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Habitat-related variation in composition of the essential oil of *Seseli rigidum* Waldst. & Kit. (Apiaceae)

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Plant specialised metabolites like essential oils are highly variable depending on genetic and various ecological factors. The aim of the present work was to characterise essential oils of the species *Seseli rigidum* Waldst. & Kit. (Apiaceae) in various organs on the individual and populational levels. Geographical variability and the impact of climate and soil type on essential oil composition were also investigated. Individually sampled essential oils of roots, aerial parts and fruits of plants from seven populations were analysed by GC-FID and GC-MS. The investigated populations showed high interpopulational and especially intrapopulational variability of essential oil composition. In regard to the variability of essential oils, different chemotypes were defined. The essential oils of *S. rigidum* roots represented a falcarinol chemotype, oils of aerial parts constituted an α-pinene or α-pinene/sabinene chemotype and fruit essential oils can be characterised as belonging to a complex sabinene/α-pinene/β-phellandrene/falcarinol/germacrene B chemotype. At the species level, analysis of variance (ANOVA), principal component analysis (PCA) and canonical discriminant analysis (CDA) showed that the plant part exerted the strongest influence on the composition of essential oils. Climate had a high impact on composition of the essential oils of roots, aerial parts and fruits, while influence of the substrate was less pronounced. The variations in main compounds of essential oils based on climate or substrate were complex and specific to the plant part.

1. Introduction

The volatiles of plants constitute a relatively large group of natural plant products and represent a complex mixture of various compounds, mainly terpenes, fatty acid degradation products, phenylpropanoids, amino acid-derived products and hydrocarbons of various biogenetic origins. They are primarily involved in defence against herbivores and pathogens, attraction of pollinators and signalling between organisms (plant-plant communication), but also show a range of biological effects on animals and humans (Dudareva et al., 2013; Maffei et al., 2011). The essential oils represent stored plant volatiles, usually a mixture of monoterpenes, sesquiterpenes and phenylpropanoids. In plants, terpenes are biosynthesised in compartmentally separated, but connected pathways: the plastidic methylerythritol phosphate (MEP) pathway for monoterpenes and the cytosolic mevalonic acid (MVA) pathway for sesquiterpenes. The high diversity of terpenes in plants is partly attributable to the presence of terpene synthases that can synthesise multiple products from a single substrate (Degenhardt et al., 2009; Dudareva et al., 2013). Terpene biosynthesis is influenced by numerous factors, primarily the species genotype, but also by various ecological factors (Figueiredo et al., 2008; Lakušić et al., 2012; Niinemets et al., 2004).

Species of the genus *Seseli* have essential oils in endogenous secretory canals which are located in all vegetative (root, stem, leaf) and reproductive (fruit) organs (Metcalf and Chalk, 1950). Several studies have shown that essential oils of *Seseli* species have antimicrobial (Marčetić et al., 2012; Stojkovic et al., 2009) and antifungal (Milosavljević et al., 2007) activity. One of the investigated species of the genus is *Seseli rigidum* Waldst. & Kit. (Apiaceae) (Marčetić et al., 2012, 2013; Šavikin-Fodulović et al., 2006; Stojkovic et al., 2009; Todorova et al., 2013), a herbaceous perennial plant

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native to Southeast Europe (Ball, 1968) that is a facultative serpentinophyte adapted to calcareous and serpentine habitats (Vicić et al., 2014). Given that almost all previous investigations (Marčetić et al., 2012; Šavikin-Fodulović et al., 2006; Stojkovic et al., 2009; Todorova et al., 2013) of the essential oils of *S. rigidum* were based on composite samples (one mixed collective sample from one population), we have only a general picture of essential oil composition, without any information about the chemical variability and specificity of populations occupying different habitats and different geographical areas. The only exception was a study conducted by Marčetić et al. (2013), who investigated the variability of root essential oil in *S. rigidum* from seven natural populations in Serbia and demonstrated discrimination of three groups of populations based on climatic differences.

In light of the results of Marčetić et al. (2013) and the well-known fact that terpene biosynthesis is influenced by various ecological factors, the aims of our research were: a) to characterise essential oils of the species *S. rigidum* in various organs on the individual and populational levels; b) to investigate the geographical variability of essential oil and define the chemotypes; and c) to explore the impact of plant part, climate and soil type on essential oil composition.

2. Results

2.1. Characterisation of *S. rigidum* essential oil

In order to define the chemical variability of *S. rigidum* essential oil, the chemical composition of 206 individual samples from seven natural populations was investigated. The chemical characterisation of essential oil and statistical analysis were performed on the species level and on the level of plant organs; roots, aerial parts and fruits.

On the species level, in all analysed samples the content of essential oils was 0.1–4.7%. One hundred and thirty-nine components were identified in the essential oils, which represented 82.5–100% of the total oils. Monoterpenes (0–97.5%), polyacetylenes (0–95.3%) and sesquiterpenes (0.7–78.1%) were the main compounds of *S. rigidum* essential oil. Falcarinol (0–95.3%), sabinene (0–69.1%), α-pinene (0–65.6%), limonene (0–43.4%), β-phellandrene (0–37.5%), germacrene B (0–33.3%), carotol (0–21.9%), germacrene D (0–19.9%) and β-sesquiphellandrene (0–19.7%) were the dominant constituents of essential oil in the species *S. rigidum* (Suppl. 1, Table 1).

The content of essential oils from the roots of 68 individual samples of *S. rigidum* was 0.1–1.0%. Analysis of the root oils revealed 68 identified compounds, which represented 82.5–98.5% of the oil. Polyacetylenes were dominant in the root oil (29.9–95.3%), but sesquiterpenes (0.7–41.5%) and monoterpenes (0–26.3%) were also present. The main constituents were: falcarinol (29.4–95.3%), α-pinene (0–15.4%), δ-amorphene (0–12.6%), sabinene (0–11.4%), 3-butyl-phthalide (0–11.3%) and β-sesquiphellandrene (0–10.5%) (Suppl. 1, Table 2).

The examined 68 individual samples of aerial parts of *S. rigidum* contained 0.2–1.5% of essential oil. Ninety-six components were identified in the oils, which represented 93–100% of the total. The oil of aerial parts was composed mainly of monoterpenes (63.3–97.5%) and to a lesser extent of sesquiterpenes (2.5–29.7%). The dominant compounds were α-pinene (2.5–65.6%) and sabinene (0.7–61.9%). Limonene (0–43.4%), β-phellandrene (0–20.4%), caryophyllene oxide (0–11.6%) and bornyl acetate (0–11.2%) were also present (Suppl. 1, Table 2).

The examined 70 individual samples of fruit contained the greatest quantity of oil (0.3–4.7%). Sixty-seven compounds were identified, which represented 84.2–100% of the total oil. As in the oil of aerial parts, monoterpenes (15.2–88.9%) and sesquiterpenes (8.0–78.1%) were dominant in the fruit oil, but there was also a significant amount of polyacetylenes (0–35.6%). The main compounds in fruit oil were: sabinene (0–69.1%), α-pinene (0.8–55.7%), β-phellandrene (0–37.5%), falcarinol (0–35.6%), germacrene B (0–33.3%), carotol (0–21.9%), germacrene D (0.6–19.9%), β-sesquiphellandrene (0–19.7%), (*E*)-caryophyllene (0–18.3%) and limonene (0–16.0%) (Suppl. 1, Table 2).

2.2. Geographical variability of *S. rigidum* essential oil

It is not unusual for the same species sampled on different locations to have different essential oil composition. The biosynthesis of essential oils is under the influence of numerous abiotic and biotic factors (Figueiredo et al., 2008; Lakušić et al., 2012). To provide insight into variations of essential oil due to the geographical distribution of natural populations and define the chemotypes of *S. rigidum* essential oils, 206 individual samples from seven populations were investigated.

The roots of 68 individuals from seven natural populations of *S. rigidum* showed quite similar content of essential oil. The greatest amounts of oil in the roots were found in individuals from Ovčar Banja (0.3–1.0%), the Brdjanska Gorge (0.4–0.9%) and Golubac (0.3–0.9%) (Fig. 1A). The polyacetylene falcarinol was the main compound in the root oil of all individuals. The highest and relatively constant content of falcarinol was determined in individuals from the Brdjanska Gorge (75.4–95.3%), while there was pronounced intrapopulational variability in the individuals from Maglič (29.4–88.7%), Golubac (43.2–86.5%) and the Gornjak Gorge (45.7–84.7%). The essential oil of *S. rigidum* roots can be characterised as belonging to a falcarinol chemotype. Besides falcarinol, the root oil of individuals from Golubac contained intrapopulationally variable α-pinene (0.9–15.4%), individuals from Maglič contained δ-amorphene (0–12.6%) and individuals from the Gornjak Gorge contained sabinene (t-11.4%) and β-sesquiphellandrene (0–10.5%) (Fig. 1).

In the essential oil content of aerial parts (68 individuals examined), intrapopulational variability was pronounced in specimens from the Grza Canyon (0.2–1.1%), Golubac (0.4–1.4%) and the Brdjanska Gorge (0.8–1.5%) (Fig. 2A). The main compounds found in the essential oil from aerial parts–α-pinene, sabinene and limonene–showed intrapopulational and interpopulational variability. Owing to high concentrations of α-pinene and sabinene in oils of the aerial parts, these oils can be characterised as belonging to an α-pinene or α-pinene/sabinene chemotype. High amounts of α-pinene were found in the oils from populations from Western Serbia (regions with a humid climate): the Brdjanska Gorge (40.3–65.6%), Ovčar Banja (53.8–61.7%) and Moravica (7.9–65.4%) (Fig. 2B). These populations represented an α-pinene chemotype. In the oils of individuals of these populations the content of sabinene was low, not higher than 15% of the total oil (Fig. 2C). The oils of individuals from Moravica differed from the others in exhibiting a high and variable limonene concentration (2.9–43.4%) (Fig. 2D).

On the other hand, the oils of individuals from populations from Eastern Serbia (regions with a semi-arid climate)–Golubac, the Gornjak Gorge and the Grza Canyon–and oils of specimens from Maglič (Western Serbia) contained smaller amounts of α-pinene (22.7–52.6, 7.7–49.4, 23.6–54.7 and 2.5–42.5%, respectively), but had significantly higher concentrations of sabinene (0.9–43.9, 1.0–40.4, 11.8–45.2 and 0.7–61.9%, respectively), and can be characterised as belonging to an α-pinene/sabinene chemotype. Oils of individuals from the Gornjak Gorge also had significant and variable content of β-phellandrene, from trace amounts up to 20.4% (Fig. 2).

Previous investigations (Marčetić et al., 2012; Šavikin-Fodulović