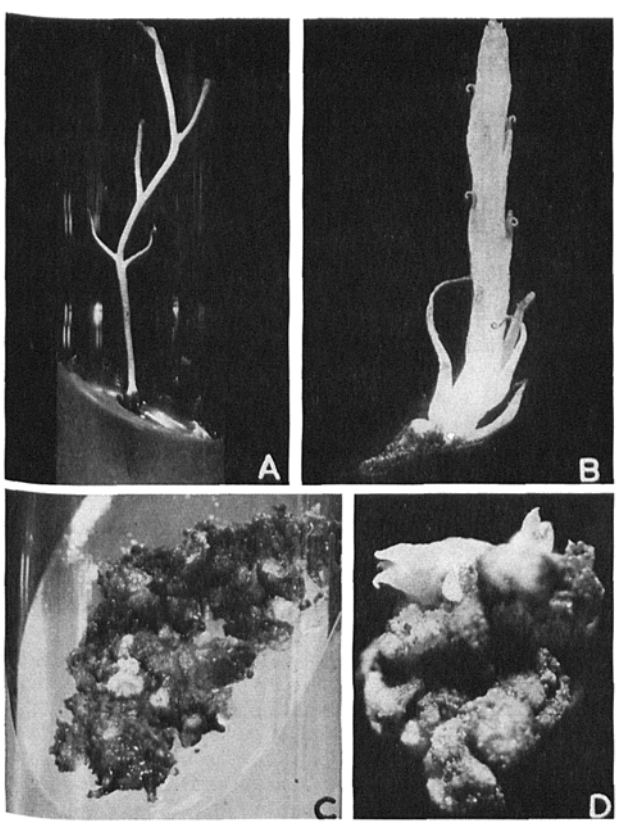
Cultivation of Embryos of *Drosophyllum lusitanicum* Link - an Insectivorous Plant

There has been growing interest in recent years in the understanding of nutrition and flowering of insectivorous angiosperms in axenic cultures[1-3]. This note reports our preliminary results on the cultivation of seeds and embryos of *Drosophyllum lusitanicum* Link. The seed is pyramidal in shape and has a thick, black seed coat. The mature embryo is small and fully organized. The bulk of the seed is occupied by the cellular, starchy endosperm.

Modified White's medium containing 2% sucrose and solidified with 0.8% agar but without IAA[4] served as the basal medium (WB). Mature seeds were surface sterilized with chlorine water and planted on WB as well as on WB + 500 mg/l each of beef extract, casein hydrolysate and yeast extract separately.

Seeds failed to germinate on any medium, even after 8–10 weeks in culture. Neither soaking in running water for 36 h nor treatment with dilute sulphuric acid prior to culture facilitated germination. HARDER[5], however, reported prompt germination in the same species. It is not clear whether he used fresh, dry or immature seeds. He does not mention the percentage of germination either.

As a logical step we excised the embryos under aseptic conditions and planted them on the following media: WB; WB + casein hydrolysate (CH); WB + CH + IAA (1 ppm); WB + CH + coconut milk (CM, 15% v/v); WB + kinetin (1 ppm); WB + yeast extract.



(A) A seedling obtained from excised embryo grown for 6 weeks on WB. ×0.88. (B) Same as A but on WB + kinetin (1 ppm). Note the fleshy shoot and scale-like leaves. × 2.2. (C) Callus mass developed on WB + CH (500 mg/l) + CM (15% v/v). × 1.6. (D) Differentiation of shoots from a subculture on WB + CH + CM, × 1.8.

On WB as well as on media containing CH and CH + IAA and yeast extract, the embryos germinated and produced seedlings in 6–8 weeks (Figure, A). The root was well developed and the shoot bore linear leaves. The leaves were characteristically reverse-circinate at the tips and had glistening, capitate, stalked or sessile glands all over the surface. The plants attained a maximum length of 7–8 cm in 10 weeks. Flowering, however, has not been observed. On a medium fortified with kinetin, root growth was totally inhibited and the shoot became highly condensed and succulent. The leaf bases were fleshy and remained closely adhered to the stem whereas the tips were green and curved (Figure, B).

Embryos planted on WB + CH + CM responded differently. Instead of germinating, the embryo developed into an actively proliferating dark-brown, friable callus after 10 weeks of planting. On this brown mass, patches of pearly-white tissue appeared which were isolated and subcultured (Figure, C). Active divisions occurred on a medium containing either CH + CM or CH + kinetin (1 ppm) + IAA (1 ppm). This tissue has been maintained through several passages for the past 18 months. Aceto-carmine squash preparations of the callus revealed mitotic stages, single cells, aggregates and tracheid-like elements. Only 12% of the subcultures differentiated into shoots and roots after 2 weeks in culture on WB + CH + CM (Figure, D).

Thus, it has been possible to overcome the dormancy of seeds by culturing the excised embryos. By changing the composition of the nutritive medium, the growth pattern of the organized embryos has been completely altered. The altered morphogenesis of embryos of *Drosophyllum* is similar to that observed in cultured embryos of phanerogamic parasites[6,7].

Résumé. Les semences de *Drosophyllum lusitanicum* Link, cultivées en milieu semi-solide de White (WB) et également dans des milieux auxquels on a ajouté de nombreux facteurs de croissance, n'ont pas germé, mais les embryons incisés ont commencé à germer tout de suite et ils se sont développés lorsqu'on les a semés sur le WB, et sur le WB + CH + IAA ou le WB + kinetin ou encore sur le WB + l'extrait de levure. Sur le WB + CH + CM, une callosité friable s'est produite. Celle-ci a été maintenue à travers plusieurs passages au cours de 18 mois. Une différentiation en pousses et racines a été observée dans 12% des tissus de calles sous-cultivés.

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