Tissue culture from hypocotyls

Leonardo Galindo

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Seed germination

1. Sterilize seeds with 20% bleach + a drop of Tween-20 (100ul) for 20minutes, in falcon tubes shaking occasionally. Rinse 3 to 6 times with sterile distilled water (until no more tween detergent is evident).
2. Place sterile seed on germination media in small petri dishes. Place 10 seeds per dish. Place petri dish (with lid removed) in a sterile upside-down **Magenta box**. Incubate at 25ºC with 16h light/8h dark for 6 days.

Shoot/Root Regeneration

3. Cut 3-6mm hypocotyl segments from 6-day-old flax seedlings. Note: DO NOT allow explants to dry out. Immediately place them onto F3 medium in **a regular petri dish** for shoot initiation (no Magenta box is needed). Put 16explants/ plate. Incubate for 10-14 days (Same condition).
4. Transfer the explants onto W3 medium (**In regular petri dish** and no Magenta box) for shoot regeneration. At this stage the explants should have developed callus tissue at the cut ends. Whole explants or just the callus tissue with hypocotyl section removed should be subcultured onto fresh W3 medium every two weeks.
5. As shoot appear, those that elongate (or do not bleach out) should be transferred onto shoot elongation media (W4 in **only Magenta box** with lid close, 1 inch thick) for 2 weeks. Take note of where the shoot has arisen. If the shoot has regenerated from hypocotyl tissue, rather than the callus tissue, it is likely Not transgenic.
6. Transfer shoots onto rooting medium (in **only Magenta box** ,1 inch thick, with lid closed).
7. Once roots have formed, transfer shoots into soil. Maintain a high humidity (**Close the tray with a yellow lid**) for several weeks until the shoots have started to grow and they feel firmly anchored in the soil.

Note:

1. It is important to transfer the explants from F3 to W3 medium within 10-14 days.
2. All hormones should be filtered sterilized (0.22uM).
3. LS modified basal medium (Phytotechnology Labs, product No.L1477) contains a buffering agent and is already adjusted to pH.5.6. Therefore no further pH adjustment is required.

Media:

1. Germination media

½-strength LS modified basal medium = 2.765 g in 1L if using Phytotech LS.

1% sucrose

0.7% agar (Not the selected the Agar, the agar used to do tissue culture is in the tissue

culture cabinet –phytoblend agar-, agar from sigma for plant tissue culture can also be used).

2. Shoot Initiation and Selection Medium (F3)

LS modified basal medium

3% sucrose

0.7% agar

1mg/l BA（6-Benzylaminopurine, promote the plant development） make 0.1mg/ml stock or 100mg/l stock, first dissolve in a few drops of 1M KOH or NaOH or 95% Ethnol and then add water to the proper volume and stir or gently heated, adjust the PH to 5.0 ,then sterile with 0.22uM filter in the benchtop).

0.1mg/l NAA（Naphthaleneacetic acid, promote the root growth）make 0.1mg/ml stock or 100mg/l stock, first dissolve in a few drops of 1M KOH or NaOH or 95% Ethnol and then add water to the proper volume and stir or gently heated, adjust the PH to 5.0 , then sterile with 0.22uM filter in the benchtop）

3. Shoot Regeneration and Selection Medium (W3)

LS modified basal medium

2.5% sucrose

0.7% agar

0.02mg/l BA

0.001mg/l NAA

4. Shoot Elongation Medium (W4)

LS modified basal medium

1% sucrose

0.7% agar

5. Rooting Medium

½-strength LS modified basal medium

1% sucrose

0.7% agar

0.2 mg/l IBA