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Influence of cytokinins on *in vitro* morphogenesis in root cultures of *Centaurium erythraea*—Valuable medicinal plant

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ABSTRACT

The purpose of this work was to acquire more information on the capacity of *in vitro* grown *Centaurium erythraea* Gillib. normal and hairy root cultures to simultaneously regenerate adventitious buds and to evaluate the variations induced on regeneration response by treatments with six cytokinins. Explants from normal and hairy root cultures were cultured on half-strength MS medium (1/2 MS) with kinetin (KIN), N^6-benzylaminopurine (BA), 6-γ,γ-dimethylallylaminopurine (2IP), N-(2-chloro-4-pyridyl)-N′-phenylurea (CPPU), 1-Phenyl-3-(1,2,3-thiadiazol-5-yl)urea (TDZ) and 6-[4-Hydroxy-3-methyl-but-2-enylamino]purine (ZEA), used alone in six different concentrations. The average number of adventitious buds per explant was promoted by all of cytokinin treatments. Urea-type cytokinins, TDZ and CPPU were more effective for the induction the morphogenesis of adventitious buds than adenine-type cytokinins. We found that the 1.0 μM CPPU induced the largest number (25.6, 18.2, respectively) of adventitious buds in normal and hairy root culture. TDZ-supplemented media induced highest number of adventitious buds (24.2) from normal root explant, but from hairy root explant average number of buds is lower (20.5). Regenerated shoots were excised and placed on 1/2 MS medium without hormone. The rooted plantlets were successfully acclimatized in greenhouse conditions.

1. Introduction

Regeneration processes in differentiated or undifferentiated tissues have attracted the attention for their potential of application as tools for plant propagation of important plant species. It is known that the adventitious buds and/or somatic embryos differ according to a number of endogenous and exogenous factors. Morphogenetic processes or organ development in plants include division, growth and differentiation of cells. Those processes are genetically determined and regulated by a number of endogenous and exogenous factors Ezhova (2003). Root cultures are generally suitable systems from the study and production of secondary metabolites (Kim et al., 2002; Sudha et al., 2003). However, they can be used also as model systems in the research focused on the effect of various substances on morphogenesis in root culture, e.g. plant growth regulators (Bálványos et al., 2001).

Cytokinins play a fundamental role almost in all plant developmental process. Some studies showed that synthetic phenylurea derivates such as TDZ and CPPU have higher cytokinin activity to induce adventitious buds than adenine-type cytokinins such kinetin KIN and BA (Huettaman and Preece, 1993). It has been shown that TDZ provided an efficient stimulus for the induction of *in vitro* shoot regeneration in several plant species (Uchida et al., 2003; Ipecki and Gozukirmi, 2004; Gu and Zhang, 2005).

The development and adoption of plant cell culture methods for a number of medicinal plant species have lead to the production of useful secondary plant compounds on large scale (Raju et al., 2004).

Centaurium erythraea is a winter-annual plant of the Gentianaceae family, growing throughout Atlantic countries of South Europe and North Africa. Aerial parts of this plant are a rich source of bitter secoiridoid glucosides and xanthones and have been put to numerous uses. In pharmacopoeias of many countries it is known by the common name Centaury and used as a appetite-stimulating, digestive, hepatic, febrifugal and tonic (van der Sluis, 1985). The populations of this plant are greatly endangered by collecting for preparing many preparations like tinctures, decoction, infusion tonic, lotion, powder, tea etc. *C. erythraea* inscriptions in the most red lists of endangered plants. (Holub et al., 1979). Due to destructive harvesting and lack of proper cultivation, the wild population of this medicinally important plant has declined very fast. Therefore, there is an urgent need to develop an appropriate protocol for mass propagation and conservation of this endangered

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medicinal plant. In general, most reports on induction of organogenesis and/or somatic embryogenesis in *C. erythreae* involve of leaf, seedlings and callus tissues (Barešova, 1988). A quick, efficient and novel shoot regeneration methodology from root tissue has been developed from *C. erythreae.* These methods avoid an intermediate callus phase, and may reduce the risk of somaclonal variation. There are only few reports on *in vitro* morphogenesis from root explants (Vinocur et al., 2000; Chaundhri et al., 2004; Shahzad et al., 2007). Hairy roots, obtained after genetic transformation of host cells by *Agrobacterim rhizogenes*, are genetically stable and posses high potential to produce the secondary metabolites than the normal roots (Doran, 2002). Hairy roots have induced in many plants by transformation with *A. rhizogenes* Ri T-DNA. This DNA segment carries a set of genes that encode enzymes which control auxin and cytokinin biosynthesis. In this fact, hairy roots are characterized by fast growth, lateral branching and genetic stability on hormone-free medium (Giri and Narasu, 2000). On the other hand, normal root cultures need an exogenous plant growth regulators supply and growth very slowly. Also, this balans of plant growth regulators induces different morphogenic responses in hairy root cultures comparing with normal root cultures. Hairy root induction and shoot regeneration from hairy root are important prerequisites for successful production of transgenic plants as well as secondary metabolites using *A. rhizogenes* (Hu and Du, 2006). The advantage of using root cultures in than they growth fast and are relatively easy to prepare and maintain, show a low level of variability and can be easily cloned to produce a large supply of experimental tissue. Previously we have report protocol of *A. rhizogenes* transformation and regeneration of transgenic plants of *C. erythreae* (Subotić et al., 2004). Also, employing the same nutrient medium with addition of different auxins or carbohydrates, Subotič et al. (2006) defined

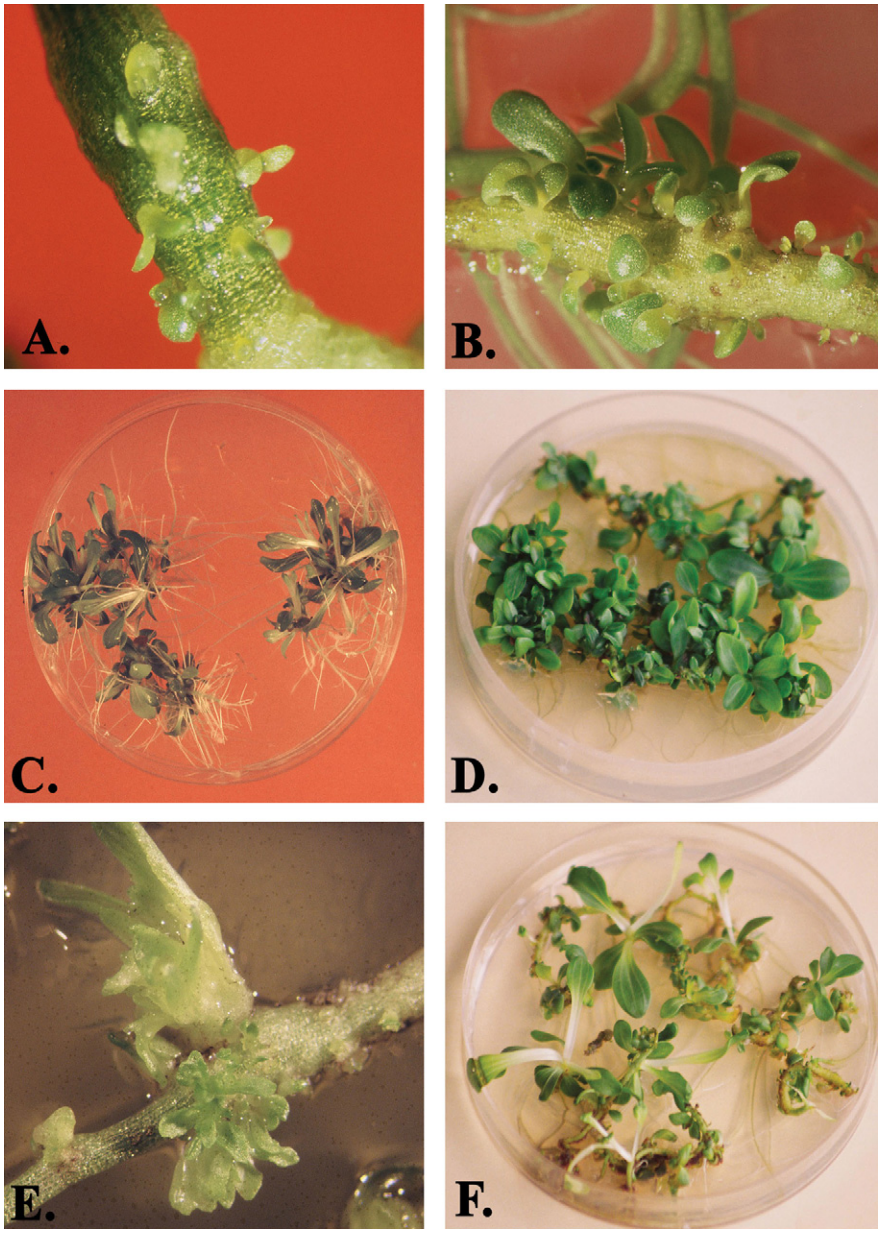

Fig. 1. Morphogenetic responses of normal and hairy root culture of *C. erythraea* on 1/2 MS medium supplemented with different cytokinins. (A and B) Adventitious buds developed on normal and hairy root explants on medium with 3.0 μM CPPU, after 10 and 20 days in culture, respectively. (C) Hairy root culture with well developed adventitious buds on medium with 1.0 μM BA. (D) Adventitious buds achieved in normal root culture on medium with 3.0 μM CPPU. (E) Differentiation of fasciated and distorted buds on medium with 3.0 μM TDZ. (F) Normal root culture after 30 days on medium supplemented with 3.0 μM BA.