Indexing

Banana Tissue Culture Cycle

In 2006, the Department of Agriculture, Republic of the Philippines, recognized the need for planting materials and awarded IPB with a grant to produce seeds and vegetatively propagated planting materials of different crops.

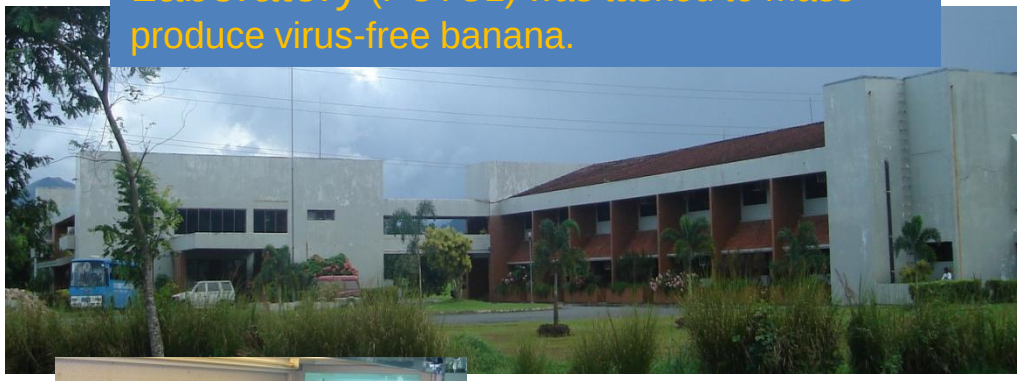
Institute of Plant Breeding (IPB), CA, UPLB

Department of Agriculture (DA) Philippines

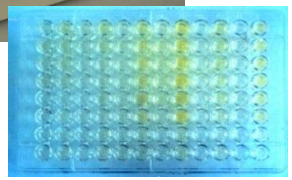
PCTCL

BIOTECH

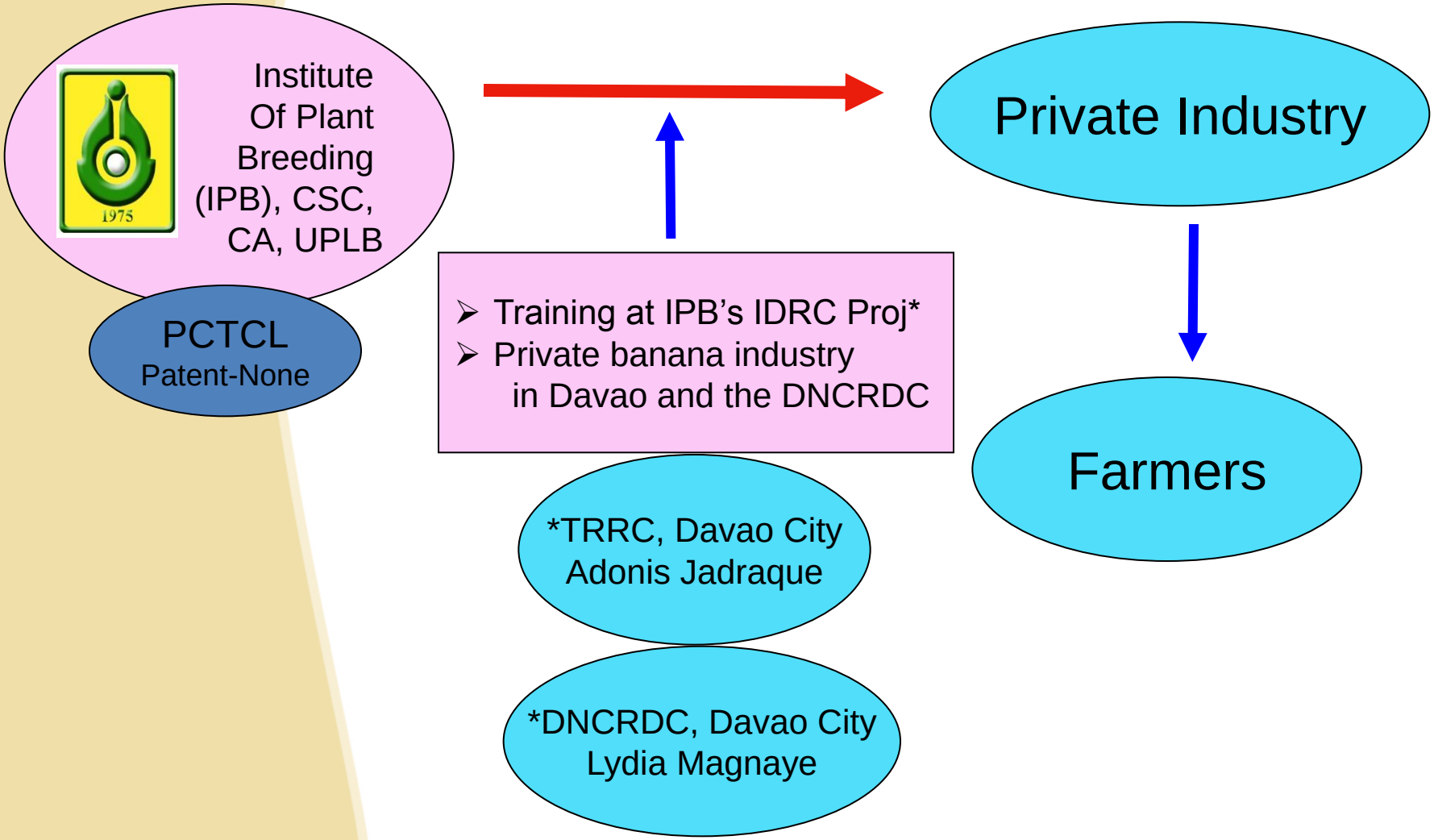
The Plant Cell and Tissue Culture Laboratory (PCTCL) was tasked to mass produce virus-free banana.



Virus-free indexing was done by BIOTECH, UPLB.

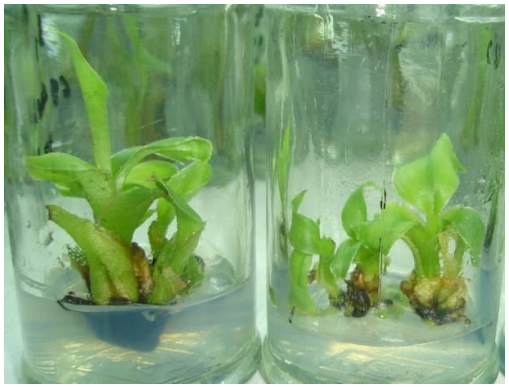


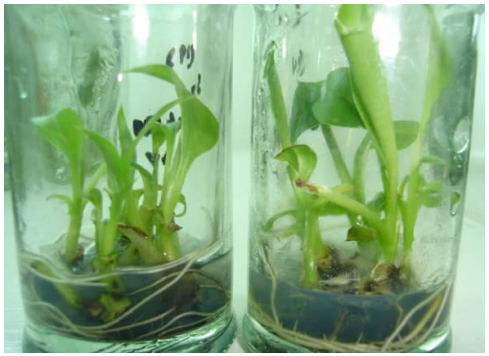
IPB –PCTCL Banana Tissue Culture Technology Commercialization

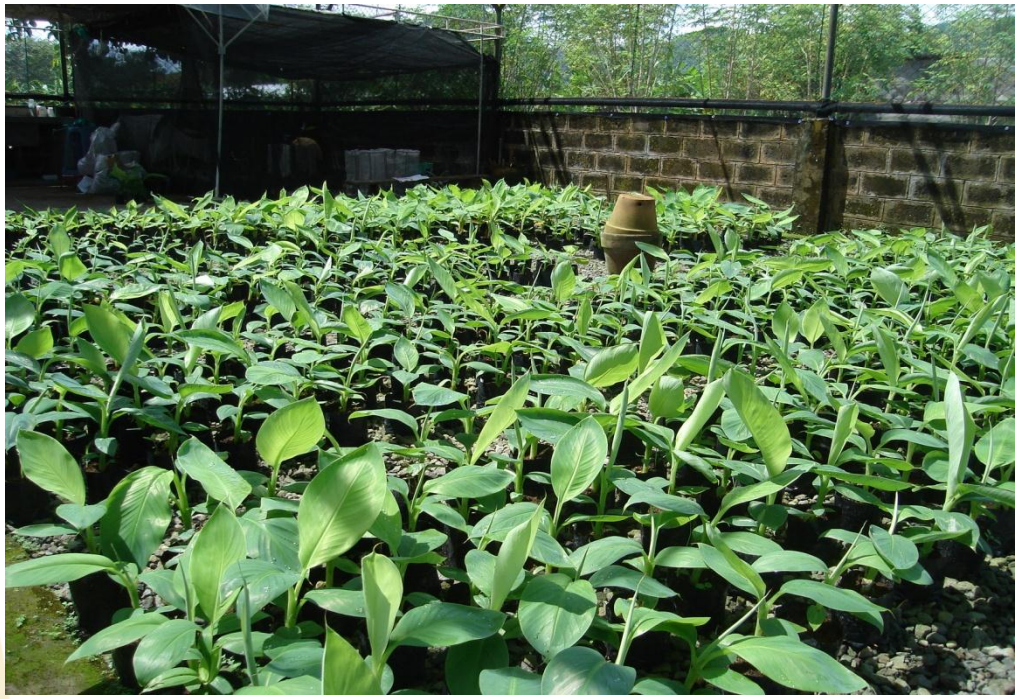


Plantlets produced by banana corporations are now estimated at over

100M every year with > PhP 1 billion market value.







The BANANA TISSUE CULTURE TECHNOLOGY

- currently used by other laboratories for various aspects of research, e.g. disease resistance;
- actively pursued to produce virus-free planting materials for the development and expansion programs for abaca

Patena, et al.,2008

Abaca Tissue Culture Lab at Visayas State University (VSU), Baybay, Visca, Leyte

FIDA Tissue Culture Laboratory at Legaspi City, Albay

FIDA Abaca Tissue Culture Laboratory in Leyte

Biggest Beneficiaries

- Commercial banana companies
- Entrepreneurs
- LGUs
- Farmers/Growers

Congratulations

AWARDS

- **1980. UPLB College of Agriculture 1st Recognition Award for Outstanding Research Team**
- **1988. UP Presidential Award for Outstanding Research Team**
- **2010. DOST-NAST Outstanding Tech Commercialization Award (Gregorio Y. Zara Medal) for the TC System for Banana**
- **2016. College of Agriculture, UPLB Outstanding Research Team**
- **2016. UPLB Outstanding Research Team**

LF Pateña * 14 Best Paper/Poster Awards, 1990 OYS, 1998 TOWNS, 2000. Featured in **one chapter of the book, "Ten Outstanding Filipino Scientists, Lilian Formalejo Pateña - Plant Biotechnology Whiz**", p. 179-202." ANVIL Publishing, Inc. Philippines, by **Queena N. Lee-Chua**, Ph.D.,

WHAT IS

PLANT TISSUE CULTURE?

PLANT TISSUE CULTURE

- technique wherein different plant parts are excised from the parent plant and grown in a defined medium under aseptic condition and controlled environment

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PLANT TISSUE CULTURE

Callus culture

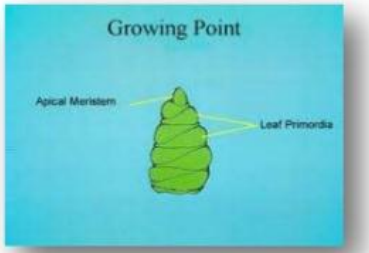
Suspension culture

Pollen culture

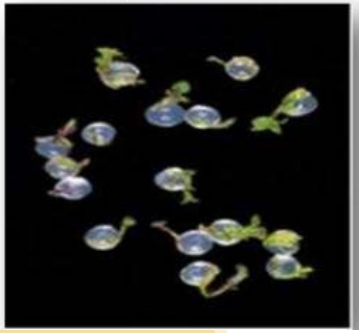
Ovule culture

Root tip culture

Shoot tip culture



Protoplast culture



Leaf primordial culture

Flower culture

I. BASIC PRINCIPLES OF PLANT TISSUE CULTURE

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PLANT TISSUE CULTURE

CONCEPTS/ PRINCIPLES

1. Plasticity
2. Totipotency

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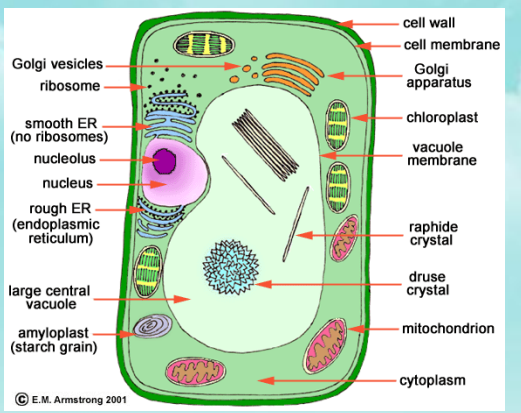
PLASTICITY



- the ability of a plant cell wall to stretch irreversibly

- auxin, first isolated by Dutch botanist Frits Went in the 1920's, increases wall plasticity

- auxin stimulates shoot growth

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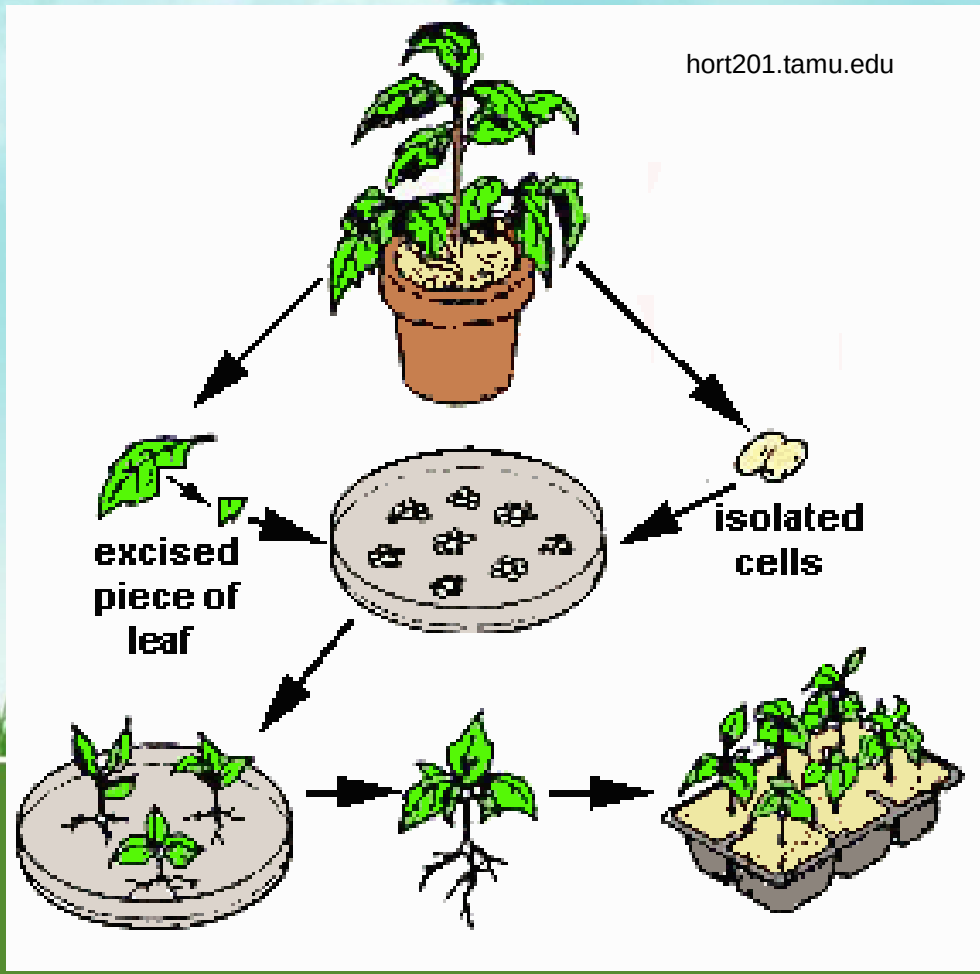
TOTIPOTENCY



- ability of the a plant cell to maintain its genetic potential & regenerate into whole plant

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PLANT TISSUE CULTURE

Requirements

1. Explant
2. Culture medium
3. Culture environment

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PLANT TISSUE CULTURE

Requirements

1. Explant

a. **Cell, with or without cell wall (protoplast)**

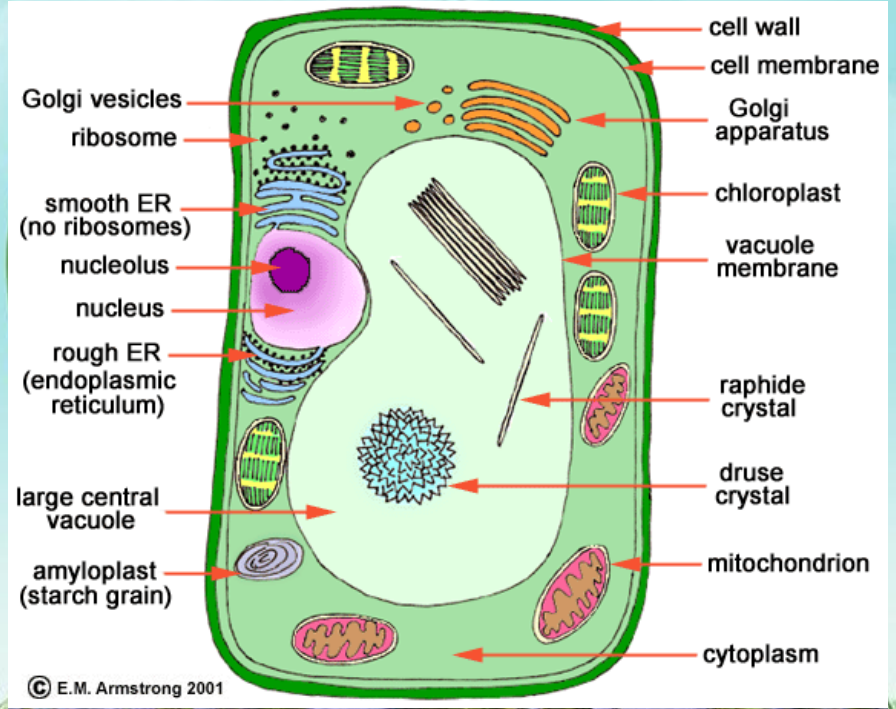
b. **Tissue – a group of cells that form a structure**

c. **Organ – leaf, shoot, root**

d. **Whole plant - embryo**

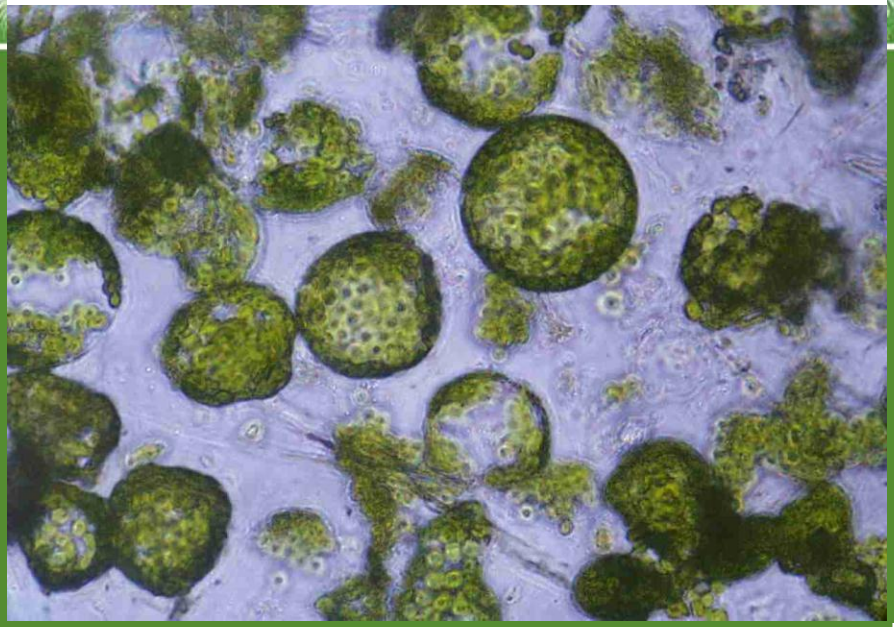
PLANT TISSUE CULTURE

Requirements



1. Explant

a. Cell, with or without cell wall (protopalst)



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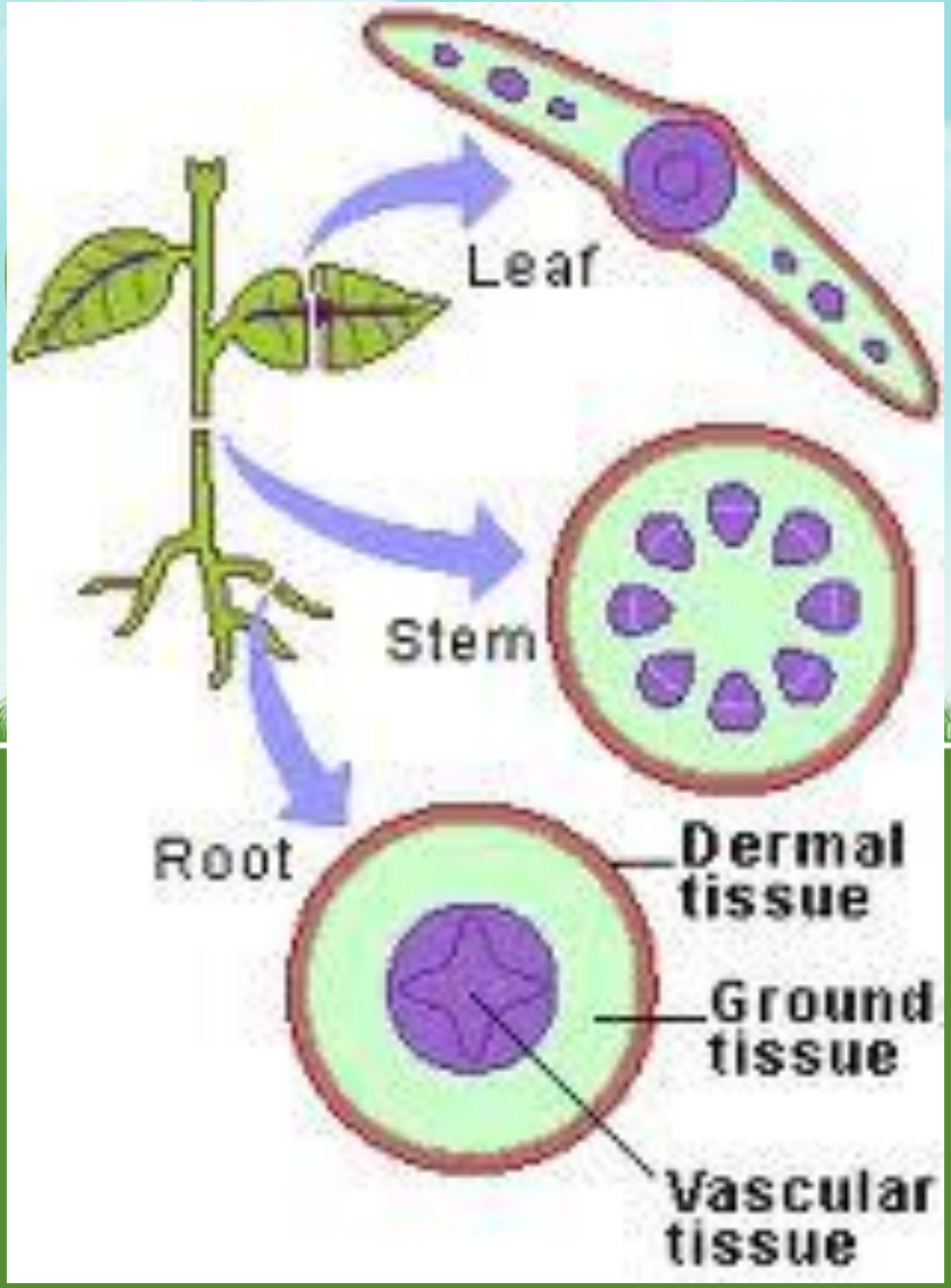
PLANT TISSUE CULTURE

Requirements

1. Explant

b. Tissue



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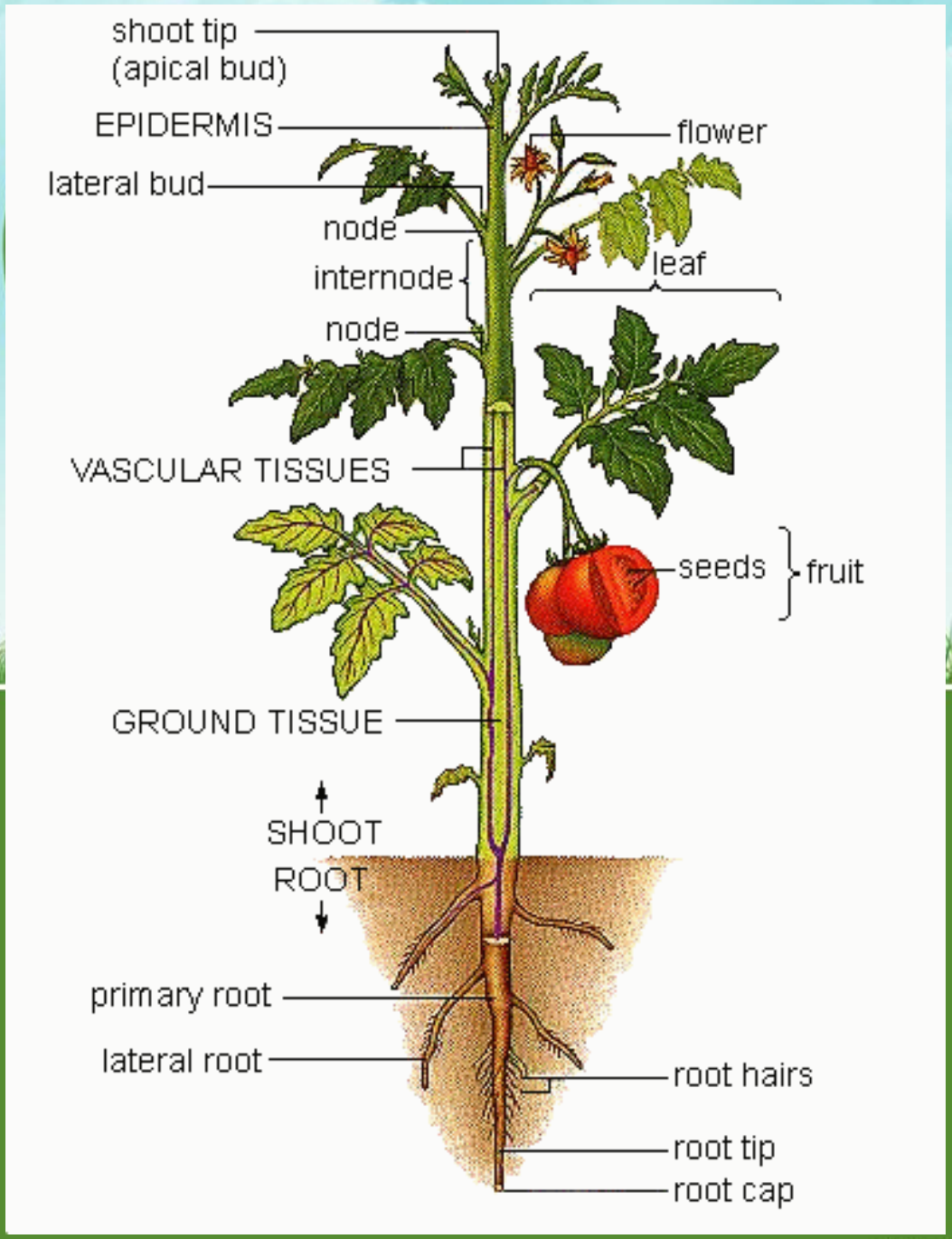
PLANT TISSUE CULTURE

Requirements

1. Explant

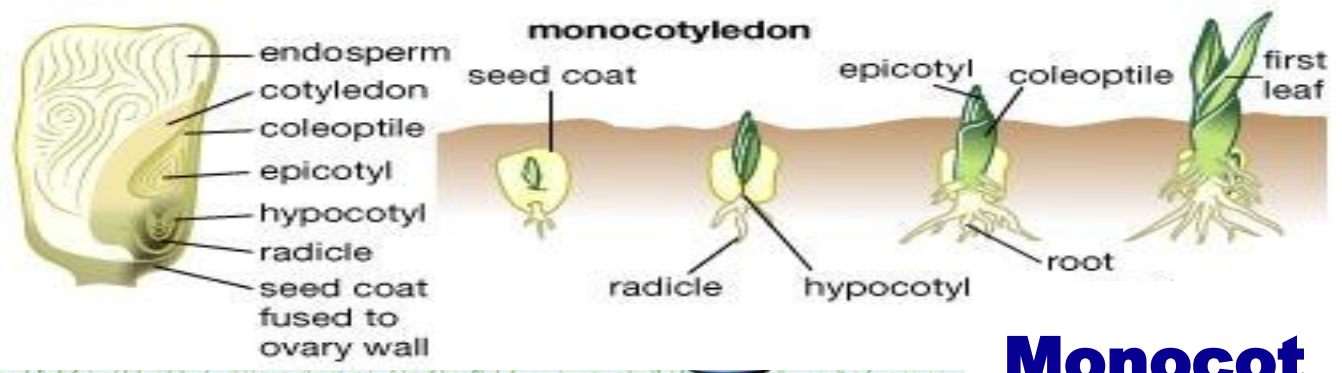
c. Organ



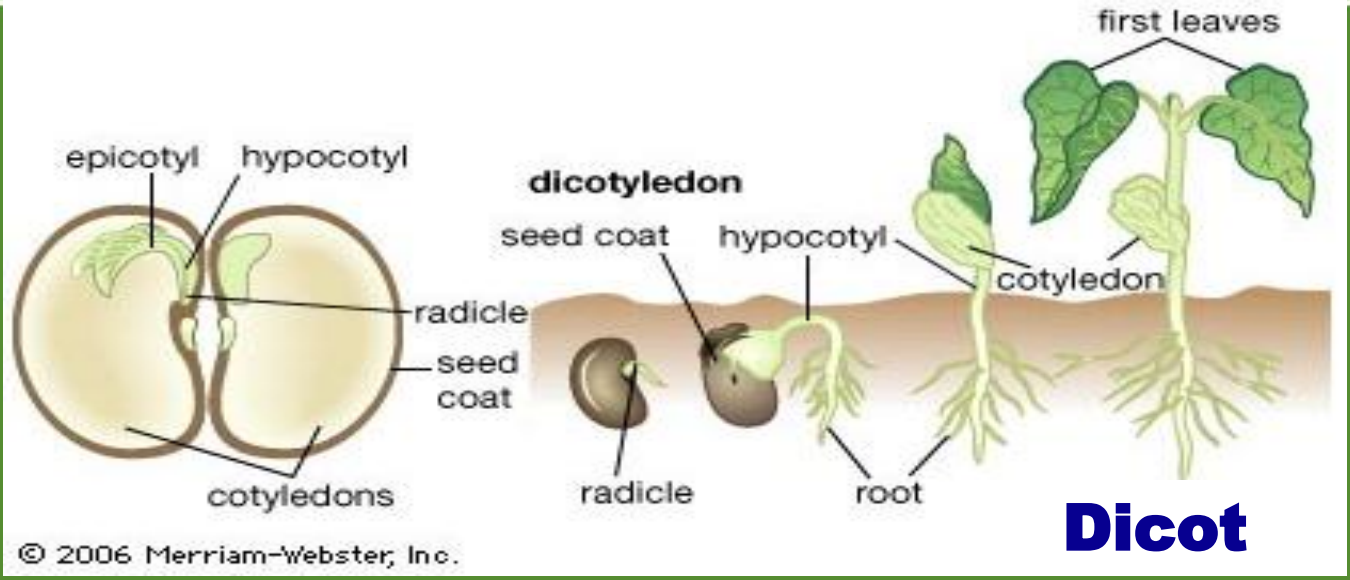
PLANT TISSUE CULTURE

Requirements

1. Explant – d. Whole plant (embryo)



Monocot



Dicot

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PLANT TISSUE CULTURE

Requirements

2. Culture medium

1. MS
2. B5
3. BP

1. Essential elements (macro & micronutrients
2. Organic supplements (vitamins, amino acids, yeast extract
3. Carbon source (sucrose)

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Murashige and Skoog (MS) Medium (1962)

Dr. Toshio Murashige, developer of the MS medium, Ph.D. adviser of National Scientist Dr. Ramon C. Barba, moved to University of California Riverside (UCR) from University of Hawaii. He is now Professor Emeritus at UCR.

PLANT TISSUE CULTURE

Requirements

2. Culture medium

Others

1. Gelling agent (agar, gelrite, etc)
2. Plant growth regulators
   - a. auxins
   - b. cytokinins
   - c. gibberellins
   - d. abscisic acid
   - e. ethylene

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PLANT TISSUE CULTURE

Requirements
2. Culture medium

Plant growth regulators:

a. Auxins (IAA, IBA, 2,4-D, NAA, Dicamba, Picloram)
b. Cytokinins (zeatin, BAP, 2iP, kinetin, Thidiazuron)
c. Gibberellins (GA_3)
d. Abscisic acid (ABA)
e. Ethylene

PLANT TISSUE CULTURE MEDIUM

BA 1 NAA 0	BA 1 NAA 0.5
BA 0 NAA 0	BA 0 NAA 0.05

Requirements
2. Culture medium

shooty

callus

control

rooty

Auxin (NAA)- Cytokinin (BA) interaction

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PLANT TISSUE CULTURE

3. Culture environment

a. temperature
b. pH
c. light (quality & duration)
d. gaseous environment
e. Osmotic pressure

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II. APPLICATIONS OF PLANT TISSUE CULTURE

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APPLICATIONS OF PLANT TISSUE CULTURE

1. Micropropagation
2. Tool in genetic transformation
3. Generation of variability through:
   a. Somatic hybridization
   b. Somaclonal variation
4. Embryo rescue/ culture

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APPLICATIONS OF PLANT TISSUE CULTURE

5. Haploid production
6. Production of virus-free plants
7. Production of pharmaceuticals
8. Germplasm conservation & exchange

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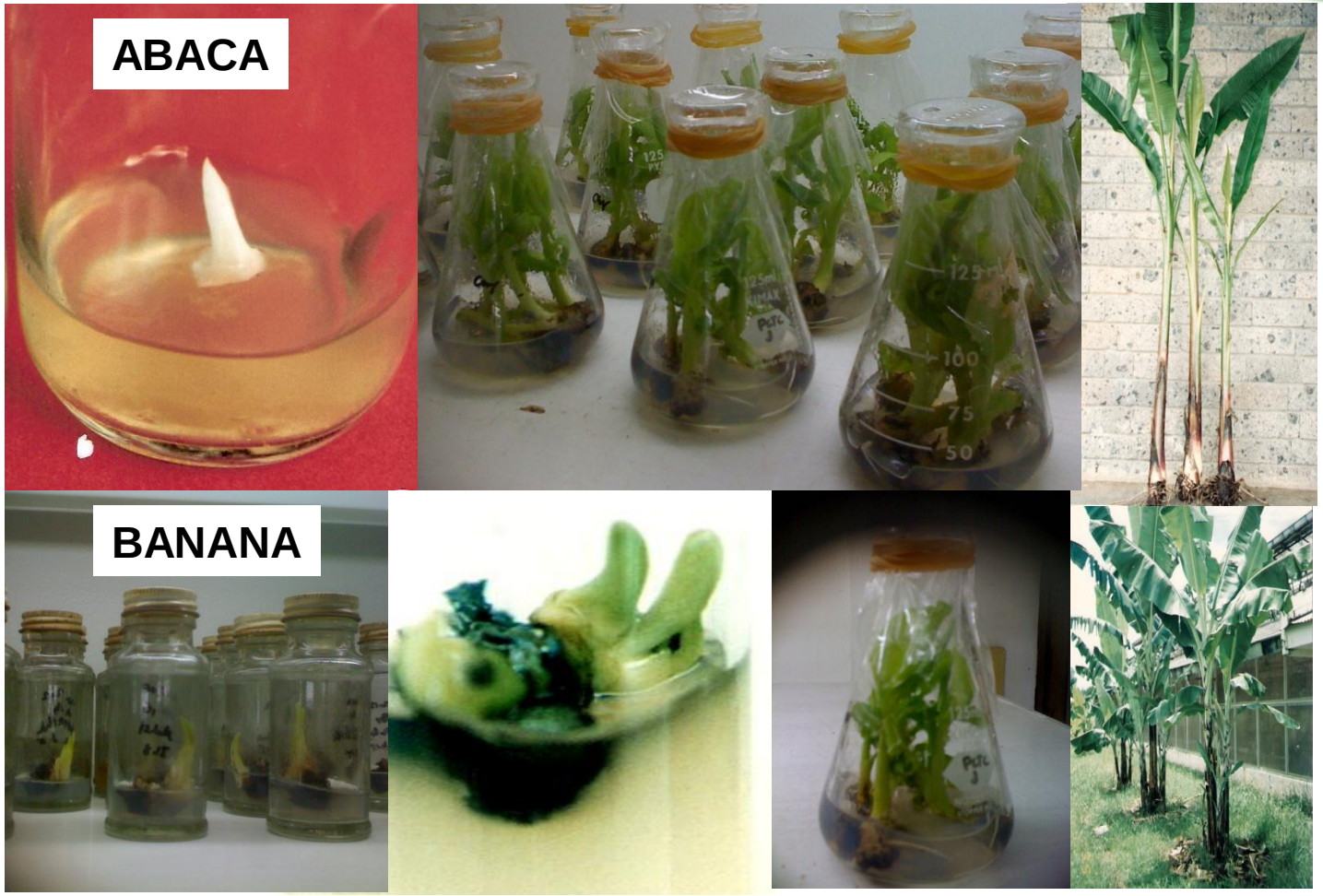
APPLICATIONS OF PLANT TISSUE CULTURE

1. Micropropagation

APPLICATIONS OF PLANT TISSUE CULTURE

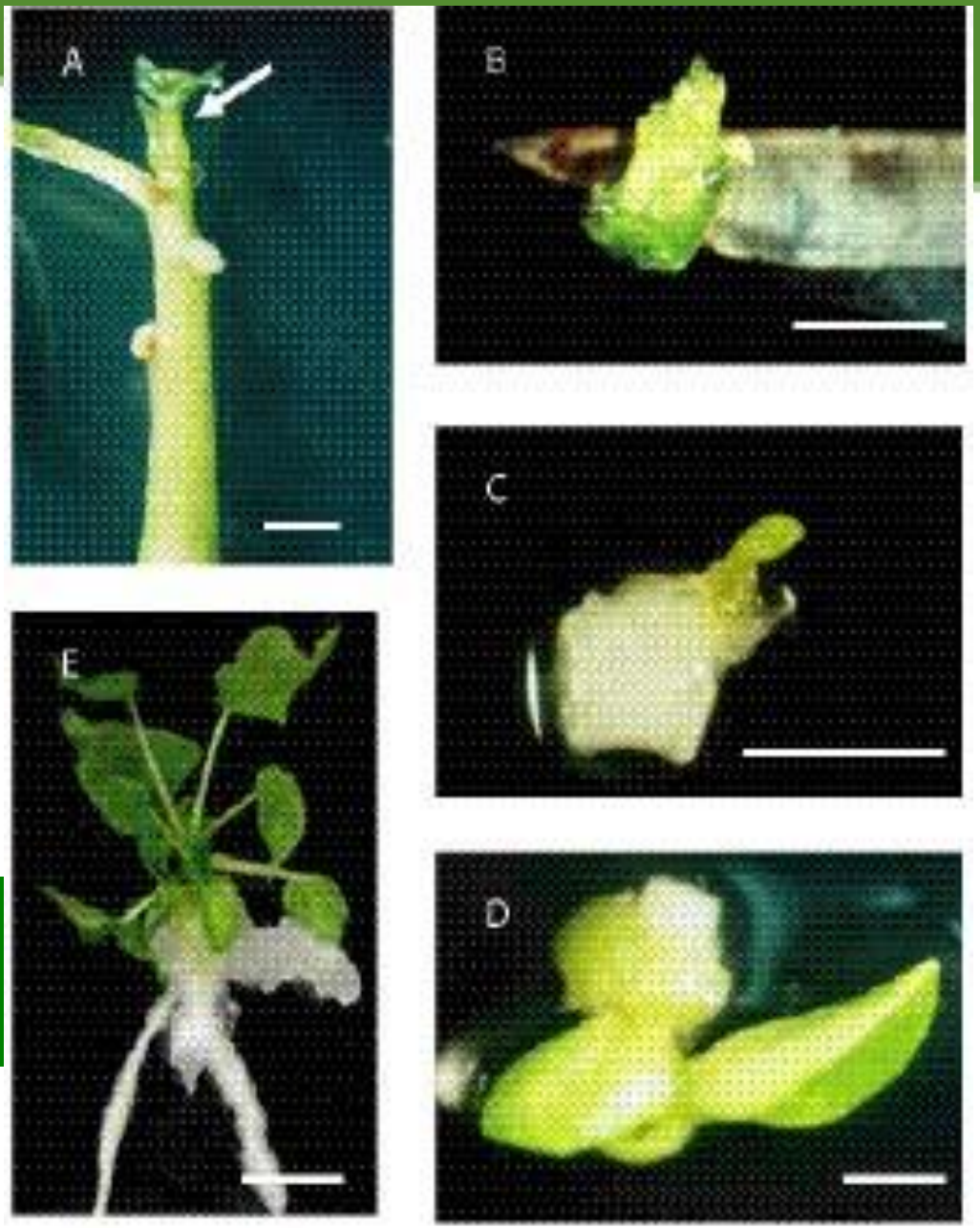
1. Micropropagation



APPLICATIONS OF PLANT TISSUE CULTURE

2. Tool in genetic transformation

Delayed ripening in papaya



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APPLICATIONS OF PLANT TISSUE CULTURE

2. Tool in genetic transformation

Bt Corn and Bt Talong

Bacillus thuringensis

Golden Rice

3. Generation of variability through:

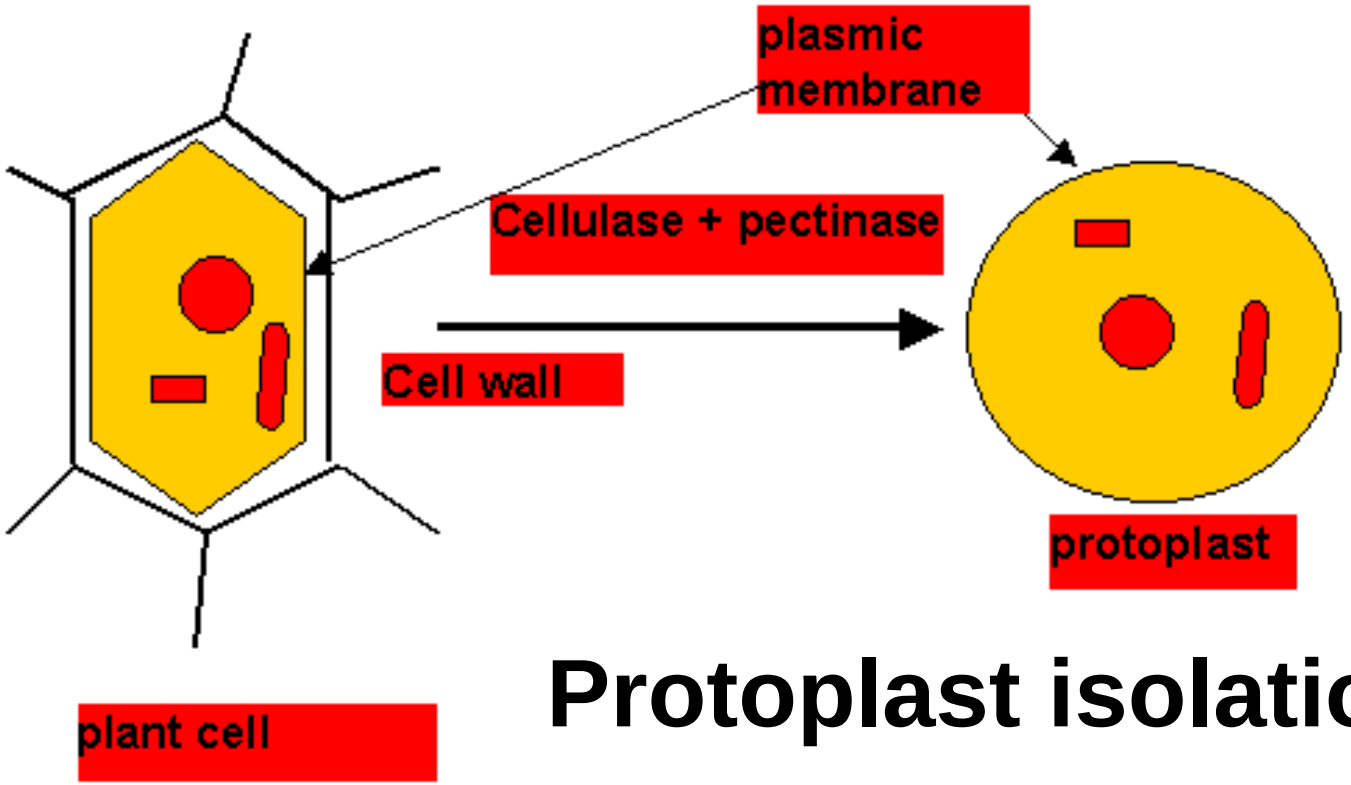
a. Somatic hybridization

- **development of hybrid plants through the fusion of protoplasts of somatic cells of two different plant species/ varieties**

- **somatic cells = cells associated with structure or functions other than reproduction**

3. Generation of variability through:
a. Somatic hybridization

When a plant cell loses its cell wall, it takes a spherical shape from a polygonal one resulting in the loss of the very rigid cell wall.



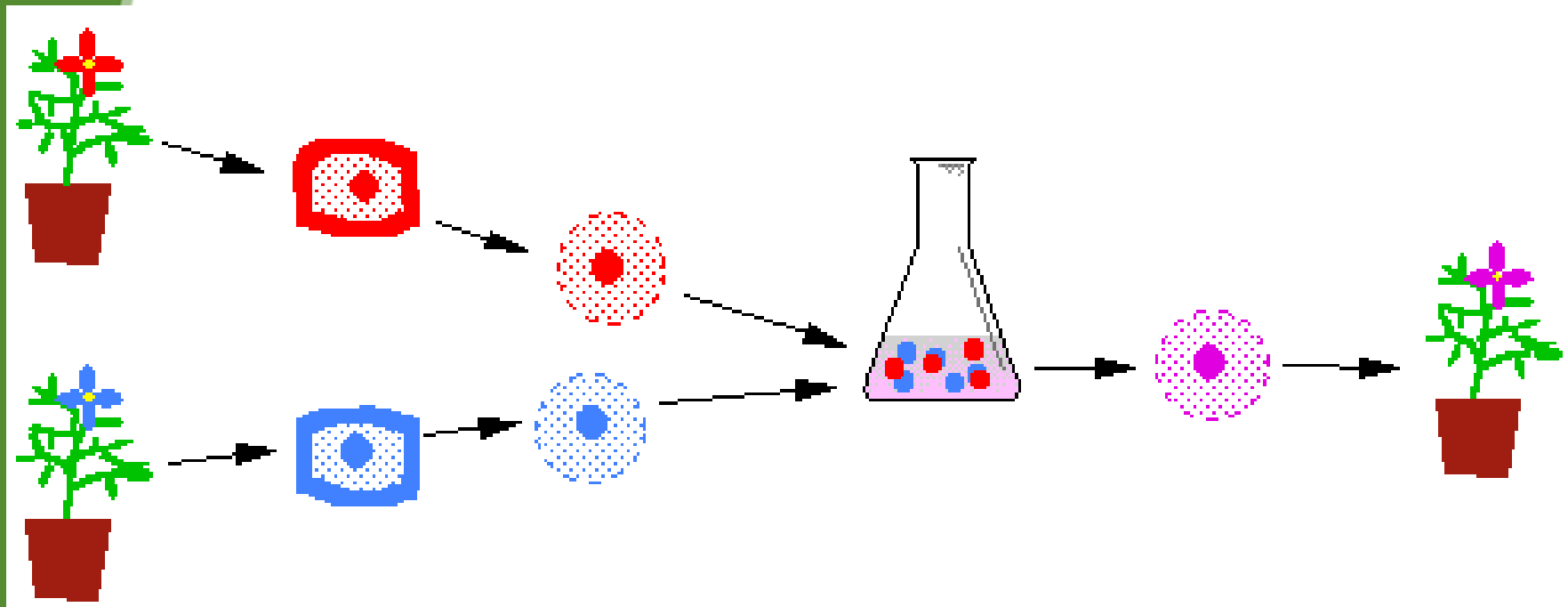
Protoplast isolation

3. Generation of variability through:
a. Somatic hybridization

Fused protoplast (left) with chloroplasts (from a leaf cell) and coloured vacuole (from a petal).

Protoplast or somatic fusion

To create new genotypes of plants



Somatic cell hybridization

3. Generation of variability through: b. Somaclonal variation

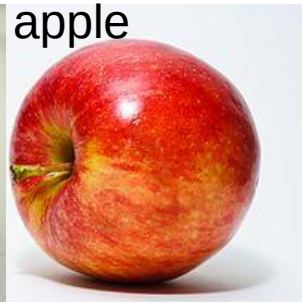
Somaclonal variations can be selected for:

- ✓ **disease resistance,**
- ✓ **improvement of nutritional quality,**
- ✓ **adaptation of plants to stress conditions,**
- ✓ **resistance to herbicides etc.**

3. Generation of variability through:
b. Somaclonal variation

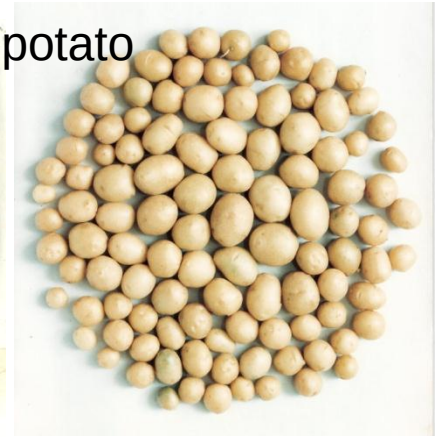
Somaclonal variation has been observed in plants such as:

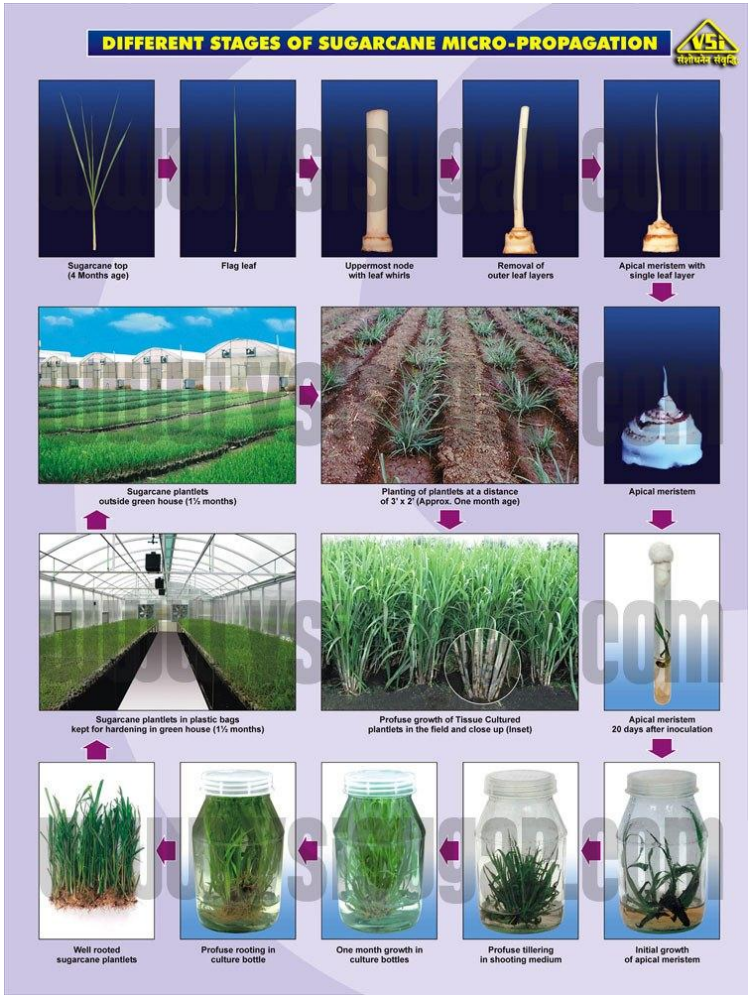
apple



tomato

potato





sugarcane

OTHER APPLICATIONS OF PLANT TISSUE CULTURE: 4. EMBRYO RESCUE

Embryo Rescue

- culture of embryos resulting from cross-pollination of distantly related species
- embryos are cultured to provide nutrients for their growth, or else they will die

PAPAYA EMBRYO RESCUE

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OTHER APPLICATIONS OF PLANT TISSUE CULTURE: 4. EMBRYO CULTURE

MAKAPUNO COCONUT TISSUE CULTURE

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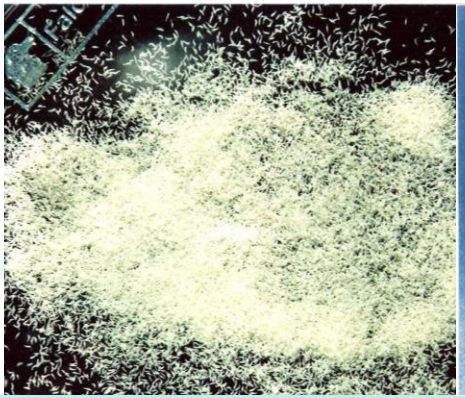
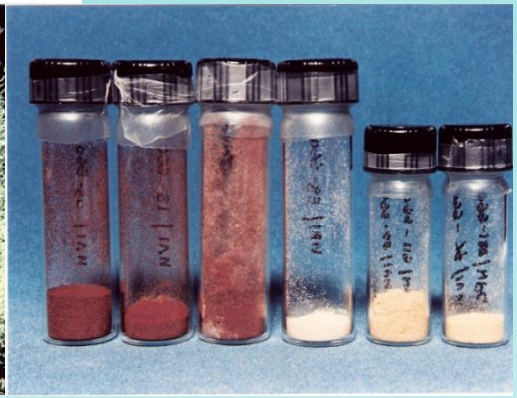
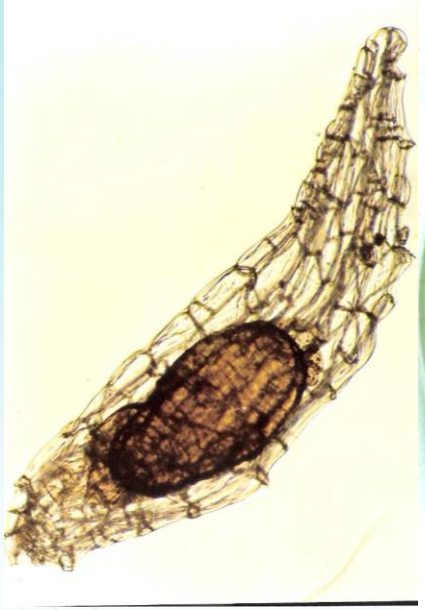
OTHER APPLICATIONS OF PLANT TISSUE CULTURE: 4. EMBRYO CULTURE

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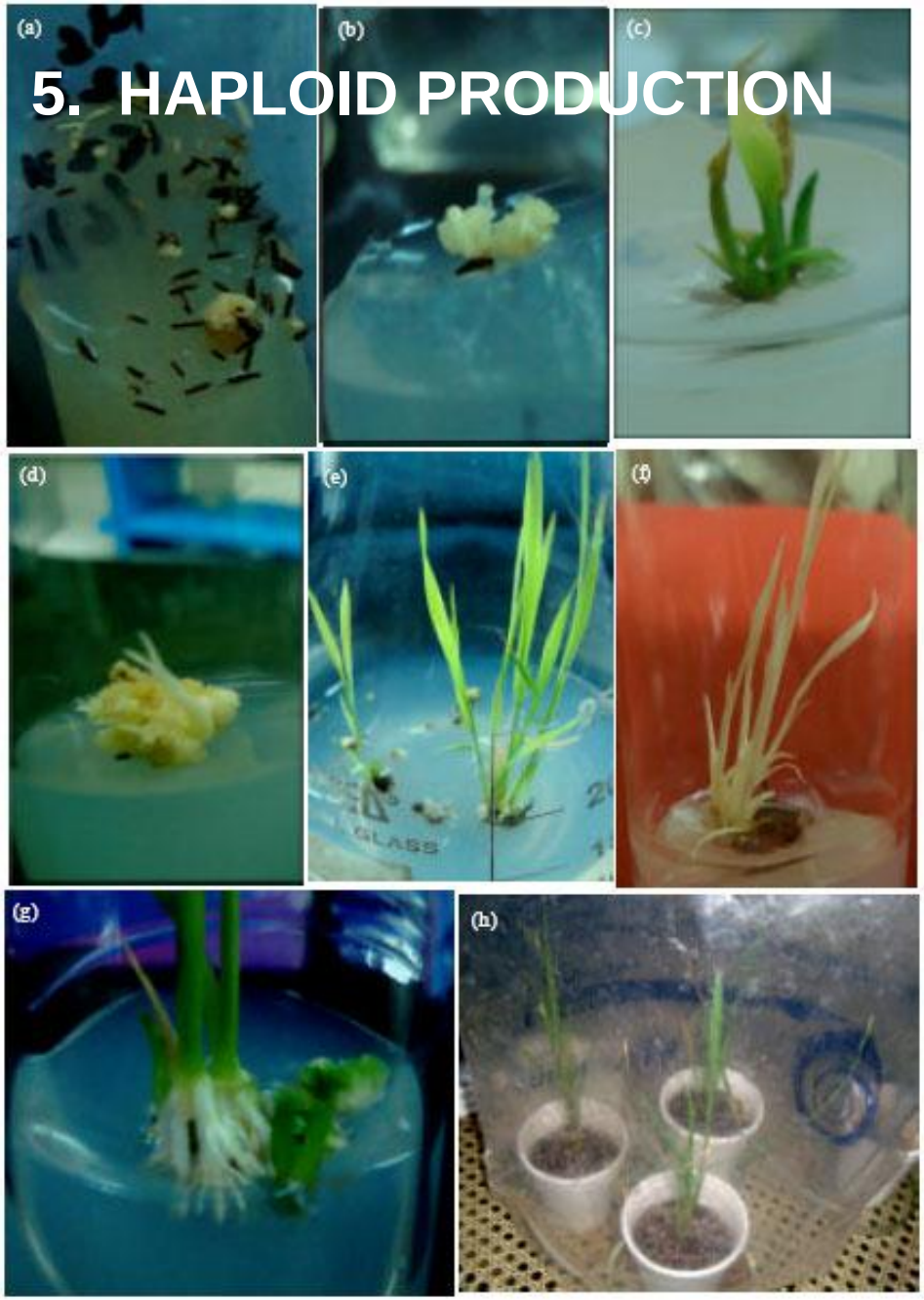
OTHER APPLICATIONS OF PLANT TISSUE CULTURE:

5. HAPLOID PRODUCTION

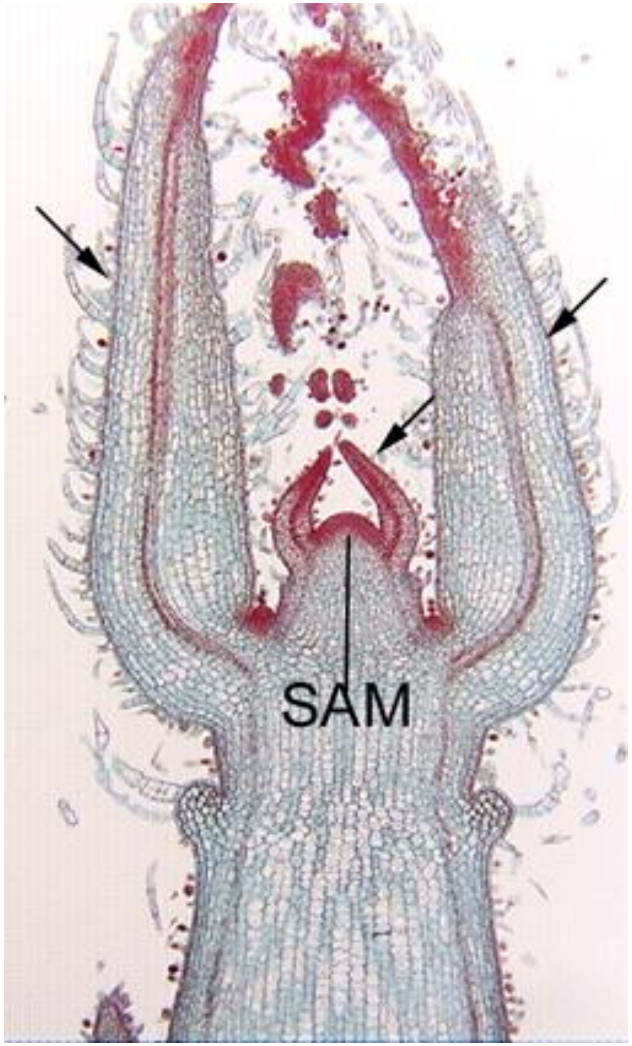
Anther Culture Response in Boro Rice Hybrids (2011)

Chaitali Sen & R.P. Singh

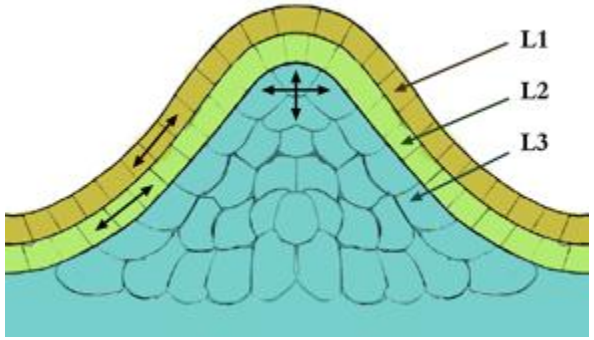
(a) Callus induction,
(b) Callus proliferation,
(c) Initiation of green shoots
(d) Initiation of albino shoots,
(e) Elongation of green shoots,
(f) Elongation of albino shoots,
(g) Initiation of roots,
(h) Hardening and acclimitazation of green plants



6. Production of virus-free plants



Shoot Apical Meristem (SAM)

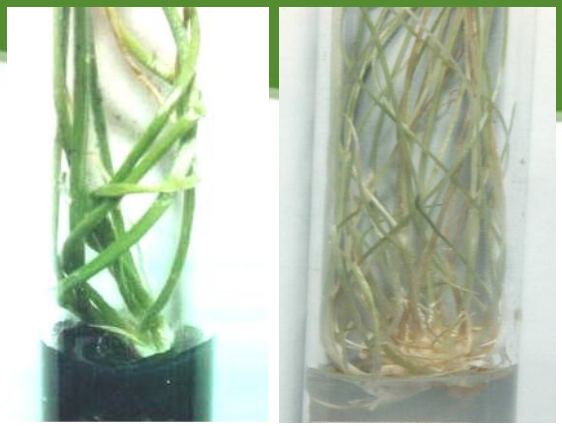


Tunica-Corpus model of the shoot apical meristem (growing tip). The epidermal (L1) and subepidermal (L2) layers form the outer layers called the tunica[]. The inner L3 layer is called the corpus. Cells in the L1 and L2 layers divide in a sideways fashion, which keeps these layers distinct, whereas the L3 layer divides in a more random fashion.

Excision of SAM using a microscope

Virus-free Garlic

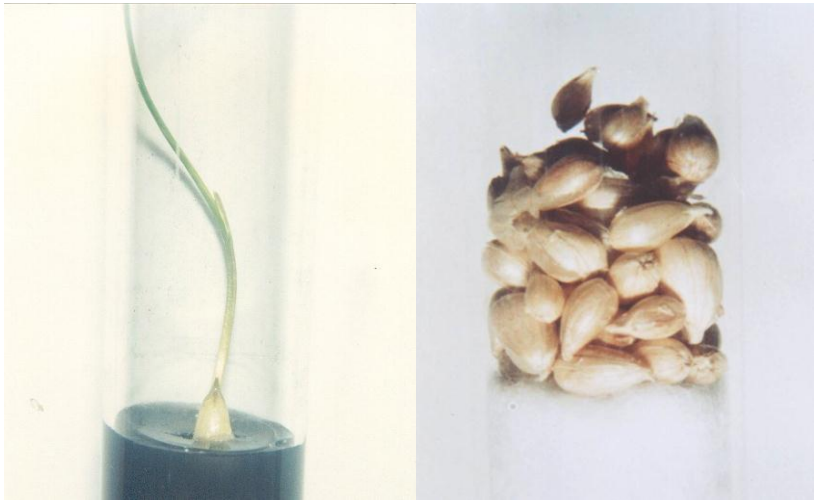
1
Garlic bulbs
Cloves separated
Washed
Pre-treatment (ref or RT)



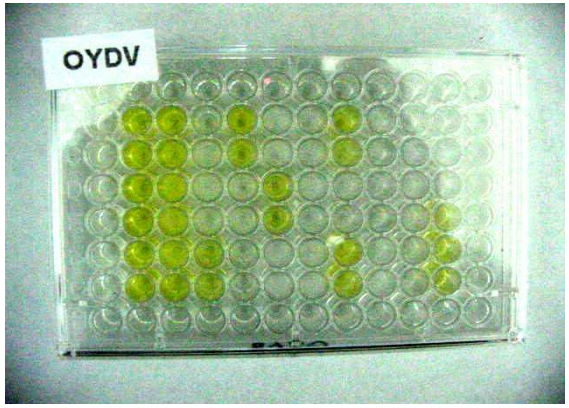
5 Multiple shoots
index

2
Garlic bulbs under thermotherapy
50°C (≤1 hr)

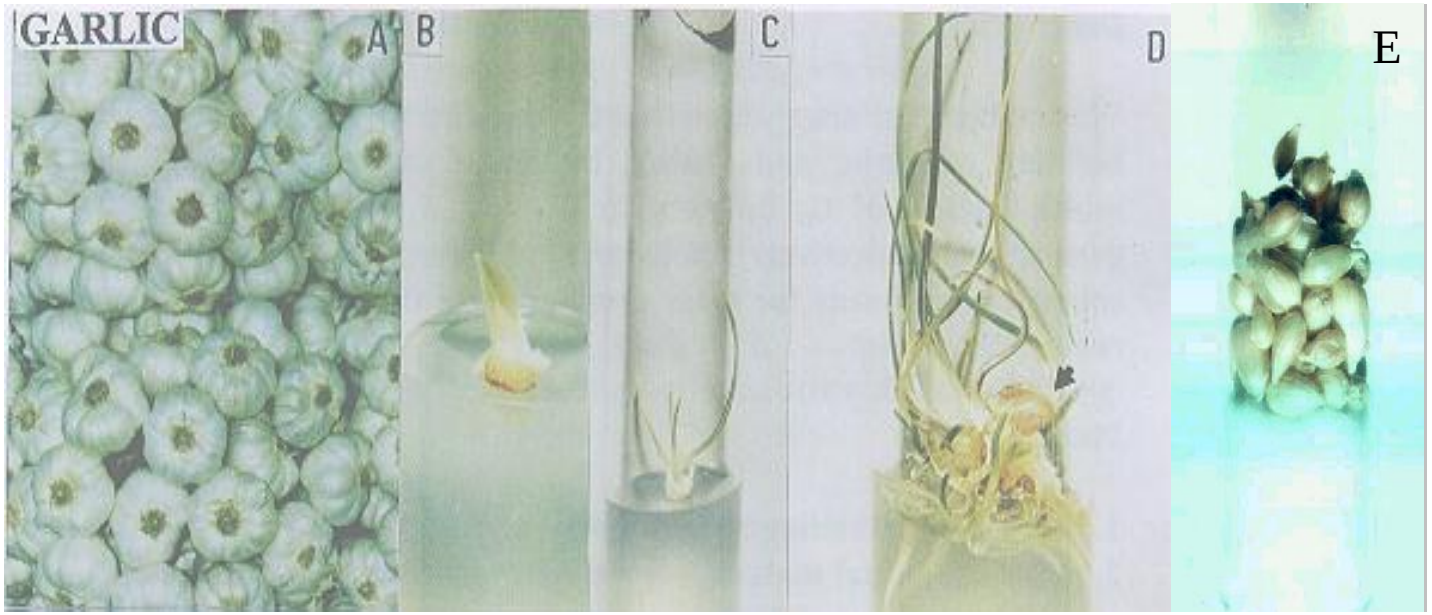
Shoot tip (left); meristem (right) culture



6 Bulblets (G_o) with shoot (left); dormant (right)



ELISA Color Reaction:
None – negative, Yellow - positive



Virus-free certification using ELISA

MASS PROPAGATION OF VIRUS-FREE AND TRUE-TO-TYPE SHALLOTS

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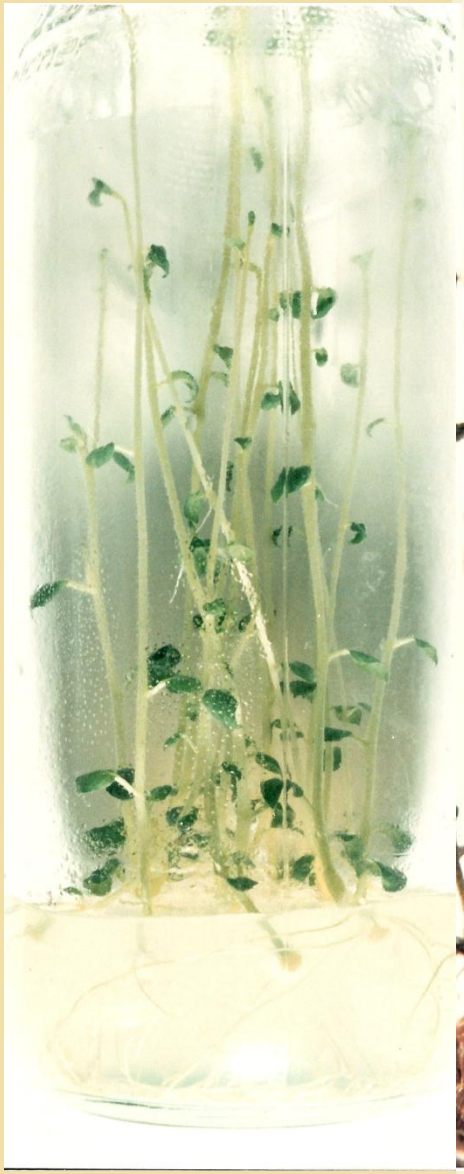

MASS PROPAGATION OF VIRUS-FREE AND TRUE-TO-TYPE WHITE POTATO (IPB, MADC)

Patena 2016

ABACA

BANANA

RAPID PROPAGATION OF VIRUS-FREE AND TRUE-TO-TYPE ABACA AND BANANA BY TISSUE CULTURE, SEROLOGY AND MARKERS*

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7. Production of pharmaceuticals

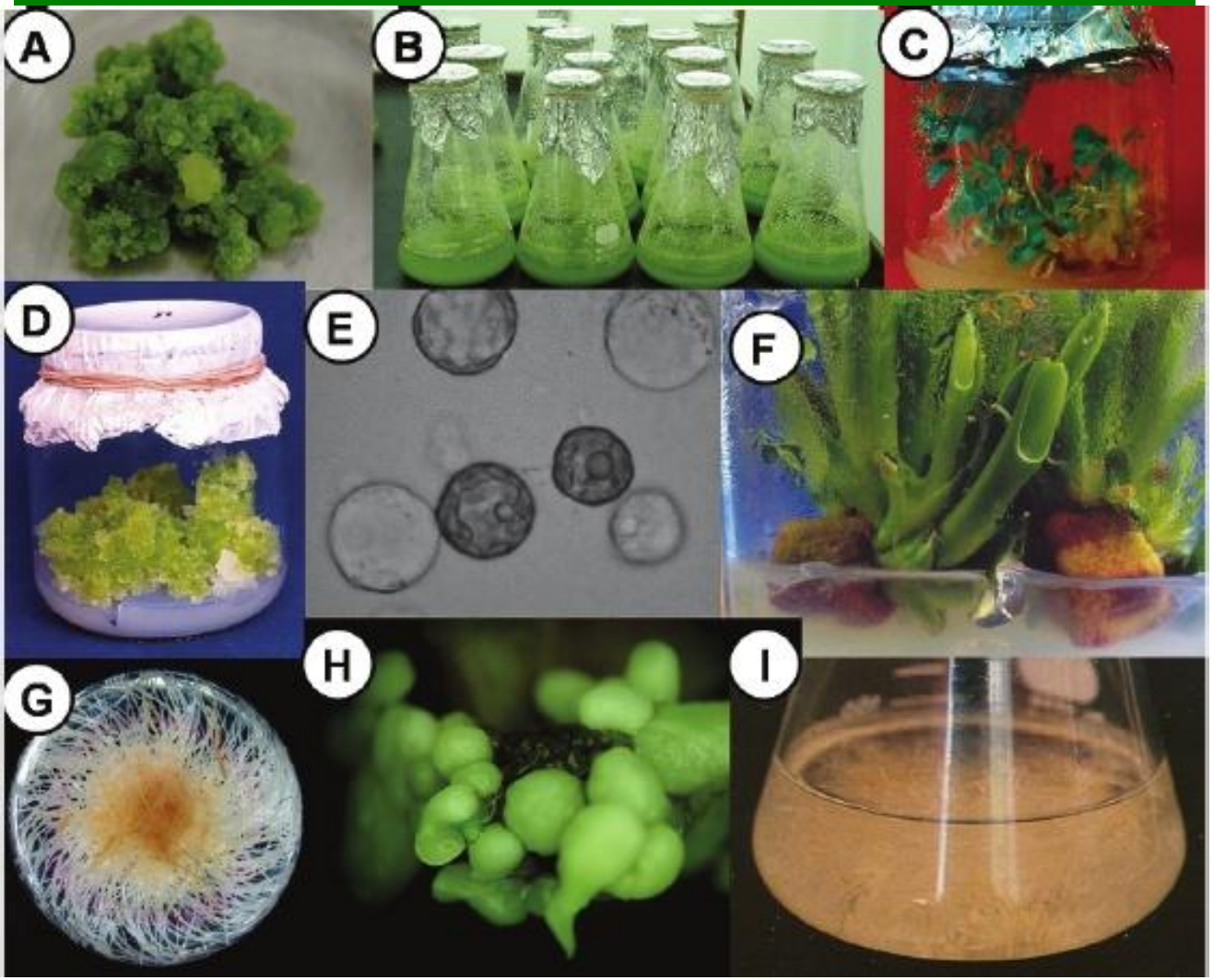
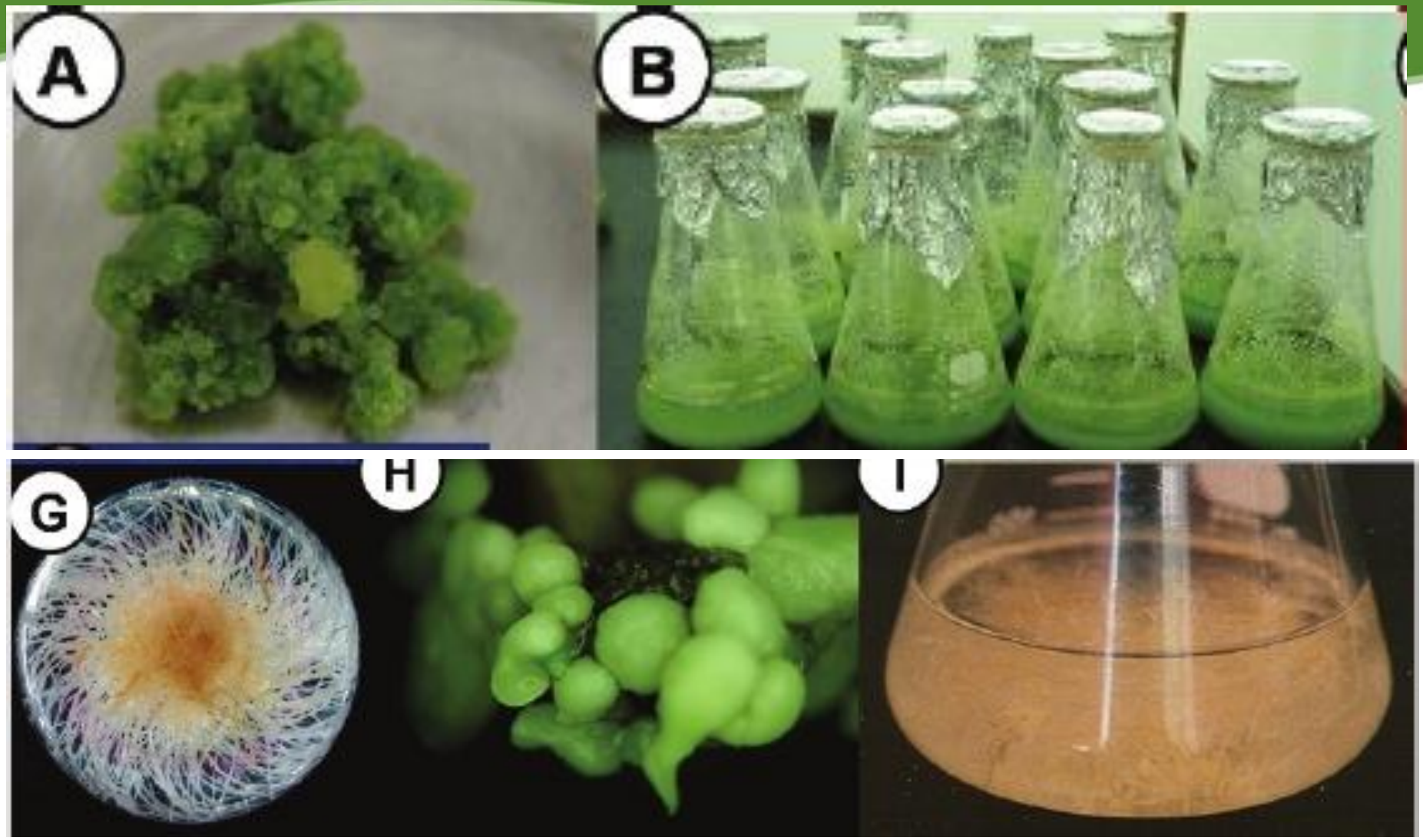


Fig. 50.1. A. Callus from Catharanthus roseus. B. Suspension culture from C. roseus . C. Regeneration of plantlets from C. roseus callus. D. Tumors from C. roseus . E. Protoplasts from C. roseus . F. Micropropagation of Agave tequilana . G. Hairy roots from C. roseus. H. Somatic embryogenesis of Coffea canephora. I. Root culture from C. roseus. Pictures A–E, G–I from the authors' laboratories. Picture F from the labora-tory of Dr. Manuel Robert all of them at Centro de Investigación Cientifica de Yucatán

7. Production of pharmaceuticals



OTHER APPLICATIONS OF PLANT TISSUE CULTURE: 8. GERMPLASM CONSERVATION AND EXCHANGE

8.1. Germplasm conservation

- **8.1.1. Slow growth**
- **8.1.2. Cryopreseravtion**

8.2. Germplasm exchange

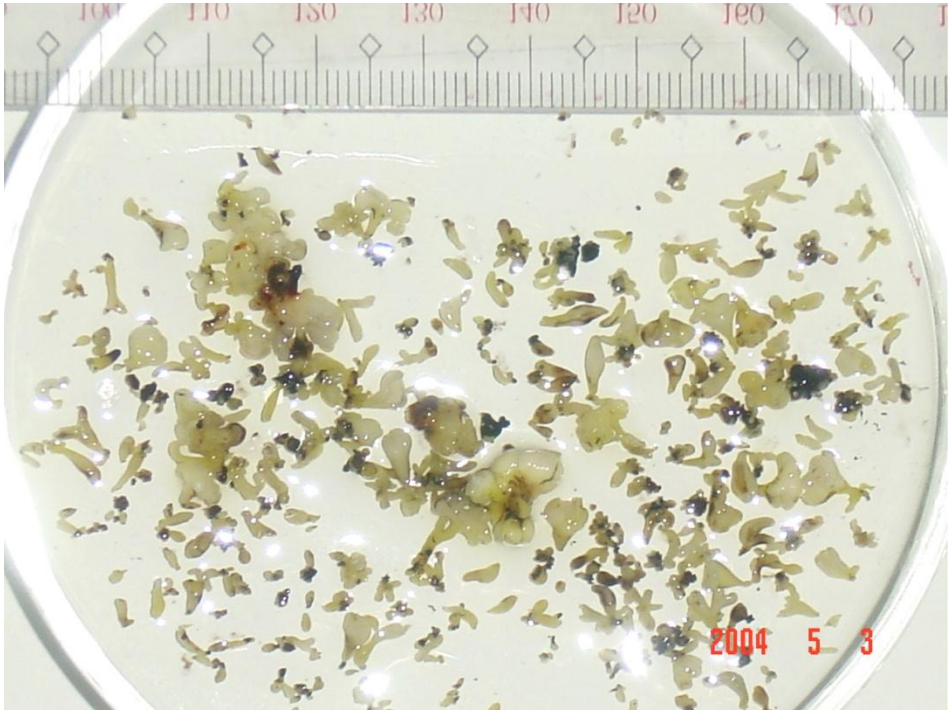
- **8.2.1. Land, air, sea**
- **8.2.2. Requirements**
- **8.2.3. Packaging**

OTHER APPLICATIONS OF PLANT TC:
8.1. GERMPLASM CONSERVATION

8.1.1. Slow growth

Sweet potato

Cassava



Somatic embryos from nucellar tissue explants of mango

OTHER APPLICATIONS OF PLANT TC:
8.1. GERMPLASM CONSERVATION

8.1.1. Slow growth

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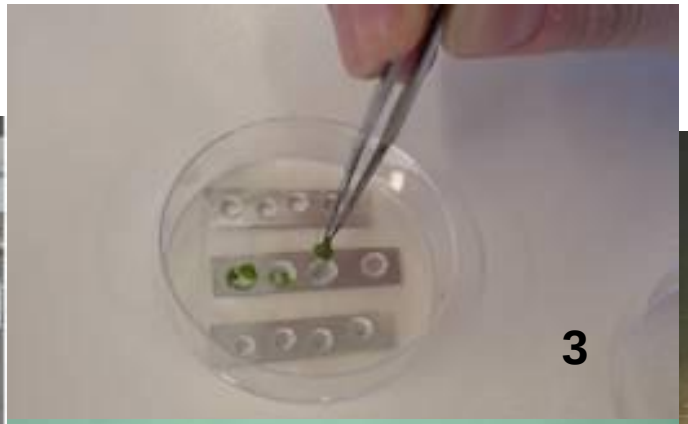
Cryopreservation

- preservation in liquid nitrogen (-196°C) of plant germplasm
- vegetative cells in state of absolute quiescence (all physical & biochemical reactions are put on hold)

Cryopreservation

1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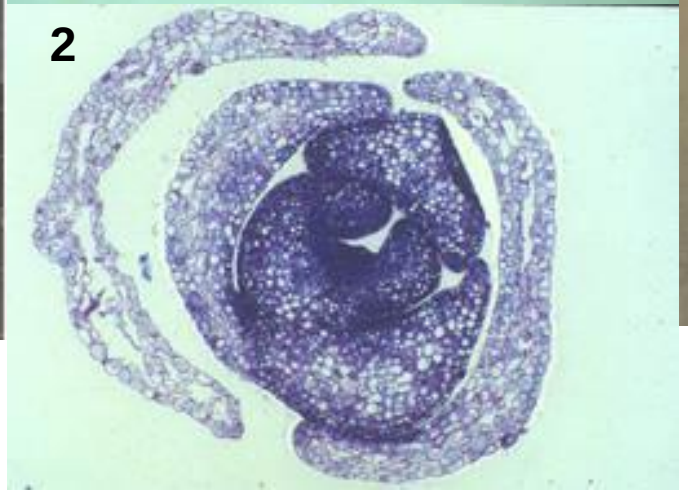
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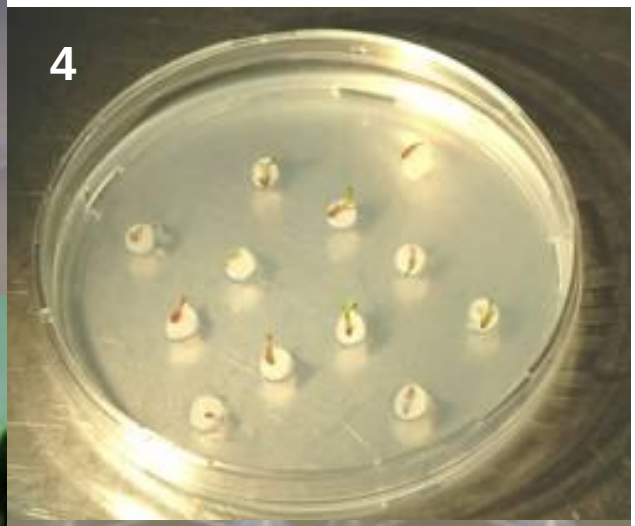
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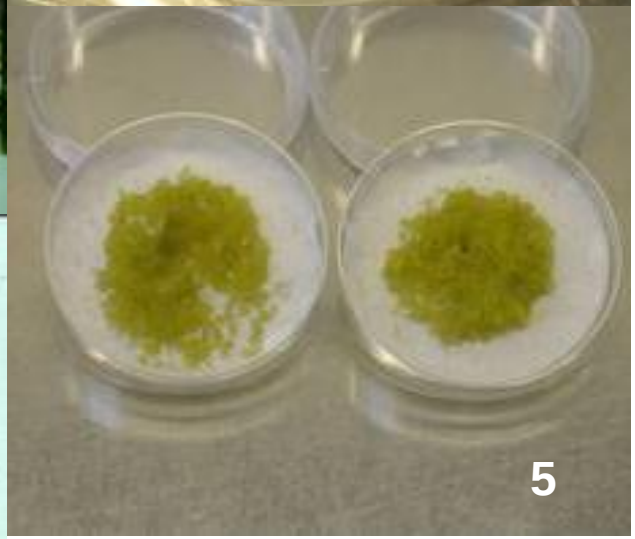
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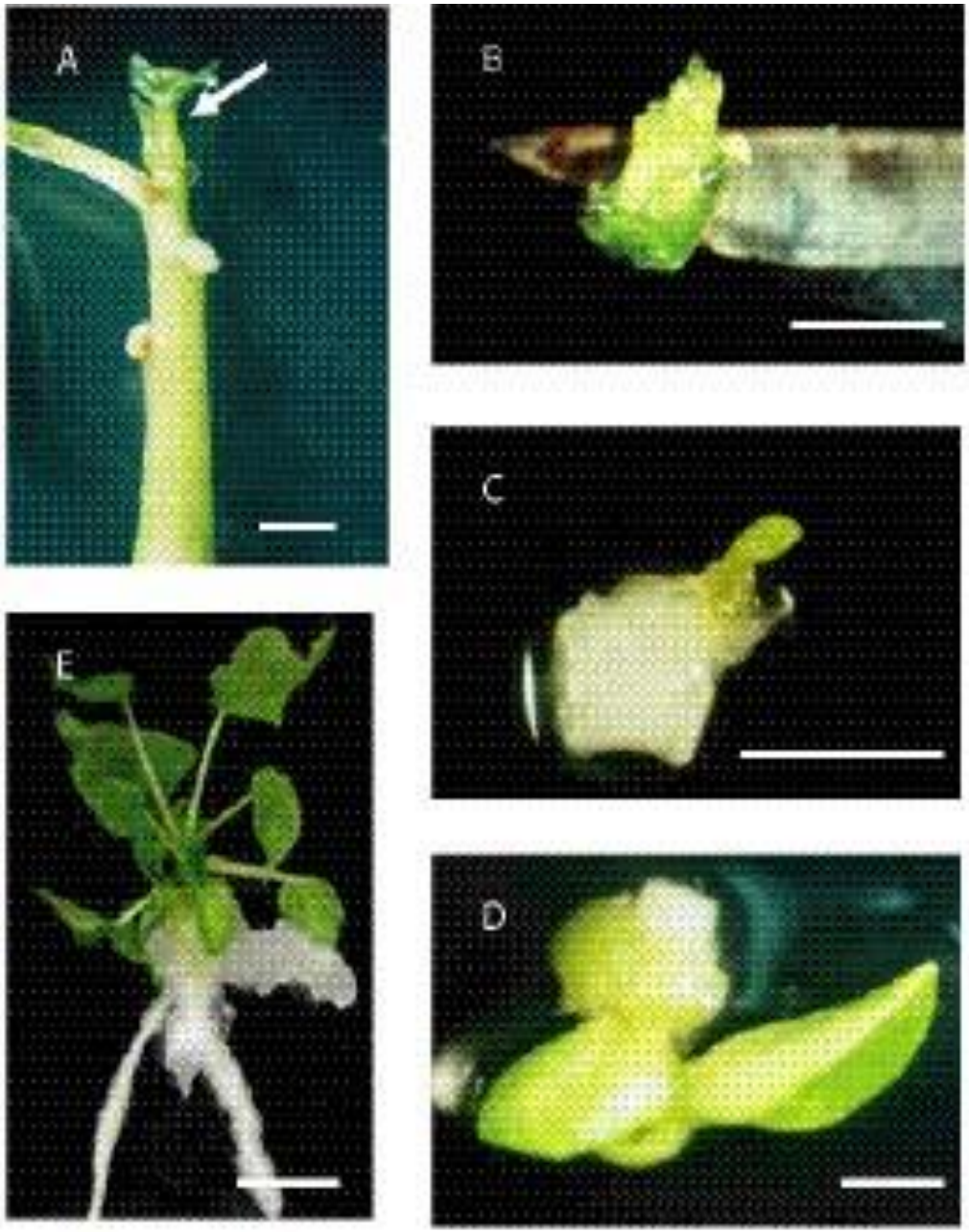
Growth of cryo-preserved papaya shoot tips



Yuan-long WANG, Ming-jen FAN, And Song-iuan LIAW (2004)

OTHER APPLICATIONS OF PLANT TC:

8.2. GERMPLASM EXCHANGE

8.2.1. Cultures in bottles transported via land, air, sea

Land – using cars, trucks, etc

Air - using airplane,

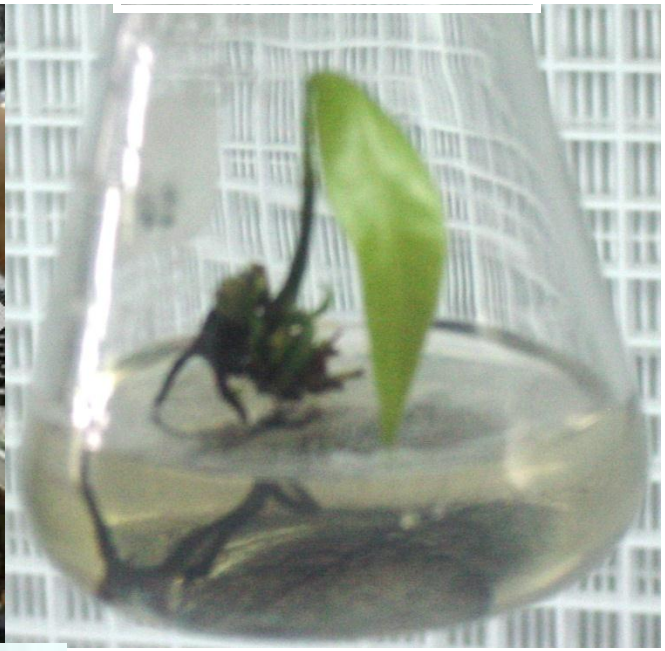
Sea – using boast, ships

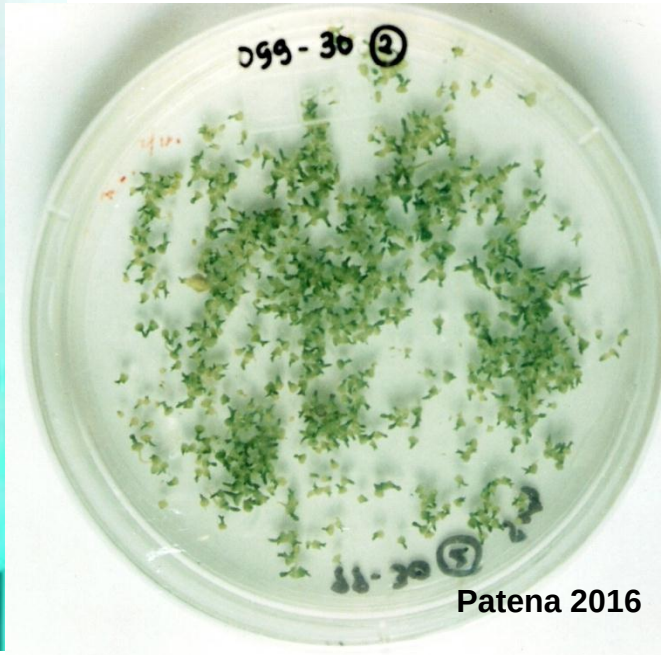
Phytosanitary certificate

- an official document issued by the plant protection organization of the exporting country to the plant protection organization of the importing country
- certifies that the plants or plant products covered by the certificate have been inspected according to appropriate procedures and are considered to be free from quarantine pests and practically free from other injurious pests, and that they are considered to conform with the current phytosanitary regulations of the importing country

- facilitates trade but it is not a trade document.

8.2.3. Packaging





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OTHER APPLICATIONS OF PLANT TC:
8.2. GERMPLASM EXCHANGE

8.2.3. Packaging

Paper

Plastic

- Tightly packed
- Proper label (fragile, this side up)
- Secured with tape or tie
- Correct adresses (from and to)

OTHER APPLICATIONS OF PLANT TC:
8.2. GERMPLASM EXCHANGE

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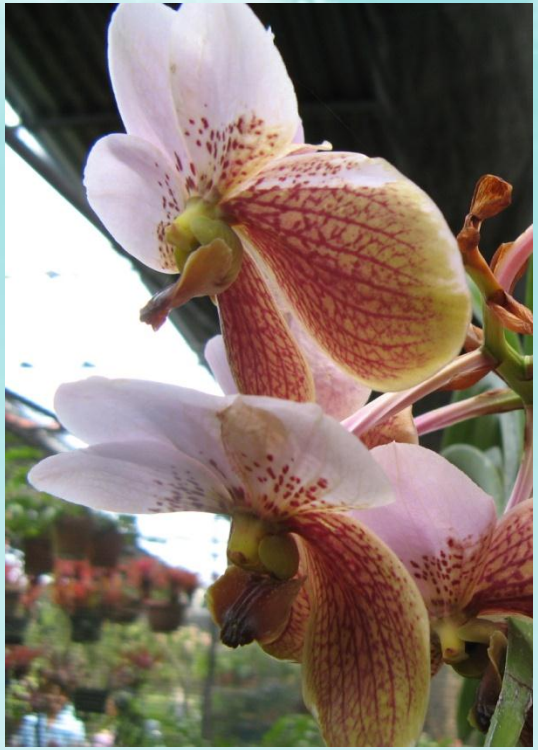

Thank You

“Whoever is careless with the truth in small matters cannot be trusted with important matters”

— Albert Einstein —