

Induction of Haploid Plants from Anther Culture in *Datura ferox* L.

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Anthers of *Datura ferox* L. were cultured *in vitro*. Androgenic embryoids were formed in Nitsch's medium supplemented with coconut milk (15% v/v) in 35-45 days after inoculation. The stages in the development of androgenic embryoids followed more or less the same pattern as that of seed embryos. The plantlets that developed from anthers were all haploid ($2n=12$).

Since Guha and Maheshwari (1964) reported a method for the production of haploid embryos from *Datura* pollen, induction of haploid plants from anther culture has been achieved with varying degrees of success in different genera. The species of *Datura* have responded favourably for experimental androgenesis as evidenced from the induction of haploids in *D. innoxia*, *D. stramonium* (Guha and Maheshwari 1966; 1967) *D. metel* (Narayanaswamy and Chandy, 1971), and *D. meteloides* and *D. wrightii* (Kohlenbach and Geier, 1972). The development of haploid plants from anther culture in *D. ferox* L. is reported here.

MATERIALS AND METHODS

Immature anthers excised from young floral buds were sterilized with 1% calcium hypochlorite solution and planted on Nitsch's basal medium supplemented with coconut milk (15% V/V) and solidified with 0.9 per cent Difco agar. Sucrose (2%) was used as carbon source and no exogenous auxins

were added. The cultures were kept exposed to constant cool white fluorescent illumination at a temperature of $25 \pm 1^\circ\text{C}$ and relative humidity of 60-70 per cent. Squash preparations of anthers under incubation were examined at intervals to study the early ontogeny of the pollen embryoids.

RESULTS AND DISCUSSION

Cultured anthers showed varied response. Young anthers containing microspore mother cells failed to grow and degenerated in 10-15 days after culturing. The immature anthers with pollen grains at the uninucleate stage of development increased in size considerably and in about 35-45 days, the anther wall collapsed at the line of dehiscence and small plantlets emerged. It was observed that masses of cells developed from single pollen grains and they were liberated through ruptured exine. The cells of the globular embryo presumably continued to divide irregularly for a time and eventually organised themselves into nor-

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mal torpedo shaped structures resembling the zygotic embryos. In about 6 - 8 weeks plants with well developed root and shoot systems were formed. Of the several hundred anthers cultured, only 4 - 6 per cent resulted in the development of plantlets. The number of plantlets in an anther varied from 1 - 4. In some anthers, only callus tissue was formed by the proliferation of cells of the anther wall and the connective. Acetocarmine squashes of root-tips of the plantlets confirmed them to be haploids ($2n = 12$).

The development of androgenic embryos in *D. ferox* follows more or less the same developmental sequence as observed in *D. innoxia* by Guha and Maheswari (1967). It begins with an increase in cell number and volume, the exine bursts and liberates masses of cells, which represent the globular stage of embryoids. Soon organ differentiation in the form of root, shoot and cotyledons begin to manifest followed by heart and torpedo stages of development as in zygotic embryos. In the present study, however, the

early ontogenetic sequences in the pollen embryogeny were not clear and there were indications to show the development of proembryoids by the divisions of vegetative cell alone.

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