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Phytochemical and morphological variation of *Stachys lavandulifolia* Vahl. populations as affected by genotype × year interaction

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ABSTRACT

In this study, 40 genotypes of the genus *Stachys* were collected from the three locations of Damaneh, Shahrekord, and Semirom in central Iran. Essential oil content and morphological characteristics were studied over two consecutive years in a randomized complete block design (RCBD). Result of analysis of variance revealed significant differences among most of the morphological traits studied and the essential oil content in terms of genotype, cultivation year, and genotype × year interaction. Coefficients of genotypic variation revealed a high diversity in both essential oil content and days to flowering, suggesting the possibility for improving essential oil by means of selection particularly in the first year. The essential oil content of the genotypes examined ranged from 0.087% to 3.15% in the first year and 0.095% to 1.16% in the second year. Based on the two-year evaluation conducted, eight genotypes with high essential oil yields were selected from the three sampling locations to determine their essential oil compounds. GC–MS analysis revealed *Z*-chrysanthenyl acetate (30.34%–63.73%), linalool (0.6%–10.82%), caryophyllene oxide (4.04%–12.91%), spathulenol (0.98%–4.53%), viridiflorol (0.36%–11.49%) and *trans*-caryophyllene (3.39%–11.95%) to be the major components. The highest positive and negative correlations were obtained between α-terpineol with linalool (0.96) and caryophyllene oxide (–0.78), respectively. Finally, Damaneh8 and Shahrekord6 were introduced as genotypes with high essential oil yields and high *Z*-chrysanthenyl acetate content. In conclusion, many of the studied traits showed improvements in the second year although the genetic variation was lower in comparison with the first year.

1. Introduction

The genus *Stachys*, comprising more than 450 species, is recognized as one of the largest genera of the Lamiaceae family (Mohammadhosseini et al., 2016). Thirty-four species of *Stachys* have been recognized in Iran, 13 of which are endemic to the region (Mozaffarian, 2008). One of the endemic species is *S. lavandulifolia* Vahl. whose aerial parts have been used as herbal tea (Ebrahimabadi et al., 2010).

Many *Stachys* species are known as “mountain tea” and applied in yogurt, jelly, and spices (Conforti et al., 2009). Moreover, *S. lavandulifolia* has been used as an herbal remedy in the treatment of several complaints (Goren et al., 2011; Flamini et al., 2005) Anxiolytic, antidepressive, appetite stimulant, and analgesic effectts have also been reported for the *S. lavandulifolia* extract (Khadivi-Khub et al., 2014).

Essential oils have been considered as one of the main group of secondary metabolites in medicinal plants (Duru et al., 1999). Both the composition of plants and their essential oil yields are affected by genetic and environmental factors (Gholami Zali and Ehsanzadeh, 2018). This is perhaps the reason underlying the differences observed between metabolites obtained from plants in wild habitats and field grown ones. Identifying and understanding the likely genetic relationships among plants are crucial for their improvement recovery programs due to the new insights provided into their genetic variation. The knowledge thus gained can be exploited to introduce high essential oil yield plants for industrial and food applications (Mondini et al., 2009).

In Iran, *S. lavandulifolia* has found wide applications in both pharmaceutical and food industries. For instance, this plant has been used as herbal tea and appetizer (Khadivi-Khub et al., 2014). Nowadays, high grazing and drought stress pressures have faced this endemic medicinal plant in their natural habitats with the risk of extinction. It is, therefore, necessary to select populations of the species with high essential oil yields for the conservation of the genetic resources of this medicinal plant and enhanced beneficial applications (Tohidi et al., 2017). For this purpose, assessment of inter- and intra-genetic variations of the different populations in terms of their morphological and essential oil yield potentials may provide new insights toward further domestication of this endemic plant species. The results thus obtained in this study

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were validated by evaluating the genetic × environment interaction in a two-year experiment.

Domestication of wild species with high essential oil yields and valuable secondary metabolites and their cultivation in the field are of great importance to both producers and industry. Much research has been devoted to these aspects of medicinal plants (Askari et al., 2009). *S. lavandulifolia* is a perennial species that regenerates annually. It is basically cross pollinated, which results in a high degree of intra-population variation which can provide high variation for screening the plants with desirable traits. Most studies of *S. lavandulifolia* were performed on the variations in the morphological and essential oil components of the plants collected from natural habitats (Khadivi-Khub et al., 2014; Ramezani et al., 2002). Moreover, the morphological and secondary metabolites of these plants are reported to have been affected by their genetics, environment, and genetic × environment (Patel et al., 2016).

Based on the above observations, the present study was designed and implemented to evaluate different *S. lavandulifolia* populations under experimental field conditions and in different years in order to gain insights for selecting the populations with high yield and essential oil content. This is while the data obtained from field experiments might be used by plant breeders in improving the valuable traits of the species. Moreover, the essential oil compounds in high essential oil genotypes determined in the present two-year experiment can help researchers identify and introduce new genotypes to meet industrial demands. Finally, the present study is unique and unprecedented in its kind as there are no published reports on field experiments that investigated essential oils, morphological variation, and plant traits in *S. lavandulifolia* through time in different years.

2. Materials and methods

2.1. Plant materials and field experiment

This experiment was conducted at the research station of the College of Agriculture, Isfahan University of Technology, Isfahan (32°32 N and 51°32 E, 1630 m asl) at the two agronomic years of 2015 and 2016. Plant materials used consisted of the seeds of 40 genotypes collected from the three regions of Shahrekord, in Charmahal and Bakhtiari Province, as well as Semirom and Damaneh, in Isfahan Province, Iran. The seeds were sown in similar conditions in a randomized complete block design (RCBD) with three replicates including 10 plants for each genotype in the plot. The experimental field had a semi-arid climate with an annual average temperature of 9.1–23.4 °C and an annual average rainfall of 122.8 mm. The soil was silt clay loam (51.2% sand, 19% clay, and 29.8% silt) with pH 7.45.

2.2. Phenotypic evaluation

The agro-morphological characteristics of plant height, plant wet weight, plant dry weight, leaf length, leaf width, sepal length, sepal width, and the number of floret; days to flowering; and days to seed emergence were measured based on each replicate performance. For the other characteristics, data were collected from five randomly selected plants in each replicate and their means were considered for analysis. The morphological characteristics were measured as plant height, plant fresh weight, plant dry weight, leaf length, leaf width, sepal length, sepal width and the number of florets.

2.3. Phenotypic data analysis

The data collected during the two years of study were subjected to analysis of variance (ANOVA) using SAS software (version 9.1). The means of the genotypes were compared using the least significant difference (LSD) tests ($p < .05$). Using the relation below, the genotypic and phenotypic variances well as heritability were calculated to specify the most important trait explaining the variation (Shojaiefar et al., 2015).

$$\sigma_g^2 = (MSG - MSE)/r$$

$$\sigma_p^2 = \sigma_g^2 + \sigma_e^2$$

$$h^2 = \sigma_g^2/\sigma_p^2$$

$$GCV\% = \sqrt{\frac{\sigma_g^2}{\overline{x}}}$$

$$PCV\% = \sqrt{\frac{\sigma_p^2}{\overline{x}}}$$

In these formulas:

MSG = Genetic mean squares	MSE = Environmental mean squares	r = rep
σ_g^2 = Genetic variance	σ_g^2 = Phenotypic variance	$\overline{X}$ = mean
GCV = genotypic coefficients of variation	PCV = phenotypic coefficients of variation	

The data were then used to create a genetic distance matrix with a squared Euclidean distance measure using the STATGRAPHICS ver 16.2.04. Subsequently, the genotypes were clustered using the Ward method. Pearson's correlation coefficient was employed to find out the correlations between the traits.

2.4. Essential oil isolation

Young aerial parts of the *S. lavandulifolia* genotypes were collected in the morning and allowed to dry in the shade at 25 °C for four days. For each hydro-distillation turn, 40–60 g of the sample was used. The round-bottom flask of a Clevenger-type apparatus was used to extract essential oils to which 500 mL of distilled water was added and boiled for six hours. The distilled essential oil was obtained using diethyl-ether as collecting solvent (v/v). Then, the essential oil was collected in a glass container. The oil yield was calculated based on dry matter of the samples. The essential oils were kept in 4 °C before GC/MS analysis.

2.4.1. Gas chromatography/mass spectrometry (GC/MS) analyses

The essential oil components were determined via GC/MS analysis using an Agilent Technologies 7890 gas chromatograph instrument with a fused silica HP-5MS capillary column (30 m × 0.25 mm × 0.25 μm) and helium as the carrier gas at a flow of 0.8 mL/minute. The oven temperature was kept at 60 °C for 4 min, and then increased at the rate of 4 °C/min to 280 °C. The chromatographer was coupled to a Hewlett-Packard 6890 mass spectrometer as a selective detector. Retention indices (RI) were calculated by injecting to the samples a series of *n*-alkanes (C5–C24) in hexane was injected using the same analytical conditions. Identification of the oil components was carried out by comparison of retention indices (RI_s) relative to *n*-alkanes obtained by HP-5MS column, with those mentioned in the literature as well as comparing of their mass spectra with those recorded in Willey (Chem Station data system) and NIST 08 (National Institute of Standards and Technology). The percentage of compounds was achieved according to the peak areas of the total oil components (Ebrahimabadi et al., 2010).

2.4.2. Statistical analysis for essential oil compounds

Cluster analysis was performed on the main components of the oil using STATGRAPHICS ver 16.2.04 and calculation of correlation among the compounds was done using SAS ver. 9.2.