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].

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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.

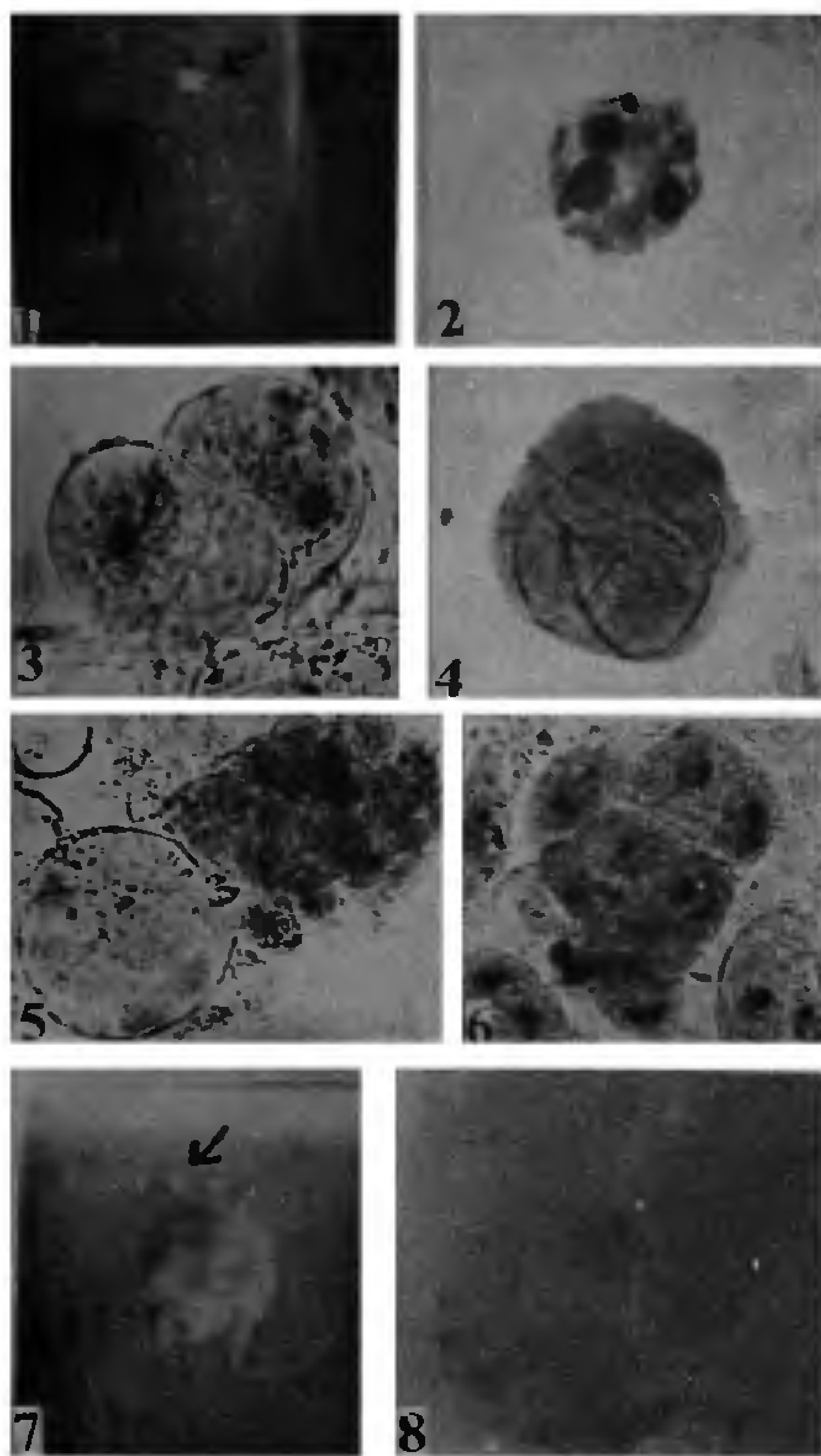
Attempts have also been made to regenerate plantlet from these calli after transferring to different media. On MS + NAA (2 mg/l) + BAP (0.02 mg/l) + LA

TABLE 1

*Comparison of the frequency of proliferation and induction of androgenesis in anther of C. arietinum L. var. B-108, cultured on MS medium with different growths adjuvents**

Medium	No. of anthers cultured	No. of callusing anther	% of callusing anther	No. of Pollen studied	No. of binucleate Pollen	No. of multinucleate/multicelller Pollen	No. of embryoid	% of multinucleate/multiceller Pollen
MS	259	—	—	450	11	—	—	—
MS+2,4-D (1 mg/l)	352	—	—	525	10	—	—	—
MS+2,4-D(2 mg/l)	453	6	1.33	621	35	1	—	0.16
MS+2,4-D(4 mg/l)	457	11	2.40	717	52	4	—	0.55
MS+2,4-D(2 mg/l) +coconut milk (10% V/V)	528	127	24.05	1985	211	22	3	1.26
MS+2,4-D(4 mg/l) +coconut milk (10% V/V)	483	95	19.66	1021	103	10	—	0.97
MS+NAA(2 mg/l) +BAP(0.02 mg/l) +coconut milk (10% V/V)	675	66	9.78	1532	226	12	—	0.78
MS+NAA(4 mg/l) +BAP(0.02 mg/l) +coconut milk (10% V/V)	526	47	8.93	1265	135	6	—	0.47
MS+IAA(1 mg/l) +CH(100 mg/l)	456	18	3.94	1139	121	3	—	0.26
MS+IAA(2 mg/l) +CH(100 mg/l)	539	61	11.31	845	106	7	—	0.82

* The data based on 250-675 anthers cultured on each medium and observation on pollen taking 20 anthers from each medium.



Figures 1–8. 1. Callus proliferation (arrow marked) from cultured anther on MS+2,4-D (2.0 mg/l) +coconut milk (10% v/v). **2.** Trinucleate condition of the pollen developmental stage. **3.** Bicellular condition of the pollen developmental stage. **4.** Tetra cellular condition of the pollen developmental stage. **5.** Multinucleate condition of the pollen developmental stage. **6.** Pollen embryoid. **7.** Rhizogenesis (arrow marked) from anther derived callus of *C. arietinum* L. **8.** Anther derived callus cell showing haploid ($n = 8$) chromosome number.

(500 mg/l) the anther derived calli showed rhizogenesis (figure 7).

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HYPOPHOSPHOROUS ACID—ITS USE IN THE SELECTIVE EXTRACTION OF RNA FROM FIXED ANIMAL TISSUES

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It was earlier reported[1,2] that both cold conc. phosphoric acid and meta-phosphoric acid can extract RNA selectively from fixed mammalian tissues. Polyphosphoric acid has also been found suitable for extracting RNA from fixed tissue sections (Dutt, unpublished). This communication presents a rapid and reliable procedure for the selective extraction of RNA from fixed animal tissues employing hypophosphorous acid.

Concentrated hypophosphorous acid (H_3PO_2) was used at 5° C. Paraffin sections (8 μm) of tissues, such as liver, kidney, testis, ovary, adrenal and submandibular gland of a white rat as well as liver and kidney of a frog, *Rana tigrina*, were used. These tissues were fixed in 10% buffered neutral formalin, paraformaldehyde and acetic acid-alcohol (1:3). Sections after deparaffinisation were treated with the cold acid for 60, 90, 120 and 240 min. Acetic acid-alcohol-fixed liver and kidney were also treated with cold (5° C) hypophosphorous acid for 30–40 min. The sections were then rinsed with water and stained with 0.5% aqueous solutions of brilliant cresyl blue and methyl green for 2 min. Stained sections were then dried