Comparative determination of antimicrobial activity of endemic species from genus *Stachys* during the process of *ex situ* conservation

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PLANT PHYSIOLOGY

INTRODUCTION

Stachys bulgarica

Stachys scardica

Conservation status: endangered (EN)

Balkan endemic species included in The Red Data Book of Bulgaria

No available data about their *in vitro* propagation and *ex situ* conservation

Scarce information about their chemical composition and biological activity

Contain phenylethanoid glycosides

Long history of use in ethnomedicine for inflammatory diseases, infected wounds, etc.

OBJECTIVE

The aim of the present work is to develop an effective protocol for *ex situ* conservation of *S. bulgarica* and *S. scardica* and comparative determination of antimicrobial activity of methanolic extracts from *in situ* grown, *in vitro* cultivated and *ex vitro* adapted plants.

MATERIAL AND METHODS

***In vitro* shoot cultures were induced from ripe dried seeds, collected from *in situ* growing wild plants and multiplicated on basal MS medium. Then the effect of different concentrations of 6-benzylaminopurine (BA) on the *in vitro* multiplication of both species was examined. The antimicrobial activity of the methanolic extracts obtained from *in situ*, *in vitro* cultivated and *ex vitro* adapted plants from the two species was tested against three gram-positive bacteria *Staphylococcus aureus, Staphylococcus epidermidis, Cutibacterium acnes*, six gram-negative bacteria *Pseudomonas aeruginosa, Proteus mirabilis, Proteus vulgaris, Acinetobacter calcoaceticus, Enterobacter cloacae, Escherichia coli* and the yeast *Candida albicans* by agar disk diffusion method.**

RESULTS

Ex situ conservation of *Stachys bulgarica* and *Stachys scardica*

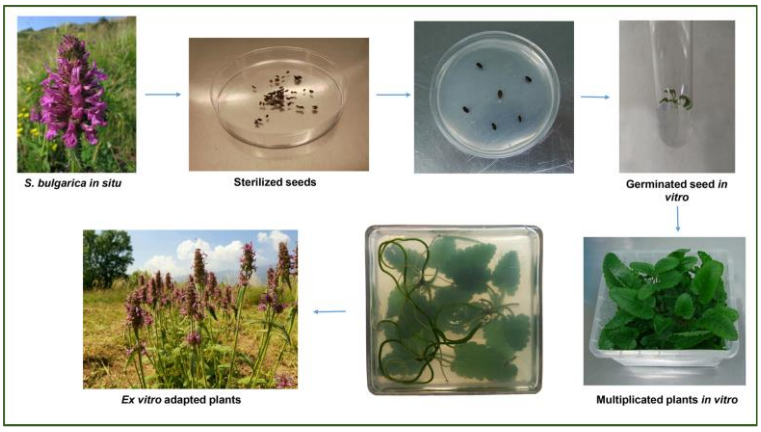

Figure 1. *In vitro* regenerated *S.bulgarica* plants and subsequent adaptation *ex vitro*

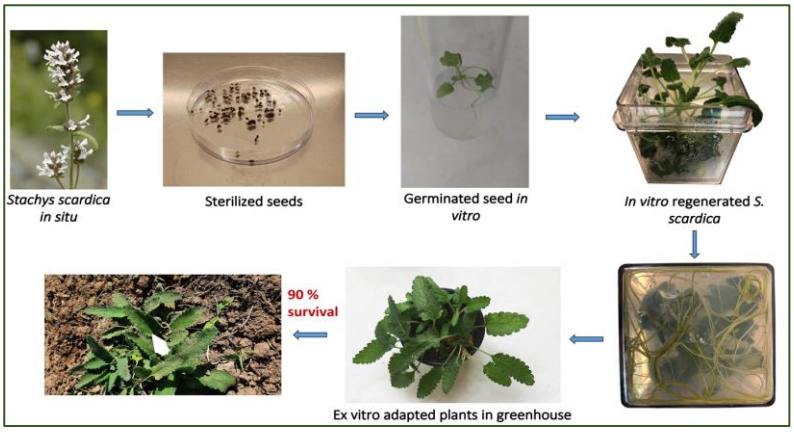

Figure 2. *In vitro* regenerated *S.scardica* plants and subsequent adaptation *ex vitro*

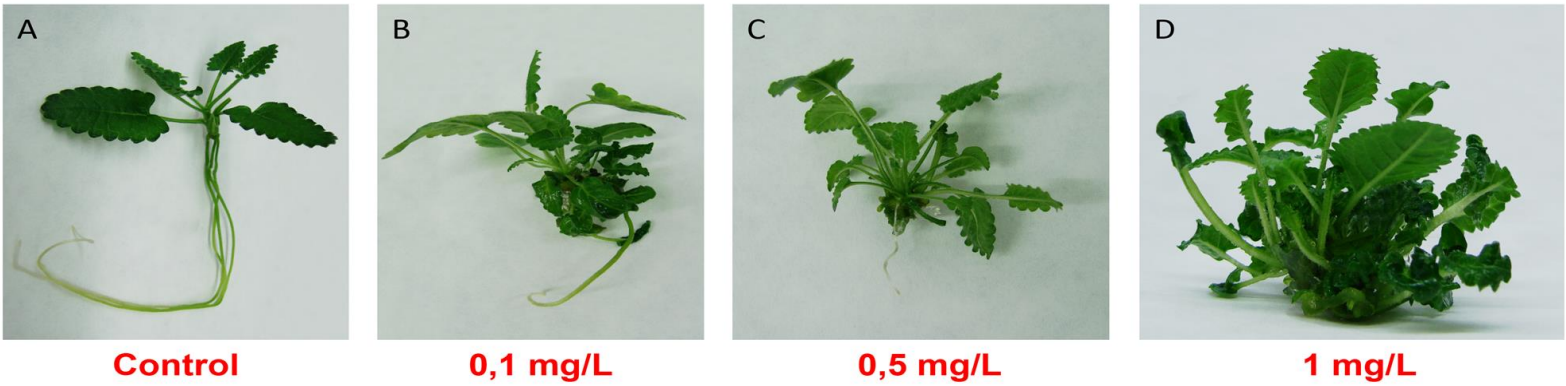

Figure 3. *In vitro* propagated *S. bulgarica* plants. A) Control plant, in vitro cultivated on MS medium; B) *In vitro* cultivated plant on MS medium supplemented with 0.1 mg/L BA; C) In vitro cultivated plant on MS medium supplemented with 0.5 mg/L BA; D) In vitro cultivated plant on MS medium supplemented with 1.0 mg/L BA

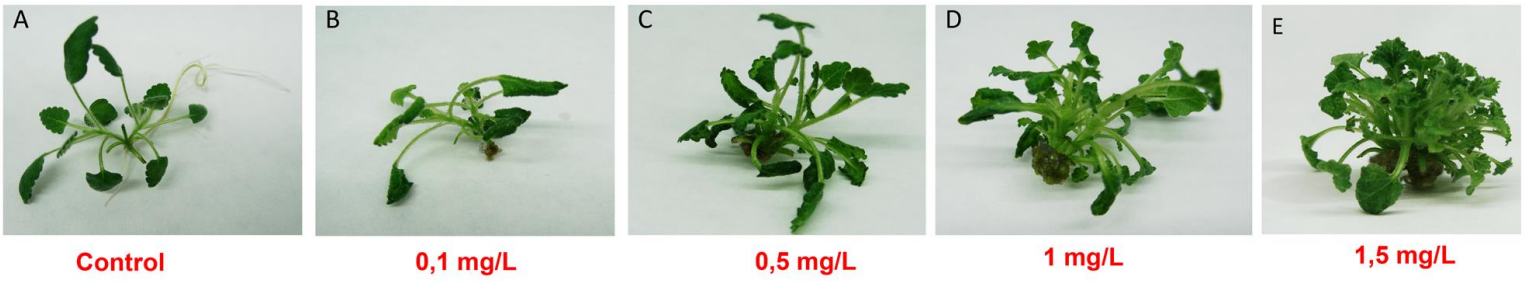

Figure 4. In vitro propagated *S. scardica* plants A) Control plant, in vitro cultivated on MS medium; B) In vitro cultivated plant on MS medium supplemented with 0.1 mg/L BA; C) In vitro cultivated plant on MS medium supplemented with 0.5 mg/L BA; D) In vitro cultivated plant on MS medium supplemented with 1.0 mg/L BA, E) In vitro cultivated on MS medium supplemented with 1.5 mg/L BA.

Variants	Number of shoots	Root formation	Root length(cm)	Degree of callus formation
Control	1.25 ± 0.43	+	8.75 ± 1.48	-
BA 0.1 mg/L	1.75 ± 0.83	+	5.5 ± 0.8	-
BA 0.5 mg/L	3.25 ± 0.43	-	-	-
BA 1.0 mg/L	8±0.71	-	-	+[1]

Table 1. Influence of different concentrations of cytokinine BA (0,1 – 1,0 mg/L) on *in vitro* multiplication of *S. bulgarica.*

Variants	Number of shoots	Root formation	Root length (cm)	Degree of callus formation
Control	3 ± 1.26	+	5.73 ± 2.54	–
BA 0.1 mg/L	2.8 ± 1.6	–	–	–
BA 0.5 mg/L	3.2 ± 0,4	–	–	+
BA 1.0 mg/L	6.4 ± 1.2	–	–	+
BA 1.5 mg/L	16.2 ± 2.04	–	–	+[1]

Table 2. Influence of different concentrations of cytokinine BA (0,1 – 1,5 mg/L) on *in vitro* multiplication of *S. scardica.*

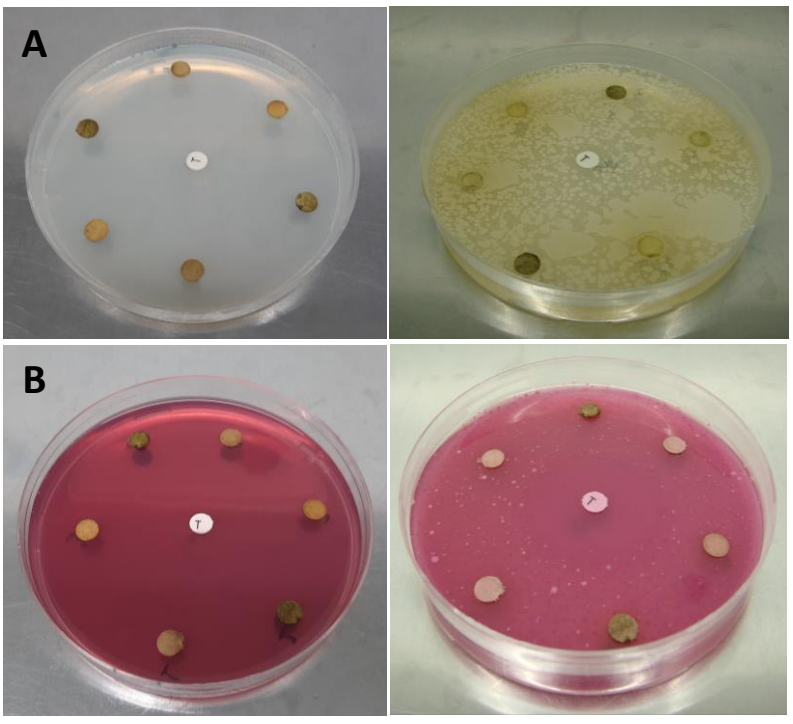

Figure 5. Testing antimicrobial activity of 100 mg/ml methanolic extracts from *in situ*, *in vitro* cultivated and *ex vitro* adapted *S. bulgarica* and *S. scardica* plants by agar disk diffusion method - A) *Acinetobacter calcoaceticus* at 0h and after 24 hours incubation; B) *E. coli* at 0 h and after 24 hours incubation

CONCLUSIONS

All tested concentrations of BA stimulated shoot development but most effective were 1.0 mg/L BA for *S. bulgarica* and 1.5 mg/L for *S. scardica* respectively.

***Ex vitro* adaptation was accomplished in greenhouse and in experimental field as well. A collection of *in vitro* cultivated and *ex vitro* adapted plants was established.**

The methanolic extracts from *in situ*, *in vitro* cultivated and *ex vitro* adapted *S. bulgarica* and *S. scardica* plants had no inhibitory effect on the tested microorganisms at concentration of 100 mg/ml.

Higher concentrations of the methanolic extracts should be tested in order to establish a significant antimicrobial activity.

ACKNOWLEDGEMENTS

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