Plant Tissue culture, Micropropagation of Pitahaya, Dragon fruit.

Plant Tissue Culture

- Micropropagation is the production of whole plants from small sections of plant such as a stem tip, node, meristem, embryo, or even a seed
- Plant tissue culture is basically the same thing, except that it implies the use of callus tissue generated from plant cells cultured in-vitro.

- Micropropagation and plant tissue culture are used to produce large numbers of plants from small pieces of the stock plant in relatively short periods of time.
    - Why does micropropagation work?
        - Plant cells have the ability to reproduce the whole plant from single cells. This is called totipotency.
        - Totipotency is the ability of a single cell to express the full genome in the cells to which it gives rise by cell division.

Plant Tissue Culture

Why do we do it?

- To regenerate plants from single cells or plant tissues
- To cultivate under sterile conditions (=in vitro)
- To produce large quantities of identical plants
- To cultivate without the impact of environmental conditions

Plant Tissue Culture

- To save species from extinction
- To isolate disease from plants
- To produce plants with enhanced stress or pest resistance
- To create new plant varieties
- To make money

Plant Tissue Culture

- The Basics of Micropropagation
- The Four stages
  - Stage one – Explant establishment or initiation
  - Stage two – Multiplication
  - Stage three – Rooting
  - Stage four – Acclimatization or hardening off

What part of the plant can be used for micropropagation?

- Virtually any part of a plant can be used.
- Meristems, shoot and root tips, leaf tissue, anthers, embryos, flowers, virtually all parts of a plant can be used.

Surface Disinfestation

- Typically !0% Bleach Solution for 20 minutes.
- Surfactant oftentimes utilized.
- Can treat with antioxidant solution.
- Other materials can be used.
- A Balancing act not to damaging tissue while at the same time getting a “clean” culture.
- Endophytic organisms? Dr. Martin Stoner.

Plant Tissue Culture

- Meristematic tissue - which are undifferentiated cells from shoot and root tips that have not been programmed for their ultimate development
- Parenchyma cells – the most common type of plant cell, which can regenerate and differentiate to initiate the growth of new and varied tissue and organs
- Adventitious growth is the development of new shoots, buds, roots, or leaves from atypical or unusual locations

Plant Tissue Culture

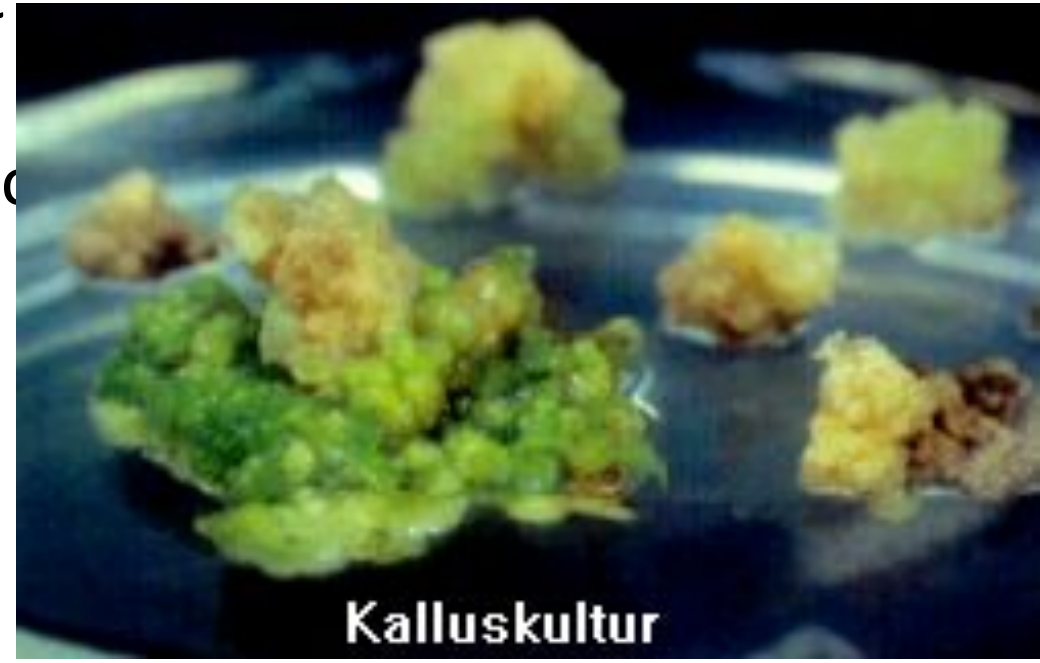
- A limitation is that each plant is propagated differently and not every plant will respond the same way.
- Each genus, species and variety may require a different tissue which will obtain the best results.
- Different plants may require very different media as well as environmental conditions.
- Some plants are better suited for a tissue culture system than others.

Plant Tissue Culture

- Tissue Culture/Micropropagation has two general forms of plantlet production.
  - Callus production
  - Micro cuttings

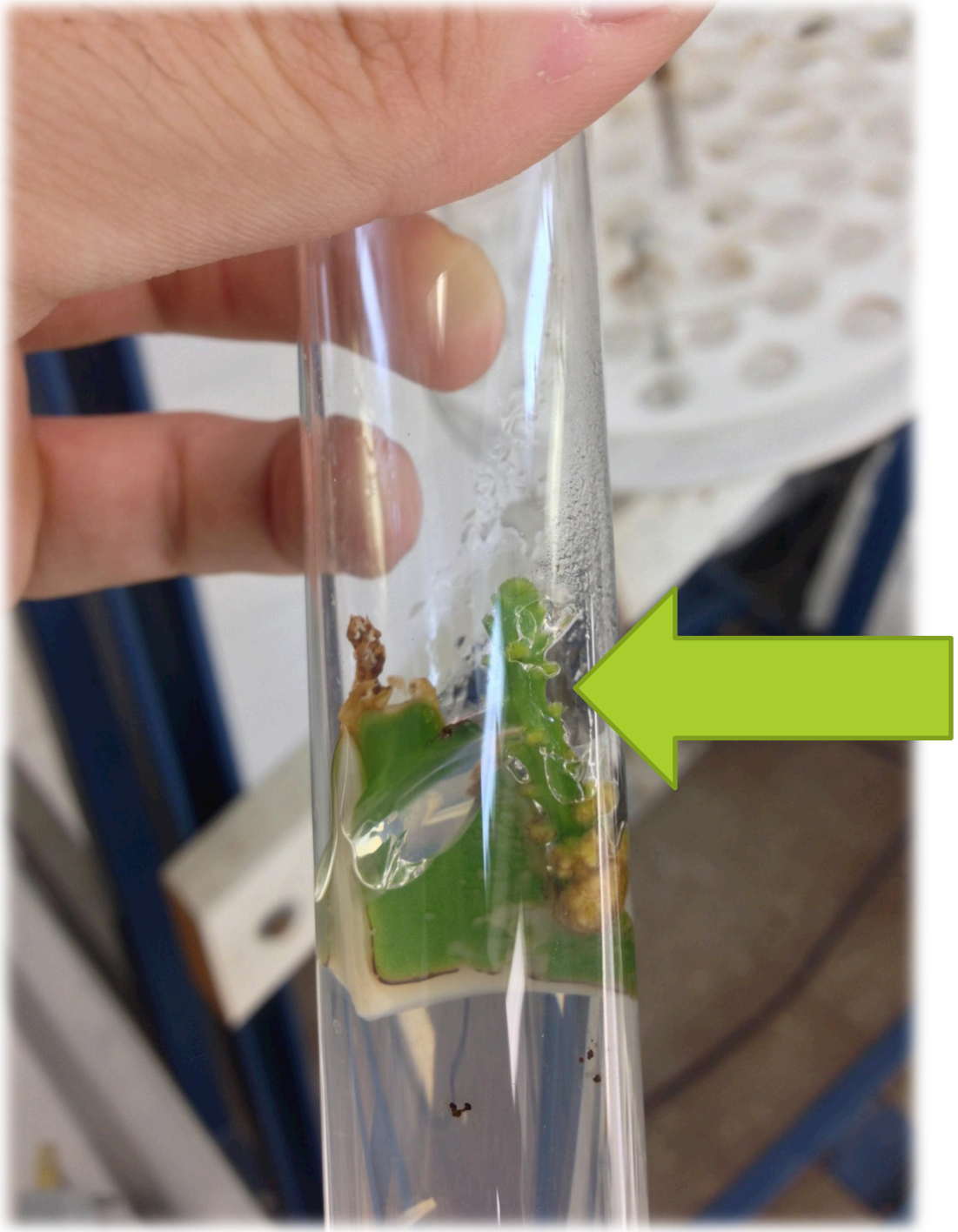
Plant Tissue Culture

- Callus production is the dedifferentiation of a plant cell into callus.
- Callus is then expanded into a larger mass of undifferentiated cells.
- Callus is then activated, by selective use of plant hormones to differentiate to produce, shoots, roots and ultimately, plantlets



Micro cuttings

Somehow, this culture is Growing underneath the Surface of the medium! What a survivor.



This one is by far the Largest shoot so far.

Major Constituents

- Salt Mixtures
- Organic Substances
- Natural Complexes
- Growth Regulators
- Inert Supportive Materials

Macro-nutrient salts

- NH_4NO_3 Ammonium nitrate
- KNO_3 Potassium nitrate
- $CaCl_2$ -2 H_2O Calcium chloride (Anhydrous)
- $MgSO_4$ -7 H_2O Magnesium sulfide (Epsom Salts)
- KH_2PO_4
- Too much NH_4 + may cause vitrification, but is needed for embryogenesis and stimulates adventitious shoot formation

Micro-nutrient salts

- FeNaEDTA or (Na_2EDTA and $FeSo_4$)
- H_3BO_3 Boric Acid
- $MnSO_4$ - 4 H_2O Manganese sulfate
- $ZnSO_4$ - 7 H_2O Zinc sulfate
- KI Potassium iodide
- Na_2MoO_4 - 2 H_2O Sodium molybdate
- $CuSO_4$ - 5 H_2O Cupric sulfate
- $CoCl_2$ - H_2O Cobalt chloride

2. Organic Compounds

- Carbon Sources
  - 1. Sucrose (1.5 to 12 %, 3 % I reference point)
  - 2. Glucose (Sometimes used with monocots)
  - 3. Fructose
- White Vitamins
  - - Thiamine 1.0 mg/l
  - - Nicotinic Acid and Pyroxidine 0.5 mg/l
  - - Glycine 2.0 mg/l
- Vitamin C (antioxidant) 100.0 mg/l

Organic compounds cont.

- Amino Acids and Amides
- Amino acids can be used as the sole source of Nitrogen but normally too expensive
- 2 amino acids most commonly used
  - L-tyrosine, enhances adventitious shoot form.
  - L. Glutamine, may enhance adventitious embryogenesis

Salt Mixtures

- M.S. (Murashigi and Skoog)
- Gamborg
- Nitsch and Nitsch (Similar to M.S.)
- White
- Knudson

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Natural Complexes, Can Tissue Culture be done Organically?

- -Coconut endosperm
- -Fish emulsion
- -Protein hydrolysates
- -Tomato juice
- -Yeast extracts
- -Potato agar

Plant Growth Regulators/Hormones

- Auxins
- IAA
- IBA
- NAA
- 2, 4-D
- Cytokinins
- BAP or BA
- Kinetin
- TDZ
- Zeatin

Growth regulators

- -auxin
- -cytokinin
- -gibberellin
- -abscisic acid
- -ethylene

Gelling Agents

- -Agar- extract from Marine red agar
  - -Phytagar
  - -Taiyo
  - -Difco-Bacto
  - -TC Agar
  - -Agarose
- -Hydrogels
- -Gelatin
- Gelrite

Equipment, Sterile Conditions

- Autoclave
- Laminar Flow Hood

Growing Area