FRUITING PLANTS FROM *IN VITRO* GROWN LEAF TISSUE OF *RAUVOLFIA SERPENTINA* BENTH.

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COMPLETE studies involving embryoid and plantlet differentiation in *in vitro* grown tissues and their rearing up to complete plants in potted soil are very few. So far, such studies have been made with carrot[1,2] and tobacco[3-5] only. We now report, the raising of fruiting plants of *Rauvolfia serpentina* Benth., a perennial.

The leaf callus explants grown on a modified Murashige and Skoog's[6] agar medium, under controlled conditions of light, temperature and humidity,[7] showed the best differentiation of organized bodies in those cultures receiving kinetin (0·5 mg./l.), or adenine or adenine sulphate (both 10 mg./l.) along with either casein hydrolysate (500 mg./l.) or yeast extract (200 mg./l.) and in presence of coconut milk (10% v/v). The pH of the media were adjusted to 5·5. However, these substances when even used singly were found effective, but to a much lesser extent. The presence of 2, 4-dichlorophenoxyacetic acid was deleterious to organogenesis in every case.

Two ontogenetic pathways were observed in the regeneration of plants from leaf callus, namely, (1) the adventive embryogenesis in which some of the stages of zygotic embryogenesis were recapitulated and (2) the differentiation of the bipolarized meristematic nodules in which the shoot and the root-growing points were established on their two poles (Fig. 1, A, B). These two types of *regenerants* were formed from meristemoids which occurred as isolated pockets of meristematic cells in the same proembryogenic tissue. The earliest stage in the differentiation of a *regenerant* that could be noticed was a twelve-celled meristemoid (Fig. 2). The other organized bodies differentiated in cultures included roots, shoot-buds and certain malformations.

The *regenerants* on transfer to fresh nutrient agar, of the same composition, grew into plantlets. When they produced 3 to 4 pairs of leaves and a good root system was formed, they were taken out from aseptic nutrient agar cultures, washed thoroughly in running tap-water and subsequently cultured in an inorganic nutrient solution (modified Knop's solution). Plantlets regenerated from leaf callus (Fig. 3, A) and seedlings raised from cultured excised embryos (Fig. 3, B) of *R. serpentina* looked exactly similar in their root and shoot systems. The plants were later transplanted into potted soil (sand and soil 1 : 1), with added nutrient solution. Finally, the growing plants were transplanted into manured soil in pots and placed under field conditions; where they grew normally and vigorously. And in about a year's growth they, like seed-plants, produced flowers only (Fig. 4, A), but during subsequent flushes of flowering, fruits were also formed (Fig. 4, B). Thus, it is quite safe to conclude that the tissue-plants were normal in every respect.

This study has demonstrated the possibility of getting a large number of genetically identical plants from a small lump of tissue for clonal propagation of *R. serpentina* by tissue culture technique.

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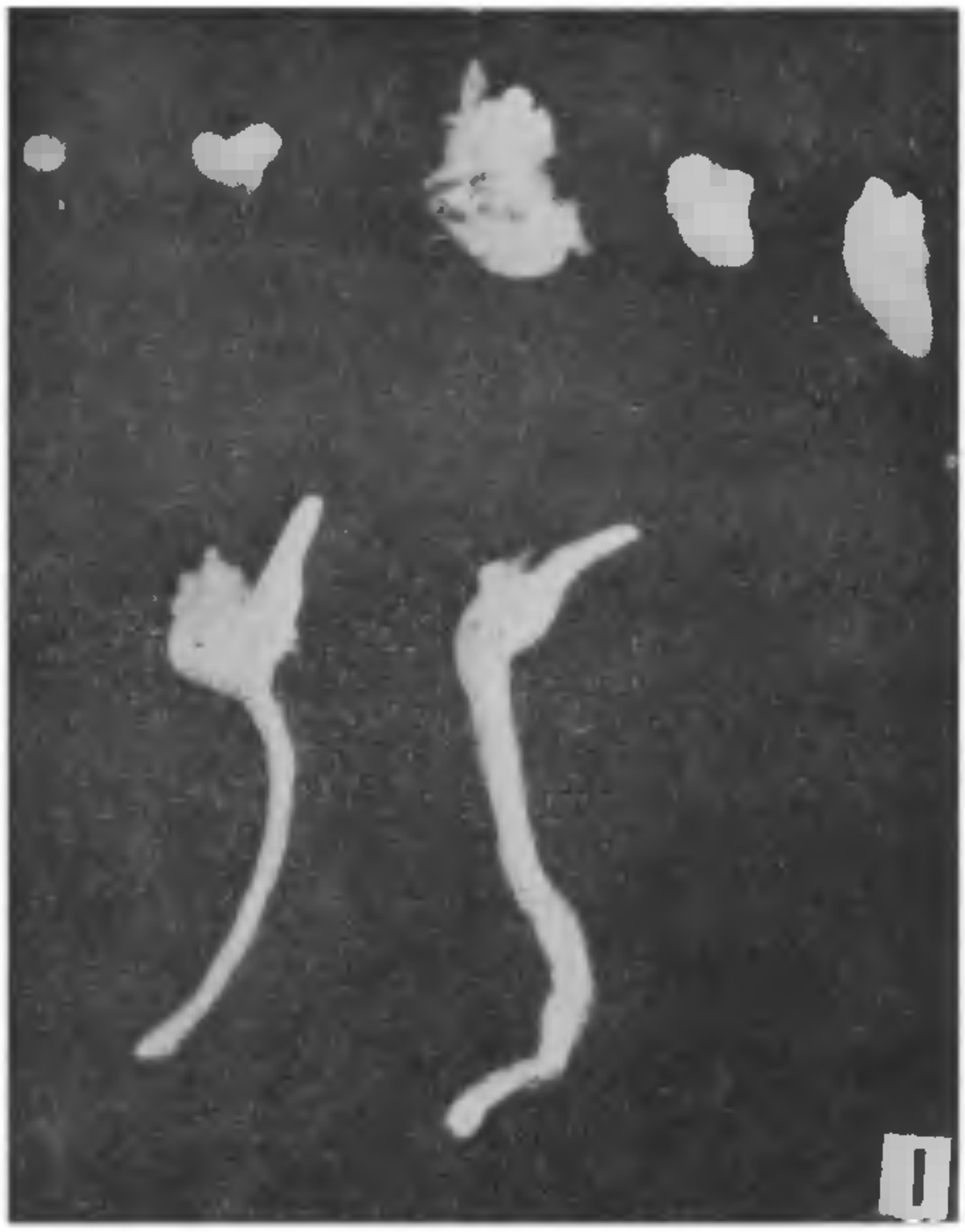

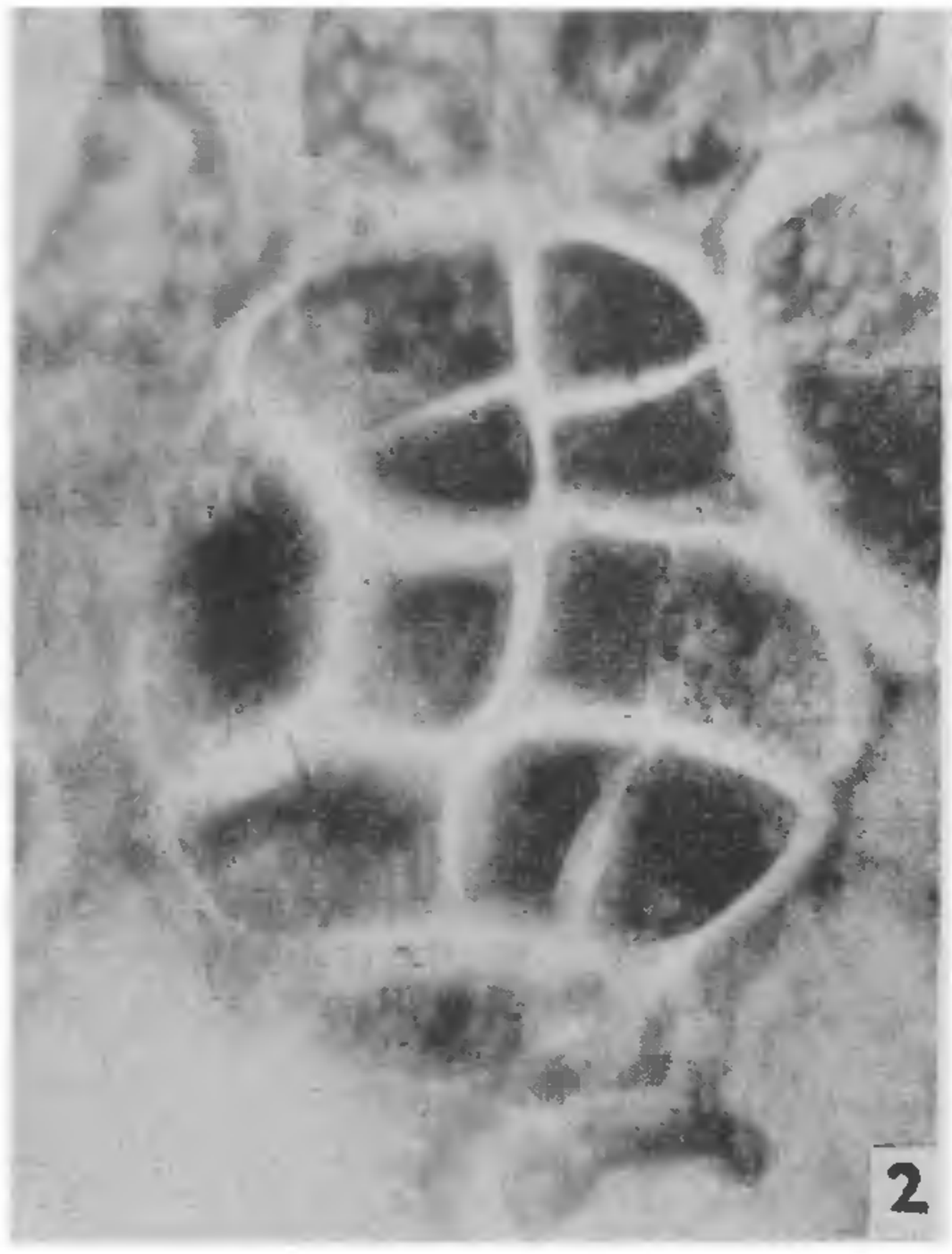

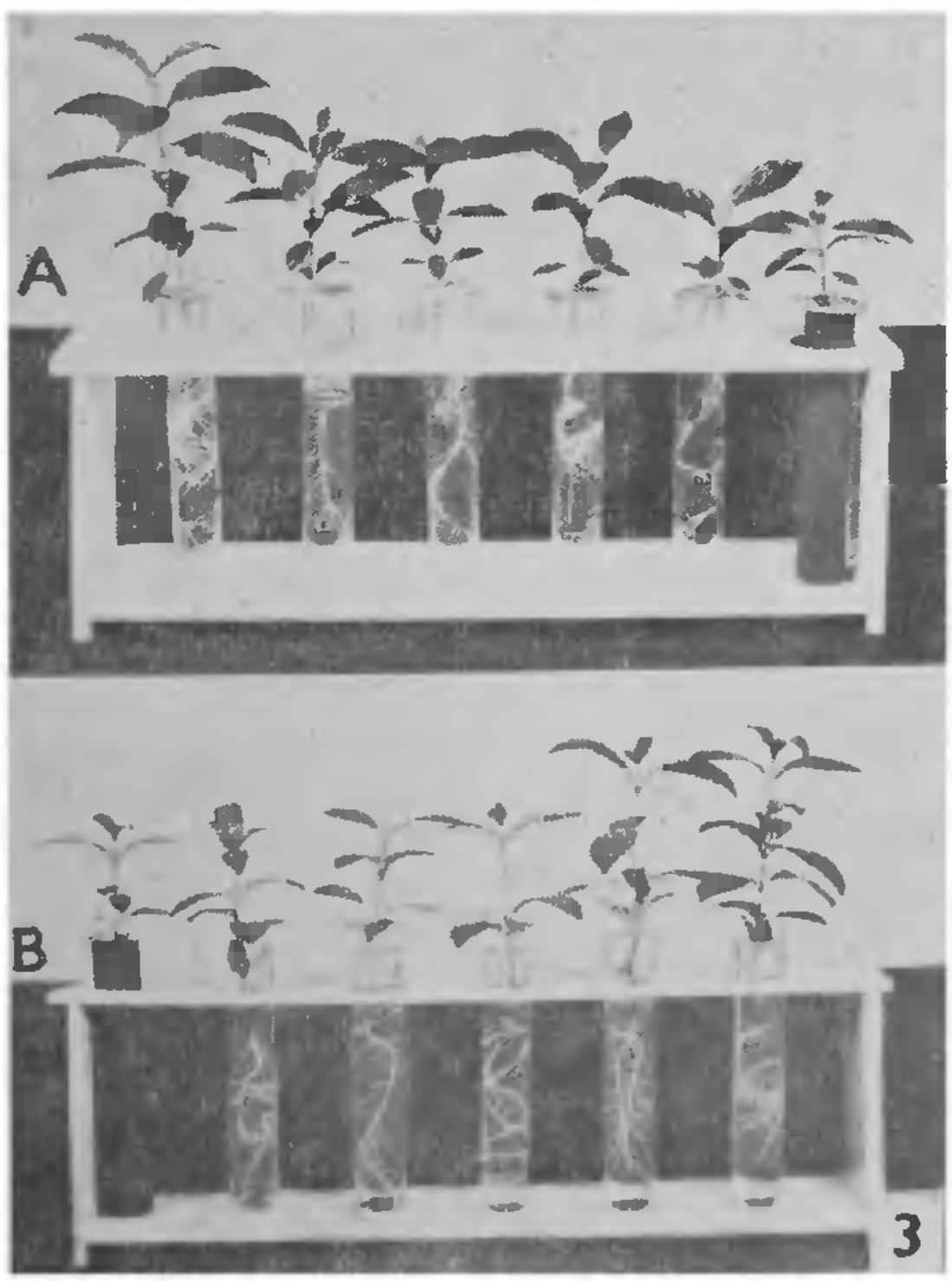

FIGS. 1–4. Fig. 1. *Regenerants* differentiated in proembryogenic tissue. A. Embryoids at different developmental stages from globular to, more or less, torpedo. B, Young plantlets developed from bipolarized meristematic nodules (Both × 5·4). Fig. 2. A 12-celled embryoid in cross-section, differentiating in proembryogenic tissue. (× 1170). Fig. 3. Regenerated plants of *R. serpentina* growing in an inorganic nutrient solution. A, plants raised from leaf callus. B, Plants raised from excised seed embryos. (Both × 0·13 approx.). Fig. 4. A, Pot culture of 1-year-old callus-regenerated *R. serpentina* plant in flowering. (× 0·22 approx.) B, A magnified view of the fruit bearing inflorescence of the same plant during the second flush of flowering. (× ·45 approx.).