SHORT NOTE

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Tissue and cell culture of phytoecdysone-producing plants as a perspective source of ecdysteroids

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Biochemical investigation of various plant species of Russian flora on the presence of ecdysteroids revealed a number of species with high ecdysteroid content. Among those *Serratula coronata* L. (Asteraceae) and *Ajuga reptans* L. (Lamiaceae) were of special interest.

In vitro cultures of these species were established to develop biotechnology for ecdysteroids production alternative to original plants. Callus was induced from different organs of the juvenile plants grown in aseptic conditions on Murashige and Skoog hormone-free agar medium under artificial lighting. Plant hormones 2,4-dichlorphenoxyacetic acid and benzylaminopurine proved the most suitable regulators for callus induction and growth. Originally 443 callus strains of *S. coronata* and 105 callus strains of *A. reptans* were obtained manifesting variety of genetic and epigenetic features of explants.

Preliminary identification of phytoecdysteroids has been made by analytical RP-HPLC in agreement with authentic standards. Column Diasorb-130/C16T (250 × 4 mm ID) was eluted at 0.7 ml/min with water : acetonitrile : tetrahydrofuran (100 : 16 : 4). Initial extraction from dried callus tissue was performed using methanol, ethanol 70% or hot water. Methanolic extraction and subsequent purification of samples on C16 DIA-PACK cartridges appeared the most suitable procedure.

Analysis of above 100 specimens of *S. coronata* and 30 specimens of *A. reptans* callus tissue revealed a number of strains producing 20-hydroxyecdysone (20HE) in amounts comparable to original plants (0.073% – *S. coronata* strain, 0.03% – *A. reptans* strain). Moreover, both callus cultures produced a number of minor ecdysteroids which in some strains showed higher amounts than those found in field collected or cultivated plants. These data are of particular interest since then tissue and cell culture may be a favourable source for minor ecdysteroids production. The spectrum of ecdysteroids produced by callus tissue of *S. coronata* included 20HE, inokosterone, makisterone A, cyasterone, ecdysone and some compounds to be identified further. Callus cultures of *A. reptans* contained 20HE, cyasterone and certain compounds to be identified. The content of the major plant ecdysteroid 20HE in callus tissues of *S. coronata* and *A. reptans* in general was lower compared to amounts of minor components produced. Some of the strains did not produce 20HE at all. For example, the content of ecdysteroids in *S. coronata* strain G1H was as follows (% of dry weight): 20HE – 0.023; inokosterone – 0.024; makisterone A – 0.1; cyasterone – 0.019; ecdysone – 0.006. *A. reptans* strain C3P contained (% of dry weight): 20HE-O; cyasterone – 0.31.

Significant modifying effect of plant auxins (2,4-dichlorphenoxyacetic acid, indole-3-acetic acid, indolebutyric acid and α-naphthalene acetic acid) on growth and synthesis of ecdysteroids in callus tissues of *S. coronata* has been observed but it was α-naphthalene acetic acid that improved both processes.

So investigation resulted in selection of the callus strains with high level of ecdysteroids production for further establishing cell suspensions to study biosynthesis, accumulation and accurate identification of ecdysteroids produced.