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# INDUCTION OF POLLEN EMBRYOGENESIS AND CYTOLOGICAL VARIABILITY IN *ARACHIS HYPOGAEA* L. THROUGH ANTHER CULTURE

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## ABSTRACT

**Regeneration of haploids by tissue culture in groundnut would facilitate the rapid production of homozygous lines. Response of genotypes to different media was studied. Murashige and Skoog [1] medium supplemented with 3% sucrose or coconut milk, gave the best callus proliferation and rhizogenesis. Variable chromosome numbers, ranging from n=20 to n=40, were observed in the callus cells. The responding anthers revealed early segmentation of pollen, multicellular pollen and pollen embryo in all the three genotypes.**

**Key words:** *Arachis hypogaea* L., anther culture, pollen embryogenesis.

Groundnut is a rich source of oil and proteins, and efforts are being made for their improvement. It can be achieved by crossing genotypes belonging to different growth habits, followed by selection. But this would take a long time to develop a new genotype with desirable composition. The induction of haploids in large numbers would facilitate the mutation and haploid breeding programme. However, successful induction of pollen embryogenesis and the subsequent regeneration of complete plants in groundnut is limited. The present investigation aims to understand the effect of various factors enhancing culture response to excised anthers and the induction of pollen embryogenesis in three genotypes of groundnut.

## MATERIALS AND METHODS

Young flower buds of groundnut (*Arachis hypogaea* L. cv. M 13, U 2-1-26 and $F_1$ of the cross M 13 x U 2-1-26) were collected from the field-grown plants at 4.00 P.M. The buds were surface-sterilized with 70% alcohol for 1 min and then with 0.1% mercuric chloride for 10 min and rinsed twice with sterilized water. The anthers were excised in aseptic conditions in Klenzaids laminar flow chamber and inoculated in Borosil glass tubes (25 x 150 mm)

containing 10 ml medium. The Murashige–Skoog culture medium [1], supplemented with 2 mg/litre of 2,4-dichloro-phenoxyacetic acid (2,4-D), 0.5 mg/litre of kinetin, and different concentrations of sucrose (3% and 8%) and coconut milk (70 ml/litre), was used. The cultures were then stored under alternate light (approx. 5000 lux) and dark conditions at $25 \pm 2^{\circ}C$.

For cytological studies, the actively growing callus and responding anthers were pretreated with saturated colchicine for 3 h, then fixed in acetic acid : alcohol (1:3), stained with 1% acetocarmine, and studied under Zeiss microscope.

## RESULTS AND DISCUSSION

The data on some of the factors affecting callus induction and pollen embryogenesis in excised anthers are summarised in Table 1.

**Table 1. Effect of various media on callus induction in cultured anthers of different genotypes of *Arachis hypogaea* cultured for 10 weeks**

| Medium | Anthers | Incubation condition | M 13 | | U 2-1-26 | | $F_1$ (M 13 x U 2-1-26) | |
|---|---|---|---|---|---|---|---|---|
| | | | total tubes incubated | callusing % | total tubes incubated | callusing % | total tubes incubated | callusing % |
| $M_1$ | Young | Dark | 9 | 100 | 10 | 100 | 10 | 100 |
| | | Light | 9 | 100 | 10 | 50 | 10 | 90 |
| | Mature | Dark | 9 | 100 | 10 | 100 | 10 | 50 |
| | | Light | 9 | 100 | 10 | 50 | 10 | 80 |
| $M_2$ | Young | Dark | 10 | 10 | 10 | 100 | 10 | 100 |
| | | Light | 10 | 30 | 10 | 80 | 10 | 80 |
| | Mature | Dark | 10 | 100 | 10 | 100 | 10 | 100 |
| | | Light | 10 | 0 | 10 | 60 | 10 | 50 |
| $M_3$ | Young | Dark | 9 | 0 | — | — | 10 | 0 |
| | | Light | 9 | 0 | 10 | 0 | 10 | 0 |
| | Mature | Dark | 9 | 0 | — | — | 10 | 0 |
| | | Light | 9 | 0 | 10 | 0 | — | — |

$M_3$—MS + 2,4-D (2.0 mg/litre) + kin (0.5 mg/litre) + sucrose (8%). See text for $M_1$ and $M_2$.

### CULTURE OF EXCISED ANTHERS

Anthers from mature and young buds, when excised and cultured on various media, showed different growth response. There are very few reports on androgenesis in grain legumes and the frequency of regeneration of haploid plants is very low in *Arachis* [2, 3].

The present investigations have shown that by manipulation of media and light/dark conditions, the callusing frequency can be considerably enhanced. Higher callus induction was observed on medium 1 (M1): MS + 2,4-D (2.0 mg/litre) + kin (0.5 mg/litre) + sucrose (3%), followed by medium 2 (M2): MS + 2,4-D (2.0 mg/litre) + kin (0.5 mg/litre) + sucrose (3%) + coconut milk (70 ml/litre). Callus induction was very slow on the medium supplemented with 8% sucrose. The response of different genotypes studied to all the three media was comparable. Comparative study of anthers kept under light and dark indicated that the frequency of callus induction was 100% under dark, whereas it varied with different media and genotypes under light. The callus formed under dark was non-friable, while that in light was friable. Most of the cultures underwent rhizogenesis (Fig 1: 1–3) in all the three genotypes under light as well as dark conditions. The anther derived callus was mostly compact and slow growing.

FACTORS AFFECTING POLLEN EMBRYOGENESIS

The pollen cultured in dark and light underwent various modes of development, showing an overall increase in size, early segmentation, multicellular pollen and pollen embryogenesis (Fig. 1: 4–6) in all the three genotypes. Frequency of embryogenesis was almost equal in the media supplemented with 3% sucrose and coconut milk, whereas medium supplemented with 8% sucrose induced more embryogenesis (Table 2). The pollen in 3% sucrose medium underwent further development to different shaped embryoids.

**Table 2. Pollen embryogenesis in *Arachis hypogaea* anthers cultured on different media**

| Medium | Genotype | No. of pollen grains studied | Uninucleate | Bicellular | Multi-cellular | Pollen embryo | Pollen embryo-genesis % |
|---|---|---|---|---|---|---|---|
| $M_1$ | M 13 | 1000 | 705 | 262 | 12 | 9 | 0.90 |
| | U 2-1-26 | 976 | 609 | 19 | 13 | 8 | 0.82 |
| | $F_1$ (M 13 x U 2-1-26) | 1105 | 696 | 23 | 15 | 9 | 0.81 |
| $M_2$ | M 13 | 1445 | 792 | 31 | 15 | 12 | 0.83 |
| | U 2-1-26 | 1575 | 881 | 29 | 19 | 14 | 0.89 |
| | $F_1$ (M 13 x U 2-1-26) | 1396 | 833 | 21 | 20 | 12 | 0.86 |
| $M_3$ | M 13 | 872 | 546 | 122 | 19 | 10 | 1.15 |
| | U 2-1-26 | 1020 | 642 | 125 | 26 | 17 | 1.67 |
| | $F_1$ (M 13 x U 2-1-26) | 985 | 499 | 150 | 29 | 13 | 1.32 |

CYTOLOGICAL STUDIES

The cytological examination of fast growing callus showed a wide spectrum of chromosome numbers. The chromosome number (Fig. 1: 7–9) varied from n = 20–40 (diploid

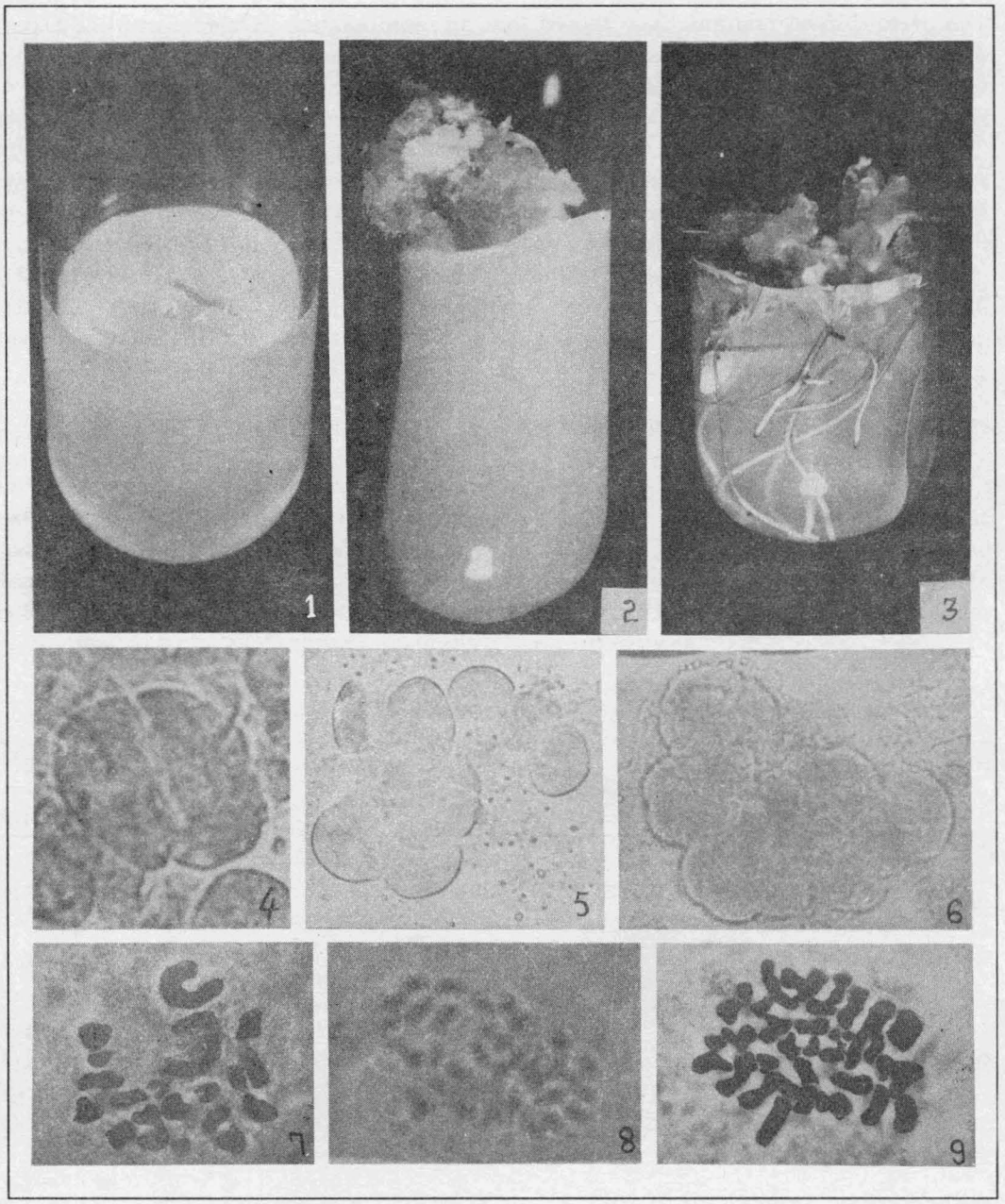


Fig. 1. Embryogenesis and variation in ploidy status in cultured anthers of *Arachis hypogaea*. Young anthers 7 days after inoculation (1) (note the swelling of anthers); 3-week-old culture showing callus formation (2); rhizogenesis after 6 weeks (3); direct segmentation in pollen (4); and multicellular embryoids (5, 6). Anther-derived callus showing n=20 (7), n=30 (8) and n=40 (9).

number 2n = 40). The other chromosome numbers observed were n=26 and n=30. Haploid cells were not observed in old calli.

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