Somatic Embryogenesis In the Threatened Cactus *Turbinicarpus pseudomacrochele* (Buxbaum & Backeberg)

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ABSTRACT

Plant regeneration through indirect somatic embryogenesis from medullar tissue discs of greenhouse grown adult plants is described. Induction of somatic embryogenesis was achieved after a 4-week period on agar solidified Murashige and Skoog (MS) medium supplemented with L2 vitamins, 3 mg/l 2,4-dichlorophenoxyacetic acid, 2 mg/l 1-naphthalene acetic acid, 2 mg/l kinetin, 500 mg/l L-glutamine, and 250 mg/l casein hydrolyzate. Germination of somatic embryos was evident after 16 weeks on the same basal medium, solidified with the agar substitute Phytagel (5 g/l), and without growth regulators.

Turbinicarpus pseudomacrochele is a Mexican threatened member of the Cactaceae family, and it is included in the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) Appendix I (Hunt, 1992). Over collection and habitat destruction have significantly reduced wild populations of this appreciated ornamental and collectable species of a high degree of endemism (Anderson et al., 1994).

Most of the species contained in the taxonomic Line "B" *Strombocacti,* which comprises 10 genera and 27 species are appreciated by collectors around the world; however, only three of them have been propagated *in vitro.* Axillary shoot proliferation in *Aztekium ritteri* and *Leuchtenbergia principis* was reported by Rodriguez-Garay and Rubluo (1992) and Starling (1985), respectively. Also, successful somatic embryogenesis was reported for *Ariocarpus retusus* (Stuppy and Nagl, 1992).

In this paper the indirect somatic embryogenesis of the cactus species *Turbinicarpus pseudomacrochele is* reported, including photographic evidence of different stages of the embryogenic process from an early proembryo to a whole plantlet.

Medullar tissue discs from greenhouse grown adult plants (kindly donated by the National Institute of Ecology, Mexico) served as the explant material, to ensure the absence of preexisting meristematic tissue (areolas). For induction of somatic embryogenesis, disc explants were placed in plastic petri dishes (100 mm x 15 mm) containing 25 ml of freshly prepared MS basal salts (Murashige and Skoog, 1962), with the addition of L2 vitamins (Phillips and Collins, 1979), 3 mg/l 2,4 - dichlorophenoxyacetic acid, 2 mg/l naphthalene acetic acid, 2 mg/l kinetin, 500 mg/l L-glutamine, 250 mg/l casein hydrolysate, and solidified with 8 g/l agar. All cultures for embryo

induction were incubated for a four-week period at 27 ±2°C with a 16 h photoperiod under fluorescent light (25 μmol•s^{-1}•m^{-2}). After four weeks, a creamy-yellowish embryogenic callus was produced, containing primarily proembryos, as well as globular and well-defined embryo structures (Figures 1a and 1b). For somatic embryo germination, cultures were transferred to the same basal medium without growth regulators, and solidified with 5 g/l of the agar substitute Phytagel™ (Sigma Chemical Co. Cat. No. P-8169), under the same incubation regime. After 16 weeks, somatic embryos started to germinate and developed typical plantlet morphology (Figures 1c and 1d) identical to some members of the *Cactaceae*, Subtribe *Thelocactinae*, Line "B" *Strombocacti*, in which the cotyledons are coalescent and the root is napiform (Bravo-Hollis and Sánchez-Mejorada, 1991).

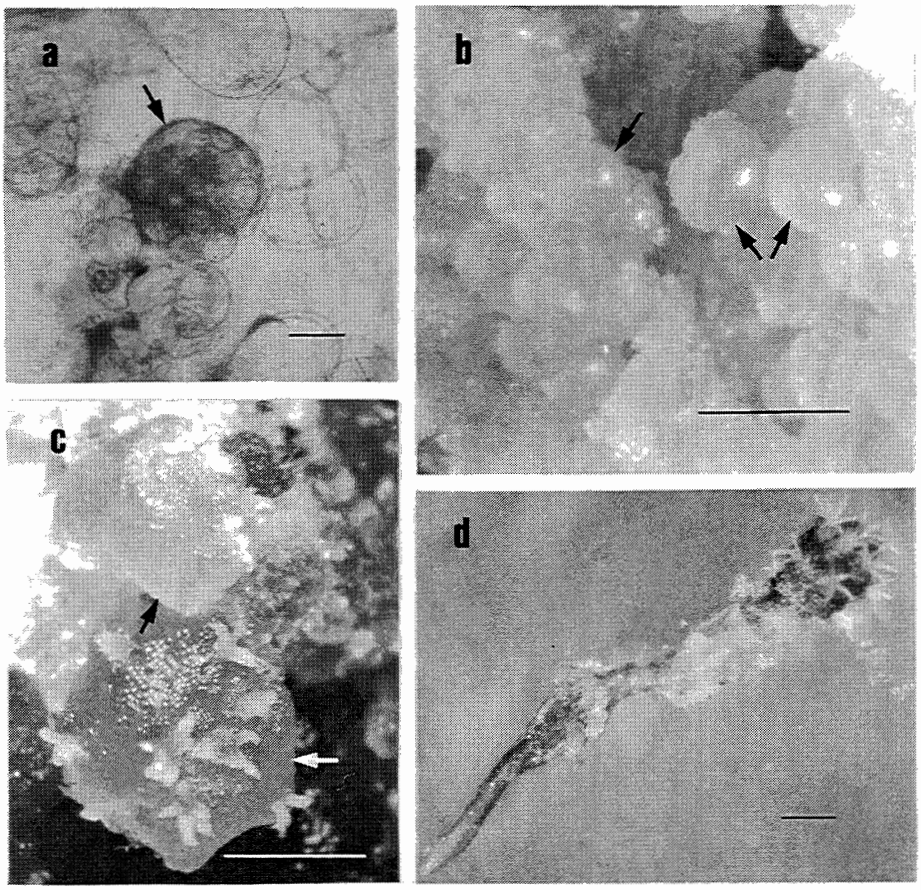

Figure 1. Somatic embryogenesis in *Turbinicarpus pseudomacrochele*
(a) Limited-celled proembryo (arrow). Bar = 0.1 mm.
(b) Embryogenic callus and globular stage structures (arrows). Bar = 1.0 mm.
(c) Germinating somatic embryos (black and white arrows). Bar = 1.0 mm.
(d) Plantlet from somatic embryogenesis. Bar = 1.0 mm.

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