

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

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INTRODUCTION & OBJECTIVE



S. thracica Davidov



S. bulgarica Degen & Nejceff



S. scardica Griseb.



- ❖ The genus *Stachys* is considered as one of the largest genera from the Lamiaceae family. Its representatives have long history of use in the ethnomedicine and recently have become object of research all over the world. *S. thracica*, *S. bulgarica* and *S. scardica* are Balkan endemic species included in The Red Data Book of Bulgaria with conservational status “endangered”. There is no available data about their in vitro multiplication and ex situ conservation which complicates their investigation.
- ❖ The aim of the present work is the phylogenetic identification of the endemic species and determination of the genetic stability of in vitro and ex vitro adapted plants.

MATERIAL & METHODS

The phylogenetic identification was achieved by the application of DNA barcoding approach. DNA was isolated from single plants and the conserved markers *rbcL*, *matK* and *ITS* were amplified and sequenced. The obtained fragments were compared with the BOLDSYSTEMS database that is enriched in sequences from different *Stachys* species. The performed sequences alignments allowed generation of phylogenetic trees for each of the marker genes. In order to study the genetic stability of the plants during their acclimatisation from in vitro to *ex vitro* conditions the sequence-related amplified polymorphism (SRAP) markers was performed.

RESULTS

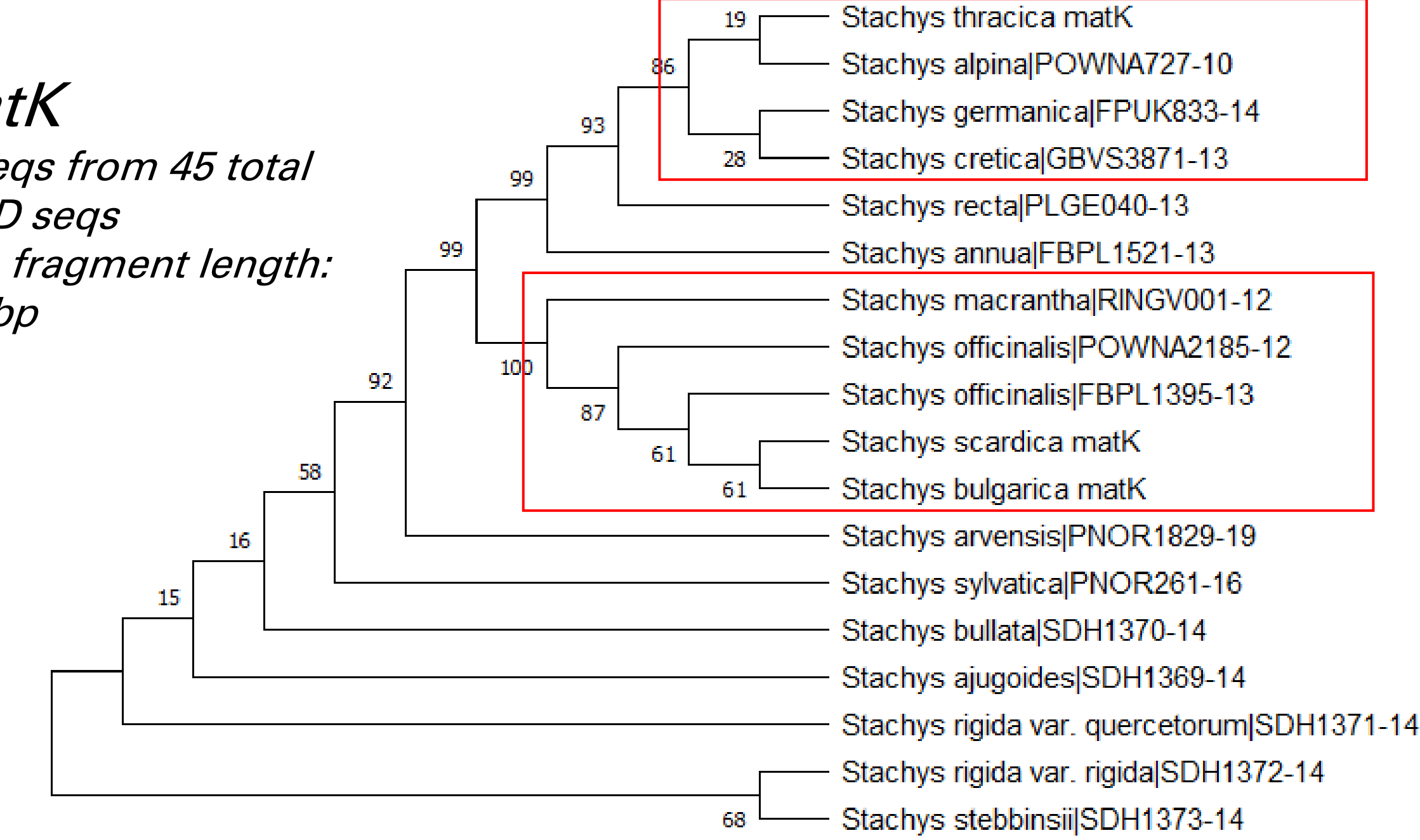
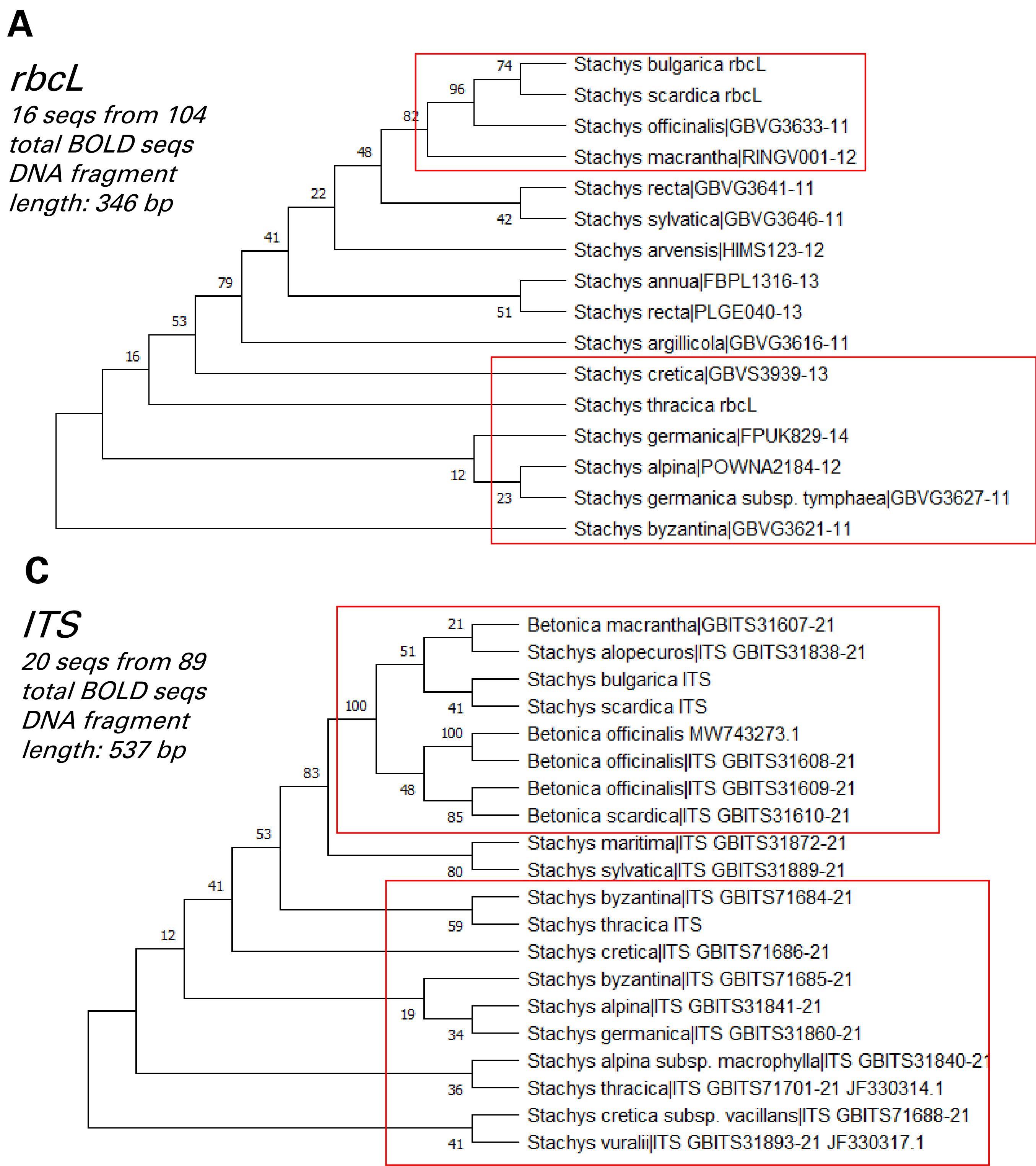


Fig. 1. DNA barcoding for taxonomic studies. A) *rbcL* marker (chloroplast genome). B) *matK* marker (chloroplast genome). C) *ITS* marker (nuclear genome). Phylogenetic trees based on the DNA markers are constructed by MEGA-X software after applying the Maximum Likelihood method and Tamura-Nei model analysis. The sequenced DNA fragment was compared with fragments from the BOLD database. For focused view, only nearby species to the studied *Stachys* species are shown. The length of the analyzed DNA fragments is indicated. In red rectangular frames are highlighted *S. thracica*, *S. bulgarica* and *S. scardica*.

CONCLUSIONS

Phylogenetic identification

- In all the three markers, *S. thracica* was placed in a cluster together with *S. alpina*, *S. germanica* and *S. cretica*, and *S. byzantina* and *S. vuralii* were included in *rbcL* and *ITS* trees, respectively.
- According to all the markers, *S. bulgarica* and *S. scardica* were clearly clustered with *S. officinalis* (or *Betonica officinalis*), and interestingly, despite their different phenotypes, they were not identified as different species when using *rbcL* and *matK* markers (also seen for *trnH* marker). Application of *ITS* managed to distinguish *S. bulgarica* and *S. scardica* as individual species, which supports the use of *ITS* as most appropriate marker for these two species.

Genetic stability

- No differences between the SRAP profiles of *in vitro* cultivated and *ex vitro* adapted *S. thracica* and *S. scardica* plants were observed and only 0.4 % differences were observed in *S. bulgarica* which indicated that all three species preserve their genetic stability during the process of *ex vitro* adaptation.

ACKNOWLEDGEMENTS

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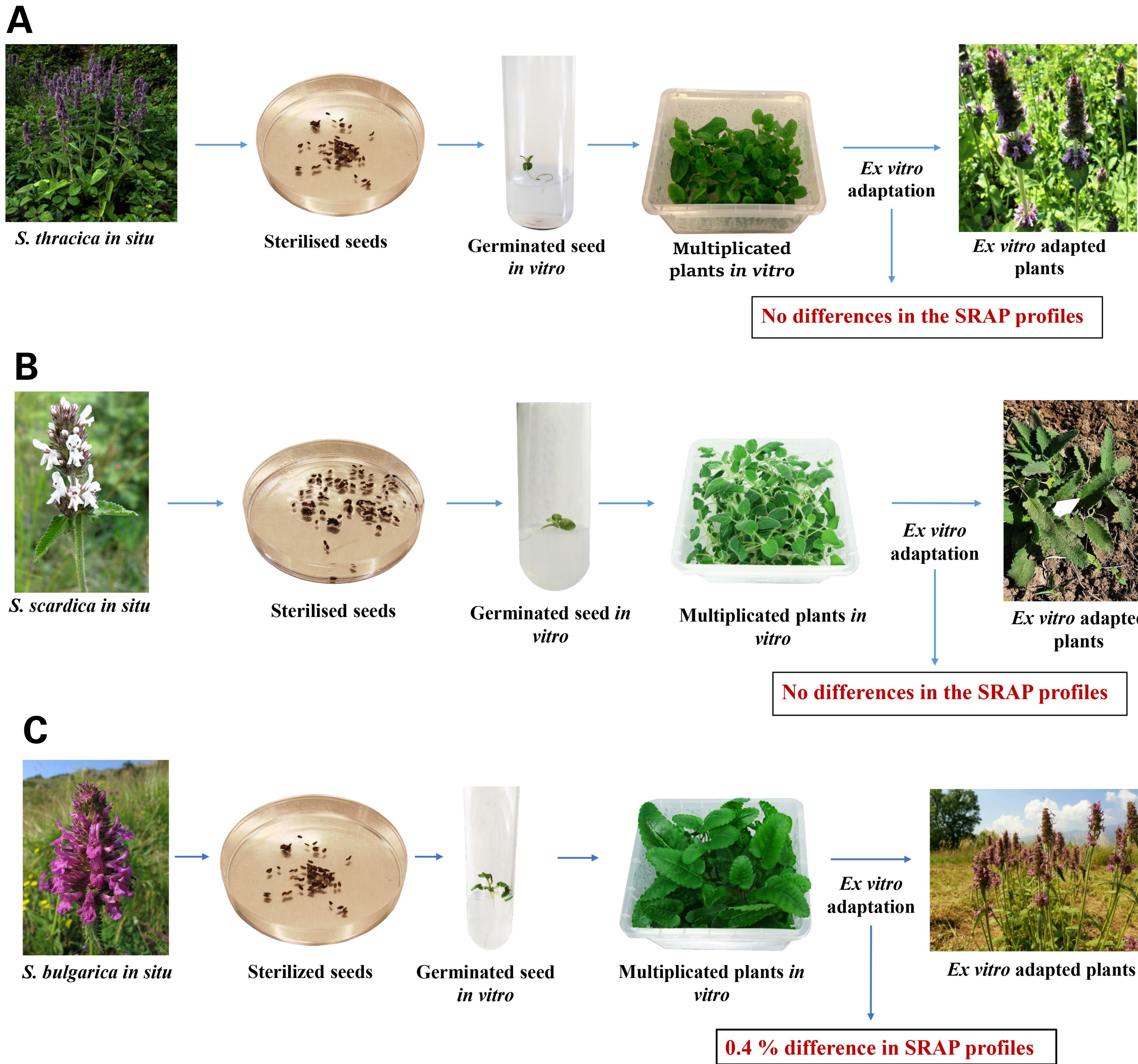


Fig. 2. Ex situ conservation of Balkan endemic species from genus *Stachys* and determination of the genetic stability of in vitro cultivated and ex vitro adapted plants by the application of SRAP analysis – sequence related amplified polymorphism. A) *S. thracica*; B) *S. scardica*; C) *S. bulgarica*