

Propagation of planting materials for vegetatively propagated crops

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Seed Enterprises Management Institute
University of Nairobi

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SEMI's SEED PRODUCTION COURSE 28th May to 3rd June 2017

Overview

- ▶ Propagation techniques
- ▶ Emphasis Tissue culture and its applications



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Propagation techniques

1. Cuttings, in fruit trees and sugarcane, bamboo
2. Runners in straw berry
3. “Eye” of the potato tuber
4. Division of the crown in some grasses and legumes
5. Grafting in roses and fruit trees
6. Layering
7. Splits in pyrethrum
8. Seed
9. Tissue culture

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Tissue culture

- ▶ The term used “for the process of growing cells artificially in the laboratory” to produce clones in which the cells have the same genotype
- ▶ Involves the culture of cells, tissues and organs under favourable conditions that would allow for multiplication and regeneration of whole plants in a sterile environment
- ▶ The process is totipotent, producing true to type genotype, allows cells to differentiate and the living plants to re-differentiate
- ▶ Commercial Plant tissue culture was made possible by the development of the artificial growth medium commonly known as Murashige and Skoog (MS) (Murashige and Skoog, 1962)

Requirements

- ▶ Appropriate tissue (some tissues are better than others)
- ▶ A suitable growth medium (solid or liquid) to provide nutrients
- ▶ Aseptic (sterile) conditions (to avoid pathogens/contaminants)
- ▶ Growth regulators (auxins and cytokinins are critical)
- ▶ Frequent sub culturing (to avoid nutrient exhaustion and build up of metabolites)
- ▶ Warmth and good lighting

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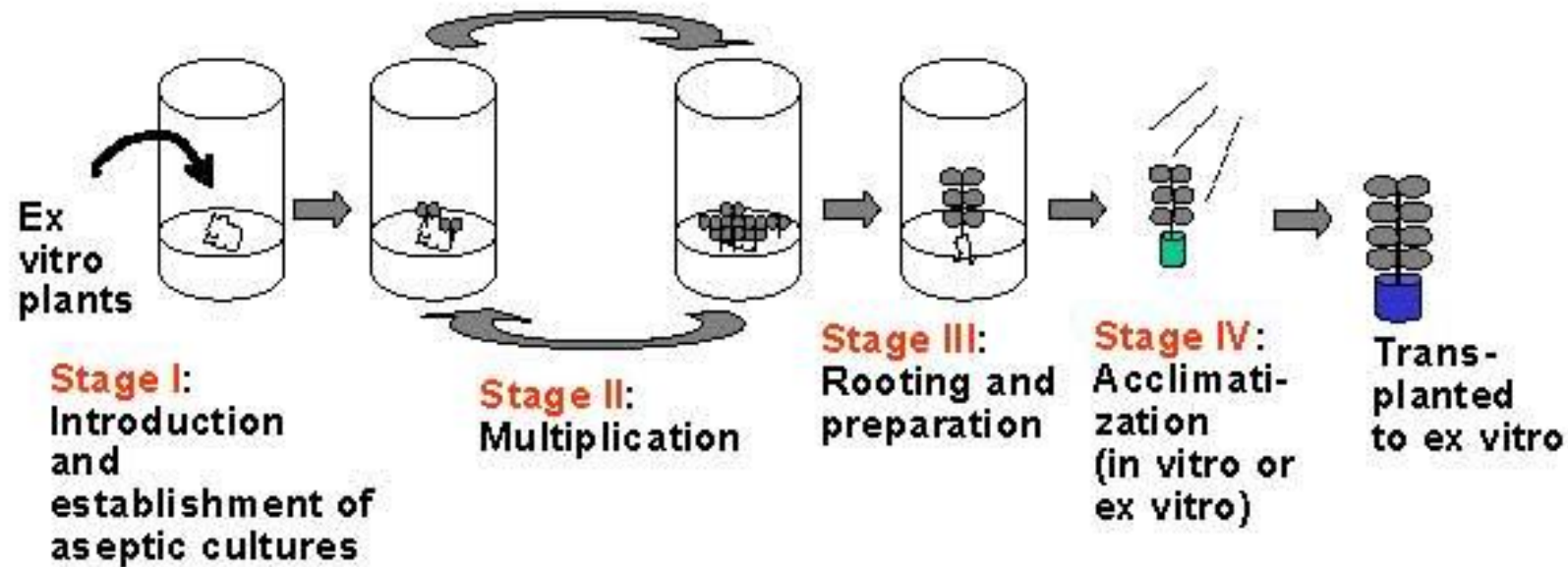
Explant: Culture Starting tissue

- ▶ Organ cultures
 - ▶ Meristem culture
 - ▶ Shoot tip culture
 - ▶ Root tip culture
 - ▶ Seed culture
 - ▶ Hypocotyl
 - ▶ Auxillary bud
 - ▶ Leaf culture
- ▶ Callus culture
- ▶ Haploid cultures
 - ▶ Ovule culture
 - ▶ Anther culture
- ▶ Protoplast culture
- ▶ Embryo culture.
 - ▶ Zygotic embryo culture, Nucellar embryo culture



Stages in micro propagation

Four stages in micropropagation



Multiplication of the regenerated plants



Multiplication involves cutting and sub-culturing the regenerated plants sometimes the callus formed is divided and sub cultured in new media

Rooting of multiplied/regenerated plantlets



The plantlets are transferred into media with a different balance of hormones to allow rooting (more auxin than cytokinin)

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Hardening in the greenhouse



Each plantlet receives an individual touch until it is grown and marketed

Plant tissue culture media

- ▶ Nutrient base that provides the cells food for growth. Must have:
- ▶ Macronutrients
- ▶ Micronutrients
- ▶ Organic substances
- ▶ Vitamins
- ▶ Plant hormones (growth regulators)
- ▶ Carbon source (sucrose)
- ▶ Undefined natural substances
- ▶ Gelling agents (Agar)



Each plant species has its own media requirements that must be established through trial and error

Potential sources of contamination

- ▶ Microorganisms are referred to as contaminants in tissue culture. They interfere with plant growth in vitro
- ▶ Sources of microbes in a tissue culture laboratory are:
 - ▶ Air,
 - ▶ water,
 - ▶ growth media,
 - ▶ people,
 - ▶ equipment and
 - ▶ plant material

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Provision of aseptic conditions (3 levels)

- ▶ All operations in tissue culture must be sterile. i.e. done in the absence of actively dividing micro-organisms.
- ▶ **Work area sterilization**
 - ▶ Laminar flow hood (filters)
 - ▶ Open flame
 - ▶ Dry heat (Oven) (100-150 °C)
 - ▶ Chemical sterilization (Sodium hypochlorite, 95% ethanol, commercial disinfectants eg. Lysol and zephiram.
 - ▶ U.V. light.
- ▶ **Media sterilization**
 - ▶ Autoclave (Moist heat) 121°C
 - ▶ Filter Sterilization (using suction and microfilters)
- ▶ **Explant sterilization**
 - ▶ Chemical sterilization (Sodium hypochlorite, 95% ethanol)
- ▶ Glassware and culturing equipment should be sterilized using the above methods

Glassware used for Tissue culture



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Factors that affect plant tissue culture

- ▶ Growth media – balance of nutrients, hormones and carbon source
- ▶ Environmental factors – light, temperature , photoperiod, sterility, media
- ▶ Explant source-- younger plants or plant parts are more preferred
- ▶ Genetics-- different plant species respond differently to the TC process

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Plant Tissue culture Applications I

For mass propagation and production of chemical compounds

- ▶ One explant can produce thousands of plantlets in a year e.g bananas can produce up to 2500 plantlets
- ▶ Taking an explant does not destroy the mother plant
- ▶ Once established young plants can be continuously supplied throughout the year regardless of the weather conditions

Plant Tissue culture Applications II

**Production of disease free plants,
disease elimination and establishment
of tissue banks**

- ▶ Production of virus free plants such as Irish potato, sweet potato, Cassava, garlic, tomato, grapes, cocoyam, passion fruit (through Callus or embryogenesis)
- ▶ Production of bacteria free plants citrus, bananas, pyrethrum



Plant Tissue culture Applications III

For Plant breeding purposes (clonal propagation)

- ▶ Tissue culture allows fast selection for crop improvement - explants are chosen from superior plants, then cloned
- ▶ Tissue culture clones are 'true to type' as compared with seedlings, which show greater variability

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Plant Tissue culture Applications III-b

Genetic modification

- ▶ Introducing desired genes (stretch of DNA) into plant cells and selecting the transformed tissues or plants
- ▶ Done in two ways: Indirect or direct gene transfer
- ▶ Indirect : Agrobacterium mediated gene transfer, Ti plasmids, T- DNA
- ▶ Direct: Polyethylene glycol (PEG), Electroporation, Microinjection, Microprojectile bombardment (Biolistic)

Concerns in genetic engineering of plants

- ▶ Negative impact to environment
 - ▶ weediness of weedy relatives
 - ▶ resistant pest lines
 - ▶ Bt crop toxins destroy beneficial insects
 - ▶ Contamination of the “centers of origin”
- ▶ Safety to humans & animals
 - ▶ Health and allergy related risks
- ▶ Bioethics
- ▶ Intellectual Property Rights

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Plant Tissue culture Applications IV

Germplasm conservation

- ▶ Establishment of frozen plant tissue banks which can be regenerated through tissue culture process (*in vitro* collection, characterization and indexing of germplasm (conventional and molecular techniques))
- ▶ Safe movement or shipment of germplasm: TC makes it easy to meet international phytosanitary plant export requirements; plant cultures in acceptable media are easier to export than are soil grown plants



THANK YOU

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