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PLANT REGENERATION

An important aspect of plant genetic engineering is the introduction of genes into plant cells and recovery of transgenic plants. Such modified cells must be competent to undergo dedifferentiation, cell division and the organization of organ structures. Although adventitious organs often arise on intact plants, the most common technique in plant genetic manipulation is the introduction of genes into explants, followed by *in vitro* culture on a medium for organ regeneration. Explants from leaf, stem, root, petiole, immature embryos, hypocotyls, cotyledons, microspore or thin cell layers are all utilized in this technique. Any tissue with living cells capable of dedifferentiation can serve as an explant.

Under appropriate culture conditions such explants, or cultured cells and tissues, will organize embryonic structures or primordia which can develop into shoots, roots, flowers or embryos. In many cases these structures have a single cell origin. The term of totipotency is used to describe cells which are capable of sustained cell division and organization of an intact plant or plant organ. The earliest demonstration of cell totipotency was due to the work of Reinert 1958a and Steward et al. 1958. They demonstrated the development of carrot plants from cultured cells by the process of embryogenesis. The developmental pattern recapitulated all the stages characteristic of zygotic embryogenesis.

The regeneration of plants through organogenesis, (organization of monopolar structures) was demonstrated by White in (1939) and conditions for such regeneration from tobacco callus *Nicotiana tabacum* L., were defined by Skoog and Miller in (1957). At least in this species auxins and cytokinins regulate organ differentiation. High levels of auxin relative to cytokinins promoted root differentiation, while the reverse favored shoot development. In addition, direct differentiation of flower buds from epidermal cell layers of *N. tabacum* and other species was demonstrated, Tran Thanh van K. 1973.

These examples clearly demonstrate the developmental plasticity of cells in tobacco explants or callus. The discovery of the relationship between *in vitro* plant morphogenesis and plant hormones and techniques for the *in vitro* culture of plant cells and explants are important factors in the advances made in plant genetic engineering.

Objective

The objective of this exercise is to examine the relationship between auxins and cytokinins and organ regeneration in leaf explants of *Nicotiana tabacum* L. cv Wisconsin #38.

Procedure

Culture medium: You will be provided with culture medium containing the following hormone combinations. This is the same as the Co-cultivation media from the previous lab, without BAP, NAA or acetosyringone.

A	0.5 mg/l 2,4-D
B	2.0 mg/l BAP
C	2.0 mg/l BAP + 0.5 mg/l 2,4-D
D	2.0 mg/l 2,4-D + 0.5 mg/l BAP
E	Control (no hormones)

Plant Material:

Sterilize the leaf material provided by first washing in distilled water for 1-2 minutes; then immersing in 70% ethanol for 1 minute, with occasional agitation. Decant the ethanol and rinse the tissue x 2 with distilled water. Immerse the tissue in 20% Javex containing 0.02% Tween 20 and place the container in the flow chamber. Agitate occasionally. After 20 minutes decant the liquid under the flow chamber and rinse x 3 with sterile distilled water. These operations must be performed carefully to avoid contamination. Once the material is placed in chlorox all operations must be in the flow chamber.

NOTE: No antibiotics will be included in this medium; cf Exercise #1. With sterile forceps transfer the tissue to sterile petri plates and with a sharp scalpel remove all surfaces that are damaged during sterilization. Cut the leaf into strips and discard the midrib. Cut remainder of the lamina into small segments. With a sterile forceps transfer 4-5 segments to each type of agar plate. Label as leaf explants and seal the dishes with parafilm. Place the cultures in a cupboard at room temperature.

Observations

Your job is to observe the cultures weekly. Determine if there is bacterial or fungal contamination. What is the source of contamination?

Determine the pattern of growth for each treatment. Record the time of organ initiation and the number of organs per explant. Examine the pattern of callus development in each treatment and compare the callus type (friable or compact).

Record the effect of auxin and cytokinins on organ initiation. Would you expect the hormone requirement for organ induction to vary with species and genotype? Explain.

What are some of the cellular changes which are associated with callus formation from specialized parenchyma cell?

From your observations, is callus development necessary for organ initiation? In terms of genetic manipulation what do you think would be the advantage of enhanced callusing prior to organ regeneration?

References

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