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IN VITRO GROWTH OF OVARIES OF *LINARIA MAROCCANA* HOOK.

ALTHOUGH La Rue[1] was the first to achieve some success in growing angiosperm flowers in artificial media, it was really Nitsch[2] who established the *in vitro* technique on a firm footing and showed that the culturing of ovaries under controlled conditions was perhaps the best means of understanding the physiology of fruit. Anataswamy Rau[3] has recently employed this method for studying the effect of colchicine on the early development of the embryo and endosperm in *Phlox drummondii*.

The present study deals with the influence of some growth substances on the fruit and seed development of *Linaria maroccana*, which is grown here as a garden plant. The ovaries were sterilized with 10% calcium hypochlorite solution and inoculated in 10 ml. of nutrient medium whose pH was adjusted between 5·5 and 6. The cultures were kept in diffused light at room temperature (17–21° C.).

Both pollinated and non-pollinated ovaries were tried. The latter did not grow in cultures (except producing some callus and roots) even with the addition of growth substances (Fig. 7). Subsequent study was therefore confined to ovaries picked two days after pollination (Fig. 1). They responded well to Nitsch's basic medium (Nitsch[2]) coupled with vitamins (White[4]), although the fruits thus obtained were smaller in size than controls in field. Following this, it was decided to study the effect of various growth substances on fruit development. Kinetin (0·1 mg./l. to 1·0 mg./l.), in combination with IAA (5 mg./l.), IBA (5 mg./l.), 2, 4–D (2 mg./l.) and adenine (5, 10 mg./l.), was incorporated in the basic medium. While some fruits were slightly larger than those on the basic medium, none of them equalled or surpassed the natural size. The optimum average length of the fruit obtained in culture was 2·3 mm. in comparison to 5·0 mm. in nature (Figs. 2, 4–6). In another set of cultures, the addition of yeast extract (0·25%, 0·5% and 1·0%) gave better fruit growth than all the preceding media, with an average length of 3·2 mm. (Fig. 3). The percentage of fruit setting was also higher in field (90–95%) than in cultures (40–80%). Though fruits of fully normal size could not be obtained in cultures, the general pattern of fruit growth was more or less similar. In both cases the maximum

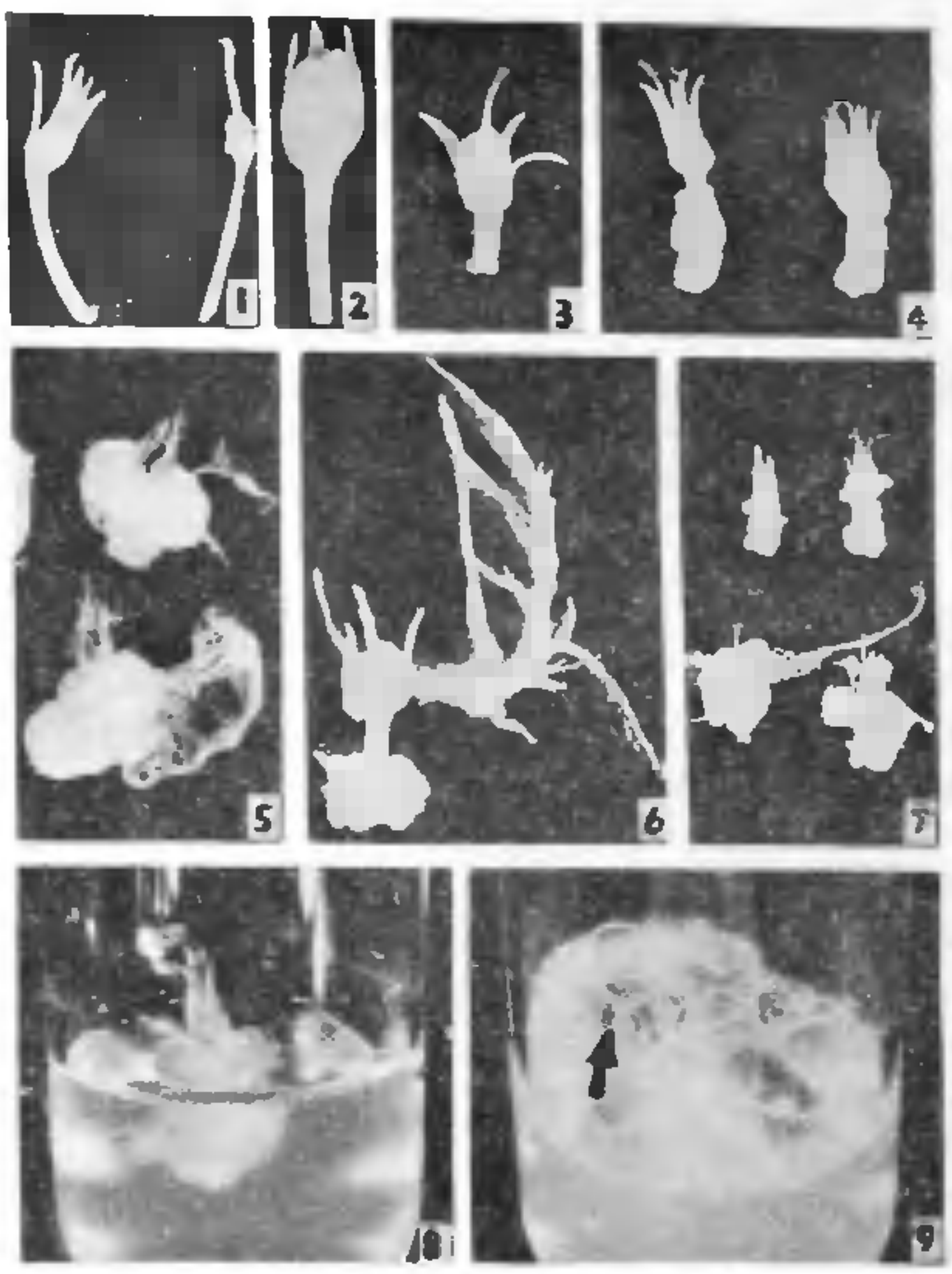

FIG. 1. Ovaries, at the stage of inoculation, excised two days after pollination, × 1·5.

FIG. 2. Control fruit *in vivo*, 12 days after pollination, × 1·5.

FIG. 3. Fifteen days' growth of fruit in Nitsch's basic medium + Vitamins (NBV) + yeast extract (0·5%). Note callus tissue at the base of the pedicel, × 1·5.

FIGS. 4–5. 22- and 38-day old fruits, cultured in NBV + kinetin (0·1 mg./l.) + IAA (5 mg./l.), showing formation of callus and roots from the pedicel and the base of the ovary, × 1·5.

FIG. 6. A fruit (32-day old) with callus and well developed root system grown in a medium comprising NBV + kinetin (0·4 mg./l.) + IAA (5 mg./l.), × 1·5.

FIG. 7. Non-pollinated ovaries (32-day old) cultured in NBV + kinetin (0·5 mg./l.) + IBA (5 mg./l.). The ovaries failed to grow but callus and roots arose from the pedicel part, × 1·5.

FIG. 8. Test-tube fruit (27-day old), grown in NBV + kinetin (0·1 mg./l. + IAA (5 mg./l.), showing enormous callus growth from the base of the ovary, × 1·5.

FIG. 9. Another test-tube fruit (marked by an arrow), showing excessive root formation in 38 days. The medium comprised NBV+kinetin (0·5 mg./l.) + IAA (5 mg./l.), × 1·5.

size of the fruit was attained 10-12 days after pollination, but the maturity of the fruit was hastened in artificial culture. Mature fruits with black seeds are formed 21-23 days after pollination in the field, whereas in cultures maturity is attained in 15-17 days after pollination. The pericarp of the artificial and control fruits comprises a similar number of cell layers. However, the pericarp of the artificial fruits is more translucent and the black seeds can be seen through the intact fruit wall. This was because of an absence of the usual thickening of the cells in the test-tube fruits.

At the time of inoculation of ovaries, the ovules invariably showed the undivided zygote and two to six endosperm cells. The development of the embryo and the endosperm proceeded similarly in cultured and control fruits, but there was a greater deposition of starch grains in the endosperm tissue of the former. Seeds obtained from *in vitro* fruits were fully viable although they took a slightly longer time (66 hours) to germinate as compared to control seeds (48 hours). However, cultured fruits always showed a greater proportion of aborted ovules. In many cases the ovules developed a black seed-coat but were empty inside.

Callus Formation on Ovaries.—The ovaries cultured on the basic medium showed no morphological changes in the pedicel. However, if yeast extract was added to the basic medium, a disc-like patch of callus arose from the cut end of the pedicel (Fig. 3). Its growth ceased after about two weeks and thereafter the cells became brownish to blackish in colour. In many cultures the basic medium was fortified with kinetin in conjunction with IAA, IBA, 2,4-D or adenine. In every case there was an excessive proliferation of the cortical cells from the cut portion of the pedicel, resulting in the formation of a callus. This was followed by the appearance of several small patches of callus all along the pedicel and also at the base of the ovary. Their rapid growth results in a coalescence of the callus groups, eventually leading to a hypertrophy of the whole stalk (Figs. 4, 5). Callusing is also common both on the inner and outer surfaces of the calyx lobes. In a few cases the ovary wall and the base of the style showed some localized meristematic activity. The callus tissue comprises three types of cells. The most common are small actively dividing parenchyma cells which often contain starch grains. Isolated cells, or more often a group of them, developed tracheidal thickenings. Lastly, some of the parenchyma cells enlarged enormously and became highly vacuolated.

Rooting of Ovaries.—In many ovaries, after about 18-20 days of growth, root primordia were initiated within the callus; only one or at the most two of them continued to grow and branch profusely (Fig. 6). Root hairs were not confined to a particular zone but were present throughout the entire length of the root (Figs. 8, 9). Some of the root branches became greatly swollen. This localized growth was brought about by the meristematic activity of the cortical cells leading to the formation of a 'secondary callus'. The ovaries cultured on the basic medium, or with yeast extract and kinetin *plus* adenine combination, failed to give any rooting response. Nitsch[1,5] too observed rooting from the pedicel of tomato ovaries. He considered that during the initiation of roots, the growth of the fruit was retarded, but after the root system was well established, it helped in the maturation of the young fruit. However, in *Linaria* the fruit ripened much before the root system was fully developed.

The use of kinetin in conjunction with some growth substances induced excessive callus and root formation, without any appreciable effect on fruit development. The chief cause of a reduced fruit size in test-tube cultures seemed to be a lower seed setting. It is hoped that it might be possible to get over this difficulty in subsequent trials.

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Dept. of Botany, R. C. SACHAR.
University of Delhi, B. BALDEV.
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EMBRYOLOGY OF *AERVA TOMENTOSA* FORSK.

In the past few years, we consistently noted the absence of male flowers in *Aerva tomentosa*. For closer study the plants were grown in the University Botanic Gardens. The spikes were carefully examined after every two or three days. Here again, there was no trace of male flowers. But the plants continued to produce normal viable seeds. It was therefore