Plant genetic resources conservation : case study of Teucrium spp. in Croatia

Jeran, Nina; Grdiša, Martina; Varga, Filip; Liber, Zlatko; Šatović, Zlatko

Source / Izvornik: **58. hrvatski i 18. međunarodni simpozij agronoma : zbornik radova, 2023, 147 - 152**

Conference paper / Rad u zborniku

Publication status / Verzija rada: **Published version / Objavljena verzija rada (izdavačev PDF)**

Permanent link / Trajna poveznica: https://urn.nsk.hr/urn:nbn:hr:204:785897

Rights / Prava: In copyright / Zaštićeno autorskim pravom.

Download date / Datum preuzimanja: **2024-12-27**

Repository / Repozitorij:

Repository Faculty of Agriculture University of Zagreb

Plant genetic resources conservation: case study of *Teucrium* spp. in Croatia

Nina Jeran[1,2], Martina Grdiša[1,2], Filip Varga[1,2], Zlatko Liber[3], Zlatko Šatović[1,2]

[1]*University of Zagreb Faculty of Agriculture, Svetošimunska cesta 25, Zagreb, Croatia (njeran@agr.hr)*
[2]*CEKOM 3LJ, Ugljane 115 C, 21240 Trilj, Croatia*
[3]*University of Zagreb Faculty of Science, Rooseveltov trg 6, 10000 Zagreb, Croatia*

Abstract

The genus *Teucrium* is a source of beneficial bioactive phytochemicals and its use is well known in the traditional medicine of many countries. Studies on the wild populations of *Teucrium* species are scarce. Croatian flora numbers 12 accepted species of this genus, including a recently discovered hybrid between *T. montanum* and *T. polium*. This study aimed at sampling of *T. montanum* and *T. polium* in Split-Dalmatia County in an area where the two species grow in sympatry and the occurrence of hybrids is more likely. We collected six populations of both *T. polium* and *T. montanum* and found potential hybrids at five sites. The goal of our future research is to confirm the hybrids at the genetic level. This work contributes to *in situ* conservation by providing new insights into the status of wild populations of *Teucrium* species, supports *ex situ* conservation by including seed accessions to the Collection of Medicinal and Aromatic Plants; and enables their further characterization and evaluation for use in future breeding programs.

Keywords: biodiversity, medicinal and aromatic plants, germander, Lamiaceae, ecogeographical survey

Introduction

Croatian flora is one of the richest in Europe, owing to its particular geographical position on the border between the continental and Mediterranean areas, separated by a higher mountain belt. One of the most numerous in species is the Lamiaceae family, which is also considered one of the richest in medicinal plants (Nikolić and Rešetnik, 2007). Among them are the species of the genus *Teucrium*. The genus includes perennial shrubs, subshrubs and herbaceous perennials, some evergreen, rarely annual or biennial, growing mostly on open rocky soils with plenty of sun.

Although this genus is cosmopolitan with more than 250 species, it is mainly distributed in the Mediterranean area (96% of the total number of species), throughout Europe and in Central Asia (Euro+Med, 2006; Gagliano Candela et al., 2021). The East Mediterranean seems to be the centre of origin of the genus, while the Western Mediterranean is its recent centre of speciation (Navarro and El Oualidi, 2000).

The genus *Teucrium* in Croatia includes 12 accepted species (Table 1.), including one hybrid (*T. x rohlenae* K. Malý), which was recently recorded in the Croatian flora, and a total of seven subspecies. One of the species is endemic to the Balkans (*T. arduinii* L.) (Nikolić, 2022), while others are distributed in various parts of the Europe, N Africa, Middle East and W Asia (Euro+Med, 2006).

The species of the genus *Teucrium* are mainly used in traditional medicine of the Middle East and the Mediterranean region, as well as in some Balkan countries. Since ancient times, the species of this genus have been used against a wide range of diseases (including gastrointestinal, respiratory, urinary, circulatory, diabetes, etc.), due to their biological properties, such as antimicrobial, anti-inflammatory, antispasmodic, anti-malaric, etc. However, the negative side effect of excessive consumption has also been recognized, as the presence of neo-clerodane diterpenoids can lead to liver damage and should therefore be avoided (Gagliano Candela et al., 2021).

Table 1. Species of genus *Teucrium* in Croatia.

	Distribution	
	Croatia	Entire area
Teucrium arduinii L.	coast and its hinterland, southern islands	Balkans
Teucrium aureum Schreb.	very rare	W Mediterranean
Teucrium botrys L.	sporadical records throughout Croatia	W Mediterranean, Balkans, UK, NW Africa
Teucrium chamaedrys L.	abundant throughout the whole Croatia	Mediterranean, middle Europe, Balkans, NW Africa, W Asia
Teucrium flavum L.	entire coast, hinterland and islands	Mediterranean (E to W), NW Africa
Teucrium fruticans L.	entire coast and islands, but less frequent	W Mediterranean, NW Africa
Teucrium marum L.	Lika County (only one record)	middle Mediterranean countries (France, Italy, SE Balkans), Tunisia
Teucrium montanum L.	coast, hinterland, islands at higher elevations	Mediterranean and middle European countries, Balkans, Middle East
Teucrium polium L.	coast, hinterland, islands, (abundant)	W Mediterranean, N Africa SW Asia
Teucrium scordium L.	coast, hinterland, islands and continental Croatia	almost whole Europe (except far N), Middle East, and N Africa
Teucrium scorodonia L.	higher mountains	W, middle and N Europe, Balkans, Tunisia
Teucrium x rohlenae K. Malý	Dalmatian hinterland (two records)	Montenegro

Although known in folk tradition for their medicinal properties, in Croatia they are among the less frequently used medicinal plants. Folk medicine of Bosnia and Herzegovina and Croatia mentions the use of *T. polium* and *T. arduini* for stomach disorders, *T. montanum* for liver and stomach disorders, *T. chamaedrys* for spleen disorders, cough, diarrhea, metabolic disorders and as a diuretic, *T. marum* for biliary disorders and *T. scordium* for diarrhea (Redžić, 2007).

Recent studies on chemical diversity continue to confirm the species of this genus as a valuable source of useful bioactive compounds. A phytochemical analysis of the essential oil of *T. montanum* and *T. polium* revealed that sesquiterpenes such as germacrene D, β-caryophyllene, and β-pinene are the most important compounds in the essential oils of the aerial parts (Zbiljić et al., 2021). Another study showed that *T. polium, T. flavum, T. montanum,* and *T. chamaedrys* have similar essential oil compositions characterized by a high content of sesquiterpenes with antiphytoviral activity (Bezić et al., 2011). In addition, the species *T. arduini, T. chamaedrys, T. montanum,* and *T. polium* are a rich source of various natural acetylcholinesterase inhibitors and antioxidants that may be useful in the prevention and treatment of Alzheimer's disease and other related disorders (Vladimir-Knežević et al., 2014). As aromatic plants, the insecticidal activity of *Teucrium* species essential oils has also been confirmed in several studies, focusing on *T. polium* (reviewed in Gagliano Candela et al., 2021).

There are very few systematic studies on natural *Teucrium* populations. In accordance with the scope of our project,

the research area of this study was limited to Split-Dalmatia County. Six *Teucrium* species have been previously recorded in this area (*T. scordium, T. fruticans, T. flavum, T. chamaedrys, T. montanum* and *T. polium*), the last two of which are among the most common (Nikolić, 2022). Also, ethnobotanical surveys revealed that along most of the Croatian coast, *T. montanum* is mainly used (under the name 'trava iva') in the form of an infusion as a panacea 'for cleansing the body' and also for common cold; while on the islands and in the lower coastal areas, where *T. montanum* is rare, *T. polium* is used instead under the same name (Luczaj et al., 2021). Otherwise, the habitats of *T. polium* and *T. montanum* overlap, just as their flowering time, from June to August. Both species are semi-woody, evergreen small shrubs.

Interestingly, two populations of individuals with intermediate morphological and anatomical characteristics of both *T. montanum* and *T. polium* were discovered relatively recently at two sites in Croatia, in Split-Dalmatia County. Previously, this taxon had been detected only at one site in Montenegro and described as *T.* × *rohlenae* K. Malý. All morpho-anatomical analyses supported the separation of two species, *T. polium* and *T. montanum*, and showed the intermediate position of the new natural hybrid in the flora of Croatia found at the two mentioned sites (Zbiljić et al., 2021).

Considering that we can expect hybrid individuals in all areas where *T. montanum* and *T. polium* grow sympatrically, the main objective was to investigate their potential habitats in the wider area of Split-Dalmatia County and to sample these species in order to gain deeper knowledge about their hybridization patterns and frequency. We focused on sampling populations of the species *T. polium* and *T. montanum* in parts of their distribution area where these two species grow sympatrically and the occurrence of hybrid individuals is more likely.

Material and Methods

Ecogeographical survey and collection of plant material of *T. montanum, T. polium* and *T.* × *rohlenae* for morphological and genetic analyses was performed within Split-Dalmatia County (Figure 1.). Prior to the collection missions the analyses of available data were undertaken, in order to find potential locations with the most suitable habitats for targeted species. The first collecting mission was carried out in June 2021. Besides *Teucrium* species, plant material of two other important medicinal and aromatic species (*Salvia officialis* L., *Helichrysum italicum* Roth.) was also collected if present at any of the examined locations, for further analyses. Voucher specimens were deposited in the Herbarium ZAGR of the Faculty of Agriculture, University of Zagreb.

Seeds collecting was performed during expedition in September 2022 and was carried out at confirmed localities as well as some newly found. Along with the seeds of *Teucrium* species (*T. montanum, T. polium*, *T. chamaedrys* and *T. flavum*), seeds of other available important medicinal and aromatic species (e.g. *Salvia officialis, Satureja montana* etc.) were also collected. After the expedition, seeds were cleaned out of the plant material and prepared for storage. Altogether 18 seed accessions were stored in the Collection of Medicinal and Aromatic Plants of the Department of Seed Science and Technology, University of Zagreb Faculty of Agriculture. Within the Collection, plant genetic resources of medicinal and aromatic plants are systematically collected, characterised, maintained, evaluated, documented and regenerated for future introduction into agricultural production and breeding programmes (Šatović et al., 2016). Recorded plant species locations were entered in Flora Croatica Database (Nikolić, 2022).

Results and Discussion

Within the area of Split-Dalmatia County, we found a total of six sites where both *T. polium* and *T. montanum* populations co-occur, and an occurrence of hybrids resembling *T. x rohlenae* at five of these sites (Figure 1). One additional location where all three species were found was only about 1 km distant from the previous site, so we assume it as the same population. The sites recorded in this study confirm two previously reported sites for Croatia (Zbiljić et al., 2021) and three that were not previously recorded. We sampled approximately 25 individuals per population of both *T. polium* and *T. montanum*, as well as an available number of hybrid individuals (1-25), depending on the site.

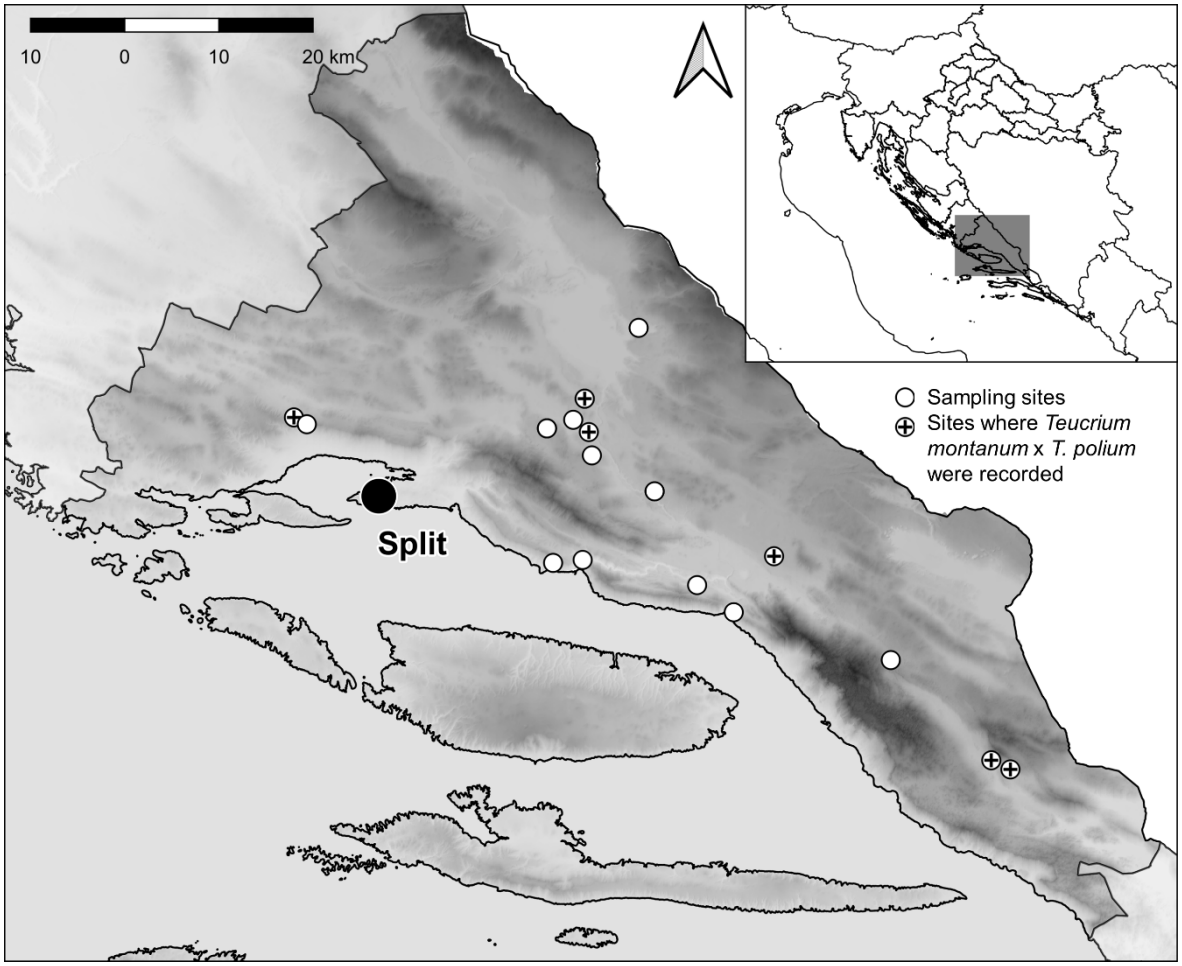

Figure 1. *Map of sampling locations in Split-Dalmatia County showing locations where putative hybrids were detected.*

The very high incidence of hybrids at sites where *T. polium* and *T. montanum* co-occur (five of the six sites) suggests that these two species hybridize frequently under natural conditions where their ecological requirements are met.

In addition, we also observed 'hybrid' individuals that were morphologically more similar to either *T. polium* or *T. montanum*. We hypothesize that this is the result of back-crosses between hybrids and their parental species. The goal of our future research is to confirm the hypothesis of the existence of hybrids and their back-crosses on the genetic level, using two complementary molecular methods: Simple Sequence Repeats (SSRs) and Amplified Fragment Length Polymorphisms (AFLP).

Zbiljić et al. (2021) performed detailed morpho-anatomical analysis, which indicated to the existence of natural hybridization between *T. polium* and *T. montanum.* This hypothesis was also confirmed by biochemical analysis of essential oil, which separated the two species *T. polium* and *T. montanum* and revealed the intermediate position of the putative hybrids (Zbiljić et al., 2021). On the other hand, some other studies show that the essential oil composition of the two species is not clearly distinguishable, as they contain similar compounds and a similar pattern of variability (Bezić et al. 2011; Marčetić et al. 2018). Therefore, more research on the phytochemistry of these species is needed. Natural hybrids may also have potential value for agronomy. Therefore, future research should focus on analyzing their phytochemical diversity and evaluating their potential for future breeding programs.

One of the long-term goals of plant genetic resource conservation and sustainable use of valuable species is their introduction into agricultural production. Currently, there are only a few studies documenting the cultivation of *Teucrium* species in Europe and Mediterranean countries, and these are related to their use as ornamental plants, due to their decorative properties. Some *Teucrium* species are used as ornamentals with functional benefits, e.g., *T. fruticans* has been shown to be a salt-tolerant species while retaining its ornamental value (Cassaniti et al. 2009).

T. scorodonia is cultivated in many places as an ornamental plant in gardens and also naturalizes in some regions (Gagliano Candela et al., 2021). To the best of our knowledge, there is no organized cultivation in Croatia. However, there is a potential for cultivation of the species of this genus, which can be used both as an ornamental and aromatic plants.

Conclusion

The main result of this study was finding of hybrid individuals between *T. montanum* and *T. polium* at three new sites in Split-Dalmatia County.

The professional contributions of this work include a broader knowledge of different *Teucrium* species and the status of their natural populations valuable for *in situ* conservation. In addition, this study contributes to *ex situ* conservation by including the collected accessions in the Collection of Medicinal and Aromatic Plants and thereby enabling their further characterization and evaluation, as well as their use in future breeding programs, taking into account the potential for cultivation of Croatian native species.

Acknowledgments

This work is part of the project CEKOM 3LJ (KK.01.2.2.03.0017) financed by ERDF.

Literature

Bezić N., Vuko E., Dunkić V., Ruščić M., Blažević I., Burčul F. (2011). Antiphytoviral activity of sesquiterpene-rich essential oils from four Croatian *Teucrium* species. Molecules. 16(9): 8119-8129. https://doi.org/10.3390/molecules16098119

Cassaniti C., Li Rosi A., Romano D. (2009). Salt tolerance of ornamental shrubs mainly used in the Mediterranean landscape. Acta Horticulturae. 807: 675-680.

Euro+Med 2006+ [continuously updated]: Euro+Med PlantBase - the information resource for Euro-Mediterranean plant diversity. Published at http://www.europlusmed.org (accessed date: 2022/11/15)

Gagliano Candela R., Rosselli S., Bruno M., Fontana G. (2021). A Review of the Phytochemistry, Traditional Uses and Biological Activities of the Essential Oils of Genus *Teucrium*. Planta Medica. 87: 432-479.

Luczaj L., Jug-Dujaković M., Dolina K., Jeričević M., Vitasović-Kosić I. (2021). Insular Pharmacopoeias: Ethnobotanical Characteristics of Medicinal Plants Used on the Adriatic Islands. Frontiers in Pharmacology. 7 (12): 623070.

Marčetić M., Zbiljić M., Lakušić D., Lakušić B. (2018). Variability of essential oil of different populations of *Teucrium montanum* L. (Lamiaceae) from Balkan Peninsula. Botanica Serbica. 42 (Suppl. 1): 133.

Navarro T. and El Oualidi J. (2000). Sinopsis of *Teucrium* L. (Lamiaceae) in the Mediterranean region and surrounding areas. Flora Mediterranea. 10: 349–363.

Nikolić T. and Rešetnik I. (2007). Plant uses in Croatia. Phytologia Balcanica. 13: 229-238.

Nikolić T. ed. (2022). Flora Croatica Database (http://hirc.botanic.hr/fcd). Faculty of Science, University of Zagreb (accessed date: 2022/11/15).

Redžić S. (2007). The ecological aspect of ethnobotany and ethnopharmacology of population in Bosnia and Herzegovina. Collegium Antropologicum. 31: 869-890.

Šatović Z., Carović-Stanko K., Grdiša M., Jug-Dujaković M., Kolak I., Liber Z. (2016). Conservation of Medicinal and Aromatic Plants in Croatia. In: Environmental and Food Safety and Security for South-East Europe and Ukraine (Vitale K., ed.). Springer Netherlands.

Vladimir-Knežević S., Blažeković B., Kindl M., Vladić J., Lower-Nedza A. D., Brantner A. H. (2014). Acetylcholinesterase inhibitory, antioxidant and phytochemical properties of selected medicinal plants of the Lamiaceae family. Molecules. 9: 19 (1): 767-82.

Zbiljić M., Lakušić B., Marčetić M., Bogdanović S., Lakušić D. (2021). Morphological and chemical evidence of *Teucrium* × *rohlenae* K. Malý (Lamiaceae), a new hybrid in Croatia. Acta Botanica Croatica. 80 (1): 48-55.