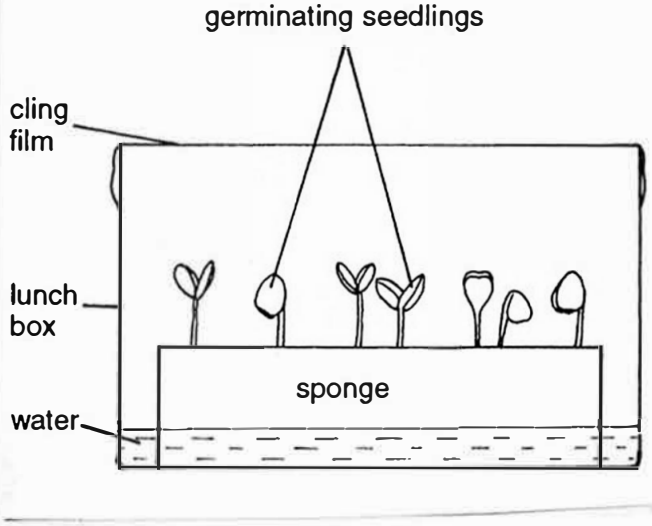
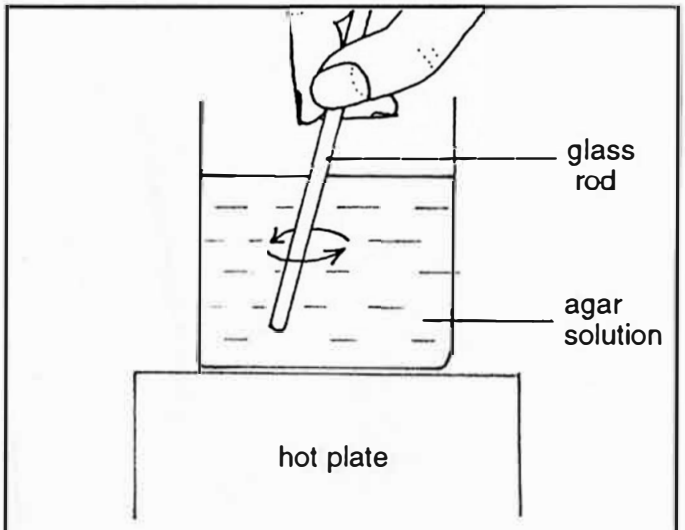
Fast tissue culture

With *Sinapis alba* and/or rapid-cycling *Brassica rapa*

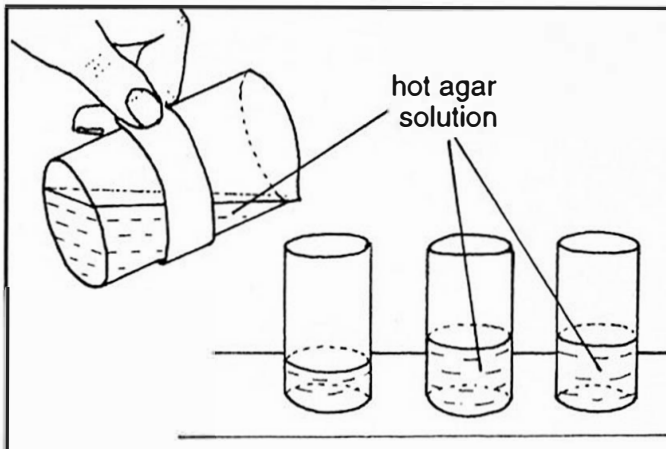
Read these instructions carefully before you start.



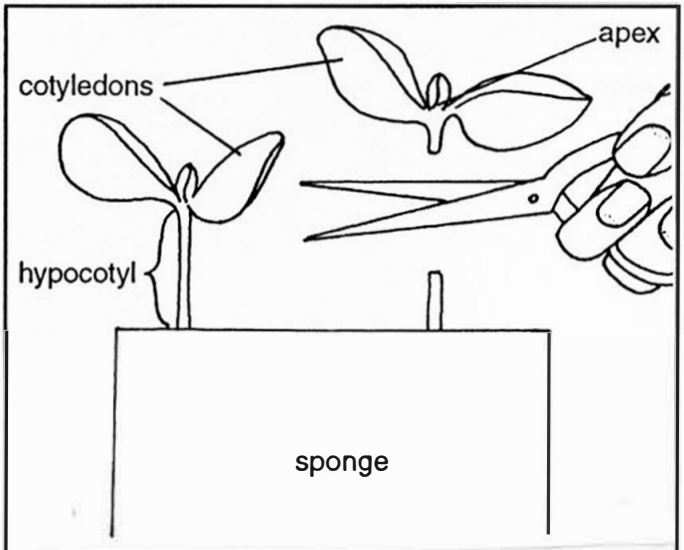
1. Sprinkle some white mustard (*S. alba*) or rapid cycling brassica seeds (*B. rapa*) on a damp sponge. Germinate them in a warm, light place until the cotyledons have just started to unfold.



2. Weigh out 2.5 g of agar and add to 250 cm^3 of distilled water. Heat and stir until the agar dissolves



3. Before the dissolved agar cools, pour the solution into several test tubes, so that there is about 2 cm of agar in the bottom of each. Allow the agar to cool and solidify in each tube.

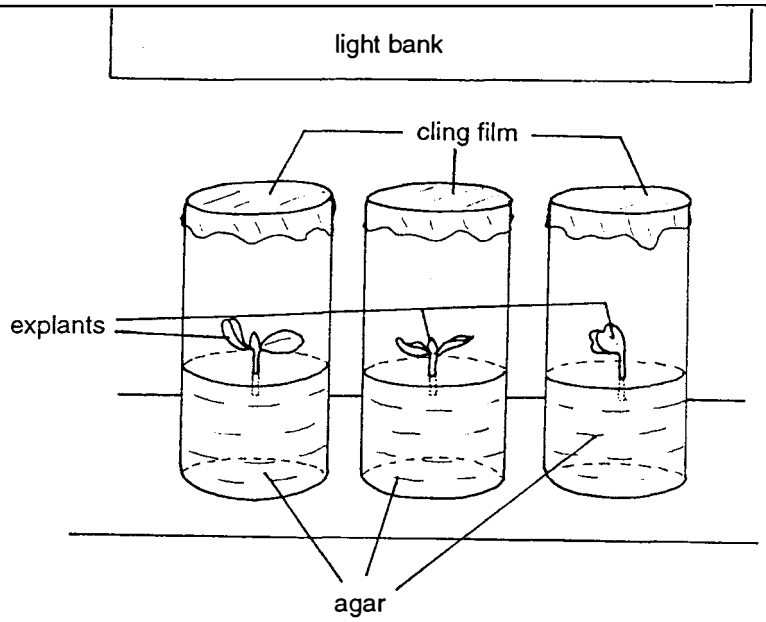


4. Cut off the tops of all your seedlings. Make sure that you decapitate them just below the shoot apex, as shown. These tops are now your 'explants'. You can discard the rest.

5. Put one explant in each test tube. Push the cut end of the shoot into the agar - but make sure that the cotyledons are not touching the surface.

Stopper the tubes with a clear lid (or use cling film), and place the tubes in a rack under a light bank or on the windowsill. Observe daily.

N.B. do not open the tubes again from now on.



Questions/further work:

1. What, if anything, would you expect the seedling explants to obtain from the agar?
2. Short-necked test tubes or McCartney bottles are ideal for this experiment. If you use long test tubes you should pour more agar into each tube than is suggested in step 3. Why is this?
3. Why should you cover the tubes with a transparent lid e.g. clingfilm?
4. Why should you not open the tubes again once you have set them up?
5. It is always best to try and obtain quantitative data from experimental work. Suggest what measurements can be made as you watch the explants grow.
6. Suggest why the cotyledon/apex explants can continue to grow when they are isolated from the rest of the seedling with so little nutritional support from the agar medium.
7. Compare your results in this experiment with those obtained from growing the shoot apex and/or isolated cotyledons on their own. What further information would you gain by doing this?

With acknowledgements to Mark Hanley-Browne, Charterhouse School, Dr Mick Fuller, University of Plymouth and Fran Fuller, South Devon College.

Science &Plants for Schools

Fast tissue culture: Teachers' Guide

Plant tissue culture refers to the growth of individual cells, tissues or, as in this case, organs on an artificial medium. The aseptic techniques which are usually associated with tissue culture experiments are not necessary here. This is because the explants will continue to grow on agar alone. Serious bacterial or fungal contamination is unlikely because the medium contains no sugar and the time scale is so short.

Seedlings *of Sinapis alba* and rapid-cycling *Brassica rapa* grow very quickly. Under ideal conditions (20-26°C under a light bank), the seedlings sown in step 1 could be ready for use in step 2 within 2-3 days. New leaves should appear on explants within 2-3 days and even new roots or flower buds within 7-10 days.

Notes on the questions:

1. The agar provides water and support for the seedling explants.

2. It would be difficult to 'plant' the explants in the agar at the bottom of longer tubes.

3. Maintain humidity/prevent moisture loss; prevent contamination; allow light in, etc.

4. **Safety:** Despite the low risk of contamination, tubes should remain closed throughout in case any potentially dangerous micro-organisms start growing on the agar. All tubes should be disposed of safely at the end of the experiment, preferably by placing in an autoclave at 120°C and 15 p.s.i. for 15 minutes.

5. The time taken for each explant to start growing a new leaf, new roots, or develop flower buds, can be recorded. A new leaf/root can be defined by size/length (e.g. record a new leaf only when it reaches 3 mm long). The percentage of explants showing a response (there is usually a high degree of variability) can be recorded. Large sample sizes should enable students to show statistically significant differences between various treatments.

6. The cotyledons are photosynthetic organs and contain their own stores of nutrients. Only one cotyledon attached to the shoot apex is sufficient to ensure continued growth without adding nutrients to the medium.

7. Perhaps some indication of the extent to which development of the explants is dependent on the presence of apex, hypocotyl or cotyledons.

Ideas for investigation: e.g. for Sc1 investigations at GCSE, or A-level projects.

1. Vary the growth conditions e.g. light/dark, different wavelengths of light, different temperatures. Compare material from different species.

2. Remove one or both cotyledons and compare growth with that of complete explants. Try growing isolated cotyledons only.

3. Explant response is enhanced by the addition of nutrients to the agar. Ready-prepared mixes, e.g. Murashige and Skoog, are available from Philip Harris, Sigma etc. Ask for 'MS salts - without added sugar'. The plasticity of explants grown on a range of media throws light on plant development and plant repair mechanisms.

Acknowledgements: Mark Hanley-Browne, Charterhouse School, Dr Mick Fuller, University of Plymouth and Fran Fuller, South Devon College. The work on the protocol described here was carried out with the help of Dr David Hanke of the Dept. of Plant Sciences, University Of Cambridge.