

### Preparation of 100 ml callus induction medium

The medium consists of inorganic salts and vitamins of MS medium, 30 g/L sucrose, 100 mg/L myo-inositol, 1 mg/L 2,4-D and 8 g/L agar Using:

- powdered MS medium (salts and vitamins) 4.4 g/L
- Sucrose
- Myo-inositol stock solution (10 mg/ml).
- 2,4-D stock solution (1 mg/ml).
- Agar

The pH is 5.8

### Calculations

#### MS Powder

$$\begin{array}{lcl} 4.4 \text{ g} & \longrightarrow & 1000 \text{ ml} \\ X \text{ g} & \longrightarrow & 100 \text{ ml} \end{array}$$

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

#### Sucrose

$$\begin{array}{lcl} 30 \text{ g} & \longrightarrow & 1000 \text{ ml} \\ X \text{ g} & \longrightarrow & 100 \text{ ml} \end{array}$$

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

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

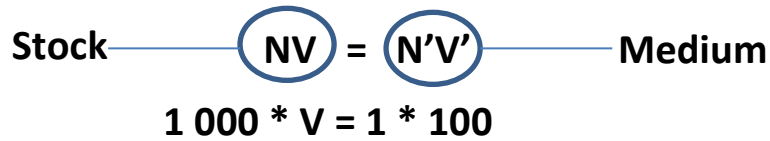
$$NV = N'V'$$

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



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

**2,4-D** stock conc. = 1 mg/ml = 1 000 mg/L



$$V = \frac{1 * 100}{1\,000} = 0.1\text{ ml}$$

### **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)

0.1 ml 2,4-D stock solution (1 mg/ml) (using 100 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 4 small jars

Autoclave for 20 minutes at 121 °C.

## Method:

- Seven-day old seedlings were used as a source for explants.
- Explants (0.5-1cm long hypocotyl sections and cotyledon along with small piece of petiole) were placed, aseptically, on callus induction medium.
- Cultures were incubated for 2 weeks at 25 °C under cool-white fluorescent light (1000 Lux irradiance) with 16-hour photoperiod.



**Hypocotyl  
segments**



**Cotyledon**

## Results

$$\text{Contamination\%} = \frac{\text{No. of Contaminated Cultures}}{\text{Total No. of Cultures}} \times 100$$

$$\text{Callus induction\%} = \frac{\text{No. of explants producing callus}}{\text{Total No. of explants}} \times 100$$

Callus color: Yellow-greenish yellow.

Callus position on explant: Callus is restricted to cut edges of explant.

### Some other measurements can describe the results including

- Fresh weight,
- Dry weight
- Compact volume of callus.
- Cytological measurements like mitotic index (percentage of cells carrying out mitosis in certain moment) can be also performed.

However, all these measurements require sacrifice of the samples.

## Comment