Plant Propagation PLS 3223/5222

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Principles of Tissue Culture and Micropropagation andTechniques for Micropropagation

Chapters 17 & 18
S. Wilson

Chapter 17 Objectives are to Understand:

1. The history of micropropagation
2. Developmental stages in micropropagation
3. Somatic embryogenesis and synthetic seed production
4. The types of tissue culture systems
5. Variation in micropropagated plants
6. The tissue culture environment

Chapter 18 Objectives are to Understand:

1. The advantages and disadvantages of micropropagation
2. General tissue culture laboratory facilities
3. Developmental stages in micropropagation
4. Procedures used for micropropagation
5. Components of the micropropagation medium

Introduction

Tissue culture- a collective term referring to procedures used to maintain and grow plant cells and organs in aseptic conditions.

- Propagation
- Genotype modification
- Production of secondary compounds
- Plant pathology
- Germplasm preservation
- Research

Totipotency

-Each living cell has the potential to reproduce an entire organism.

Advantages of Micropropagation

- Mass propagation of specific clones
- Production of pathogen-free plants
- Clonal propagation of parental stock for hybrid seed production
- Year-round nursery production
- Germplasm preservation

Applications

- Plant regeneration
- Genetic engineering
- Reforestation
- Secondary products
- Cultivars with high market value
- Propagation of difficult to root plants or plants that are typically divided

Limitations of Micropropagation

Expense	Species specificity	Production scheduling
Contamination	Variability	Acclimatization

The Tissue Culture Environment

- Vitrification
- Pathogens
- Exudation
- Habituation
- Variation

Types of Tissue Culture Systems

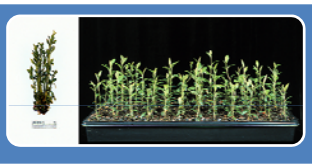
- Plantlets
- Seedlings
- Callus
- Somatic embryos

Techniques used to Regenerate Plants Through Tissue Culture

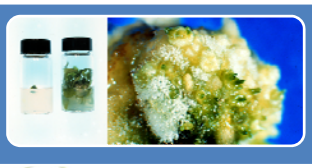
Structure	Regeneration	Explant Source
Plantlet	Axillary shoot	Meristem or shoot tip
	Adventitious shoot	Leaf pieces, stem internodes
Seedling	Seed culture	Seeds
	Embryo culture	Mature or immature embryos
Callus	Callus cultures	Vegetative tissue
	Protoplast cultures	Single cells
Somatic embryo	Direct or indirect	Embryo, seedling or leaf

Modified Table 17-1: Hartmann et al., 2011

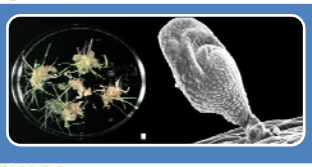
Micropropagation Methods



Shoot culture



Shoot organogenesis



Non-zygotic embryogenesis

Kane, 2002

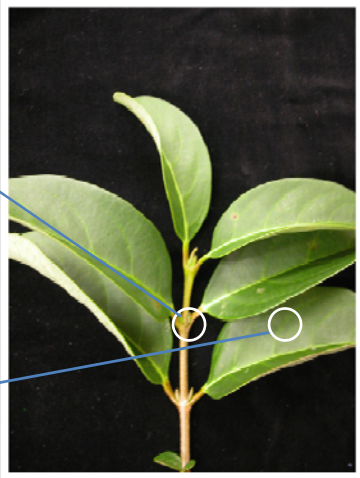
Regenerating Plants

Axillary shoot formation

- meristem culture
- axillary shoot cultures

Adventitious shoot formation

- direct
- indirect



Developmental Stages in Micropropagation

Stage I
- establishment

Stage II
- multiplication

Stage III
- root formation

Stage IV
- acclimatization

Stage I Objectives

To successfully place an explant into aseptic culture and an in vitro environment that promotes stable shoot production

Explant source selection

Explant disinfestation

Culture medium

Stabilization

Laboratory Facilities


Laminar flow hood


Auto-clave


Media prep room


Culture room

Media Preparation

Inorganic Salts

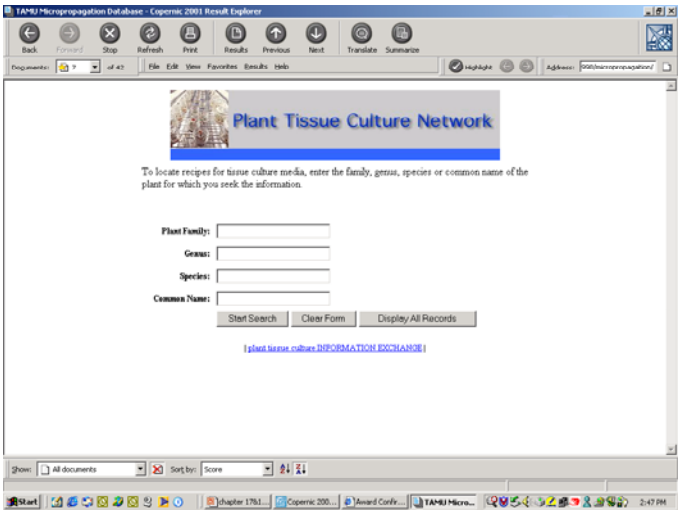
Organic Compounds

- sucrose
- vitamins
- hormones

Supports

- agar
- membrane boats
- cellulose plugs

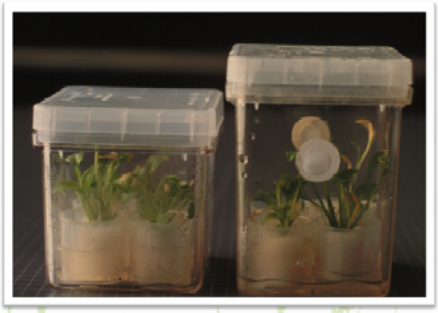
http://horticulture.tamu.edu:7998/micropropagation/



Containers

Control of the Tissue Culture Environment

- Temperature
- Light Intensity
- Photoperiod
- Light quality
- Air exchange



Light

Air Exchange

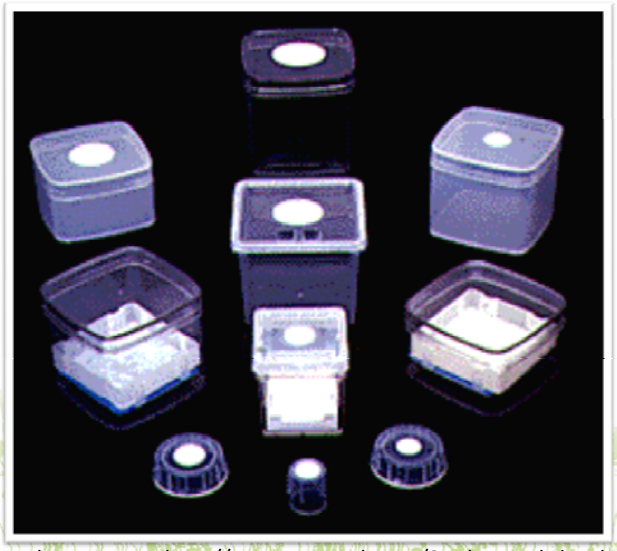


Photo source: http://www.osmotek.com/StudyVessels.html

Stage II Objectives

-To maintain the culture in a stabilized state and multiply microshoots to the number required for rooting.

- Growth regulators
- Subculturing
- Propagation ratio

Subculturing

-transferring the explant to a fresh medium

Explant/Propagule

- the piece of the plant used to initiate the micropropagation process

Stage III Objectives

-To root microcuttings and prepare them for transfer to ex vitro conditions

In vitro rooting

Ex vitro rooting

Stage IV Objectives

-To shift from a heterotrophic (sugar-requiring) to an autotrophic condition

Acclimatization

In vitro vs. ex vitro anatomy and physiology

Organogenesis

- The formation of organs, such as leaves, shoots, or roots, from cells or tissues.

- The process of developing adventitious shoots and/or roots

Indirect Organogenesis

Somaclonal Variation

- genetic variation induced in plants produced from populations of cells in culture

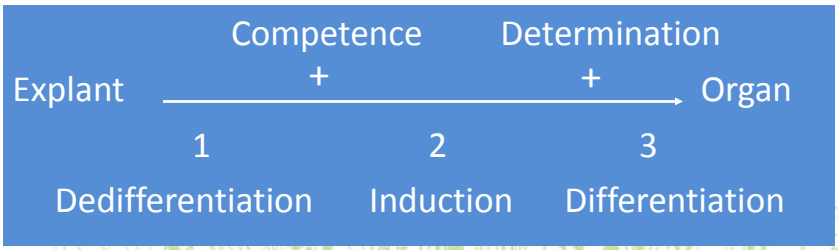
Primary Explant → Callus → Organ Primordium

Dedifferentiation

- process of reverting to a non-specialized or undifferentiated state

Differentiation

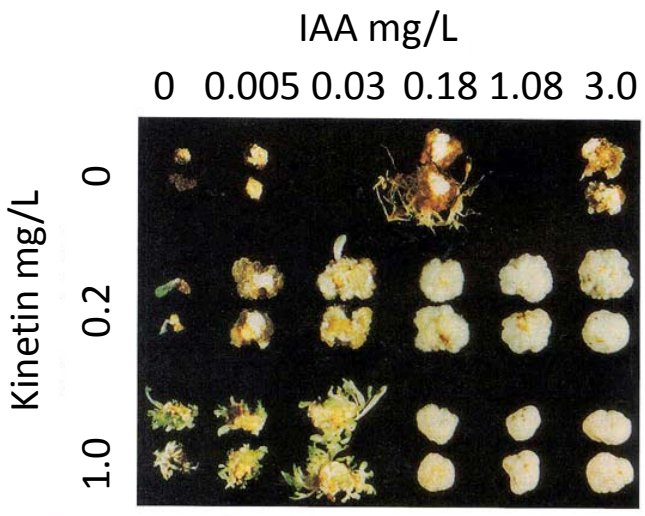
- process of initiating the growth of new and varied tissues or organs for specialized functions



Adventitious Shoot Formation
Diploid Plant Regeneration

1. Leaf pieces
2. Thin layer epidermal strips
3. Fragmented shoot apices
4. Cotyledons and hypocotyls
5. Young needle fascicles
6. Immature inflorescences on flower stems
7. Bulb scales

Effects of Cytokinin and Auxin



Raven et al., 1999

Callus

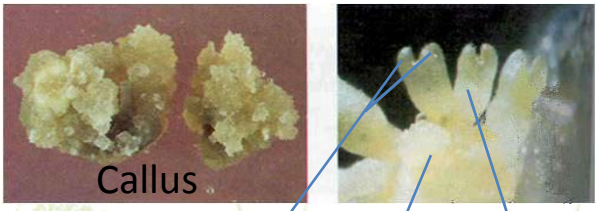
- Cell division of nondifferentiated parenchyma cells

Produced on explants in vitro as a response to wounding and medium supplementation with growth hormones

Seeds, stems, roots, leaves, storage organs, or fruits can be excised, disinfected and induced to form callus

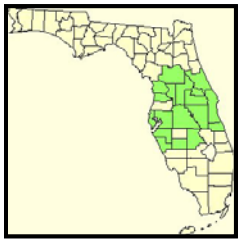
Somatic Embryogenesis

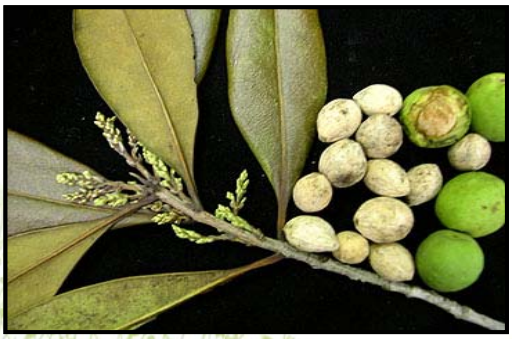
- The development of embryos from vegetative cells rather than from union of male and female gametes.



Mauseth, 1998

Osmanthus megacarpus





Osmanthus Embryo

Osmanthus Seed Germination

Trt.	Scarify	GA	Warm Stratify	Cool Stratify
1	No	No	No	No
2	Yes	No	No	No
3	Yes	Yes	No	No
4	Yes	No	No	Yes
5	Yes	No	Yes	No
6	Yes	No	Yes	Yes
7	Yes	Yes	No	Yes
8	Yes	Yes	Yes	No
9	Yes	Yes	Yes	Yes

Vegetative Propagation of *Osmanthus*

IBA:NAA (Dip n Grow)	% Rooting
No hormone	25.0
500 IBA: 250 NAA	40.0
1,000 IBA: 500 NAA	35.0
5,000 IBA: 2,500 NAA	46.7
10,000 IBA: 5,000 NAA	45.0

A Possible Candidate for Somatic Embryogenesis?

Photo source: Vendrame, 2004

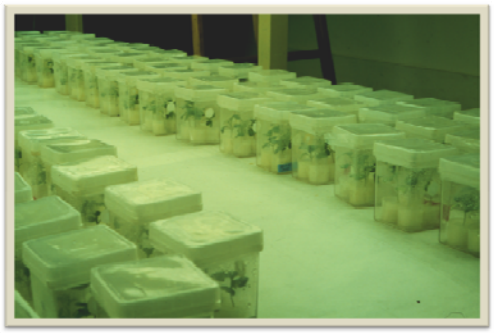
Commercial-Scale Micropropagation Agristarts, Apopka, FL




Weekly Production Goals Estimated by Propagation Ratio 80,000 units/week

Micropropagation Technology

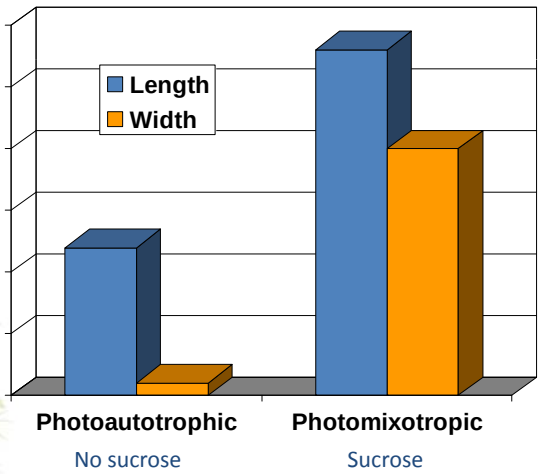
- Automation
- Natural sun
- Biotechnology



Responses of Plants to the In Vitro Environment

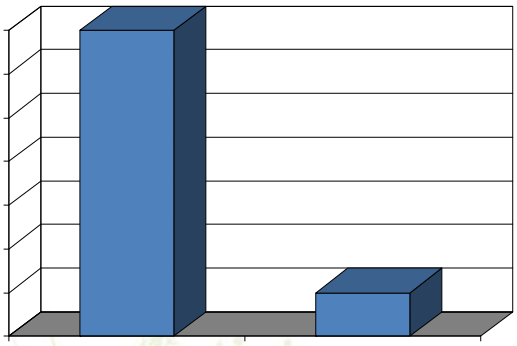
- Reduced wax formation
- Stomatal malfunction
- Low chlorophyll content in leaves
- Low percent dry matter
- Restricted leaf expansion
- Low stomatal density on leaves
- Inferior vascular development
- Low photosynthetic ability

Stomatal Pore Size (µm)



Zobayed et al., 2000

Wax Content ($g \cdot m^{-2}$)



Zobayed et al., 2000

Cross Section of Roots In vitro and Ex vitro

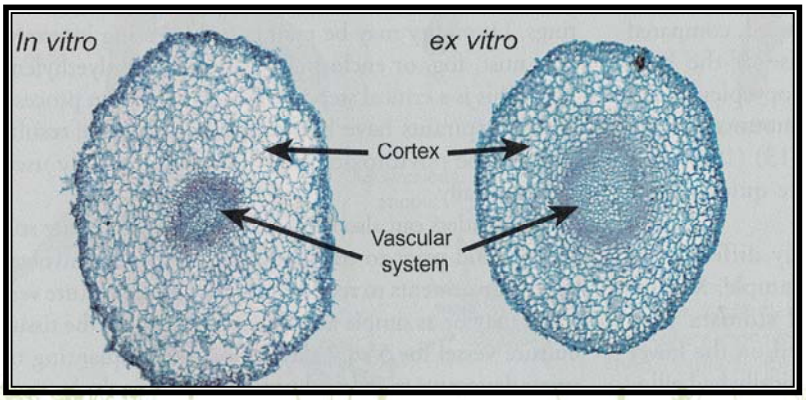
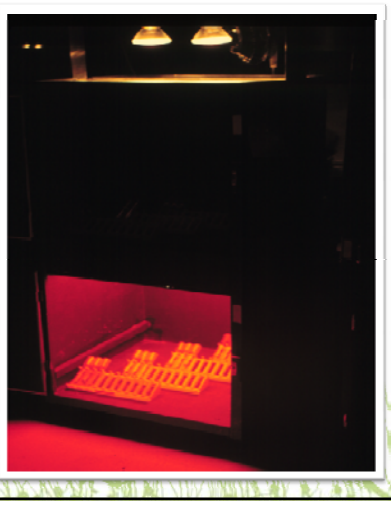
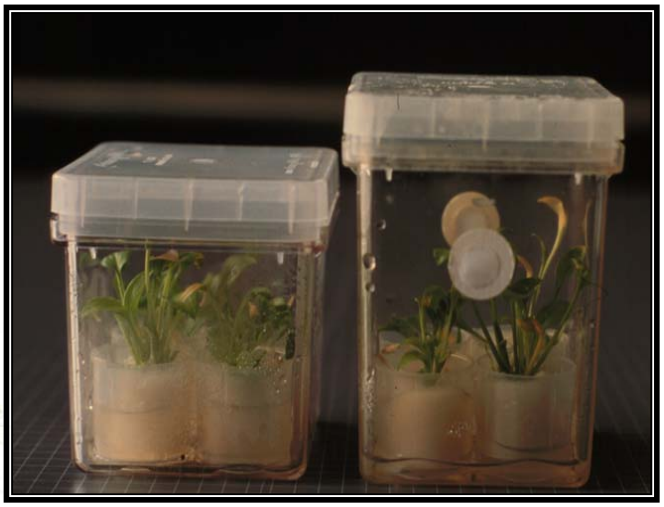


Fig. 17-12: Hartmann et al., 2011

IPT-Transgenic Tobacco Responses to End of Day Red and Far-red Light



Micropropagated Hosta- Storage at Light Compensation Point



Light Quality Growth Chambers

White **Red** **Blue** **Dark**

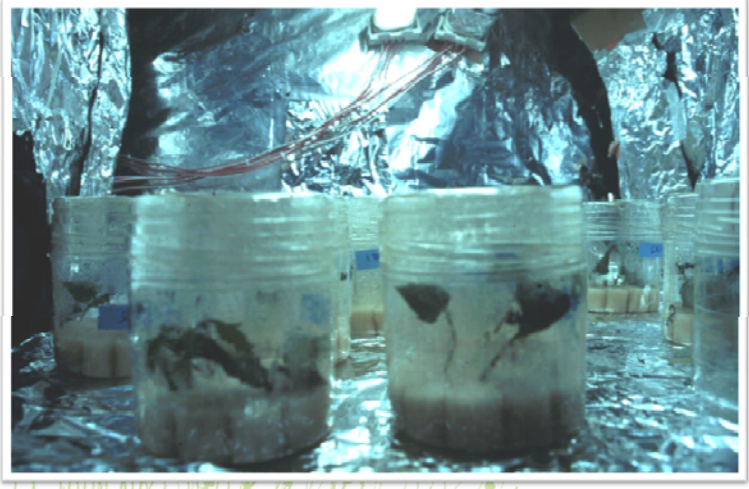
12 Weeks Storage at 10 °C and 60 days in Greenhouse

Suc.

No Suc.

White Red Blue Dark

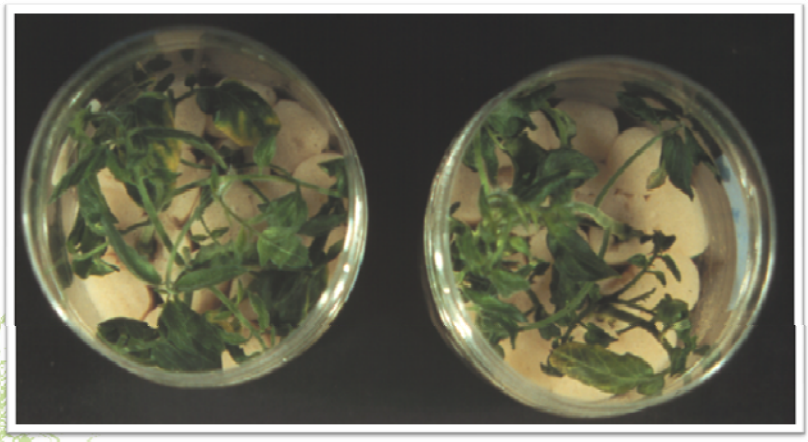
CO_2-Enriched Growth Chamber



Sweet Potato Day 17

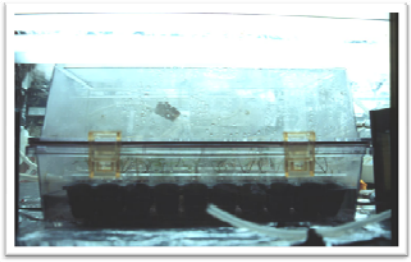
Photoautotrophic Photomixotrophic

Tomato Day 17



Photoautotrophic Photomixotrophic

Forced Ventilation System



Effects of CO_2 Enrichment

No sucrose and forced CO2

With sucrose and natural ventilation

Traditional method in agar with sucrose

Sea Oats

Liquid-No Sucrose

Liquid-3% Sucrose with CO2

Liquid-3% Sucrose no CO2

AGAR-3% Sucrose

16-3 EK 11-1

Web Lecture

Objectives are to Understand:

- The history of micropropagation
- Advantages of micropropagation
- Five stages of shoot culture

Dr. Mike Kane

Video	Video
• Demonstration of sterile technique to divide micropropagules	• Agristarts, commercial micropropagation

Web Lecture

Dr. Wagner Vendrame

Objectives are to Understand

- The advantages of micropropagation
- Somatic embryogenesis applications
- Embryo conversion

Final Exam

80% New Material:

- Lecture from chapters 14-18
- Web lectures and videos

20% Review Material:

- Seed Propagation (10 pts)
- Vegetative Propagation (10 pts)