In vitro clonal propagation of *Nephrolepis*

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Ferns are cultivated as ornamental plants because of their evergreen foliage. The present investigation deals with the successful application of tissue culture techniques as a powerful tool for the rapid and mass propagation, on a commercial scale, of two species of *Nephrolepsis*. *In vitro* grown *N. cordifolia* Presel and *N. exaltata* on B_5 medium (Gamborg *et al.* 1968) were used for stolon explants.

Stolon segments grown on B_5 medium containing IBA (indolebutyric acid) produced greenish proliferations along their entire margin within 2 weeks. These proliferations were produced due to the initiation of meristematic growth centres. These meristematic growth centres consisted of cells in their undetermined stage of development. At this crucial stage, they were transferred to B_5 medium supplemented with 6-benzylaminopurine. The incorporation of this cytokinin in the medium induced further growth and development of these meristematic cells, resulting in the formation of adventitious buds. Each bud primordium developed a typical shoot apical meristem. Each bud on transfer back to IBA medium regenerated into a complete plantlet. Thus, it became apparent that these adventitious buds behaved as 'asexual vegetative propagules'. Adopting this technology, numerous buds were produced at a time, thereby achieving more rapid clonal propagation. About 10,000 *Nephrolepis* plants were produced from a single stolon explant within 6 months.

To bring down the cost of production of ferns raised by tissue culture, sucrose and Difco-bacto agar from the medium were replaced by sugar and ordinary agar. Both these *Nephrolepis* species grown by this technique exhibited uniformity. Tissue culture has revolutionised fern propagation and because of its profitability has gained much popularity among the commercial fern growers.

Gamborg, O. L., Miller, R. A. and Ojima, K. 1968. Nutrient requirements of suspension cultures of soybean root cells. *Exp. Cell. Res.* **50**, 151–158.

Cryo SEM of reproduction in pteridophytes

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Scanning electron micrographs can yield valuable data and provide three-dimensional information about biological material. Conventional preparative techniques (such as freeze drying and critical point drying), however, regularly result in distortion, shrinkage and/or collapse of the fragile reproductive organs of pteridophytes. Plate 1 shows material prepared by low temperature freezing, and illustrates how the recent development of cryo SEM can be applied to yield excellent preservation.

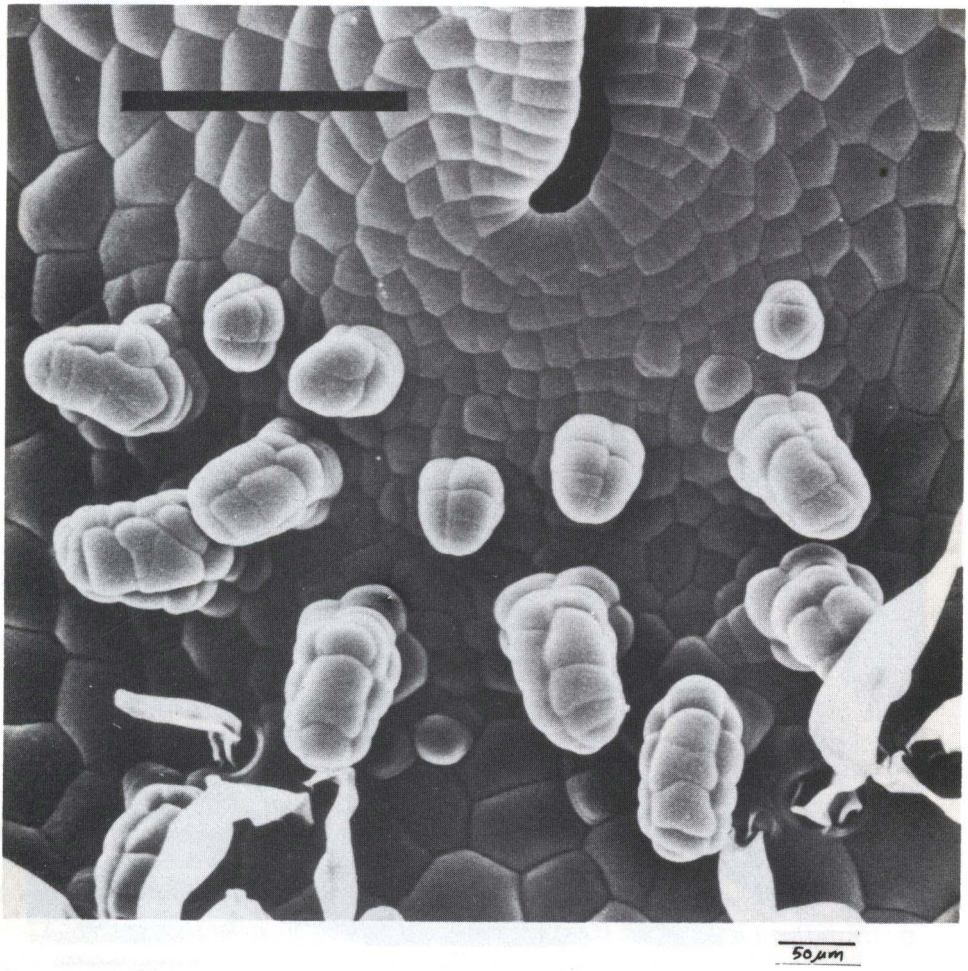

Plate 1. (a) Archegonial region of a diploid gametophyte of *Pteridium aquilinum* formed aposporously from juvenile leaf tissue. Scale = 200 µm.

https://doi.org/10.1017/S0269727000008630 Published online by Cambridge University Press

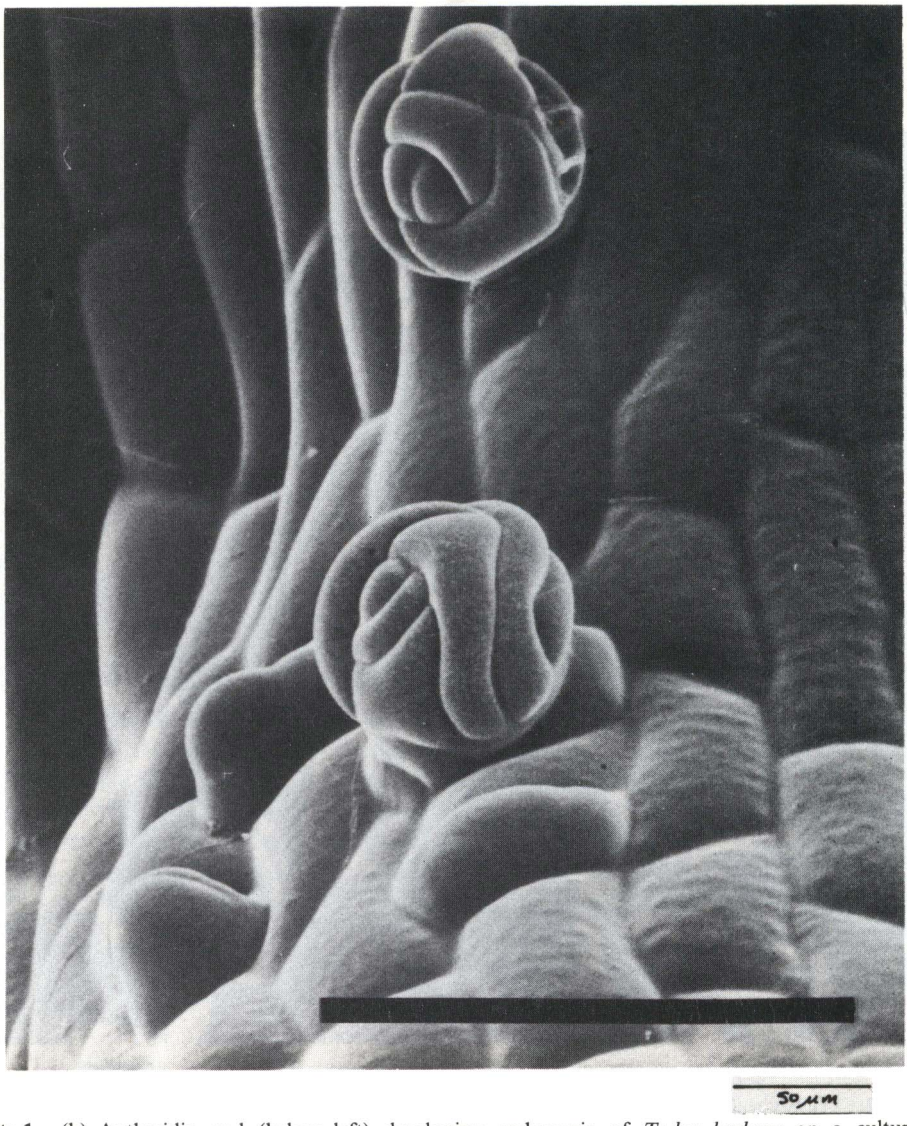

Plate 1. (b) Antheridia and (below left) developing archegonia of *Todea barbara* on a cultured gametophyte. Scale = 200 µm.

https://doi.org/10.1017/S0269727000008630 Published online by Cambridge University Press

The material shown was taken from plants in aseptic culture. Each specimen was attached to an SEM stub with quick-drying silver paint and plunged into slushed liquid nitrogen. The slushing chamber was evacuated and the stub transferred to a Cambridge S150 fitted with a cryo stage (Hexland) at −190°C. Superficial ice was removed by sublimation at −80°C and the specimens sputter-coated in a cooled pre-chamber at −190°C. They were then returned to the cryo stage for observation and photography.

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D. THE BIOLOGY OF BRACKEN (*Pteridium aquilinum*)

Isolation and regeneration of *Pteridium* protoplasts

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Protoplast isolation. Protoplasts were isolated from gametophytic tissue using the widely adopted cellulase and pectinase enzymes. Protoplast release per g of tissue increased with gametophyte age. The possibility therefore exists that young *Pteridium* prothalli produce an enzyme-resistant cell wall component, the presence of which decreases with increasing age of the prothallus. This component may take the form of a cuticular layer or a structural change in the cell wall. TEM observations of the wall indicate that there is a change in cell wall structure as the prothalli mature. Huckaby *et al.* (1982) report finding a cellulase-resistant component in outer prothallial cell walls of *Onoclea sensibilis*. Wada and Staehelin (1981) also observed an external waxy cuticle on protonemata of *Adiantum capillus-veneris*.

SEM of freshly isolated protoplasts. SEM of freshly isolated protoplasts was performed and comparisons made between freeze-dried (FD) and critical point dried (CPD) and cryo-preserved protoplasts. For biological specimens, it has been found that one of the benefits to be gained from cryopreservation is that the material can be viewed in the SEM without prior fixation. Protoplasts, however, must be suspended in an osmoticum both during and after isolation. On sublimation of a frozen protoplast suspension, the osmoticum therefore remains as crystals, obscuring much of the surface detail. The protoplasts were therefore subjected to a short glutaraldehyde fixation to allow the osmoticum to be replaced by water. The cryo-treated protoplasts were mounted on membrane filters which absorbed much of the water in the suspensions. The remaining water (ice) was removed by sublimation to expose the protoplasts.

Dehydration of most plant material has been shown to result in shrinkage and also wrinkling and collapse of cell walls. Since protoplasts possess no rigid exterior, the convolutions and wrinkles seen in FD and CPD specimens could result from