EXPERIMENTAL INDUCTION OF TRIPLOID SHOOTS *IN VITRO* FROM ENDOSPERM OF *DENDROPHTHOE FALCATA* (L.f.) ETTINGS.

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THE aseptic culture of various sporophytic and gametophytic tissues has enabled us to understand their totipotency and some of the causal factors for organ differentiation. However, not much success has been achieved in inducing morphogenesis in the endosperm tissue of angiosperms. Although tissues of unlimited growth have been obtained from endosperm of several species,[1-3] only in the singular instance of *Exocarpus cupressiformis* Labill. (Santalaceae) has this tissue differentiated shoot buds.[4] The present work was initiated to explore whether or not endosperm of some other taxa has potentiality for morphogenesis *in vitro*.

Ripe fruits of the loranthaceous parasite *Dendrophthoe falcata* ($2n = 18$) were washed with 'Cetavlon' cetrimide-concentrate (diluted to 100 times) and surface-sterilized with 90% ethyl alcohol for about five minutes. The endosperm, together with the embryo (Fig. 1, A), was aseptically excised from the fruits and implanted on modified White's agar (0·8%) medium containing 3% sucrose (WM), and on WM supplemented with indoleacetic acid (IAA), indolepyruvic acid (IPA), indolebutyric acid (IBA), naphthaleneacetic acid (NAA), 2,4-dichlorophenoxyacetic acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), adenine, benzyladenine, kinetin, 6-(γ,γ-dimethylallylamino)-purine, tricanthine, zeatin, casein hydrolysate, coconut milk, and yeast extract individually and in different combinations. Twenty-four cultures were maintained under daylight (10-20 ft.-c), at 25 ± 2° C and 50–60% relative humidity.

On WM, which served as the control, the embryo developed into a seedling and the endosperm collapsed in eight weeks after culture. On WM *plus* an auxin—IAA, IPA, IBA, NAA, 2,4-D, or 2,4,5-T—both embryo and endosperm proliferated (Fig. 1, B). The callus from embryo as well as from endosperm showed unlimited growth on WM supplemented with IBA (5 ppm). On WM enriched with 5 or 10 ppm of any one of the 6-substituted aminopurines, the embryo formed a seedling. In 80% of the cultures on WM + kinetin (5 ppm) or adenine (20 ppm) numerous shoot buds appeared adventitiously from the radicular end of the embryo, 6-8 weeks after culture. The endosperm did not show any visible morphological change, except for a negligible proliferation; though the cultures were maintained for over 20 weeks.

Unlike auxin or cytokinin alone, a combination of the two with WM induced growth and differentiation of shoot buds both from embryo and endosperm. In 90% of the cultures additional shoot buds appeared from the radicular end of the embryo, whereas only 16% of the cultures showed shoots originating from endosperm. Addition of casein hydrolysate (2000 ppm) to WM + IAA (5 ppm) + kinetin (10 ppm) or adenine (40 ppm) enhanced the percentage of cultures forming shoot buds from embryo to 95 and from endosperm callus to 35. Embryo as well as endosperm callus, formed on WM + IBA (5 ppm), when transferred to WM + IAA + kinetin (or adenine) + casein hydrolysate differentiated shoot buds in 70 and 50% cultures, respectively. Several leaves (maximum length 2·5 cm.) were formed from these buds of endosperm origin, and were similar in shape to the plumular leaves (Fig. 1, C.)

Anatomical studies revealed that the endosperm usually proliferated on the surface that lay in contact with the agar medium, and the shoot buds developed on the surface away from the medium (Fig. 1, D).

The basic chromosome number of *Dendrophthoe falcata* as determined from the aceto-carmine squashes of microspore mother cell is 9. The plumular shoots and their leaf-tip cells formed *in vitro* showed the diploid number 18. But the shoot and leaf-tips which developed from the endosperm or endosperm callus were, as expected, triploid ($3n = 27$; Fig. 1, E).

Thus, the endosperm of angiosperms, like any other tissue, retains all the genetic information and its totipotency can be evoked under suitable cultural conditions.

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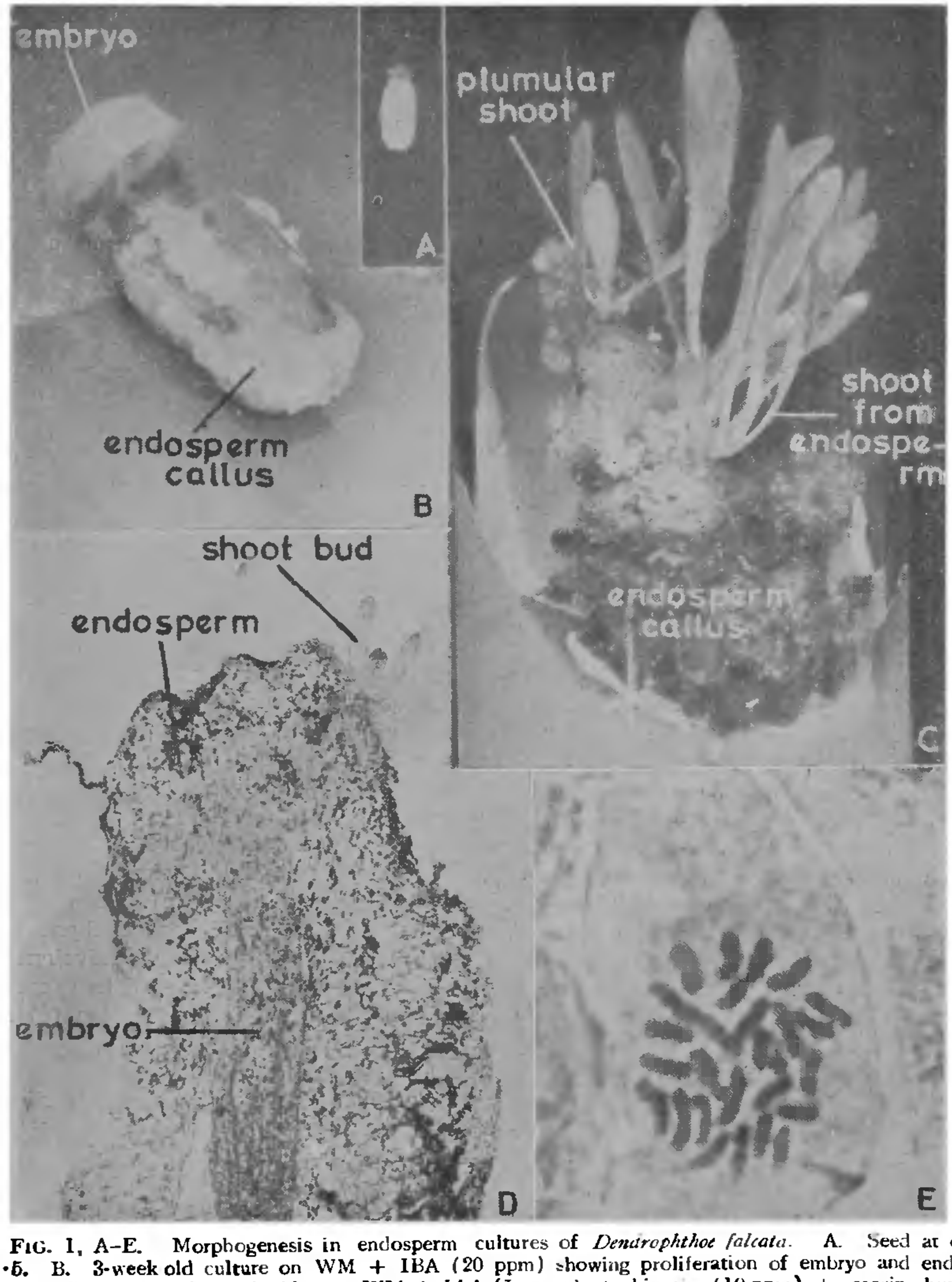

FIG. 1, A–E. Morphogenesis in endosperm cultures of *Dendrophthoe falcata*. A. Seed at culture, ×1·5. B. 3-week old culture on WM + IBA (20 ppm) showing proliferation of embryo and endosperm, × 5. C. Same, 14-week-old, on WM + IAA (5 ppm) + kinetin (10 ppm) + casein hydrolysate (2000 ppm); in addition to plumular leaves, note the development of leaves, from callused endosperm. × 2·4. D. L.s. portion of seed from a 9-week-old culture on WM + IAA (5 ppm) + adenine (40 ppm) + casein hydrolysate (2000 ppm); note superficial origin of bud from endosperm. × 15·6. E. A cell from acetocarmine squash of shoot tip formed from endosperm, showing 27 chromosomes ($3n = 27$), ×2,439.

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