Preparation of 100 ml shoot regeneration medium

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

- **powdered MS medium (salts and vitamins) 4.4 g/L**
- **Sucrose**
- **Myo-inositol stock solution (10 mg/ml).**
- **BA stock solution (1 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

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

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

BA stock conc. = 1 mg/ml = 1 000 mg/L

Stock — NV = N'V' — Medium

$$1\,000 * V = 4 * 100$$

$$V = \frac{4 * 100}{1\,000} = 0.4 \text{ ml}$$

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)

0.4 ml BA stock solution (1 mg/ml) (using 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 4 jars

Autoclave for 20 minutes at 121 ^{o}C.

Method

- Shoot regeneration was induced by placing hypocotyl explants carrying calli on regeneration medium (MS medium supplemented with 4.5 mg/L BA).
- Cultures were incubated for 4 weeks at 25 °C under cool-white fluorescent light (1000 Lux irradiance) with 16-hour photoperiod.

Results

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

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

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

Some other measurements can describe the results including

- Fresh weight of shoots

- Dry weight of shoots
- Leaf area
- Photosynthetic pigments content

Comment

Preparation of 100 ml rooting medium

The medium consists of inorganic salts and vitamins of MS medium, 30 g/L sucrose, 100 mg/L myo-inositol, 0.5 mg/L IBA and 8 g/L agar Using:

- **powdered MS medium (salts and vitamins) 4.4 g/L**
- **Sucrose**
- **Myo-inositol stock solution (10 mg/ml).**
- **IBA stock solution (1 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

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

IBA stock conc. = 1 mg/ml = 1 000 mg/L

Stock — NV = N'V' — Medium

1 000 * V = 0.5 * 100

$$V = \frac{0.5 * 100}{1\,000} = 0.05 \text{ ml}$$

Agar

8 g → 1000 ml

X g → 100 ml

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

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)

0.05 ml IBA stock solution (1 mg/ml) (using 100 µl micropipette)

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

Adjust pH to 5.8 using KOH and HCl

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

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

Divide into 4 jars

Autoclave for 20 minutes at 121 ^{o}C.