average, 5·27 per cent more seeds, although the difference is not statistically significant.

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[1] Satyabalan, K., Ninan, C. A., and Krishna Marar, M. M., *Nature*, **202**, 927 (1964).
[2] Davis, T. A., *J. Genet.*, **58**, 42 (1962).
[3] Davis, T. A., *Experientia*, **18**, 321 (1962).
[4] Davis, T. A., *J. Genet.*, **58**, 126 (1963).
[5] Petch, T., *Ann. Roy. Bot. Gard. Peradeniya*, **5**, 538 (1911).
[6] Reber, G., *Castanea*, **25**, 122 (1960).
[7] Reber, G., *J. Genet.*, **59** (1964).
[8] Davis, T. A., *Nature*, **201**, 515 (1964).
[9] Davis, T. A., and Selvaraj, C., *J. Bombay Nat. Hist. Soc.*, **61** (1964).

In vitro Production of Embryos from Anthers of *Datura*

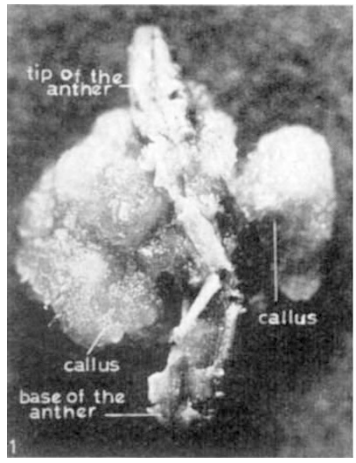
Observations of natural phenomena such as adventive embryony and production of plantlets from the leaf margins of *Bryophyllum* support the view that although cells may differentiate and appear to behave differently, their genetic potentialities remain the same. As a corollary to this view, differentiation operates not through a segregation mechanism but due to selective and programmed suppression or stimulation of genic activity.

In recent years this view has been greatly strengthened by the use of *in vitro* culture technique, and the regeneration of perfectly normal plantlets from mature tissues of the stem and root has been demonstrated even in cases where such a phenomenon has not been observed in Nature. Thus buds differentiate under suitable treatment from tobacco pith callus[1], from stem callus of potato[2] and horseradish (*Armoracia*)[2,3], from root callus of *Isatis*[4], *Convolvulus*[5] and carrot[6,7], from bud scales of *Lilium*[8] and from embryonal callus of *Cuscuta*[9] and other plants. In several other instances embryo-like microscopic structures have been reported from callus masses, although their further development into plantlets has not been observed[10,11]. To the best of our knowledge, however, thus far there has been no report of the origin of embryos from anthers. The observation which we report here was made during attempts to grow callus from *Datura* anthers for an examination of cell multiplication in liquid suspensions.

Anthers of *Datura innoxia* Mill. growing wild in the university campus were sterilized with chlorine–water and planted in various nutrient media. The basic nutrient medium comprised Nitsch's or White's minerals and 2 per cent sucrose. This was supplemented with various growth substances such as indolyl-3-acetic acid (IAA, 10^{-6} M), kinetin (10^{-6} M), casein hydrolysate (1,000 p.p.m.), yeast extract (1,000 p.p.m.) or fruit juices like coconut milk (15 per cent), cherry juice (10 per cent), grape juice (10 per cent), litchi juice (10 per cent), pineapple juice (10 per cent) and plum juice (10 per cent) singly or in combination.

The inoculated anthers varied in maturity from the archesporial stage to mature pollen grains. However, the younger anthers did not grow in any of the media and eventually dried up. Those inoculated at the pollen grain stage showed vigorous proliferation on media containing 10^{-6} M indolyl-3-acetic acid and 10^{-6} M kinetin. The proliferations seen in Fig. 1 consisted of warty outgrowths, about 1 or 2 mm in diameter, sometimes arising all around the anther. In media containing both yeast extract and casein hydrolysate, proliferation occurred to a much lesser extent. All the proliferated masses grew satisfactorily on sub-culturing and have now been maintained for about 12 weeks.

Curious behaviour was observed in anthers implanted in media containing (1) casein hydrolysate (1,000 p.p.m. + 10^{-6} M IAA + 10^{-6} M kinetin, (2) coconut milk, (3) grape juice and (4) plum juice. For a long time these anthers showed no change at all and after 6–7 weeks the cultures were about to be discarded. Then, suddenly, numerous embryo-like structures projected out of the anther from all sides (Fig. 2). The embryos possessed root-shoot axes and cotyledons. Moreover, they were green and looked like perfectly normal embryos, with the only difference that most of them were polycotyledonous with as many as 10 cotyledons. Dicotyledonous embryos were also present, but in much smaller numbers. Many of these embryos have developed into normal seedlings. The effectiveness of different media in producing embryos varies. On media containing coconut milk and plum juice almost all the anthers showed the production of embryos. In the remaining two media only about 50 per cent of the anthers did so. In the basic medium, without added growth substances or extracts, no embryos were formed. Most of the embryos appear to be located inside the pollen sacs and are free-floating. They may have arisen from the pollen grains or from the connective tissue of the anther. Further work is in progress to ascertain this point.



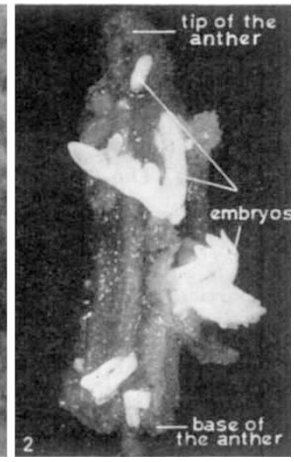


Fig. 1. Anther showing proliferation on basic medium containing IAA 10^{-6} M and kinetin 10^{-6} M
Fig. 2. Anther showing embryos from all sides on basic medium containing 15 per cent coconut milk

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[1] Skoog, F., and Miller, C. O., *Symp. Soc. Exp. Biol.*, **11**, 118 (1957).
[2] Wurm, G., *Flora*, **149**, 43 (1959).
[3] Sastri, R. L. N., in *Plant Tissue and Organ Culture—a Symposium*, edit. by Maheshwari, P., and Rangaswamy, N. S. (Intern. Soc. Plant. Morph., Delhi, 1962).
[4] Danckwardt-Lillieström, C., *Physiol. Plantarum*, **10**, 794 (1957).
[5] Torrey, J. G., *Plant Physiol.*, **33**, 258 (1958).
[6] Wiggans, S. C., *Amer. J. Bot.*, **41**, 321 (1954).
[7] Steward, F. C., *Amer. J. Bot.*, **45**, 709 (1958).
[8] Robb, Sheila M., *J. Exp. Bot.*, **8**, 348 (1957).
[9] Maheshwari, P., and Beldev, B., *Nature*, **191**, 197 (1961).
[10] Wadhi, Mridul, and Mohan Ram, H. Y., *Phyton*, **21**, 143 (1964).
[11] Konar, R. N., *Phytomorphology*, **13**, 165 (1963).

Agar-diffusion Technique for estimating Gibberellin Production by Plant Organs

So far, all published results relating to the gibberellin content of plants, or plant organs, have been obtained by various gibberellin-extraction techniques. This procedure is useful in the determination of the gibberellin content at any point in time, but it is often desirable to be able to estimate the rate of gibberellin production by various plant organs. In the course of our work on the gibberellin relationships of organs in the sunflower plant (*Helianthus*