PLANT MICROPROPAGATION

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MICROPROPAGATION

Rapid clonal in vitro propagation of plants from cells, tissues or organs cultured aseptically on defined media contained in culture vessels maintained under controlled conditions of light and temperature

MICROPROPAGATION

***In vitro* propagation**

Tissue culture propagation

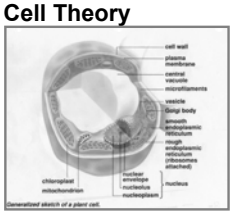
MICROPROPAGATION	MACROPROPAGATION
Small propagule	Larger propagule
Aseptic conditions	Non-aseptic conditions
Controlled environment	Less environmental control
Heterotrophic growth	Photoautotrophic growth
Rapid multiplication	Slower multiplication
Greater initial costs	Nominal costs

In Vitro Culture: Historical Perspective

How did it all begin?

Historical Perspective

Schleiden 1838
Schwann 1839 → **Cell Theory**



Cell is the basic unit of life

Each living cell of a Multicellular organism should be capable of independent development if provided with the proper external conditions

Totipotency Concept **Plant Cell**

In Vitro Culture: Early Attempts

Haberlandt 1902

Innate potential of cells

Attempted culture of isolated leaf cells

Haberlandt

Formulated plant tissue culture principles

Culture Medium: mineral salts & glucose

Unsuccessful results

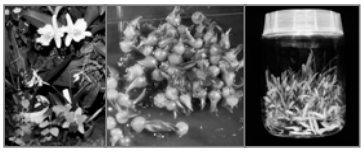
Eichhornia crassipes

***In Vitro* Culture: Early Attempts**

Knudson 1920s Asymbiotic orchid seed germination & culture

Concept of in vitro plant production

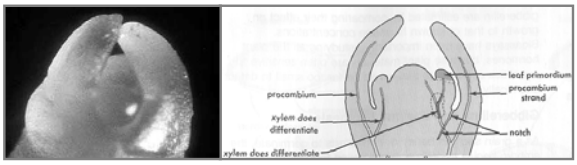
Knudson



Orchids Orchid Seedlings Seedling Culture

Toward Commercial Micropropagation 1950s

Morel & Martin 1952 Meristem-tip culture for disease elimination



Commercialization of Micropropagation 1960s

Morel	1960	Disease eradication
Wimber	1963	& in vitro production of orchids

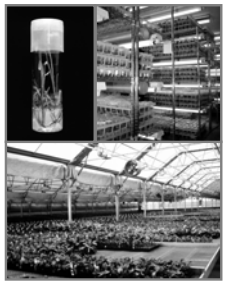
Commercialization of Micropropagation 1970s & 1980s

Murashige	1974	Broad commercial application

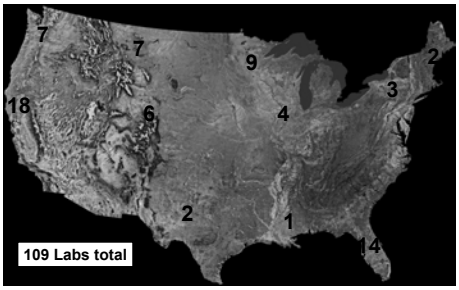
Dr. Toshio Murashige
University of California

Micropropagation: Advantages for Plant Production

- ✓ Rapid & efficient propagation
- ✓ Year-round production
- ✓ Precise crop production scheduling
- ✓ Reduce stock plant space
- ✓ Long-term germplasm storage
- ✓ Production of difficult-to-propagate species



Commercial Micropropagation Labs (2000)

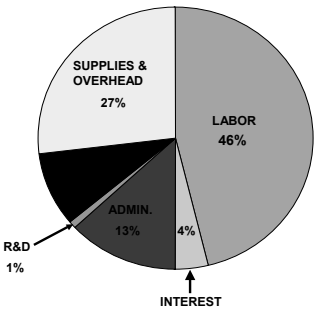


Micropropagation Production in the United States

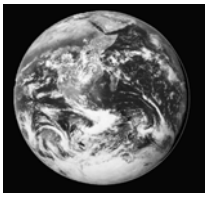
Foliage Plants	63,695,000
Greenhouse Flowers	11,297,000
Perennials	9,448,000
Trees & shrubs	15,294,000
Vegetables	12,862,000
Fruits	3,721,000
Miscellaneous	4,545,000
Total:	120,862,000

(Zimmerman, 1966)

USA Commercial Micropropagation Laboratory Costs



Commercial Micropropagation: A Global Industry



- Israel
- Japan
- India
- Malaysia
- Mexico
- Central America
- South America

Strive to reduce labor costs!

Bangkok Flower Center Thailand

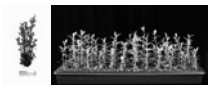
Oglesby Plants International, Inc.

1985
Lab built in Altha, FL
12,000,000 plants/yr

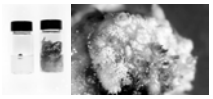
Oglesby Plants International, Inc.

Micropropagation Methods

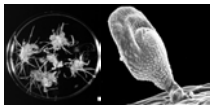
- Shoot culture



- Shoot organogenesis



- Non-zygotic embryogenesis

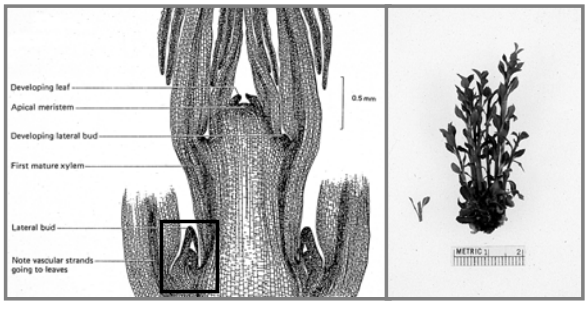


Shoot Culture

Method Overview

Clonal in vitro propagation by repeated enhanced formation of axillary shoots from shoot-tips or lateral meristems cultured on media supplemented with plant growth regulators, usually cytokinins. Shoots produced are either rooted first in vitro or rooted and acclimatized ex vitro

Shoot Culture



Cytokinin-enhanced outgrowth of lateral meristems

Shoot Culture

- ✓ Most widely used method for commercial micropropagation
- ✓ Relatively high genetic stability in the plants produced

Shoot Culture

ADVANTAGES

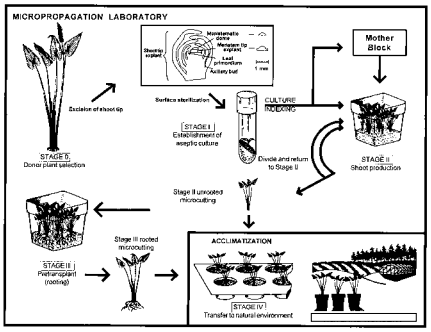
- ✓ Reliable rates and consistency of shoot multiplication
- ✓ 3 - 8 fold multiplication rate per month
- ✓ Pre-existing meristems are least susceptible to genetic changes

Micropropagation Stages

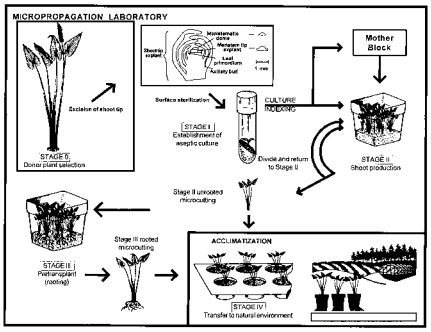
- Stage 0. Donor Plant Selection
- Stage I. Establishment Of Sterile Culture
- Stage II. Shoot Multiplication
- Stage III. Pretransplant (rooting)
- Stage IV. Transfer Natural Environment

Five stages to successfully produce plants via micropropagation

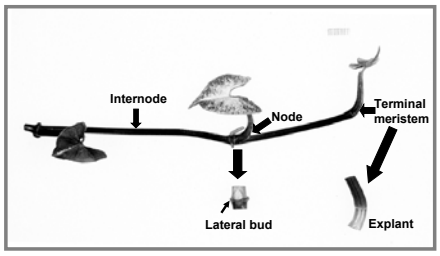
Shoot Culture: Micropropagation Stages



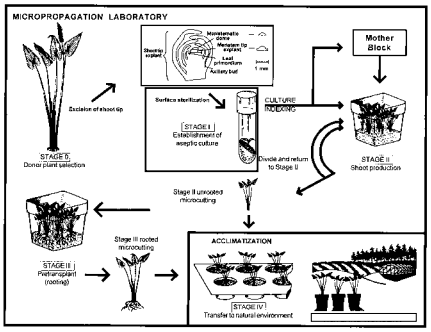
STAGE 0: Selection of Donor Plant



SHOOT CULTURE



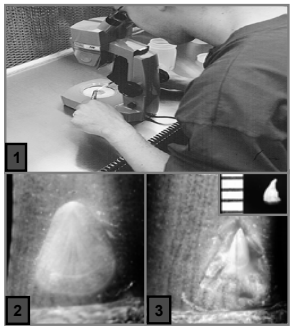
STAGE I: Establishment of Aseptic Culture



Surface Sterilization Procedure

Rinse ⟶ **Surface sterilize (bleach)** ⟶ **Agitate & rinse**

Explant Isolation



Meristem and Meristem-tip Culture

Techniques used specifically to produce *pathogen eradicated plants* not directly used for propagation

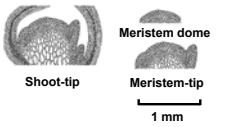
Meristem Culture

Culture of apical meristem dome

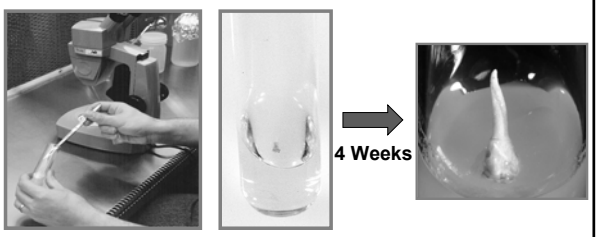
- **0.1 - 0.2 mm diameter**
- **0.2 mm in length**

Meristem-tip Culture

Culture of larger (0.2 - 0.5 mm long) meristem-tip explants



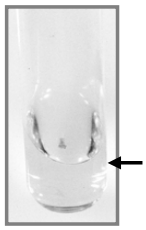
Stage I. Culture Initiation



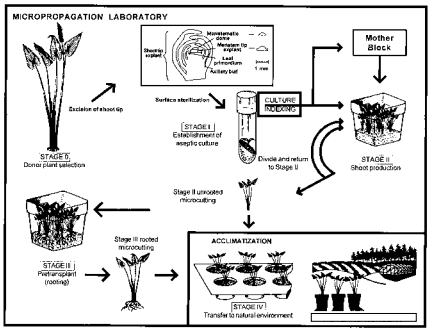
Inoculation

Stage I Culture Medium

Murashige & Skoog mineral salts
30 g/l sucrose
100 mg/l myo-inostiol
0.4 mg/l thiamine
cytokinin
auxin
agar or other gelling agent



STAGE I: Establishment of Aseptic Culture



Stage I Culture Contamination

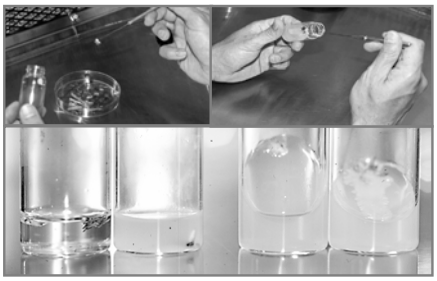
Bacterial **Fungal**

Stage I Culture Contamination

Many times what you "see" is not what you get!

Need to screen (index) for <u>cultivable</u> contaminants

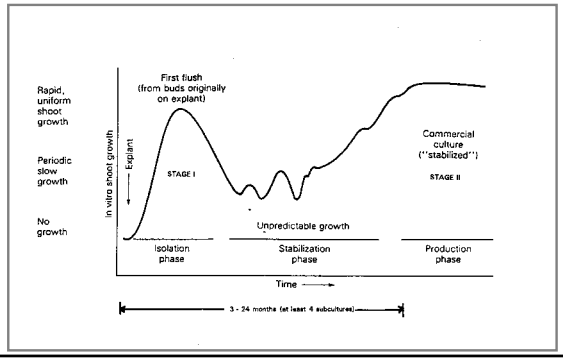
Stage I Culture Indexing



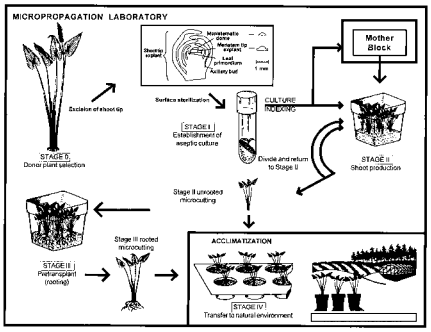
STAGE I: Establishment of Aseptic Culture

Sterile (indexed) Stage I. culture

STAGE I: Culture Stabilization



STAGE I: Establishment of Aseptic Culture



Mother Block Concept

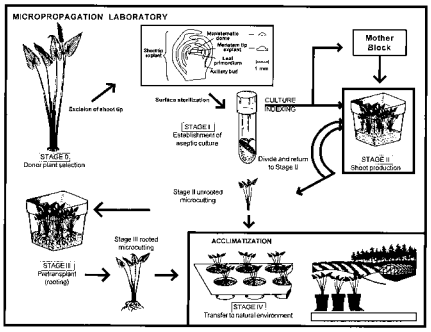
Mother Block:

A slowly multiplying indexed and stabilized set of cultures

Serve as source of cultures (explants) for Stage II multiplication

Mother Block Room

STAGE II: Shoot Production



STAGE II: Shoot Production

- ✓ Repeated enhanced axillary shoot production
- ✓ Presence of higher cytokinin level in medium to disrupt apical dominance
  - 2-isopentenyladenine (2-iP)
  - Benzyladenine (BA)
  - Kinetin
  - Thidiazuron (Dropp)

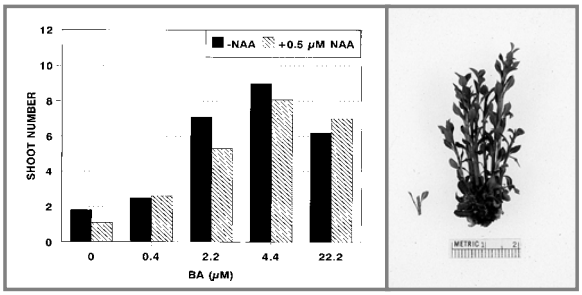
STAGE II: Shoot Production

- ✓ Stage II selection of cytokinin type and concentration determined by:
  - Shoot multiplication rate
  - Length of shoot produced
  - Frequency of genetic variability
  - Cytokinin effects on rooting and survival

STAGE II: Shoot Production

- ✓Auxin may be added to enhance shoot production/elongation (graph)
  - α - indole-3-acetic acid (IAA)
  - 1- naphthaleneacetic acid (NAA)
  - indolebutyric acid (IBA)

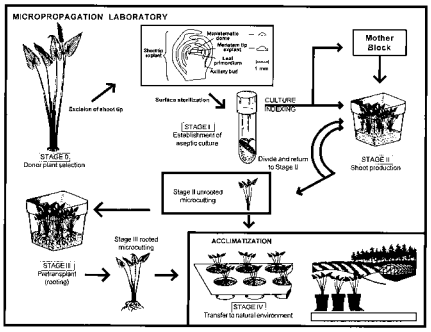
STAGE II: Shoot Production



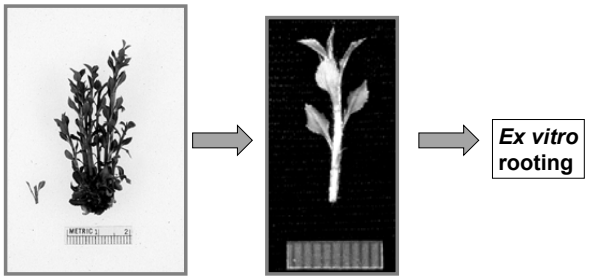
STAGE II: Shoot Production

- ✓ **Subculture shoot clusters at 4 - 5 week intervals**
- ✓ **3 - 8 fold increase in shoot numbers (4.3 x 10^7 shoots/explant/year)**
- ✓ **Number of subcultures possible is species/cultivar dependent:**

STAGE II. Shoot Microcuttings

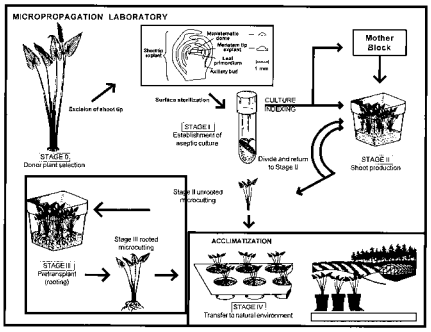


Stage II Microcuttings



Stage II Microcutting

STAGE III: Pretransplant (rooting)



STAGE III: Pretransplant (rooting)

Goals:

- ✓ **Preparation of Stage II shoots/shoot clusters for transfer to soil (prehardening)**
- ✓ **Elongation of shoots prior to *ex vitro* rooting**
- ✓ **Fulfilling dormancy requirements of storage organs**

STAGE III: Pretransplant (rooting)

Goals:

- **Adventitious rooting of individual shoots or clusters *in vitro***
  - ⊠**Stage III rooting usually not desirable**

STAGE III: Pretransplant (rooting)

- ✓ **Adventitious rooting induced in the presence of an auxin**
  - ♦ **α-indole-3-acetic acid (IAA)**
  - ♦ **1- naphthaleneacetic acid (NAA)**
  - ♦ **indolebutyric acid (IBA)**

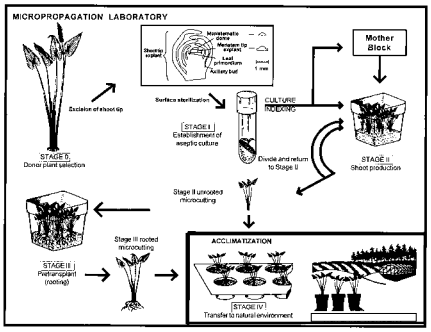
STAGE III Rooting

0 | 0.05 | 0.1 | 0.5 | 1.0

IBA (mg/L)

DAY 28

STAGE IV: Transfer To Natural Environment



STAGE IV: Transfer to Natural Environment

Ultimate success of shoot culture depends on ability to acclimatize vigorously growing quality plants from *in vitro* to *ex vitro* conditions

High humidity & low light
In vitro

Lower humidity & high light
Ex vitro

STAGE IV: Transfer to Natural Environment

Acclimatization:

Process whereby plants physiologically and anatomically adjust from in vitro to ex vitro cultural and environmental conditions

- **Two reasons micropropagated plants may be difficult to acclimatize *ex vitro*:**
  - **Low photosynthetic competence (heterotrophic nutrition)**
  - **Poor control of water loss**

STAGE IV: Transfer to Natural Environment

Planting Stage III Rooted Microcuttings

Acclimatization Structures

Propagation Dome

Humidity Tent

Automatic Mist

Fog System

STAGE IV: Transfer to Natural Environment

Fully acclimatized plantlet