Micropropagation of Canola shoots

The medium consists of inorganic salts and vitamins of MS medium, 30 g/L sucrose, 100 mg/L myo-inositol and 8 g/L agar Using:

- **powdered MS medium (salts and vitamins) 4.4 g/L**
- **Sucrose**
- **Myo-inositol stock solution (10 mg/ml).**
- **Agar**

The pH is 5.8

Calculations

MS Powder

4.4 g ⟶ 1000 ml

X g ⟶ 100 ml

$$X = \frac{4.4 * 100}{1000} = 0.44 \text{ g}$$

Sucrose

30 g ⟶ 1000 ml

X g ⟶ 100 ml

$$X = \frac{30 * 100}{1000} = 3 \text{ g}$$

Myo-inositol stock conc. = 10 mg/ml = 10 000 mg/L

Stock — NV = N'V' — Medium

$$10\,000 * V = 100 * 100$$

$$V = \frac{100 * 100}{10\,000} = 1 \text{ ml}$$

Agar

8 g ⟶ 1000 ml

X g ⟶ 100 ml

$$X = \frac{8 * 100}{1000} = 0.8 \text{ g}$$

Steps:

In 250 ml clean beaker, put 50 ml distilled water and dissolve:

0.44 g powdered MS medium (weigh using 3 digits electric balance)

3 g sucrose

1 ml myo-inositol stock solution (10 mg/ml) (using 1 ml glass Pipette or 1000 µl micropipette)

Up to 90 ml with distilled water (using 100 ml measuring cylinder)

Adjust pH to 5.8 using KOH and HCl

Up to 100 ml with distilled water (using 100 ml measuring cylinder)

Add 0.8 g Agar and boil with continuous stirring till disappearance of Agar

Divide into 2 jars

Autoclave for 20 minutes at 121 °C.

Method:

- Seven-day old seedlings were used as a source for explants.
- Explants (terminal buds along with cotyledons) were placed, aseptically, on shoot multiplication medium.
- Another group of explants were placed on MS medium (control).
- Cultures were incubated for 2 weeks at 25 °C under cool-white fluorescent light (2000 Lux irradiance) with 16-hour photoperiod.

Results

$$\text{Contamination\%} = \frac{\text{No. of Contaminated Cultures}}{\text{Total No. of Cultures}} \text{X100}$$

$$\text{Shoot regeneration \%} = \frac{\text{No. of explants producing shoots}}{\text{Total No. of explants}} \times 100$$

$$\text{Av No. of shoots per explant} = \frac{\text{Total No. of shoots on all explants}}{\text{No. of explants}} \times 100$$

	MS medium	MS + 4.5 mg/L BA
Contamination %		
Shoot regeneration %		
Av No. of shoots per explant		

Some other measurements can describe the results including

- Fresh weight of shoots
- Dry weight of shoots
- Leaf area
- Photosynthetic pigments content
- Genetic fidelity can be assessed using PCR-based techniques eg: RAPD.

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