PROJECT 4-28 GROWTH OF ISOLATED ROOTS IN STERILE CULTURE

PROBLEM

Various parts of a plant are normally linked together – leaves, stems, roots, etc. but by growing the parts separately under sterile conditions it is possible to find out if each can grow independently, or whether it is dependent on material passed from other parts of the plant. You might like to try and get tomato roots to grow cut off from the top of the plant.

INFORMATION

1. Read Section G on sterile techniques at home.

2. Tomato cultivar Grosse Lisse is a good plant to start with as seeds germinate easily and isolated roots grow well.

3. Prepare sterile Petri dishes each with 3-4 pieces of sterile filter paper in the bottom or with a watch glass wrapped in filter paper (Project 4-18). Add enough sterile water to wet the paper.

4. Sterilize seed by placing in a sterile jar and adding about 100 mL undiluted Miltons and one small drop of detergent. Leave for 35-40 minutes swirling gently about once a minute. Pour off Miltons and shake the seeds in three lots of sterile water. This sounds easy but the seeds escape when you try to pour off the solutions. While you sterilize the seeds also sterilize in undiluted Miltons, the bowl of a small plastic tea strainer and use this to catch the seeds.

5. Using sterile forceps transfer good looking seeds to Petri dishes (about 15 per dish), discard broken and abnormal ones. Seal edges with Gladwrap and place in the dark. They germinate within a week or so.

6. When roots are about 1½ cm long, using sterile instruments cut off the 1 cm tip and place one in each flask of liquid medium. Take precautions when you do this – don't leave the flask or Petri dish wide open to the air and contamination.

7. The medium in which you grow the roots is made up of minerals which the plant normally get from the soil, vitamins and sugar which are normally transported from the leave.

8. You can make up your own medium using the recipe given by Butcher and Ingram but it is complicated to prepare. You can buy it in powder form from Medos Scientific Supplies, 90 Goodwood Parade, Rivervale W.A. You require a Gibco medium – "Murashige and Skoog salts with organics including sugar but without agar". This is about $4.50 for 1 L.

9. Dispense medium 50 mL per 100 mL flask. Insert cotton wool plugs and cover tops with alfoil. Sterilize in pressure cooker and store in refrigerator until ready to use.

10. All glassware should be Pyrex and well washed and rinsed in two changes of distilled water before use.

11. Cultures grow best in the dark at 25° C. Not all roots grow well. After 10 days select the best ones and transfer them to a sterile Petri dish with some of the liquid medium. Cut into pieces as shown in diagram below and subculture into new media. Pieces grow well for a number of subcultures.

DESIGN OF EXPERIMENT

1. Even to get the initial cultures growing well is an achievement but having mastered this you might like to investigate which tomato cultivars grow best, or to try using other plants.

2. You could also compare growth in the complete medium compared with growth in distilled water, or water with 20% sucrose. If you are preparing your own media you could investigate the effect of different levels of sucrose, or of leaving out the vitamins.

3. How are you going to compare growth in different treatments? Total root length? Number of laterals?

4. If you are unsuccessful you should try to analyse the reason (a), is your medium contaminated (i.e. gone milky – bacteria; with floating or submerged mats of fluffy stuff – fungi) (b), did you dry out the root and kill it while cutting it off and transferring it? (c), maybe the strain of tomato you are using doesn't grow well.

5. Consider the cost of this experiment before starting. Many of your cultures will be contaminated so you may use several litres of medium.

REFERENCES

Butcher, D.N. and Ingram, D.S. (1976). Plant Tissue Culture (Studies in Biology No. 65). (Edward Arnold : London).

deFossard, R.A. (1979). Tissue Culture for Plant Propagators (University of New England Press : available from Department of Continuing Education, U.N>E. Armidale, N.S.W. 2351, $15).