INITIATION OF *IN VITRO* CULTURE OF THE BALKAN ENDEMIC SPECIES *STACHYS THRACICA* DAVIDOV

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Abstract

The genus *Stachys* is one of the largest genera in the family *Lamiaceae*. It is presented globally by more than 400 species, with 22 naturally distributed species in Bulgaria. *Stachys thracica* Dav. (Thracian woundwort) is a Balkan endemic plant included in the Red Data Book of Bulgaria with the national conservation status: rare. There is no available data on in vitro propagation and *ex situ* conservation of Thracian woundwort and scarce information about its chemical composition was available.

The present study aims to develop a protocol for successful sterilization of seeds and induction of *in vitro* shoot culture of *S. thracica.*

Thracian woundwort shoot culture was induced by sterilization of 100 ripe dry seeds with 70% ethanol and subsequent washing with 96% ethanol. After 14 days, 10% of 50 seeds cultivated on 0.7% water agar germinated, while on ½ MS medium no germination was observed. The sprouting seedlings were then transferred on basal MS medium, supplemented with 3% (w/v) sucrose and 0.7 g/L agar and cultivated under controlled environmental conditions. The regenerated plants had high growth index, well-developed root system and plentiful leaf biomass. A collection from *in vitro* tissue cultures, which is an approach for preservation of *S. thracica* has been established.

Keywords: *Stachys thracica, in vitro cultivation, ex situ conservation*

Introduction

The genus *Stachys* (family *Lamiaceae*) are perennial herbs, most of which grow wild in Bulgaria, Greece and Turkey. The species is listed in the Red Data Book of the Repunlic of Bulgaria under status: rare (1984). In Bulgaria *Stachys thracica* is distributed only in Strandja Mountain and Southern Black Sea coast, but there are data for populations in Sofia district.

Stachys thracica Dav. is an endangered Balkan endemic plant under the list of European ecological network Natura 2000. *S. thracica* plants contain variety of biologically active compounds: monoterpens, diterpens, sesquiterpens, iridoids, phenolic acids and flavonoids (Piozzi et al., 2011, Tundis et al., 2014). There is no available information in the literature about *ex situ* conservation of *S. thracica* and its biologically active compounds.

Plant micropropagation is an approach that ensures a rapid multiplication and preservation of the genetic potential of the initial plants. In this respect, *S. thracica* as being an endangered plant species is a suitable subject for *in vitro* cultivation, *ex vitro* adaptation, and *ex situ* conservation. The aim of this study was to develop a protocol for successful sterilization of seeds and induction of *in vitro* shoot culture of Thracian woundwort.

Material and methods

Plant material

Fully grown matured plants *Stachys thracica* Dav. (*in situ*) were collected at its natural habitat in Silistar, Sinemorets village, near Tsarevo, Bulgaria. Thracian woundwort shoot culture was induced by sterilization of 100 ripe dry seeds with 70% ethanol and subsequent washing with 96% ethanol. After 14 days germinated plants are transferred on basal MS medium (Murashige and Schook, 1962) without growth regulators.

Results and discussion

Habitats of the endemic species *Stachys thracica* are under accelerating anthropogenic impact

which is one of the prerequisites for reducing populations of the plant, despite the measures taken by state institutions. Violation of environmental balance in the habitat of the plant reduces its numbers and more specific measures for the preservation and conservation of the Thracian woundwort have to be undertaken. Introducing into *in vitro* culture aims preservation of the species, as well as its rapid propagation as a source of valuable secondary metabolites. For successful introduction into an *in vitro* culture it is necessary to establish an effective protocol for sterilization of initial plant material.

Successful shoot culture was induced by sterilization of 100 ripe dry seeds with 70% ethanol and subsequent washing with 96% ethanol. Sterilized seeds were set to medium for germination – ½ MS and a medium containing 0.07% agar (WA).

After 14 days, 10% of 50 seeds cultivated on 0.7% water agar germinated, while on ½ MS medium no germination was observed. The sprouting seedlings were then transferred on basal MS medium, supplemented with 3% (w/v) sucrose and 0.7 g/L agar and cultivated under controlled environmental conditions (lighting and temperature). The regenerated plants had high growth index, well-developed root system and plentiful leaf biomass.

Despite the low germination, *in vitro* cultivated *S. thracica* plants are characterized by active and luxuriant growth and well-shaped leaves, stems and roots. Panayotova et al. (2008) initiated the *in vitro* cultures of *S. maritima*, which also observed low germination and luxuriant growth of growth medium containing no growth regulators. *In vitro* cultures are particularly suited for study of secondary metabolites and the therapeutic potential of *Stachys thracica*, as the somatic variation and the possibility of fungal and bacterial infections are thus avoided. Micropropagation allows long-term maintenance of *in vitro* collections and the periodic harvest of plant material for research purposes, without prejudice to the populations of Thracian woundwort.

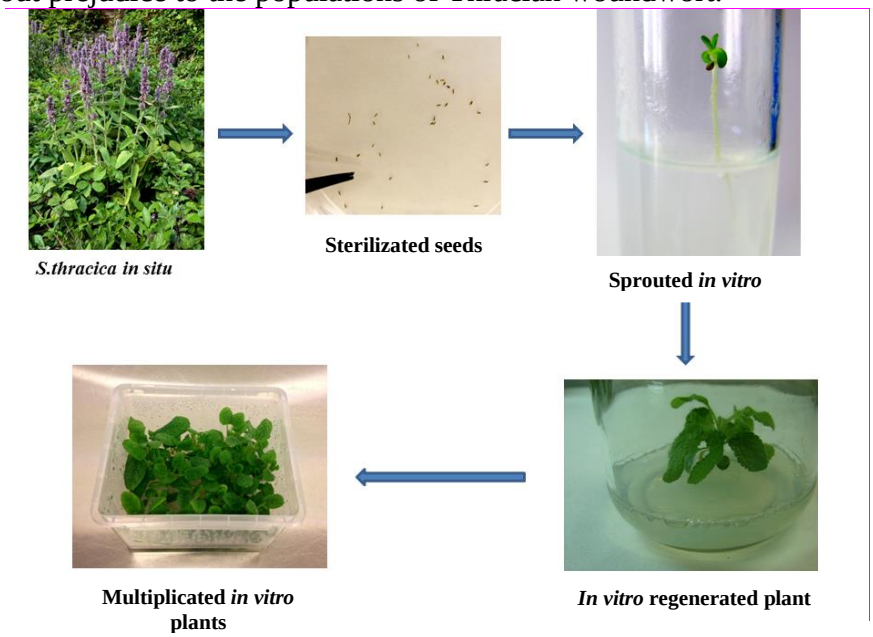

Fig. 1. Schematic representation of the procedure of introduction of S. thracica *in vitro* culture from seeds.

Conclusion

In this work we present an effective alternative method for propagation and preservation of the important medicinal plant *Stachys thracica*. A collection of *in vitro* tissue culture has been established as an approach for the conservation of Thracian woundwort. Further experiments are needed in order to determine the chemical composition of *Stachys thracica*. The present experiments could be essential for further pharmacological, physiological and biochemical studies

of the plant's secondary metabolites.

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