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Haploid Embryogenesis in Anther Cultures of Pigeon-Pea (*Cajanus cajan*)

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Summary. Pollen embryogenesis and callus showing a wide range of ploidy is induced in the in vitro cultured anthers of pigeon-pea. A suspension of pollen from such anthers incubated in drop cultures on agar medium develops further to form embryoids and colonies of callus.

Key words: Haploids – Anther culture – Grain-legumes

There is a paucity of information on the induction of androgenesis and the differentiation of complete plants from callus tissue cultures of grain-legumes, which, in fact, is holding back progress in the utilization of in vitro techniques to improve these crops. In view of the potential significance of somatic cell genetics and haploids (Reinert and Bajaj 1977, Wenzel 1980) in legume improvement programs, the present investigation has been undertaken to augment the basic information on various aspects of the establishment of haploid cultures of pigeon-pea, a grain-legume rich in dietary protein.

Anthers containing uninucleate pollen, aseptically excised from the flower-buds of field-grown plants of pigeon-pea (*Cajanus cajan* (L.) Millsp. cv. 'T 21'), were cultured on agar solidified Murashige and Skoog's medium (Murashige and Skoog 1962) supplemented with IAA (4 mg/l) + kinetin (2 mg/l), and maintained at 23-26°C in diffused light. A suspension of pollen prepared from 3-week-old cultured anthers was incubated in drops (0.1 ml) on the agar medium. For cytological studies, the androgenic pollen and callus cells were pre-treated with L-bromonaphthalene, fixed in acetic alcohol, and observed after staining with Feulgen and acetocarmine.

The anthers showed signs of growth within 4 days, and about 36% of them proliferated to form a mass of callus within 3 weeks (Fig. 5). The pollen underwent repeated nuclear (Figs. 2, 3) and cellular divisions to form embryoids (Fig. 4); however, the frequency of androgenesis was low (Table 1). In a parallel study on another grain-legume (*Phaseolus aureus*), almost a similar pattern of the induction of androgenesis, and callus formation was observed (Bajaj and Singh 1980).

A suspension of pollen obtained from 2-5 week-old cultured anthers resumed growth and formed colonies of callus (Figs. 6, 8) or embryoids (Fig. 7), when transferred in drops to the agar medium.

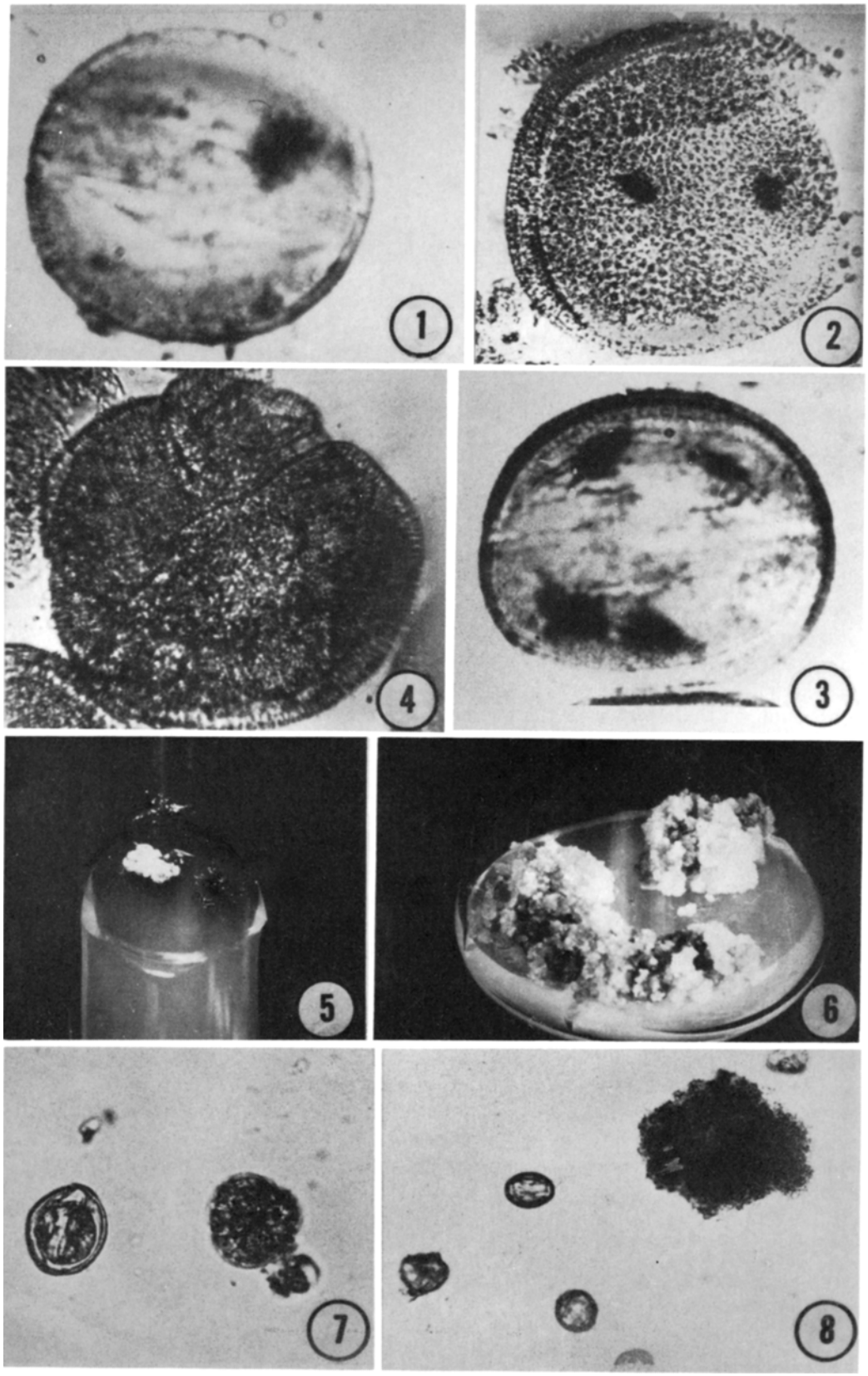
The anther-derived callus was mixoploid, with a wide range of genetic variability (Fig. 9). The chromosome number varied from 8 (n = 11) to 28 (2n = 22). The callus contained 50.8% diploids and 17.4% haploids, with the rest (32%) being aneuploids.

The reproducible induction of differentiation in callus tissue cultures of grain-legumes is still a challenge, though plants can be regenerated from excised meristems (Bajaj and Dhanju 1979). Efforts are, therefore, being made to induce differentiation in the pollen-derived callus.

Table 1. In vitro response of anthers of *Cajanus cajan* cultured for 5 weeks on MS + IAA (4mg/1) + kinetin (2mg/1)

No. of anthers cultured	No. of callusing anthers	% of callusing anthers	No. of pollen studied	No. of multinucleate/ multicellular pollen	% of multinucleate/ multicellular pollen
500	180	36	2550	46	1.8

0040-5752/80/0058/0157/$ 1.00



Figs. 1-8. Androgenesis, and callus formation in excised anthers of *Cajanus cajan* cultured on MS + IAA (4 mg/l) + kinetin (2 mg/l). **1** An uninucleate pollen from anther at the time of culture; **2, 3** bi-, and tetra-nucleate pollen obtained from a 4-week-old cultured anther; **4** a pollen-embryoid from 6-week-old culture; **5** proliferation of anthers to form callus, 3 weeks after culture; **6** mass of callus obtained from a single pollen 23 weeks after culture; **7, 8** Suspension of pollen grown in drop cultures showing the formation of embryoid and callus in Fig. 7 and Fig. 8, respectively

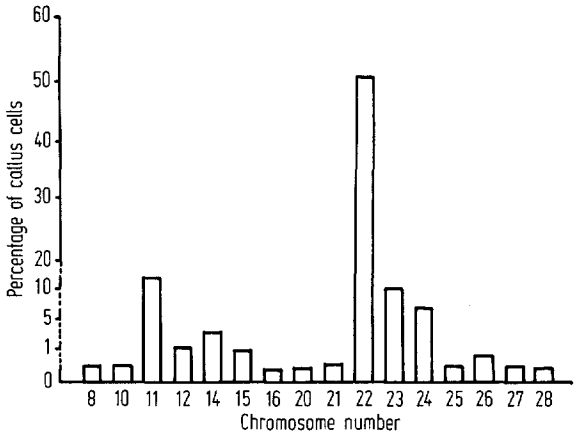


Fig. 9. Anther-derived callus showing a wide range of genetic variability, the chromosome number varied from 8-28 (Data based on 925 dividing cells)

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Literature

Bajaj, Y.P.S.; Dhanju, M.S. (1979): Regeneration of plants from apical meristem tips of some legumes. Curr. Sci. **48**, 906-907

Bajaj, Y.P.S.; Singh, Harbakshish (1980, in press): In vitro induction of androgenesis in mung bean (*Phaseolus aureus*) Indian J. Exp. Biol. vol. **18**

Murashige, T.; Skoog, F. (1962): A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. **15**, 473-497

Reinert, J.; Bajaj, Y.P.S. (1977): Anther culture: haploid production and its significance. In: Applied and Fundamental Aspects of Plant Cell, Tissue, and Organ Culture (eds.: Reinert, J.; Bajaj, Y.P.S., pp. 251-267. Berlin, Heidelberg, New York: Springer

Wenzel, G. (1980): Anther culture and its role in plant breeding. Symp. 'Plant Tissue Culture, Genetic Manipulation and Somatic Hybridization of Plant Cells'. BARC, Bombay

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