

## ***Ex situ* conservation and comparative determination of antimicrobial activity of endemic species from genus *Stachys***

Desislava Mantovska\*<sup>1</sup>, Detelina Petrova<sup>1</sup>, Lyubomira Yocheva<sup>2</sup>, Zhenya Yordanova<sup>1</sup>

<sup>1</sup>Department of Plant Physiology, Faculty of Biology, Sofia University "St. Kliment Ohridski", Sofia, Bulgaria

<sup>2</sup>Department of Biology, medicinal genetics and microbiology, Faculty of Medicine, Sofia University, "St. Kliment Ohridski", Sofia, Bulgaria

### **Introduction**

The genus *Stachys* includes more than 300 species distributed worldwide and it is considered as one of the largest genera from *Lamiaceae* family. There are 22 species in Bulgaria, 5 of which are under the protection of the Bulgarian biodiversity law. *Stachys thracica* Davdov, *Stachys bulgarica* Degen & Neic and *Stachys scardica* Griseb. are Balkan endemic species included in The Red Data Book of Bulgaria with conservation status "endangered". The natural populations of the species are comprised of few individuals and are located in regions under high anthropogenic impact.

Since ages many species from *Stachys* genus had been widely used for the treatment of inflammatory diseases such sclerosis of the spleen, coughs, genital tumors, ulcers and infected wounds (Conforti et. al., 2007).

Nowadays the antibiotic resistance is a global problem due to inefficiency of the current medicaments and their excessive usage. Overcoming this problem requires the research of alternative sources with antimicrobial activity.

The biotechnological approaches are successfully applied for *ex situ* conservation of rare and endangered plants species. The *in vitro* micropropagation allows the cultivation of plants in controlled environment, regardless of seasons and climatic changes and allows isolation of valuable secondary metabolites without disturbing the plants natural populations.

The aim of the present work is *ex situ* conservation of the endemic species *S. thracica*, *S. bulgarica*, *S. scardica* and comparative study of antimicrobial activity of extracts

isolated from *in situ*, *in vitro* cultivated and *ex vitro* adapted plants.

### **Material and methods**

*In vitro* shoot cultures from *S. thracica*, *S. bulgarica*, *S. scardica* were induced from ripe dried seed collected from their natural habitat with the permission of the Ministry of Environment and Water of Bulgaria. The seeds were sterilized with 70 % ethanol for 5 min. then washed with 96 % ethanol for 1 min. and inoculated on half-strength MS medium and on 0.7 % agar for germination. The seedlings were transferred on basal MS medium supplemented with 3 % sucrose and 0.7 % agar. Then the effect of different concentrations of BA (0.1 mg/L, 0.5 mg/L, 1 mg/L, 1.5 mg/L) on the growth of *S. bulgarica* and *S. scardica* was examined.

*Ex vitro* adaptation was carried out in phytotron chamber with controlled environmental conditions and then in greenhouse and experimental field.

The antimicrobial activity of the methanolic extracts obtained from *in situ*, *in vitro* cultivated and *ex vitro* adapted plants from the three species was tested against three gram-positive bacteria *Staphylococcus aureus*, *Staphylococcus epidermitis*, *Enterococcus faecalis*, seven gram-negative bacteria *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Proteus vulgaris*, *Acinetobacter calcoaceticus*, *Enterobacter cloacae*, *Escherichia coli*, *Klebsiella pneumoniae* and *Candida albicans*. The determination of antimicrobial activity was accomplished by agar disk diffusion method. The minimum inhibitory concentration

(MIC) was determined by micro-well dilution assay.

## Results and discussion

*In vitro* cultures from the endangered species *S. thracica*, *S. bulgarica* and *S. scardica* were successfully induced by sterilized ripe dried seeds. The regenerated *S. thracica* plants had vigorous growth and well-developed root system, but still *S. bulgarica* and *S. scardica* had poor growth index. All tested concentrations of the cytokinin BA (0.1 mg/L, 0.5 mg/L, 1 mg/L, 1.5 mg/L) stimulated shoot development in both *S. bulgarica* and *S. scardica* but most effective were 1 mg/L BA and 1.5 mg/L BA respectively. *Ex vitro* adaptation was successfully accomplished in phytotron chamber, greenhouse and experimental field with 100 % survival for *S. thracica*, 96% for *S. bulgarica* and 92% for *S. scardica*. Successful initiation of *in vitro* cultures of endangered species *S. maritima* and *S. leucoglossa* was also reported (Panayotova et al., 2008; Mantovska et al., 2019).

It has been established that most of the tested extracts from *Stachys* species showed low antimicrobial activity (Ebrahimabadi et al., 2009; Shakeri et al., 2019). In our pilot study the methanolic extracts from *in situ* grown, *in vitro* cultivated and *ex vitro* adapted *S. thracica*, *S. bulgarica* and *S. scardica* plants were more active against gram “-“bacteria rather than gram “+” and the most sensitive strains were *A. calcoaceticus* and *K. pneumoniae*.

## Conclusion

The successful initiation of *in vitro* cultures from the endangered species *S. thracica*, *S. bulgarica* and *S. scardica* is an alternative biotechnological approach for the preservation of the species. The obtained methanolic

extracts exhibit low to moderate antimicrobial activity against the tested microorganisms which is a prerequisite for further research on metabolite profiles and expanding the range of test microorganisms to obtain a clear assessment of the antibacterial potential of *Stachys* species.

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