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Haploid *Capsicum* through Experimental Androgenesis

Brief Report

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With 10 Figures

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Summary

Haploid embryos and plantlets in yet another solanaceous member viz., *Capsicum annuum* L. var. *grossum* Sendt, have been successfully reared *in vitro* through culture of young immature anthers. Exogenous auxin (IAA) was essential for the initiation of cell division in pollen oriented towards a haploid morphogenesis. Of the several hundred anthers cultured, not more than 0.1% produced viable plantlets.

1. Introduction

Induction of haploid plants from pollen grains has been realized under experimental conditions in quite a few members of both dicotyledons and monocotyledons. In particular rearing of pollen embryoids and plantlets *in vitro* and their growth to mature flowering plants on soil have been demonstrated in several solanaceous species of *Datura* (Guha and Maheshwari 1964, Narayanaswamy and Chandy 1971), *Nicotiana* (Bourgin and Nitsch 1967, Sunderland and Wicks 1971, Sunderland 1971), *Atropa* (Zenkteler 1971, Narayanaswamy and George 1972), *Solanum* (Harn 1971, Irikura and Sakaguchi 1972, Kohlenbach and Geier 1972, Zenkteler 1973), *Lycopersicon* (Gresshoff and Doy 1972, Debergh and Nitsch 1973), *Lycium* (Zenkteler 1972), and *Petunia* (Raquin and Pilet 1972). The ease with which plantlets could be produced in aseptic culture prompted us to exploit the phenomenon of pollen totipotency in the study of haploid induction in cultivated chillies. This report concerns the successful growth and development *in vitro* of haploid plants of *Capsicum annuum* L. var. *grossum* Sendt[1] ($2n = 24$), fam. *Solanaceae*, through culture of pollen grains retained within the anther.

[1] The plant was identified at the National Botanic Gardens, Lucknow, India.

2. Material and Methods

Immature anthers containing uninucleate pollen released just after the tetrad stage, were excised from young floral buds sterilized with chlorine water, and planted on LINSMAIER and SKOOG (LS) basal medium (1965). Growth supplements such as coconut milk (CM), yeast extract (YE), casein hydrolysate (CH), auxins and cytokinins were incorporated in the medium in concentrations and combinations as needed. FeEDTA was used as the source of iron and 2% sucrose as carbon source. Purified agar-agar (Indian), 0.8% was used to gel the medium. The cultures were kept exposed to constant cool-white, fluorescent illumination at a temperature of 25 ± 2 °C and relative humidity of 50–60%.

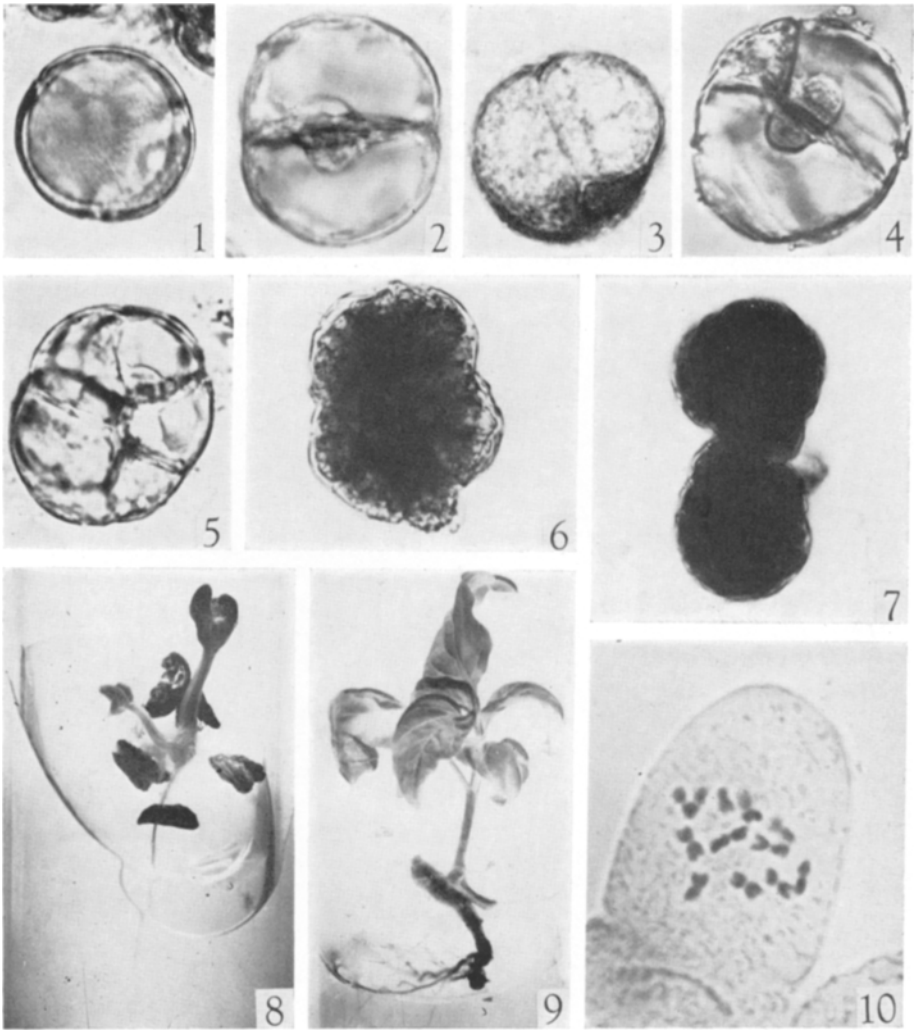
3. Results

A large number of pollen grains divided to form two daughter cells of equal size on the basal medium supplemented by CM (15%), 2,4-dichlorophenoxyacetic acid (2,4-D, 2 ppm), and kinetin (kn, 1 ppm) but failed to grow beyond the two-celled stage. On the other hand, it resulted in the proliferation of cells of the anther wall and the connective giving rise to a callus tissue. Sustained divisions in pollen grains were observed in the following media:

1. LS + kn (0.3 ppm) + IAA (3.0 ppm),
2. LS + kn (3.0 ppm) + IAA (1.0 ppm),
3. LS + CH (400 ppm) + IAA (0.5 ppm) + inositol (100 ppm).

The pollen grains may cut off a crescent-shaped diminutive generative cell closely appressed to the cell wall, which may or may not divide to form the two germ cells before degeneration sets in (Fig. 3). Subsequent divisions were initiated in the vegetative (prothallial) cell. Pollen grains that were quiescent and showing no apparent change were also observed. Occasionally, rupture of the exine of the pollen resulted in the protrusion of the intine as a balloon-shaped germ tube which might function as an embryoidal cell.

De-differentiation of pollen to form multicellular structures resembling proembryoids was observed in only 1% of the pollen grains in an anther sac. Frequent transfer of the embryoid-bearing anthers to fresh media containing CH (400 ppm), IAA (0.5 ppm), and inositol (100 ppm) was necessary to cause their further differentiation into the heart and torpedo stages of embryogeny. Of the several hundred anthers cultured *in vitro* during the period extending over a year, complete plantlets with primary root and shoot meristems bearing "cotyledonoid" leaves, were obtained in only a couple of instances. Figs. 1–8 show the ontogenetic sequences starting from a pollen grain to whole-plant morphogenesis through a series of stages reminiscent of ovular embryogeny or adventive embryony from somatic cells. The plantlets were reared on the basal medium without supplementary ingredients but retaining IAA in low concentration. In about 8 weeks a full-fledged plantlet with several leaves and primary root (Fig. 9) was ready for transfer to soil. Acetocarmine squashes of root-tips of two such plantlets that were successfully nurtured confirmed them to be haploid (Fig. 10, $2n = 12$).



Figs. 1–9. Stages in the differentiation of pollen-embryoids in *Capsicum*
Fig. 1. Normal pollen grain. ×640
Fig. 2. Pollen grain divided into equal halves. ×720
Fig. 3. Pollen grain showing degeneration of generative cell (germ cells) and division of tube cell. ×720
Fig. 4. Oblique divisions in 2-celled pollen. ×720
Fig. 5. Multicellular pollen grain. ×400
Fig. 6. Differentiating embryoid ×64
Fig. 7. Twin embryoids ×48
Fig. 8. Emergence of two plantlets from anther. ×1.2
Fig. 9. A well-developed pollen plantlet ×1
Fig. 10. Root tip cell of a plantlet showing haploid complement of chromosomes ($2n = 12$) ×1,120

4. Discussion

Exogenous auxin was essential for the initiation of cell division and sustenance of an essentially embryoidal type of growth of the pollen grain. Addition of myo-inositol and a source of reduced nitrogen in the form of casein hydrolysate was conducive for differentiation of the proembryoids into fullterm mature embryos which could germinate to form plantlets. Coconut milk was non-essential and when present in the medium with IAA, caused the proliferation of cells of the anther wall to form a compact callus mass.

Haploid morphogenesis in *Capsicum* has been realized for the first time although the production of haploid individuals has been very low, being only one in a thousand anthers cultured. The possibilities of augmenting the yield are being explored.

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