In vitro Culture of Excised Ovules of Papaver somniferum

Although the embryo (*1*), the endosperm (*2*), and the fruit (*3*) of angiosperms have been investigated by the culture technique, the young ovule, as far as I am aware (*4*), has not been reared to maturity under artificial conditions. The present report on *Papaver somniferum* outlines a technique for the successful growth in culture of ovules and also summarizes the results of some experiments with kinetin and indoleacetic acid on their development and maturation.

Young capsules of *Papaver* were excised from the plant and surface-sterilized by dipping in 90 percent alcohol and flaming. The capsule was then cut open, and a portion of it was fixed at once; from the remaining portion, ovules (*5*) were scooped out with a sterile scalpel and planted in Nitsch's medium (*3*) supplemented with 5 ml/lit of a mixture of vitamins (*6*). This served as the basic medium. In some cases the culture medium was supplemented with kinetin, which has recently been reported (*7*) to be extremely potent in inducing cytokinesis.

In the basic medium the ovules grew to maturity in 23 days, starting from the two-celled stage of the proembryo. In nature the time interval between pollination and maturation of ovules is shorter and usually extends to about 18 days. Ovules containing a two-celled proembryo (Fig. 1A) and free-nuclear endosperm were cultured on a medium containing kinetin (0.4 mg/lit) and indoleacetic acid (5 mg/lit). Ovules collected 9 days after planting showed globular embryos and cellular endosperm (Fig. 1B), with food reserves similar to those in naturally ripening seeds. From the same lot, 23-day-old cultures showed completely mature seeds, some of which had germinated *in situ* to produce seedlings (Fig. 1C) which were quite normal except that the radicle and root tip showed localized callusing.

A few interesting abnormalities were met with. In 9-day-old cultures some of the embryos showed lobes in which active meristems were present. Still other embryos completely lacked differentiation but showed radial rows of meristematic cells toward the margin (Fig. 1D). In one embryo the suspensor, which is normally a linear row of three or four cells, showed the appearance of a globular mass. In all these cases the endosperm was either arrested at the free-nuclear stage or had degenerated after reaching the cellular stage.

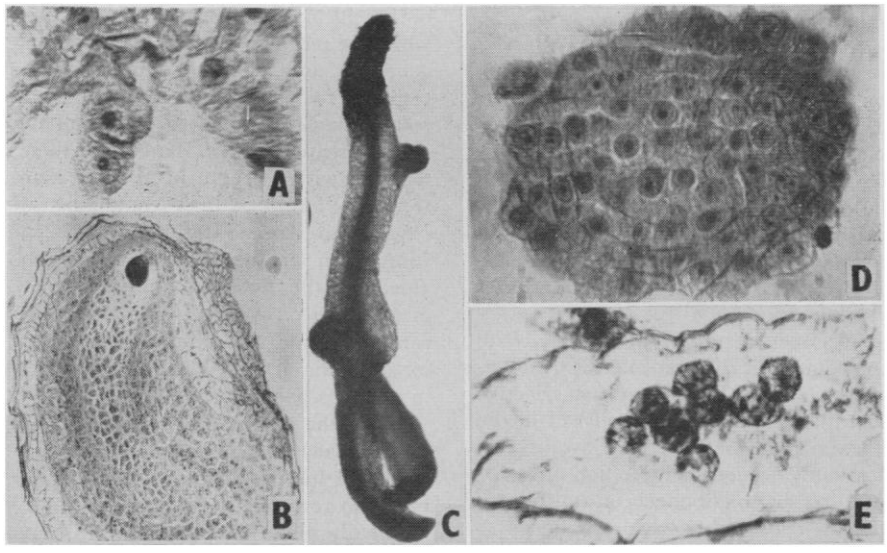

Fig. 1. Results obtained with ovules of *Papaver somniferum* cultured in Nitsch's medium supplemented with kinetin (0.4 mg/lit) plus indoleacetic acid (5 mg/lit). (*A*) Two-celled proembryo at the time of planting (× 107); (*B*) globular embryo and cellular endosperm as seen 9 days after planting (× 107); (*C*) seedling formed *in situ* in 23 days (× 14); (*D*) 9-day-old undifferentiated embryo with radially arranged meristematic cells at the margin (× 107); (*E*) eight nuclei in a single hypertrophied endosperm cell from a 9-day-old culture (× 107).

In kinetin media the endosperm showed a few other changes, like renewed divisions in apparently mature cells, accumulation of as many as nine nuclei in single hypertrophied cells (Fig. 1E), and formation of patches of meristematic cells around enormously enlarged endosperm cells. Frequently mature embryos having three cotyledons or two unequal cotyledons were encountered in kinetin media. It may be noted that recently Haccius (*8*) induced twinning of cotyledons in seeds of *Eranthis hiemalis* by treatment with 2,4-dichlorophenoxyacetic acid, α-naphthalene acetic acid, and 2,4,5-trichlorophenoxyacetic acid.

The results obtained support the view of Miller *et al.* (*7*) that kinetin is a factor in cell division. The radial rows of meristematic cells seen at the periphery of late globular embryos (Fig. 1D) as well as the renewed divisions in apparently mature cells of the endosperm appear to have been induced by kinetin.

Ovule culture provides a new tool for the study of seed physiology. While emphasis has hitherto been laid on the artificial culture of excised embryos, the present study shows that entire ovules, too, are capable of being grown to maturity. In the culture of embryos it has not proved feasible to dissect out very early stages without damaging them. The culturing of ovules involves no such difficulty. Further, it may be possible, by suitable modifications of the technique and by adding various chemicals to the culture medium, to cause the formation of adventive embryos and even the induction of parthenogenesis and apogamy (*9*).

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References and Notes

1. J. Rappaport, *Botan. Rev.* **20**, 201 (1954).
2. E. P. Sternheimer, *Bull. Torrey Botan. Club* **81**, 111 (1954).
3. J. P. Nitsch, *Am. J. Botany* **38**, 566 (1951).
4. There is a very brief and preliminary report by C. L. Withner [*Am. Orchid Soc. Bull.* **11**, 261 (1943)] on rearing orchid ovules in test tubes. Judging from his statement that "the older embryos seem to develop more rapidly, but there is no indication that younger ovules cannot be used as well," it appears that he took fairly old ovules for his study. C. D. La Rue [*Bull. Torrey Botan. Club* **69**, 332 (1942)] and P. R. White [*Arch. Exptl. Zellforsch. Gewebezücht* **12**, 602 (1932)] secured callus in cultures of snapdragon ovules. In *Erythronium americanum* it is reported that the ovules grow to 4 times their original size, but nothing further is mentioned by La Rue [*Bull. Torrey Botan. Club* **69**, 332 (1942)]
5. The ovules in any one capsule of *Papaver* are more or less at the same stage of development.
6. P. R. White, *Cultivation of Animal and Plant Cells* (London, 1954).
7. C. O. Miller *et al.*, *J. Am. Chem. Soc.* **78**, 1375 (1956).
8. B. Haccius, *Nature* **176**, 355 (1955).
9. This work was done as part of a research project on "Chemical stimulation of ovule," financed by the Indian Council of Agricultural Research. I am indebted to Professor P. Maheshwari, under whose guidance this research was conducted. I am also grateful to Dr. R. C. Sachar for active cooperation in the work and to I. K. Vasil, Manohar Lal, and S. C. Maheshwari for helpful criticism.

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