PROJECT 4-29 PLANT CALLUS CULTURE

## PROBLEM

As a plant grows the cells in various organs mature and stop dividing. However, under certain conditions such as wounding, some cells can be stimulated to divide and form a mass of disorganised (= undifferentiated) cells called a callus. This can be used in studies of plant biochemistry, mutation, regeneration of shoots and roots from the callus and plant breeding. You might attempt to grow a callus from the stems or roots of some plants in sterile culture.

## INFORMATION

1. Carrot root is a good tissue to start with. The method involves sterilizing the outside of the carrot then cutting out small pieces and placing them on a medium similar to that used for isolated roots (Project 4-28) but also containing hormones that cause cells, particularly parenchyma and cambial regions to start dividing.

2. Select a carrot about 1-1½ cm diam. home grown ones are best. Wash gently. Cut into 4 cm segments discarding top and bottom. Place in sterile jar in undiluted Miltons for 30 minutes or 5% sodium hypochlorite for 10 minutes, swirling every minute or so. Using sterile forceps transfer to three changes of sterile water in sterile jars. Place on sterile Petri dish. Make a shallow cut about 1 cm from each end then break (rather than cut) off the ends and discard. Cut out 5 cm cubes from the cambium region and place these horizontally, half embedded in the agar medium.

3. Grow in dark at about 25° C. Within two weeks you should see a callus developing and at six weeks the callus should have developed to a size that you can cut up and subculture.

4. You may wish to examine the effects of leaving out the auxin or the cytokinin, or both, and of culturing the callus in the light.

5. You may wish to extend your experiments to other tissues. Choose material that is thick and solid and likely to be sterile inside e.g. potato, artichoke, turnip.

6. You can prepare your own medium using the recipe given in Butcher and Ingram or you can buy one that only needs the hormones added. Gibco media are supplied in W.A. by Medos Scientific Suppliers (90 Goodwood Parade, Rivervale). A suitable medium for callus would be their "Murashige and Skoog salts with organics and agar". This is about $5 for enough for 1 litre. You will still need to add the hormones. Buying pure hormones is expensive but they are also available from Medos.

   Auxin

   Buy 2,4-D (= 2, 4-dichlorophenoxyacetic acid). Make up a solution of 0.04 g in 100 mL by first dissolving the powder in a 2-3 mL of absolute alcohol then pouring in all the water at once. Store in the refrigerator and use 5 mL per 1L of medium to give you a final concentration of 2 $mgL^{-1}$ which is about 9 µM.

   The cytokinin to use is Kinetin =(6-Furfurylaminopurine).

   Make up a solution of 0.04 g in 100 mL by first dissolving the powder in 2-3 mL of IN HC1 then pouring in the water all at once. Store in refrigerator. If it precipitates out try pressure cooking for 20 minutes. Add 5 mL to 1 L of medium to give you a final concentration of 2 mg $L^{-1}$ which is about 9 µM.

## DESIGN OF EXPERIMENT

1. Why do you need to sterilize the outside of the carrot and use sterile instruments to cut out your segments?

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2. How might you measure the growth of the callus.

3. Which cells in your segment do you think give rise to the callus cells?

4. What effect are the hormones in the medium having on the tissue?

5. Think about whether you can afford to do this project as while you get 100 tubes each of 10 ml media from the 1 L many of your initial cultures will be contaminated and you will use several litres of medium.

REFERENCES

As for Project 4-28

Weier, T.E. Stocking, C.R. and Barbour, M.G. (1974). Botany an Introduction of Plant Biology (5th ed.). (Wiley : New York). Chp. 20.