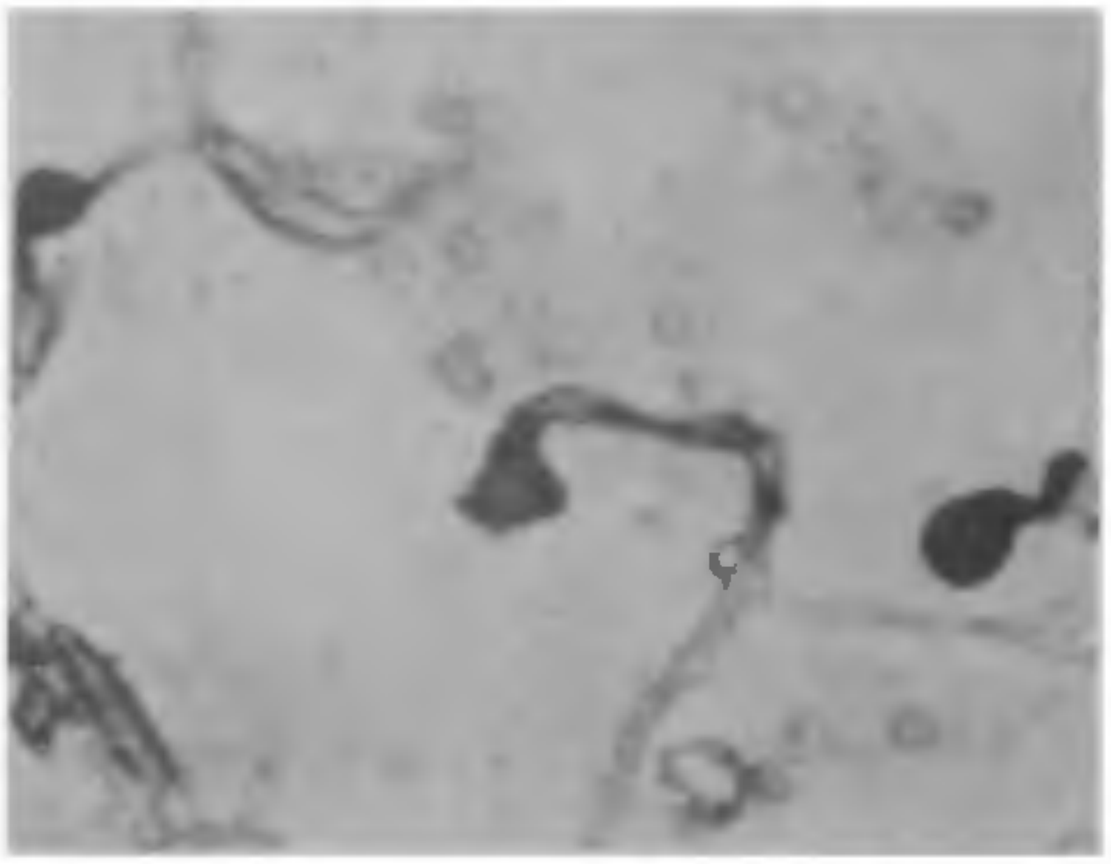

Figure 2. Appressoria of *C. falcatum* in soil.

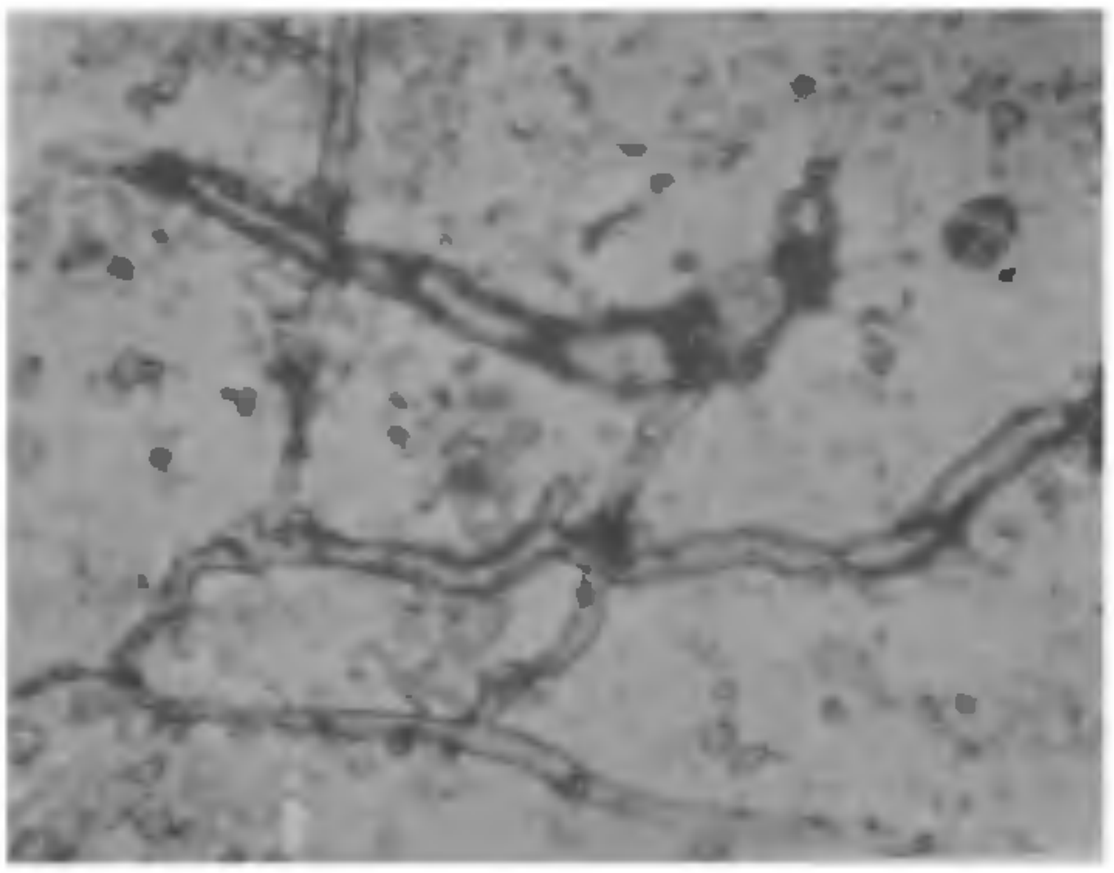

Figure 3. Thick-walled mycelium of *C. falcatum* in soil.

mycelium. This could serve an initial source of inoculum for recurrence of the disease.

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PRODUCTION OF POLLEN EMBRYOIDS IN ANTHER CULTURES OF *SAMBUCUS NIGRA* L.

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THERE is considerable literature regarding the production of androgenic haploids in families like Cruciferae, Gramineae, Ranunculaceae and Solanaceae[1]. However, there has been no work in certain other families. This prompted the authors to take up anther culture studies in *Sambucus nigra* L., an ornamental shrub belonging to the family Caprifoliaceae.

Flower buds 3-4 mm in length were selected for experimental studies. At this stage their anthers show uninucleate pollen grains (figure 1A). Flower buds were surface-sterilized with chlorine water. Five anthers from each bud were transferred aseptically to the culture tubes. The medium of Murashige and Skoog[2] containing 2% sucrose jelled with 0.8% Difco bacto-agar was used as basal medium (MS). MS was also supplemented with different concentrations of benzylamino purine (BAP – 0·3, 0·5, 1 ppm); kinetin (KN – 0·25, 0·5 ppm); 2,4-dichlorophenoxyacetic acid (2,4-D – 0·5, 1 ppm); naphthaleneacetic acid (NAA – 0·5, 1 ppm); and yeast extract (YE—500, 750 ppm) either singly or in various combinations. The cultures were grown under continuous fluorescent light at 25 ± 2°C and 55 ± 5% relative humidity. Acetocarmine squashes of cultured anthers were made at regular intervals to study the various stages of pollen development.

On MS, the anthers failed to grow. However, when MS was supplemented with 2,4-D (1 ppm); 2,4-D (1 ppm) + KN (0·5 ppm); 2,4-D (1 ppm) + YE (500 ppm); and BAP (0·5 ppm) + NAA (1 ppm), there was initiation of callus from the anthers a week after inoculation. Within the next five days there was a slight growth of the callus. Fifteen days after culture, in 20% cases callus proliferated (figure 1B) while in others it remained quiescent and failed to grow further. The callus was

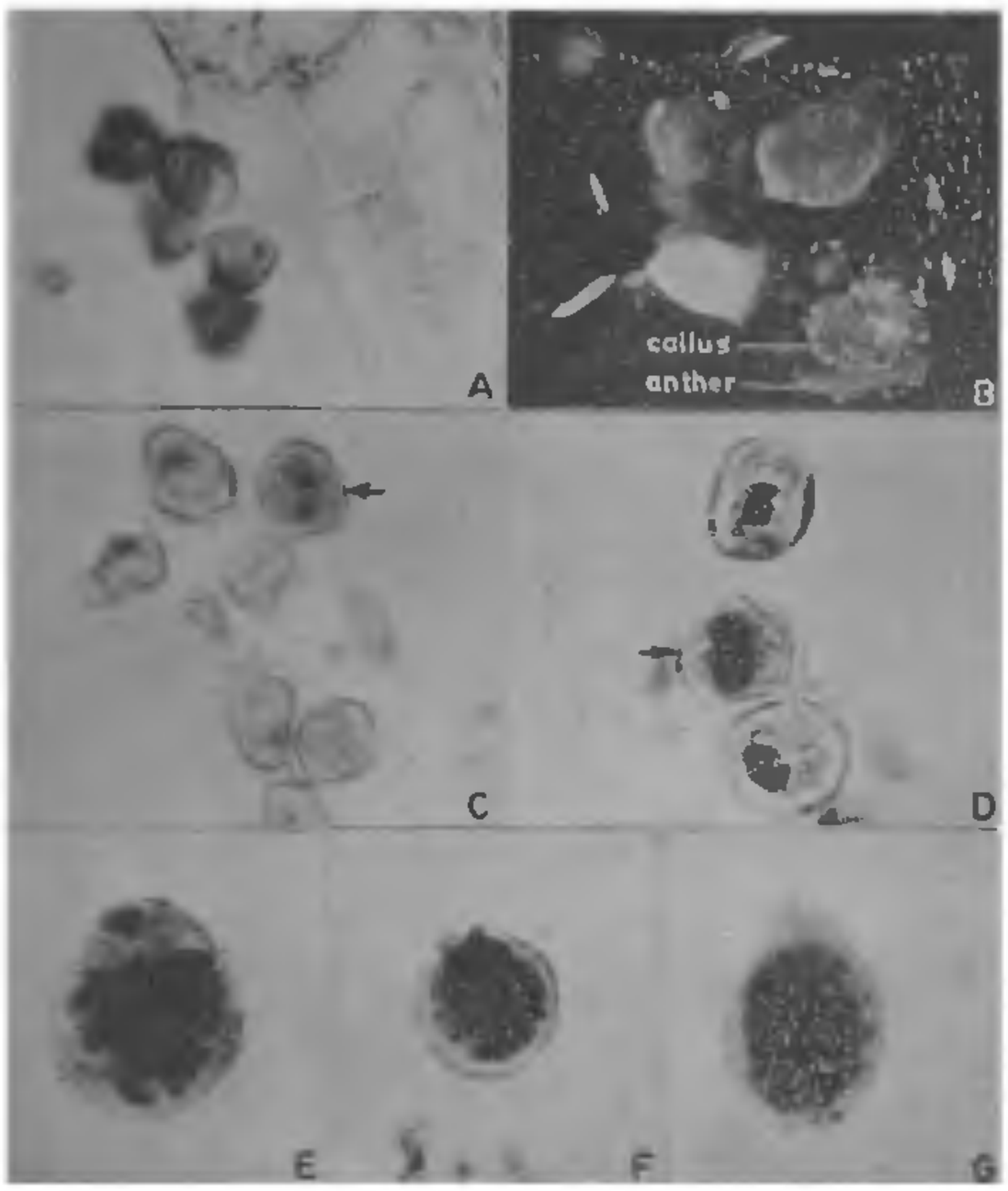

Figure 1. A–G. Anther culture in *Sambucus nigra* L. (A) T.s. portion of anther showing uninucleate pollen grains, × 466. (B) 15-day-old culture on MS + 2,4-D (1 ppm) + KN (0·5 ppm), showing the profuse callus, × 2·6 (C) T.s. portion of anther locule showing pollen grains. Note a large vegetative nucleus and a small generative nucleus in one of the pollen grains (arrow), × 933. (D) Squash preparation, showing a pollen (arrow) with two equal nuclei, × 1,866 (E) Same, from 24-day-old culture, showing a multicelled pollen grain, × 1,166 (F, G) Same, showing different stages of development of the pollen embryoids. F × 933, G × 1,166.

whitish in colour and histological preparations revealed that it originated from the connective region of the anther.

Acetocarmine squashes and histological studies of anthers at various stages of growth period, raised on MS + 2,4-D (1 ppm) + KN (0·5 ppm), showed that the pollen grains had followed two different pathways of development. Eight days after culture, in 70% pollen grains, nucleus divided to form a large vegetative and a small generative nucleus (figure 1C). However, in 20% of the pollen grains, the nucleus divided and two equal nuclei were formed (figure 1D). The remaining 10% of the pollen grains enlarged and were filled with starch grains as revealed by IKI staining. A few pollen grains were elongated during the course of division. In 14-day-old cultures, some pollen grains with four nuclei (3 large and 1 small) could also be seen. Twenty four days after culture, multicelled pollen were observed (figure 1E). These have been referred to as pollen embryoids. Some pollen embryoids ready to exude out of the pollen wall were also observed (figure 1F, G). A few of them later produced incipient plantlets.

It has been demonstrated by several workers that the addition of both an auxin and a cytokinin to the basal medium is necessary for inducing divisions in the pollen grains[3]. Similar observations have also been made in *Sambucus nigra* (present work). However, these growth adjuvants induce proliferation of connective tissue as well.

In *Sambucus nigra* (present work), the growth of the callus from the connective is fast, which account for the failure of the pollen embryoids to develop further. Further experiments are being conducted to suppress the growth of the sporophytic tissue and to obtain the pollen embryoids and consequently androgenic haploids by manipulation of growth regulators in the medium.

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EXCEPTIONAL FEATURES OF STERILITY IN *HIBISCUS ROSA-SINENSIS* CV. SCARLET

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LEWIS[1] suggested that self-incompatibility is a device to conserve eggs for compatible pollination and the oddity to this rule is *Theobroma cacao* where incompatible pollen germinates, the tube grows at compatible rates and the male gametes fertilize the egg and endosperm nucleus but still the embryo aborts. We report a similar loss of ovules on self-pollination in a cultivar of *Hibiscus rosa-sinensis* although the failure of seed set here appears to be due to inhibitory factors operating both at pre- and post-fertilization stages.

About 1000 cultivars of *Hibiscus rosa-sinensis* are known[2] but these are generally sterile. However, of the