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Cultures of transformed roots and shoots of *Salvia bulleyana* Diels as a source of bioactive metabolites.

Salvia bulleyana Diels derives from Yunnan Province and has been locally used in Traditional Chinese Medicine as a substitute of *Danshen* (micronized roots and rhizome of *Salvia miltiorrhiza*). Therapeutic potential of that species is due to the presence of two main groups of secondary metabolites – tanshinones and polyphenols. However, the restricted availability of the raw material from its natural sites restrains analysis, confirmation of therapeutic potential and its common usage. An alternative way of obtaining the plant material that can be used in such cases are plant *in vitro* cultures. Especially promising are transformed organ cultures which are acquired by genetic transformation with soil bacteria from genus *Rhizobium*.

Due to many advantages of genetic transformation and opportunities offered by *in vitro* cultivation, the aim of this study was to use selected biotechnological techniques to obtain highly productive cultures of transformed roots and shoots of *S. bulleyana*. The incorporation of bacterial DNA fragments was confirmed by PCR method using selected genes: *aux*1, *aux*2, *rol*B, *rol*C and *rol*D. Then, the most productive root clone (C4) was selected for further research. The process of optimization of growth conditions had significant impact on culture productivity. It turned out that a 40-day cultivation in the dark, in ½SH liquid medium containing half of vitamin concentration and 3% sucrose was the most favorable for biomass accumulation and resulted in two-fold increase in the polyphenol content in the plant material compared to the value obtained before optimization. Further stimulation of the biosynthesis of secondary metabolites was possible due to a 3-day exposure of hairy roots to 100 μM of methyl jasmonate. The levels of polyphenols (124,4 mg/g DW) and predominant rosmarinic acid (110,2 mg/g DW) obtained in these conditions were, respectively, 5 and 13-times higher than those reported for the roots of 2-year-old intact plant. On the other hand, in the case of transformed shoots, the highest polyphenol (39,6 mg/g DW) and rosmarinic acid (34,4 mg/g DW) contents were achieved during 5-week cultivation in liquid MS medium supplemented with 0,1 mg/l IAA and 1 mg/l BAPR with bacterial nanocellulose as a supporting material. These values were respectively, 3- and *approx.* 6-times higher than those found in the aerial parts of 2-year-old intact plants.

The technology of bioreactors is crucial for the mass multiplication of selected species in defined, optimal conditions and allows the automatization of the cultivation processes, what associates with the reduction of production costs. An attempt to scale up the culture which included cultivation in temporary immersion systems – PlantForm and Rita showed that examined cultures of transgenic roots and shoots can be successfully cultivated in both types of bioreactors. It occurred that the results depended on the type of vessel, as well as on the frequency and length of immersion of the plant material. Especially beneficial was the 5-week cultivation of transgenic shoots in the Rita system were obtained in which 751,4 mg of polyphenolic compounds, 693,5 mg of rosmarinic acid and 28,6 mg of salvianolic acid K per liter of medium. In the case of hairy root culture, the productivity obtained in both immersion systems was high, but lower than that reported for roots grown in optimized conditions in Erlenmayer flasks.

Based on the results of antioxidant tests (free radical scavenging assays, reducing capacity assay, inhibition of lipid oxidation assay) it was found that extracts prepared from transformed cultures of *S. bulleyana* were characterized by a strong antioxidant activity, which was comparable or higher than for extracts from plants obtained by conventional methods. Moreover, a promising cytotoxic potential of the rich in polyphenols hairy root extract against all cancer cell lines (especially gastrointestinal cancers) used in the experiment was demonstrated. In addition, hairy root extract exhibited moderate antimicrobial activity.

In conclusion, the selection and optimization of the culture conditions enabled to obtain efficient transformed root and shoot cultures of *S. bulleyana*. They occurred to be one of the best biotechnological sources of rosmarinic acid, in terms of productivity, and can be an effective tool for the industrial production of this compound and also other phenolic acids identified in the plant material. What is more, the hairy root extract of *S. bulleyana* exhibited strong antioxidant and significant cytotoxic activity, while being relatively safe for normal mammalian cells. It can therefore be assumed that in the future, transformed cultures of *Salvia bulleyana* may be used in the therapy and prevention of civilization diseases.