Histology (Sectioning and Microscopy) of Putative Somatic Embryos from Direct Somatic Embryogenesis of Sundew

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ABSTRACT

Sundew has been a medicinally important carnivorous plant and also a favourable ornamental plant for centuries. Owing to this, sundew is being over-exploited in certain countries, causing their populations to reduce to a critical level. To address these problems, in vitro micropropagation of sundew serves as an ideal way to regenerate and propagate these plants in large scale. Being one of the plant regeneration pathways, somatic embryogenesis possesses several advantages over other methods, rendering it to be more preferred. Previously, nodular structures were induced on Drosera x tokaiensis leaf segments on Murashige & Skoog (MS) medium supplemented with thidiazuron (TDZ) plant growth regulator. In order to confirm that these observed structures were somatic embryos, histology study was conducted. $D. \times$ tokaiensis leaves were cultured on MS medium supplemented with 1 mg/L TDZ hormone, and were then sampled and fixed in FAA fixative followed by dehydration in ethanol series. The dehydrated leaf samples were then cleared in xylene and infiltrated with paraffin wax prior to sectioning. Sections were dried, dewaxed and stained either using toluidine blue or safranin-aniline blue prior to microscopy. Microscopy observation identified competent-like cells, and embryogenic cell at one-, two-, four-, eight-cell stages and globular somatic embryos. Identification of these embryo developmental stages confirmed the somatic embryogenesis pathway of the species. However, identity of competent-like cells could not be confirmed as the morphology of competent cells is highly variable across different species. The identity could be confirmed by molecular detection of early-embryogenesis gene marker in future.