Phylogenetic identification of Balkan endemic *Stachys* species and genomic stability during *ex vitro* conservation

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Abstract

The genus *Stachys* is one of the largest in the Lamiaceae family. Representatives of the genus are among the most ancient medicinal plants used in the ethnomedicine. The Balkan endemic species *S. thracica*, *S. bulgarica* and *S. scardica* are included in The Red Data Book of Bulgaria and due to their endangered status are scarcely studied. The aim of the present work was to examine the genetic status of these three endemic *Stachys* species during the process of their *ex situ* conservation. To gain information about their taxonomic position in the genus *Stachys*, we applied the DNA barcoding approach. Nuclear (ITS) and plastid (*rbcL*, *matK* and *trnH-psbA*) DNA barcodes were generated and aligned with accessions available in the data base. In the constructed phylogenetic trees *S. thracica* was placed in a cluster together with *S. alpina*, *S. germanica* and *S. cretica*, while *S. bulgarica* and *S. scardica* were clustered with *S. officinalis*. The *ex situ* conservation was achieved by the initiation of *in vitro* shoot cultures and their subsequent adaptation in *ex vitro* conditions. To check the genomic stability of the plants during the acclimatisation from *in vitro* conditions to *ex vitro*, analysis by sequence-related amplified polymorphism (SRAP) markers was performed. No difference was detected between the SRAP profiles of *in vitro* cultivated and *ex vitro* adapted *S. thracica* and *S. scardica* plants. In *S. bulgarica*, only 0.4% fragment difference was detected. The obtained results indicated that the three *Stachys* species preserved their genetic stability during the process of *in vitro* multiplication, which is a prerequisite for conserved bioactive capacity.

Keywords

DNA barcoding, in vitro multiplication, SRAP markers, Stachys

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Presented at

International Conference on DNA barcoding and Biodiversity, 25-27 May 2022, Sofia, Bulgaria

Funding program

This work was financially supported by Project BULCode No. Д01-271/02.10.2020, National Program “European Scientific Networks“ 2020-2022, Ministry of Education and Science of Bulgaria

Conflicts of interest

The authors declare no conflict of interest.