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BRIEF COMMUNICATION

Histological analysis of somatic embryogenesis and adventitious shoot formation from root explants of *Centaurium erythreae* Gillib.

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Abstract

Direct somatic embryogenesis and adventitious shoot formation were successfully achieved from root explants of *Centaurium erythrea* Gillib. cultured on Murashige and Skoog medium with half-strength macronutrients, full-strength micronutrients and vitamins, 3 % sucrose, 0.7 % agar, 100 mg dm^{-3} myo-inositol and without growth regulators. Histological studies revealed that somatic embryos were formed directly from epidermal cells and adventitious buds were developed from meristematic cells in root cortex tissues. Somatic embryos as well as adventitious shoots developed into whole plantlets.

Additional key words: histology, organogenesis, root culture.

Numerous publications have reported that the plants could regenerate from somatic cells by somatic embryogenesis and/or organogenesis. Both processes can occur directly or indirectly, *i.e*. passing through an intermediate callus stage. Studies aimed at understanding morphogenetic differentiation have resulted in a paper describing the various factors that influence morphogenic responses in plant tissue (Tran Than Van 1981). Although extensive research has been carried out on *in vitro* organogenesis and embryogenesis in cultivated *Centaurium* species (Barešová 1988), there is little information on the development of somatic embryos and shoots or their cellular origin. Certain herbaceous plant species have been reported to be capable of forming somatic embryos (Twyford *et al*. 1996, Tylicki *et al*. 2000) and adventitious buds (Sankhala *et al.* 1996) from root explants. The aim of present work is to evaluate the histological aspects associated with the induction and development of somatic embryos and adventitious shoots from root explants of *Centaurium erythreae* Gillib. cultured in inductive conditions.

Seeds of *Centaurium erythrea* Gillib. were surface sterilized with 30 % (v/v) sodium hypochloride for 10 min, rinsed in sterile distilled water three times. Disinfected seeds were than transferred on a filter paper, placed in Petri dishes (55 × 15 mm) with 2 cm^3 of sterile distilled water for germination. Roots were excised from three-weeks-old seedlings and cut into 15 mm long pieces. They were aseptically placed on culture medium containing half-strength Murashige and Skoog (1962; MS) macronutrients, full-strength micronutrients and vitamins, 3 % (m/v) sucrose, 0.7 % (m/v) agar and 100 mg dm^{-3} myo-inositol. The pH of the media was adjusted to 5.8, prior autoclaving at 114 °C for 25 min. The cultures were maintained at 25 ± 1 °C under 16-h photoperiod (irradiance of 50 μmol m^{-2} s^{-1}). All experiments were repeated three times with 50 explants each. For whole plantlet regeneration, somatic embryos and adventitious buds were transferred on MS medium without plant growth regulators.

To obtain histological confirmation of the initiation and development of somatic embryos and adventitious buds, the root explants at different stages of development were fixed in FAA (formaldehyde:acetic acid:ethanol, 10:5:85, v/v) for 24 h. After ethanol dehydration the samples were embedded in paraffin wax (58 - 60 °C). Sections 5 μm thick were cut on rotary microtome (*Reichert*, Wien, Austria) and fixed on glass slides. Sections were de-waxed with xylene for 5 - 10 min and then stained with haematoxyline, by passing the slides through an ethanol solution (Johansen 1940). Sections

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were mounted in Canada balsam before microscopic examination.

The initial root explants began to enlarge and featured morphological changes after 5 d in culture on MS medium without grow regulators (Fig. 1*A*). Furthermore, under these conditions excised root showed somatic embryos (SE) of cotyledonary stages of development after 15 d of the first subculture (Fig. 1*B*). At the same time, also adventitious buds (Fig. 1*C*) were formed on the surface of root explants. Throughout the whole culture period, differentiation of somatic embryos and adventitious shoots were asynchronous and continuous. Successive division in all directions gave rise to small globular proembryo structures (G) from the epidermis, clearly of unicellular origin (Fig. 2*A*). Continuous division within those globular structures resulted in globular embryos (GE) formation after 7 - 10 d in culture (Fig. 2*B*), and finally gave rise to the cotyledonary stage embryos (Fig. 2*C*). The embryonic root pole did not develop in these structures since the shoots originating from them formed only lateral roots. Simultaneously with the differentiation of epidermal cells and the subsequent embryo formation, meristematic cells (MC) became more and more abundant due to the continuing division in the root cortex (Fig. 2*D*). From the MC cells, adventitious buds with apical meristem (AM) and leaf primordia (LP) differentiated after 20 d under the same conditions in culture (Fig. 2*E*). Fig. 2*F* shows well developed shoots with apical meristem, leaves (L) and provascular strands (VS). The pattern of development was independent of the tissue in which the embryogenic structures had formed, and the process was asynchronous: somatic embryos and adventitious buds at different stages of development coexisted on the same explant. Under the influence of the applied culture conditions, the embryos could not continue their normal development but progressively changed into shoot-like structures at an early developmental stage. Both somatic embryogenesis and adventitious shoot formation were also observed in tissue culture of *Rosa hybrida* L. (Van der Salm *et al*. 1996).

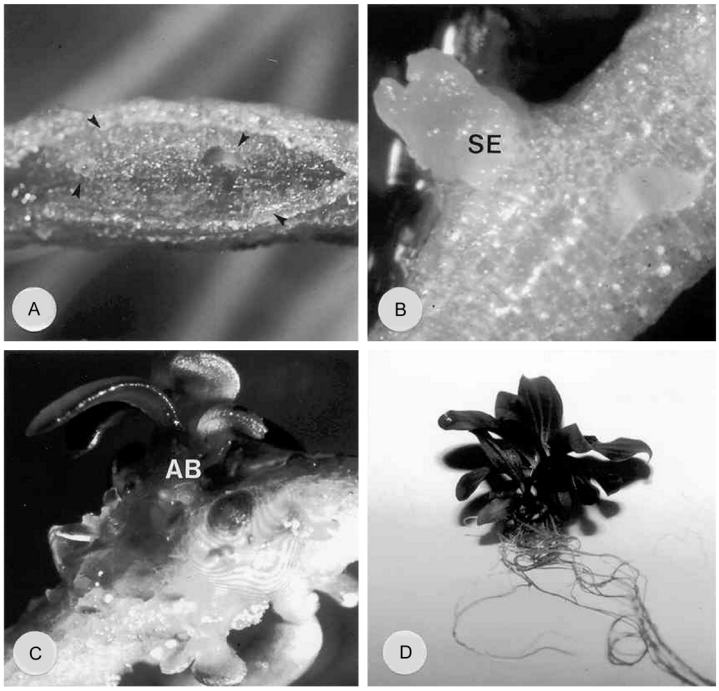

Fig. 1. Shoot formation by somatic embryogenesis on root explants of *Centaurium erythrea* Gillib. *A* - Detail of root with first visible morphological changes (*arrows*). *B* - Cotyledonary stages of somatic embryos (SE) development formed on root explants on MS after one month in culture. *C* - Adventitious buds (AB) growth on MS medium after one month in culture. *D* - Regenerated plantlet.

In this preliminary work we have shown that direct formation of somatic embryos and adventitious shoots can be obtained from root culture of *C. erythrea* using MS medium without growth regulators. Shoot formation from root explants on medium without auxin has already been reported in *Citrus mitis* (Sim *et al*. 1989). The highest yields of shoots were obtained from intact roots on whole seedlings, suggesting that endogenous auxins synthesized in shoots of intact seedlings might be essential for shoots regeneration. Further experiments concerning the effects of auxins and cytokinins on somatic embryos and adventitious shoots initiations on excised root cultures of *C. erythrea* Gillib are in progress.

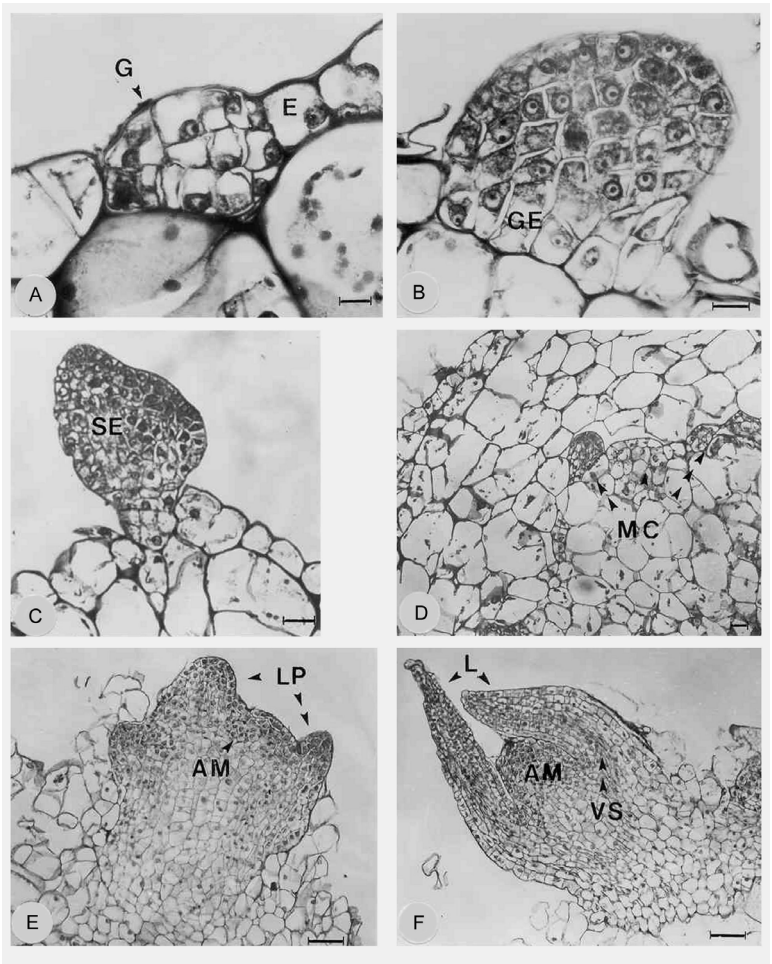

Fig. 2. Histological aspects of somatic embryos and adventitious shoots developed from root explants of *Centaurium erythrea* Gillib. *A* - Section of a one-month-old root explant with globular embryo-like structures (G) formed after division of epidermal cells (E), *scale bar* 10 μm. *B* - Globular somatic embryos (GE) of epidermal origin, *scale bar* 10 μm. *C* - Somatic embryos (SE) at the early cotyledonary stage of development, *scale bar* 10 μm. *D* - Cross section of one-month-old root with meristematic center (MC) originating from the root cortex, *scale bar* 50 μm. *E* - Adventitious buds with apical meristem (AM) and leaf primordia (LP), *scale bar* 10 μm. *F* - Well-developed shoot with apical meristem (AM), leaves (L) and provascular strands (VS), *scale bar* 10 μm.

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