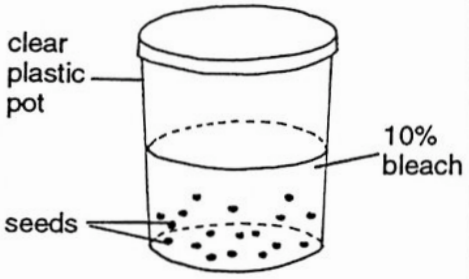
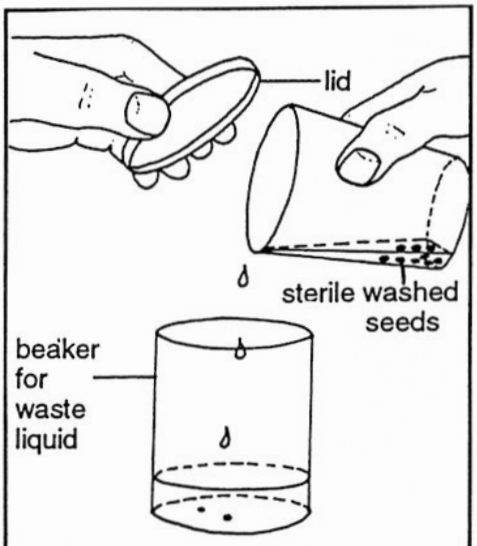
Plant tissue culture 2: the effect of sugar on the growth of root explants

Read these instructions carefully before you start.

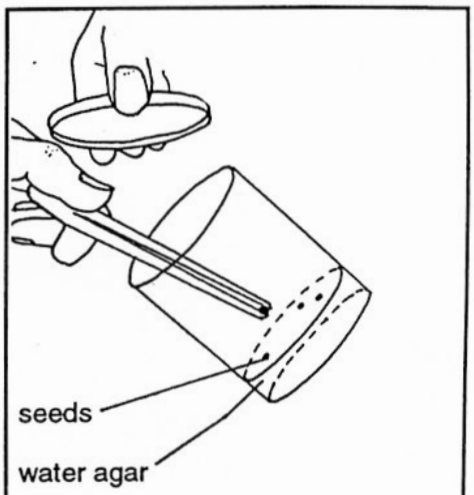
⚠ **Safety: wear eye protection when handling bleach.**
Caution: spilt bleach will affect clothing.



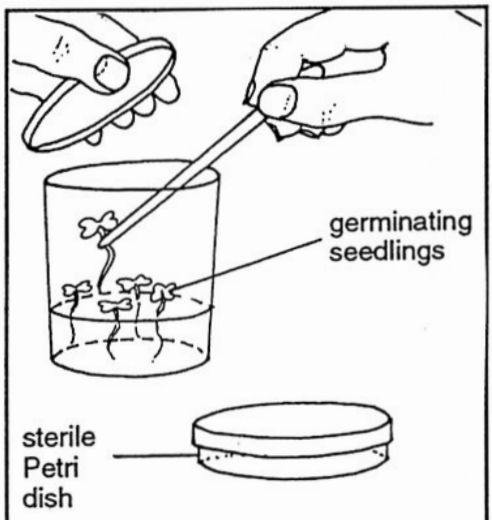
1. Wash white mustard seeds in a small volume of 10% bleach for 10 - 15 minutes.



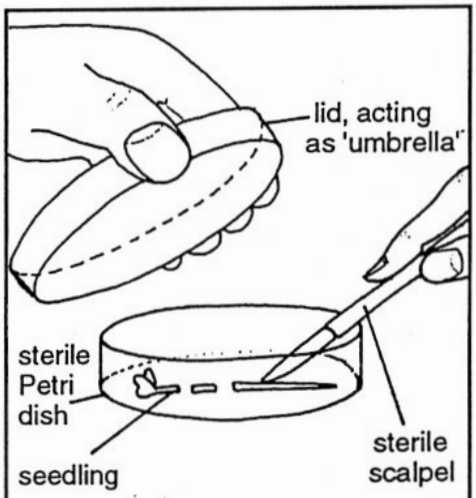
2. Pour off bleach and rinse three times with sterile distilled or boiled water. Leave seeds covered with a little sterile water. Success will depend on keeping the seeds and seedlings sterile.



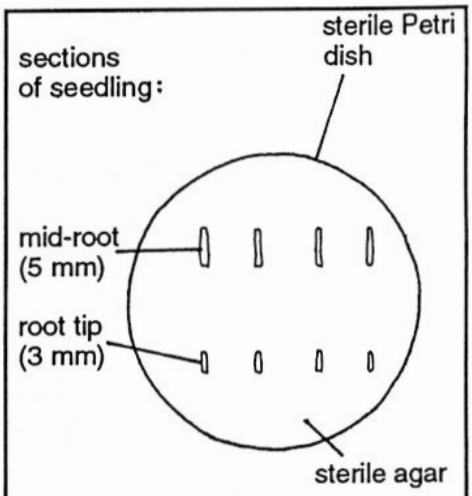
3. Using sterile forceps remove some surface-sterilised seeds from the plastic pot and place them on the surface of the water agar in a sterile plastic pot. Hold the lid over the pot to reduce accidental contamination.



4. Four days later remove the seedlings using sterile forceps and place in a sterile Petri dish. Discard any seedlings that touch other surfaces during transfer.



5. Cut 3 - 5 mm sections of mid-roots and root tips with a sterile scalpel. Hold the Petri dish lid over the base to reduce the risk of contamination by fungal spores.



6. Place the sections on the surface of the agar in a sterile agar plate using sterile forceps. Seal the sides of the Petri dish with sellotape. Examine daily.

Instructions continued:

7. Make sure that you label your plates carefully and show clearly the positions of your different explant tissues.

8. These plates will be incubated in a warm dark cupboard or incubator at approximately 20 - 25°C.

* * * * * **Important information for students** * * * * *

- **The aim is to examine the growth of root explants over a 7-day period. The addition of sugar to the medium increases the risk of fungal and bacterial contamination unless you take precautions to keep your explants and apparatus sterile. The methods outlined will give adequate sterility if the instructions are followed carefully.**

- **Metal instruments can be sterilised by dipping in ethanol and flaming them in the Bunsen flame. Make sure they have cooled down before handling living tissue, including seeds.**

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Questions/ Further work:

Q1. Why do you need to label the explant parts on your plates?

Q2. Suggest why sugar is added to the agar medium

Q3. Your explants may be kept either in the light or in the dark. Suggest why these can be kept in the dark? What do you think would happen if you kept them in the light?

Q4. Do the root tips and mid-root explants behave differently in culture? Suggest a reason for your answers.

Q5. Do root hairs develop on your explants? If so, describe where they occur.

Q6. In this investigation:

a) What is the dependent variable?
b) What is the independent variable?
c) Make a list of all the control variables which you can identify.

This practical can be used as the basis of further investigations based on the list you have made in Q6 c).

Acknowledgements to: Mark Hanley-Browne (Charterhouse School), Mick Fuller, (University of Plymouth) and Fran Fuller (South Devon College). Artwork by Linda Gray.

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Technical Notes for Student Sheet 13.

This is more demanding than the protocol outlined in *Osmosis* 10, and is more suitable for post-16 students. Some previous knowledge of aseptic technique would be an advantage.

Practical points for setting up:

1. All media and containers must be sterile. For sterilisation of media, water and glassware we recommend autoclaving for 15 mins at 121°C and 15psi. However sufficiently sterile water can be obtained by boiling distilled water in a beaker covered with foil for 15 mins and leaving it to cool.

2. To prepare the germination pots add appropriate amounts of 1% water agar and then autoclave. Baby food jars are excellent for this. Reusable transparent caps or closures for them can be bought from Sigma Chemicals. Alternatively, sterile clear pots can be bought and sterile agar can be poured into them.

3. Use four sterile media which contain 0%, 0.5%, 1.0% and 3% sucrose to investigate the effects of sucrose on root growth. Sterile Petri dishes may be bought from the manufacturers. NB 1% sucrose agar can be made by dissolving 1g sucrose in 100ml of water, adding 1g of agar and autoclaving as above.

Potentially this protocol will generate a great deal of quantitative information for students to handle, and could form the basis of highly original projects. For example, students could measure the increase in root length each day to see if the increase is linear or exponential. They could count the number of lateral roots produced by mid root explants over time, correlating this to the length of the explant.

To simplify this protocol each student could use and collect data from a single explant type on different media or look at the two explants on a single medium. Combining the results would give a comprehensive set of class data.

Safety

1. All plates must be sealed after Step 5 and not re-opened. Dispose of all plates by autoclaving as above.

2. Make sure that the ethanol container is kept away from naked flames and have a glass lid readily accessible to cover the container in case of accidental fires.

Acknowledgements to: Mark Hanley-Browne (Charterhouse School), Mick Fuller (University of Plymouth) and Fran Fuller (South Devon College).