Plant Tissue Culture

Media ingredients

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All media contains:

- Deionized and distilled water
- Macro and micro plant nutrients
- Inositol
- Vitamins
- Hormones
- Sugar
- Agar

Then:

- Media pH is adjusted
- Tubes are filled with 12 mL media and capped
- Tubes are sterilized in autoclave at 121°C for 20 minutes (at 15 psi)

Media ingredients

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Water deionizer and distiller

Tissue Culture

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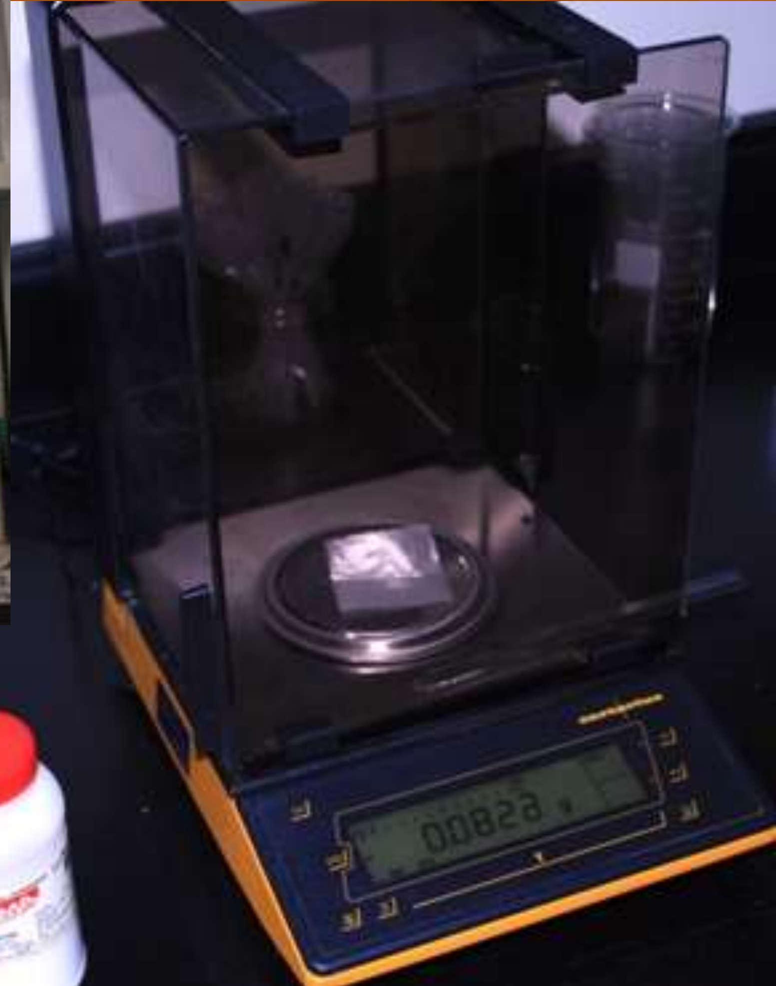

Chemicals and analytical scale

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Hotplate/stirrer with media bottle, pH meter, and autoclave

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Woody Plant Medium (WPM) for Rhododendrons

- A Stock: 4 ml (into 150 ml ddH_20 in 250 ml media bottle)
- B Stock: 4 ml
- $CaCl_2$ x 2 H_2O: 0.0254 g
- D Stock: 2 ml
- E Stock: 2 ml
- F Stock: 2 ml
- G Stock: **2** ml
- Inositol: 0.02 g
- Low Hormone Stock: 12 ml
- Sucrose: 4 g, dissolve, and then adjust volume to 200 ml using ddH_20
- pH: Adjust to 5.2 (1 to 4 drops 0.5 N NaOH)
- Agar: 1.5 g (heat until clear on hot plate)
- Dispense: put 20 ml per test tube and cap
- Autoclave: 115 psi for 20 min. (then remove racks after 1 hour and allow to cool on a sharp angle for several hours)

Media example: stock solutions reduce the time required to make commonly used media

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Preparation

- Brush off dirty clothes and hair outdoors.
- Tie back long hair and remove dirty footwear before entering Rm. 1662.
- Have laminar flow hood on for 5 to 10 minutes before opening a tube.
- Wash hands with antibacterial soap and water and dry thoroughly.
- Put on a clean lab coat and apply small amount of hand cream.
- Remove all non-essential items from laminar flow hood.
- Drip 70% ethanol to work surface and wipe dry with paper towel.
- Do NOT get alcohol into the back of hood (it harms the HEPA filter).
- Have forceps and scalpel soaking in 70% ethanol for at least 2 minutes initially and then for at least 10 seconds between transfers.
- Keep alcohol deep in hood so it does not get spilled. Lite alcohol burner away from alcohol.

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Transferring Process (reverse if left handed)

- Remove the cap from the tube and hold between two fingers.
- Move tube from left to right hand, holding between thumb & index finger.
- Flame the tool over the alcohol burner to remove the alcohol. There's no need to heat the tool.
- Pick up the surface sterilized plant tissue or seed and place it into the agar inside the tube.
- Quickly recap the tube and place in rack. Important: Seal tube with parafilm wrap.

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Minimizing contamination & health risks

- Personal hygiene is important. Work at least half way into hood.
- If surface sterilizing plant material then follow instructions carefully.
- Do not talk or breathe heavily during the transferring process.
- There should be nothing between an open tube and the back of the hood to block airflow.
- Instruments need to be in 70% ethanol for at least 10 seconds.
- Keep the tip of tools pointing downwards so fingers stay dry and do not get burned. Be careful not to dip a lit tool into the alcohol flask!
- Do NOT open any tube with fungal contamination. Staff will check for and discard contaminated cultures regularly.

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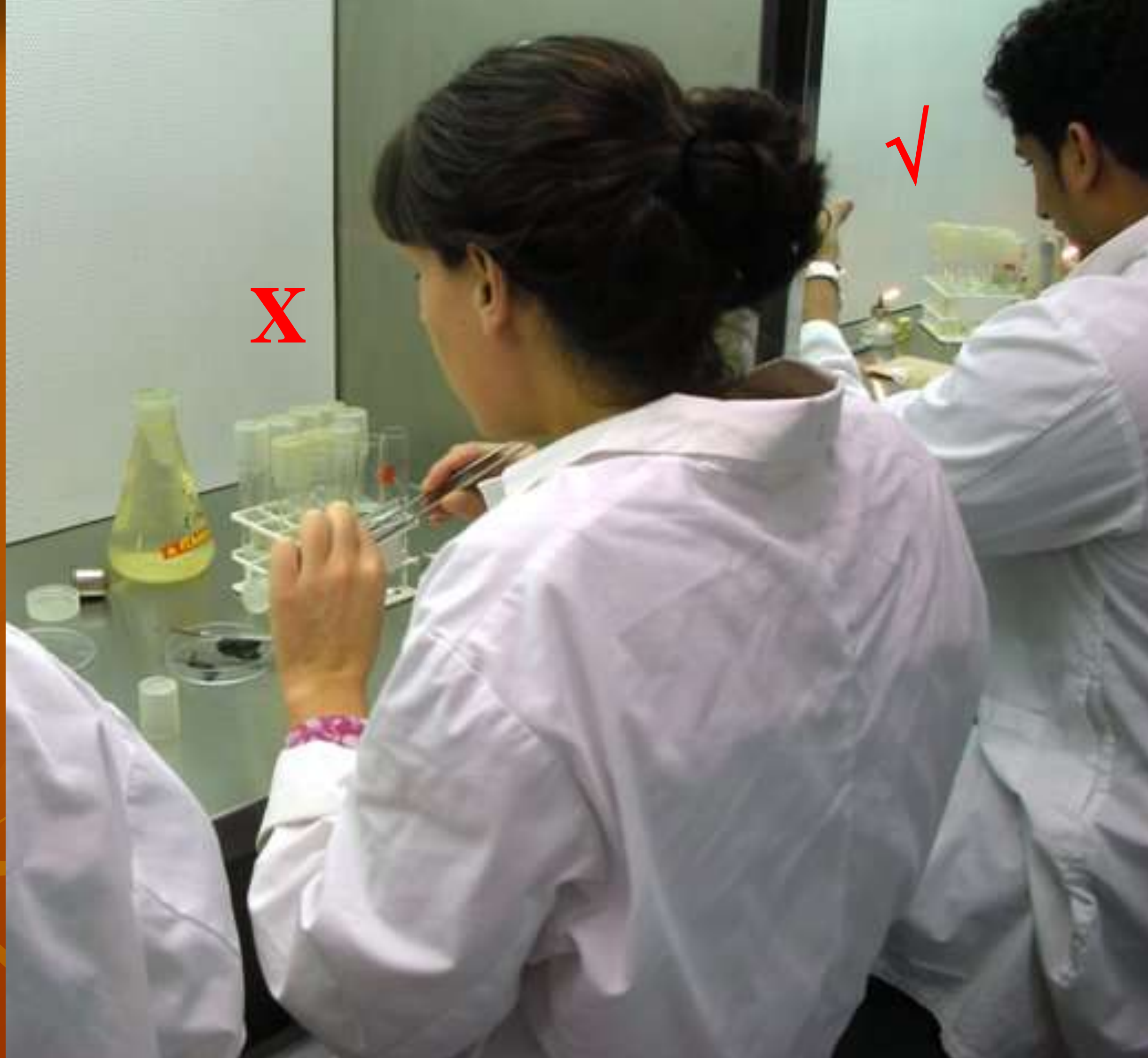

Work with hands at least half way inside the hood
Ensure nothing is blocking the air flow between back of hood and the specimen!

Sterile transfer technique

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African Violet Explant Establishment (SI)

- Soak two young leaves in 10% bleach with 1% tween added for 10 minutes maximum. Vortex.
- Rinse in three changes of sterile deionized water (5 minutes in each rinse).
- Cut center portion of two leaves out into four 1 cm X 1 cm in size in sterile petri dish (avoid midrib).
- Aseptically transfer the pieces into the agar and submerge about half-way into it.

Methods and some materials (tubes with media, 10% bleach, cultures, vortex, Parafilm)

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Bleach (10%) the African violets leaf piece for no more than 10 minutes!

Sterile transfer technique

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Parafilm tubes 2 to 3 times around to keep spores of bacteria and fungi out.

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New SII cultures

- Old culture: transplanting should be done at least every two months
- Fungal contamination.

Culture maintenance

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Effects of Varying Cytokinin and Auxin Levels on African Violet Differentiation in Tissue Culture

↓ Cytokinin (mg/l BA) ↓ ↓	Auxin: 0 mg/l NAA	Auxin: 0.1 mg/l NAA	Auxin: 0.5 mg/l NAA	Auxin: 1.0 mg/l NAA	Auxin: 2.0 mg/l IAA
0					
.01					
0.1					
1.0					

Hormonal effects

Tissue Culture

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- Roots in culture are only useful for certain plants that are very difficult to root on a mist bench or under a humidity dome.
- Even then it's only the SIII culture where we want to see roots.
- Media must be well washed off or a disease will likely kill the plant.

Hormonal effects: too high auxin to cytokinin ratio may result in only roots being formed!

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