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Androgenic Response from Cultured Anthers of a Leguminous Tree, *Cassia siamea* Lam.

Brief Report

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Summary

Anthers of *Cassia siamea*, on culture on B_5 medium supplemented with coconut milk (15%, v/v), 2,4-D (2 mg/l), and kinetin (0.5 mg/l), split open after one to two weeks of inoculation and eject a callus mass. Microscopic examination of the anthers cultured at the late uninucleate or early bi-celled stages, after 7–14 days of culture, revealed many multicellular structures at various stages of development, thus indicating the pollen origin of callus. Callus cells also showed the haploid chromosome number (n = 14).

Keywords: Androgenesis; Callus; *Cassia siamea;* Pollen embryoids.

1. Introduction

Subsequent to the discovery of induction of haploids in *Datura innoxia* through anther culture (GUPTA and MAHESHWARI 1966), this technique has been extended to over 170 species belonging to many different families (MAHESHWARI *et al.* 1982). However, very little work has been done on the production of haploids via anther culture in members of the *Leguminosae*, and in only three genera plantlet formation has been reported, namely *Pisum sativum* (GUPTA 1976), *Trifolium alexandrinum* (MOKHTARZADEH and CONSTANTIN 1978) and two species of *Arachis*, *A. hypogaea* and *A. villosa* (BAJAJ *et al.* 1981). The haploids of leguminous plants in general and of tree species in particular are of special interest because raising of pure lines in trees is severely hampered due to the long periods of juvenility in their life cycle (BONGA 1977). In the present communication, we report androgenic response from cultured anthers of the leguminous tree, *Cassia siamea*.

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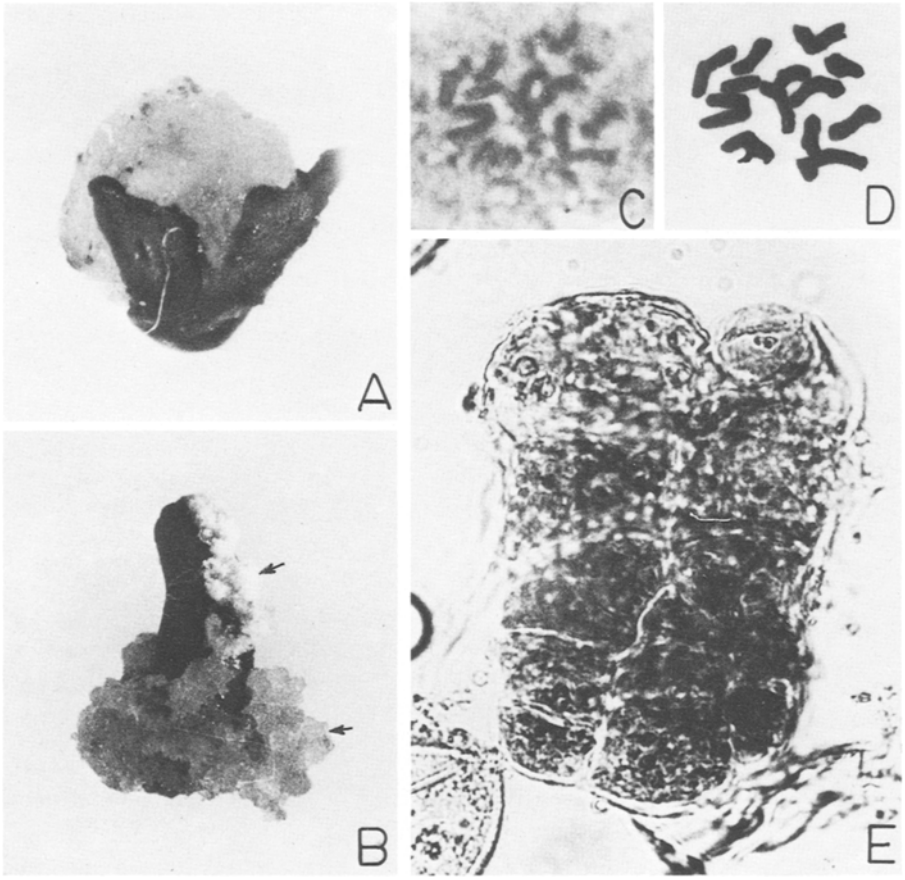
2. Material and Methods

Flower buds were collected from trees growing at the Inter-State Bus Terminal, Delhi. Being a member of the sub-family *Caesalpinoideae*, different anthers in a bud varied in size and consequently also the stage of pollen development. For this study, 0.5 to 0.6 cm long buds were selected in which the median anther contained pollen at the late uninucleate or early bi-celled stage. The anthers from these buds were implanted on B_5 nutrient medium (GAMBORG *et al.* 1968) containing 5×10^{-5} M $FeSO_4$ and 5×10^{-5} M Na_2EDTA as the iron source and 2% sucrose as the carbohydrate source. The medium was supplemented with 15% (v/v) coconut milk (CM) from green coconuts and different growth regulators at various concentrations. It was jelled with 0.9% Difco Bacto-agar and the pH adjusted to 5.8, before autoclaving. All cultures were maintained at 100–150 lux light intensity under a daily photoperiodic regime of 16 hours at 27 ± 1 °C during light hours and 25 ± 1 °C during darkness. For cytological studies, the cultured anthers were fixed periodically in formalin-acetic acid-alcohol (9:0.5:0.5) and squashes made in 2% propionocarmine.

3. Results and Discussion

About 80–90% of the anthers, on culture on medium fortified with 15% CM, 2 mg/l 2,4-D and 0.5 mg/l kinetin, showed some swelling within a week. This was followed



Figs. 1 *A–E*. Induction of androgenesis in anther cultures of *Cassia siamea* on BM + 2,4-D (2 mg/l) + Kn (0.5 mg/l) + CM (15%, v/v). *A* Emergence of callus from an anther after 2 weeks of culture. *B* Callus formation from both the gametophytic (arrow at top) and somatic (arrow below) tissues. *C* and *D* A haploid chromosome spread with idiotype. × 2,735. *E* A multicellular structure of pollen origin. × 780

by browning of the anthers and by the second week white, friable, calli emerged from the anther locules after rupturing the anther wall (Fig. 1 *A*). Simultaneously, somatic tissues of the anther, *i.e.*, the filament and wall, also started callusing. However, in the beginning it was possible to distinguish yellowish somatic callus from the white callus emerging from anther (Fig. 1 *B*). Squash preparations of such white callus, growing rapidly, showed the presence of haploid cells (n = 14; Figs. 1 *C* and *D*) indicating its pollen origin.

The examination of anthers also showed a number of multicellular structures at different stages of development. The sporophytic pathway in the pollen of this taxon starts by the formation of two identical cells in the original uninucleate pollen. It appears that subsequently these units follow one of two modes of development. Either cell enlargement and further divisions lead to early rupturing of the exine, and proliferation of callus, or there may be more organized growth leading to the development of pollen embryos with some indication of bipolarity. In the latter mode of development, the cells stain more densely and the multicellular masses may remain enclosed within the exine up to the 6 to 8-celled stage, though later they too are released (Fig. 1 *E*).

Compared to many other families of angiosperms, androgenesis has been difficult to obtain in the *Leguminosae* and many attempts have ended in partial success or total failure (NIIZEKI and GRANT 1971, IVERS *et al.* 1974, LADEINDE and BLISS 1977, MROGINSKI and FERNANDEZ 1980, TOMES and PETERSON 1981). Apart from the few instances cited in Introduction, in this group of plants positive response has been obtained in *Phaseolus vulgaris* (PETERS *et al.* 1977), *P. aureus* (BAJAJ and SINGH 1980), *Arachis correntina* and *A. villosa* (MROGINSKI and FERNANDEZ 1979), *A. glabrata* (BAJAJ *et al.* 1980 a), *Vicia faba* (HESEMANN 1980) and *Cajanus cajan* (BAJAJ *et al.* 1980 b), though in these plants success has been limited only to microspore divisions

and callus formation. In this context, the present work on *Cassia siamea* – where multicellular structures, a few of them showing some indication of resemblance proceeding towards development of pollen-embryoids – is of definite interest. However, in common with other legumes further organized growth is yet to be obtained and attempts continue in this direction to confirm whether structures such as shown in Fig. 1 *E* actually form pollen-embryos and plantlets.

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