zobial strains of the ten weeds was able to nodulate *Cicer arietinum*.

However, the pelleting of Cicer seeds with four rhizobial strains of *Lathyrus aphaca*, *L. sativus*, *Trigonella polycerata* and *Tephrosia apollinea* resulted in the formation of pseudonodules of varied size and shape containing enlarged parenchymatous cells without any bacterial infection (figures 1–3).

In one instance the histological studies of pseudonodules formed on *Cicer* under the influence of rhizobium of *Lathyrus aphaca*, consisted of a portion of primary root with its swollen parenchymatous cortex enclosing four lateral roots developed in close succession all round (figure 1). In another instance the main root which had branched off at one end bore two lateral roots with their swollen parenchymatous cortices (figure 2). The rhizobium of *Trigonella polycerata* influenced the formation of a pseudonodule consisting of a swollen cortices of the tap and lateral roots (figure 3). In this case the cause (non-homologous rhizobial strains or the type of their metabolites) for the development of pseudonodules deserves further study.

The authors are grateful to Prof. B. Tiagi for encouragement.

8 December 1982; Revised 8 February 1983

1. Beal, J. M., *Bot. Gaz.*, 1944, **105**, 471.
2. Wilde, Mary. H., *Am. J. Bot.*, 1951, **38**, 79.
3. Allen, E. K., Allen, O. N. and Newman, A. S., *Am. J. Bot.*, 1953, **40**, 429.

## DEVELOPMENT OF THE EMBRYO IN *OREORCHIS FOLIOSA* LINDL.

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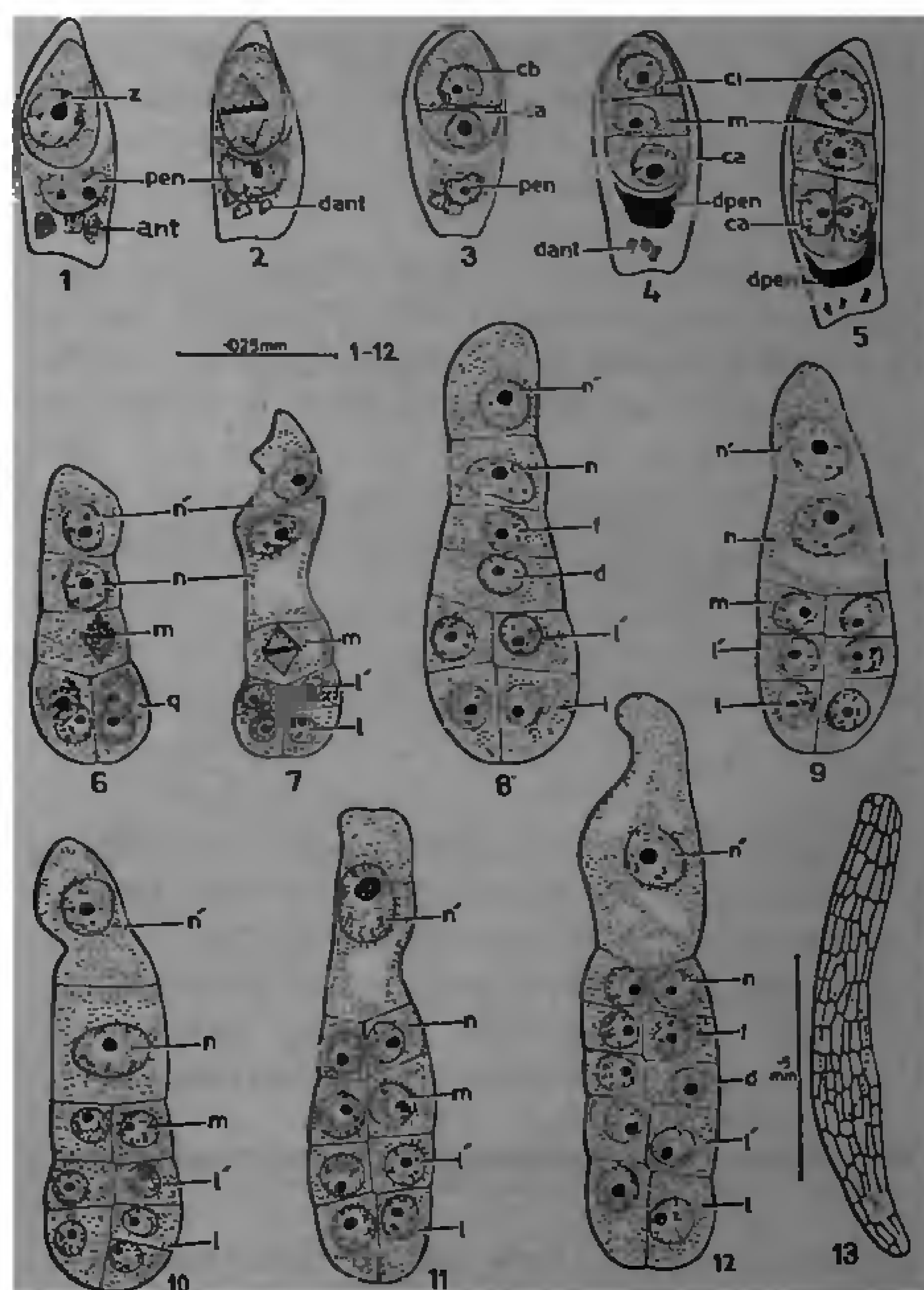
Perusal of the previous embryological works in Orchidaceae[1-6] revealed that the development of embryo in the genus *Oreorchis* Lindl., subtribe Cyrtopodiinae, tribe Epidendreae, subfamily Orchidoideae[7] is hitherto unknown and hence the present investigation was undertaken.

The zygote divides transversely resulting in a basal (cb) cell and a terminal (ca) cell (figures 1–3). The basal cell (cb) divides transversely forming a middle cell (m) and a suspensor initial (ci) cell (figure 4). The primary endosperm nucleus enlarges initially but degenerates at the three-celled stage of proembryo (figures 1–5). Longitudinal division occurs in (ca) resulting in a T-shaped proembryonal tetrad (figure 5). The two daughter cells of the terminal cell (ca) divide longitudinally, at right angles to the first (figure 6), forming a quadrant (q) the cells of which are partitioned transversely leading to an octant which contains two tiers (l) and (l') of four cells each (figures 7,8). The middle cell (m) divides transversely to form (d) and (f) (figures 6–8) whereas (ci) segments (figure 6) into (n) and (n'). Occasionally (m) divides longitudinally (figures 9–11). The cell (n') elongates and enlarges to form 1-celled suspensor (figures 9–12). It elongates considerably and embeds into the tissue of placenta. However, no branching of suspensor is observed. The seeds are numerous, minute and non-




**Figures 1–13.** **1.** Zygote, primary endosperm nucleus and antipodals. **2.** Zygote in transverse division. **3.** Two-celled proembryo. **4.** Three-celled proembryo; note the degenerated primary endosperm nucleus and antipodal cells. **5.** Four-celled, T-shaped proembryonal tetrad. **6–8.** Seven-, 11–, and 12-celled proembryos. **9–12.** Later stages in embryogeny; note enlarged and vacuolated suspensor cell *n'*. **13.** Mature seed with embryo. (ant, antipodals; dant, degenerated antipodals; dpen, degenerated primary endosperm nucleus; pen, primary endosperm nucleus; z, zygote.).

endospermic. Each seed consists of an ellipsoid embryo surrounded by a reticulate seed coat (figure 13).

The mature embryo is formed mostly by the derivatives of terminal cell and middle cell. A noteworthy feature in the embryogeny of *Oreorchis foliosa* is the participation of some of the derivatives of suspensor initial cell in the organisation of mature embryo. However, its embryogeny corresponds to the Onagrad type of Johansen[2].

The author wishes to express his gratefulness to Prof. H. C. Agrawal, Himachal Pradesh University, for his kind interest in this study and to Dr P. R. Mohana Rao, Nagarjuna University, Guntur, for his valuable suggestions.

31 March 1983; Revised 3 June 1983

1. Davis, Gwenda L., *Systematic embryology of the angiosperms*, John Wiley, New York, 1966, 528.
2. Johansen, D. A., *Plant Embryology*, Waltham, Mass, U.S.A., 1950, 340.
3. Schnarf, K., *Vergleichende Embryologie der Angiospermen*, Gebruder Borntraeger, Berlin, 1931, 354.
4. Swamy, B. G. L., *Am. Midl. Nat.*, 1949a, **41**, 184.
5. Swamy, B. G. L., *Am. Midl. Nat.*, 1949b, **41**, 202.
6. Wirth, M. and Withner, C. L., In *the Orchids—A scientific survey* (ed. C. L. Withner), The Ronald Press, New York, 1959, 647.
7. Dressler, R. L. and Dodson, C. H., *Ann. Mo. Bot. Gard.*, 1969, **47**, 25.

## *IN VITRO* INDUCTION OF ANDROGENESIS AND ORGANOGENESIS IN *CICER ARIETINUM* L.

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INDUCTION of haploids from anthers cultured *in vitro* has potential value in genetics and plant breeding[1,2]. The induction of pollens plantlets has been reported in many species[1] but from grain legumes, this information is meagre except in the case of *Glycine max*[3], *Phaseolus vulgaris*[4], *Cajanus cajan*[5], *Pisum sativum*[6] and *Phaseolus aureus*[7].

In view of the potential significance of haploids for legume improvement programmes, the present investigation has been undertaken to augment the basic information on various factors for the induction of haploidy in *Cicer arietinum* L.-grain legume rich in dietary protein. In this communication, the induction of androgenesis, development of callus and organogenesis from anther culture of *C. arietinum* L. var B–108 are reported.

Chickpeas (*C. arietinum* L. var B–108) were grown to maturity and suitable sized flower buds were excised from the field-grown plants. Flower buds were surface-sterilized in 0.1% $HgCl_2$ solution 8–9 min followed by rinsing in sterile-distilled water 8–10 times. Before the inoculation, 2–3 anthers from each flower bud were squashed in 1% aceto carmine to determine the stages of pollen development. Anthers containing uninucleate and binucleate pollen grains were placed aseptically on Murashige and Skoog's nutrient medium[8], supplemented with various combinations of growth regulators (table 1). After placing the anthers on culture medium they were kept in the dark for 3 days at 25° ± 1° C. The cultures were then incubated for 10 hr in light (1500 lux) followed by 14 hr dark period. For cytological studies, anther-derived callus and regenerated root tips were periodically fixed overnight in glacial acetic acid:absolute ethanol (1:3). The root tips or calli were washed in distilled water, hydrolysed for 10 min in 1 N HCl at 60° C followed by Feulgen and acetocarmine treatment.

When the anthers were cultured on MS + 2,4-D (2 mg/l) + coconut milk (10% v/v), callus proliferation was observed within 7–10 days and a mass of calli was formed within 18–21 days (figure 1). Uninucleate pollen showed best response in induction and proliferation of callus masses. The nature and colour of the calli were compact to friable, greenish white, turned brown if not subcultured during 3 weeks interval in MS + 2,4-D (2 mg/l) + coconut milk (10% v/v) + lacalbumine hydrolysate (500 mg/l). Ten sets of media were tested, (table 1) of which MS + 2,4-D (2 mg/l) + coconut milk (10% v/v) gave the best result in induction of callusing and androgenesis. Ther percentage of anther responded on MS + 2,4-D (2 mg/l) + coconut milk (10% v/v) for callus proliferation was 24.05 and in the same medium frequency of androgenesis was 1.26% (table 1). The pollen during androgenesis showed repeated nuclear (figure 2) and cellular divisions (figures 3–5) to form pollen embryoid (figure 6).

Cytological observation of the anther derived callus revealed that 28.1% of cells was haploid ($n = 8$) (figure 8). Deviation in chromosome numbers varying from 8–16 in the callus cells was also observed. The callus contained 28.1% haploid, 38.2% diploid with the rest 37.7% being aneuploids.

Attempts have also been made to regenerate plantlet from these calli after transferring to different media. On MS + NAA (2 mg/l) + BAP (0.02 mg/l) + LA