CELL SUSPENSION CULTURE OF ARABIDOPSIS

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1. MEDIA   (i) SOLID MS0 MEDIUM Murashige and Skoog Basal Medium, e.g., Imperial Laboratories #9-300-40 3% (w/v) Sucrose 0.8% (w/v) Agar, e.g., Sigma A7002 pH 5.8

   (ii) LIQUID MSA MEDIUM As solid MS0 medium, but supplemented with: 0.5 mg/l-1 2,4-D 0.05 mg/l-1 kinetin

   (iii) CALLUS INDUCTION MEDIUM Gamborg's B5 Basal medium e.g., Sigma G 5893 2% (w/v) Glucose 0.8% (w/v)Agar 0.5 gl-1 MES 0.5 mg/l-1 2,4-D 0.05 mg/l-1 Kinetin pH 5.7

   * All Petri dishes containing cultures are sealed with Parafilm M or the equivalent, e.g., Sigma P 7543.

2. SEED STERILISATION   (i) Immerse seeds in 10% (w/v) "Domestos" (commercial bleach) for 20 min. Wash twice with 500 ml of sterile distilled water, then leave to dry in a laminar flow cabinet overnight. (ii) Push the resulting crust of seeds through a sterile tea-strainer in order to separate the individual seeds. Store sterile seeds in a 60 mm Petri dish until required.

3. GERMINATION   (i) Lift sterile seeds with a flamed spatula onto MS0 solid media in screw-cap round glass jars, 55 mm diameter, 100 mm height. (ii) Place jars under fluorescent lights at about 22oC.

4. CALLUS INITIATION   (i) When large enough to handle, transfer plants to a sterile tile and excise the roots and chop them into sections, approximately 1 mm in length. (ii) Place these pieces onto callus induction medium in 60 mm Petri dishes and place in the same conditions as for germination.

   * Alternatively, use the largest leaves for callus initiation. (iii) Transfer each leaf to the tile, then damage it with a sterile scalpel-blade in order to initiate a wound response. * It doesn't seem to matter which face of the leaf is placed downwards on the solid medium.

5. INITIATION OF CELL SUSPENSIONS   (i) After callus formation, about 2-3 weeks, place a small piece of callus, roughly pea sized, in a 100 ml conical flask together with 20 ml of liquid MSA medium. (ii) After replacing the aluminium-foil lid, place the flask on a shaker at 40 rpm in a constant temperature room at about 22oC (either with or
After 7-10 d, the flask can be sub-cultured.

6. SUB-CULTURING  
   (i) Decant all liquid off. Add 50 ml of fresh MSA, then pour everything into a 250 ml flask.  
   (ii) When the volume of tissue has doubled, transfer 30% of the culture to a fresh flask.  
   From now on, use 100 ml of medium per 250 ml flask.

   (iii) Sub-culture at weekly intervals, even if the tissue appears to be growing more slowly. Adjust the amount of tissue which is used to inoculate a new flask in accordance with the growth rate.