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Anatomical and Morphological Properties of Trichomes in Four Iranian Native *Salvia* Species under Cultivated Conditions

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Abstract
The morphological specifications play a substantial role in classification and breeding programs of various plant taxa. In the current study, some macro- and micro-morphological features of *Salvia nemorosa*, *Salvia syriaca*, *Salvia frigida* and *Salvia virgata* (three accessions), were investigated using scanning electron microscopy and image analysis program. The completely randomized design (CRD) was used to compare the morphological properties (r=4). Macro-morphological and agronomical measurements showed that *Salvia* species have great potential for cultivation as medicinal and ornamental plants. The cluster analysis suggested that *S. virgata* and *S. frigida* located in the same group; on the other side, *S. syriaca* and *S. nemorosa* had more similarity. The micro-morphological results showed that glandular trichomes (GTs) and non-glandular trichomes (NGTs) were widely distributed over the leaf and flower surfaces. The peltate GTs with high-density covered sepal and petal and were observed with low density on the leaves. The comparison with different species showed that the highest size and density of GTs belonged to *Salvia virgata.* The GTs are large and cover a significant portion of the flower surface (3.85-18.45%). The multicellular GTs were observed in *S. nemorosa* and *S. virgata* A_1 and A_2. The uniseriate and multicellular NGTs covered the adaxial leaf surface in different species. Besides, relative variation in GTs was observed in the three studied *S. virgata* accessions (15.33- 30.58 trichomes per mm^2), which indicates the role of environmental conditions in trichomes formation. A wide range of variation in most of the micro- and macro-morphological traits of *Salvia* genotypes observed in cultivated condition.

Keywords: Breeding, Diversity, Morphology, *Salvia*, SEM, Trichomes.

Abbreviations: **GA_3**, Gibberellic Acid; **GTs**, Glandular Trichomes; **NGTs**, Non-Glandular Trichomes; **SEM**, Scanning Electron Microscopy.

Introduction

The *Salvia* genus with about 1000 species worldwide is one of the greatest genus in the Lamiaceae family (Walker and Sytsma, 2006). Jamzad (2013) recognized 61 species of *Salvia* in flora of Iran, of which 17 are endemic (28%). She described the genus as Maryam Goli or Shafabakhsh, which consists of herbs and shrubs, annual, biennial or perennial species. Most of these species are important for their essential oil content and aromatic properties. Today most species of *Salvia* widely used as ornamental (special in Europe and in the

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United States) and medicinal plants (Karabacak et al. 2009). Various extracts of aerial parts were shown to have antioxidant activity, and Rosmarinic acid and Coffeic acid were reported as the most components in their extracts (Koşar et al., 2008). The aqueous and methanolic extracts of aerial parts of *S. nemorosa* were shown to have anti-nociceptive property (Hosseinzadeh and Amel, 2000). *Salvia syriaca* provides a valuable source of bioactive natural compounds for functional foods as well as medical and pharmaceutical applications (Bahadori et al., 2017).

Many studies have explored different aspects of *Salvia* such as anatomy and morphology (Avato et al., 2005; Firat et al., 2017), diversity (Fattahi et al., 2014), domestication, variation of essential oils (Yousefi et al., 2012), antimicrobial activity and pharmacological applications (Bahadori et al., 2017). Some morphological characteristics of different *Salvia* species such as leaf size, petiole length, inflorescence length, number of verticillasters, stamen type, nutlet micromorphology, and bract shape and size reported in previous studies (Kahraman et al., 2009; Salimpour et al., 2014; Martins et al., 2017). Today macro- and micro-morphological studies of medicinal plants are handy to make a correlation between morphological and biochemical trails to reduce the breeding costs. For example, trichome features (e.g. shape, size, density) are the most useful taxonomic characteristics in some genera of the Lamiaceae such as *Salvia L.*, *Stachys* L., and *Satureja* L. (Krstic et al., 2006; Dinç and Öztürk, 2008; Kahraman et al., 2010b).

The trichomes and their bioactive compounds play a crucial role in protecting the aerial parts of plants against biotic (herbivores, pathogens, insects, other plants, etc.) and abiotic (high temperature, drought and Ultraviolet-B radiation damage) stresses (Karabourniotis et al.,1995; Bisio et al., 1999; Björkman and Ahrné, 2005; Kjær et al., 2012). The biological activity of secondary metabolites in secreted products has attracted growing attention for pesticide (Teixeira et al., 2013), pharmaceutical (Hosseinzadeh et al., 2009; Loizzo et al., 2010), food flavoring and fragrance industries (Coisin et al., 2012).

The trichomes are mostly developed out of one young epidermal cell, whether it grows only unicellular hairs or also dividing, multicellular hairs. Glandular (GTs) and non-glandular trichomes (NGTs) are widely distributed over the aerial reproductive and vegetative organs of plants in the Lamiaceae, as a family of great economic importance for the essential oil produced in their trichomes (Tissier, 2012; Rusydi et al., 2013). Regarding the structures, GTs can vary widely among species (Rusydi et al., 2013), but in general two types of GTs (peltate and capitate) are described in Lamiaceae family (Dunkić et al., 2001; Teixeira et al., 2013).

Trichome density also affects the natural enemies of herbivorous insect's population and efficiency (Krstic et al., 2006); because trichome density can affect the movement speed of natural enemies and trapping of harmful insects (Björkman and Ahrné, 2005). High trichome density can lead to high resistance against pests and diseases due to the presence of allelochemicals such as the diterpenes, sesquiterpenes, phenolic compounds, etc. Therefore, modern plant breeding programs look for pests and disease-resistance cultivars (Karabourniotis et al., 1995).

One factor that has a vital role in density, shape and mutually volatile oil quality and quantity of trichomes is geographical conditions such as latitude, longitude, height, temperature, photoperiod and etc. (Gales et al., 2010; Mossi et al., 2011; Talebi et al., 2018). These factors seem to affect the expression of genes (Krstic et al., 2006). Since GTs are the site of biosynthesis and storage of essential oils, they are linearly correlated with essential oils (Kofidis et al., 2003; Martins et al., 2017). For this reason, one of the new lines of studies is exploring ways to increase trichomes density and size (manipulate) and consequently increasing the essential oil yield (Huang et al., 2008; Kjær et al., 2012).

Many studies have investigated morphological anatomy, especially in the Lamiaceae family (Bisio et al., 1999; Avato et al., 2005; Dinç and Öztürk, 2008; Kahraman et al., 2010a). Moreover, the glandular hairs of various species belonging to the genus *Salvia*, a prestigious group with a wide range of species of economic interest, have been described (Kaya and Aksakal, 2007; Mayekiso et al., 2009). Knowledge of the existing genetic variation is vital for the selection of best species and population for conservation, cultivation, and breeding programs (Tissier, 2012).

Therefore, the objectives of this study were to (a) analysis some morphological characteristics at the flowering period, which could be used to distinguish between species and similar accessions of four *Salvia* species (*S. virgata, S. nemorosa, S. syriaca, S. frigida*), (b) investigate the type, distribution, and morphology of GTs and NGTs using scanning electron microscopy (SEM), (c) compare glandular hairs of leaves and flowers (sepal and petal), (d) compare trichomes of *S. virgata* collected from three different regions of Iran.

Materials and Methods

Plant material

Seeds of *Salvia* species were collected from various natural habitats of Iran (Table. 1). We also used some treatments (leaching, scarification, GA_3 and hot water) for germination enhancement. After selecting the best treatment for germination, seeds were leached for 24 h and soaked in 100 ppm GA_3 for 12 h. The treated seeds sowed in trays containing a mixture of coco-peat, peat moss, and perlite. After ten weeks, seedlings grew to a length of 15 cm with more than six leaves. The seedlings transplanted into field plots at Ferdowsi University of Mashhad (36º17´25´´ N, 59º35´45´´ E and 1100 m Elevation), Iran. The meteorology data of cultivating area are presented in Table 2. The experimental design was a complete randomized design (CRD) with four replications. The plots were maintained using the same cultural and irrigation practices. Leaves and flower samples collected in the flowering period for the SEM analysis.

Table 1. Description of the *Salvia* species origin used in this study.

Species	Location (city-province)	Longitude	Latitude	Elevation (m)
S. nemorosa	Buinmeyandasht - Isfahan	33º 01´ 00´´	50º 20´ 16´´	2330
S. syriaca	Aligudarz - Lorestan	33º 10´ 41´´	49º 29´ 35´´	2441
S. frigida	Fereydan – Isfahan	33º 08´ 45´´	50º 16´ 22´´	2640
S. virgata (A_1)	Darkesh - North Khorasan	37º 26´ 31´´	56º 45´ 08´´	1239
S. virgata (A_2)	Afoos - Isfahan	33º 00´ 14´´	50º 00´ 42´´	2557
S. virgata (A_3)	Eghled - Fars	30º 30´ 45´´	52º 43´ 51´´	2559

Table 2. The climate parameters of the studied area in the growth period.

Month	Temperature (ºC)	Absolute Humidity (%)	Precipitation (mm)	Wind Speed (Km/h)
May	25	41.8	0.6	8.6
June	28.2	30.2	0.1	8.9
July	28.8	25.9	0.0	9.6
Agues	25.9	21.6	0.1	8.9
September	24.3	31.7	0.0	7.3
October	14.1	41.9	0.0	6.1

Macro-morphological characteristics

Macro-morphological studies were carried out on the flowering stage (second year). Some of these features are used in *Salvia* classification, such as stamen types and fertility (Jamzad, 2013; Will and Claben-Bockhoff, 2017). Attributes such as leaf size, petiole color, petiole length (cm), internode length (cm), plant height (cm), number of flowering stem per plant, main inflorescence length (cm), secondary inflorescence length (cm), number of verticillasters, seed size, color and shape, pistil and filament length (cm) and flowering period were evaluated before harvesting. These analyses conducted evaluating eight samples per plot. Cluster analysis was done base on the stamen and pistil length (mm) and distance between stamen tip and stigma surface (mm) using Ward method.

Scanning electron microscopy (SEM)

Small pieces of fully-expanded leaves (5×5 mm), sepals (2×2 mm) and petals (2×2 mm) fixed in formaldehyde-ethanol- acetic acid (FAA) at room temperature for 24 h and dehydrated in a graded alcohol series. The specimens were then dried to a critical point with liquid CO_2, sputtered with gold, and viewed with a Philips 515 SEM at an acceleration voltage of 20 kV. Photographs were taken using *Kodak Technical Pan Film*.

Image analysis

SEM images were analyzed using Manual Microstructure Image Processing software (*MIP- Nahamin Pardazan Asia Co., Iran*) to estimate the average size, diameter (µm), area (μm^2) and trichome density (number of glands per mm^2) of five representative samples of adult leaves and flowers, which had been randomly excised from different plants.

Statistical analysis

Data were analyzed using one-way ANOVA, followed by Tukey test via JMP.8 statistical software at a significant level of $\alpha = 0.05$. Cluster analysis was done with the Ward method on 90% similarity by SAS statistical software.

Results

Macro-morphological characteristics

Twenty macro-morphological traits of *Salvia* genotypes are shown in Table 3. Also, flowering duration and the adaptability ratio of species in cultivation condition at Mashhad weather conditions after two years were determined (Table 3). According to the Tukey test ($P < 0.05$), the species showed statistical differences for all parameters except for leaf length and leaf width. In continue presented the short description of each cultivated genotypes.

Salvia virgata Jacq. This genotype produced some leaves at first weeks and then appeared flowering stem with a height of approximately 60–85 cm. The plant had 5.5 ± 0.9 stems per plant with erect stems, leaves simple 7-17 × 3-11 cm, and showed green petiole with 4-12 cm long. Main and lateral inflorescence long were 19-40 and 14-27 cm, respectively. Pistil and stamen lengths were 16.1-17.9 and 11.1-12 mm, respectively. The whole areal parts were covered with simple multi-cell trichomes. The small variation among *S. virgata* accessions was observed, but it was not significant except flowering duration (Table 3). The flowering period of this species was about 26 -41 days (Table 3). *Salvia virgata* (A3) showed the highest ratio of adaptability (91%).

***Salvia nemorosa* L**. is a resistant herbaceous perennial plant. This species height was about 52-75 cm, erect, branched, and produced more than eight stems per plant. Leaves distributed over stem or rosette-forming with 4-12 × 2.5-4 cm, green petiole was 2-5 cm long and showed the highest leaf area (1836.3 cm^2) in comparison to other genotypes. Inflorescence was 18-25 cm long. Corolla purple, pistil and stamen length were 24.9-9.1 mm, respectively and distance between stamen tip and stigma surface was 1.1 cm.

Mature nutlet was the ovoid-elongated shape and bold brown color (Table 3). Flowering started in late April and ended at first June (35 days flowering duration). The ratio of adaptability of *S. nemorosa* after two years was about 83.3%.

Salvia syriaca **L**. is an herbaceous perennial plant with the rhizome. In the first year only grew rosette leaves and did not rose above the surface of the soil, leaves were 7-9.5 × 2.5-5 cm, linear-oblong to ovate, petiole 3-5 cm long. Stems were erect with rarely branched at the base and grew to a height of 25-60 cm. This genotype had the lowest leaf area (348 cm^2) and stem per plant (1) in comparison with the other genotypes. Inflorescence had up to 40 cm length, corolla white, pistil, and stamen length were 20.9 and 9 mm, respectively and showed the highest distance between the tip of stamen and pistil. The flowering duration was about 35.3 days. Note color was maize and ovoid shape.

Salvia frigida **Boiss**. is a perennial herb with a thick woody rootstock. Stems were solitary or several, approximately 65-75 cm high. Leaves distributed over stem or rosette-forming, 8-16 × 4-8 cm, petiole color was violet and 5-8 cm long. The whole areal parts were densely covered with capitate glands. Calyx had 5-8 mm long, covered with stalk GTs, pistil and stamen had16 and 10 mm long, respectively. Seed color was the brown and ovoid shape. Flowering started in early May and finished at mid-June. The ratio of adaptability in this genotype was 51.7%.

Salvia nemorosa and *S. virgata* are flowered in the first year, but *S. frigida* and *S. syriaca* were produced rosette leaves in the first year and flourished on the second year. The study of the flowering period was shown a high variation of flowering duration (day) in species and populations (Table 3). All species have a long flowering period (26-40 days). The ratio of adaptability in species and accessions was 25 - 91% in *S. syriaca* and *S. virgata* (A3), respectively. Before harvesting, some morphological specifications were evaluated (Table 3). For the leaf area, the most significant value was found for *S. nemorosa* (1836.3 cm^2), and the lowest value was found for *S. syriaca* (348.0 cm^2). The highest plant height was found for *S. virgata* particularly for the A_1 (88.3 cm). *Salvia nemorosa* had great potential for lateral stem production (8.3 per plant), this observed for *S. syriaca* in the third year.

For the flower specifications, *Salvia* species presented statistical differences ($P < 0.05$). The results showed that *S. virgata* and *S. nemorosa* had the highest main and lateral inflorescence lengths, also main and lateral florets numbers (Table 3). Overall all species showed great potential for seed production. The distance between pistil and stigma tips in various species was 1.1 - 5.2 mm in *S. nemorosa* and *S. syriaca*, respectively.

The stamen morphology is the primary characteristic that uses for separating *Salvia* taxa from other Lamiaceae genus and supported evolution in *Salvia* (Claßen-Bockhoff et al., 2004; Jenks, 2009). Accordingly, we clusterized six *Salvia* genotypes according to the stamen and pistil length and distance between stamen tip and stigma surface (Fig. 1). The results showed that *S. virgata* (three accessions) and *S. frigida* located in the same clade, on the other hand, *S. nemorosa* and *S. syriaca* and belong to the same group. The variation in *S. virgata* accessions caused various origin and effect of environment.

Micro-morphological characteristics

The SEM results showed that the distribution of trichomes was different in various organs (Fig 2-4). The overall leaves and flowers of *Salvia* species were covered with peltate GTs. The GTs density in leaves was significantly less than reproductive organs. In all micrographs obtained, the density of GTs was found to be higher on lower epidermis than that of the upper one. Moreover, uniseriate and

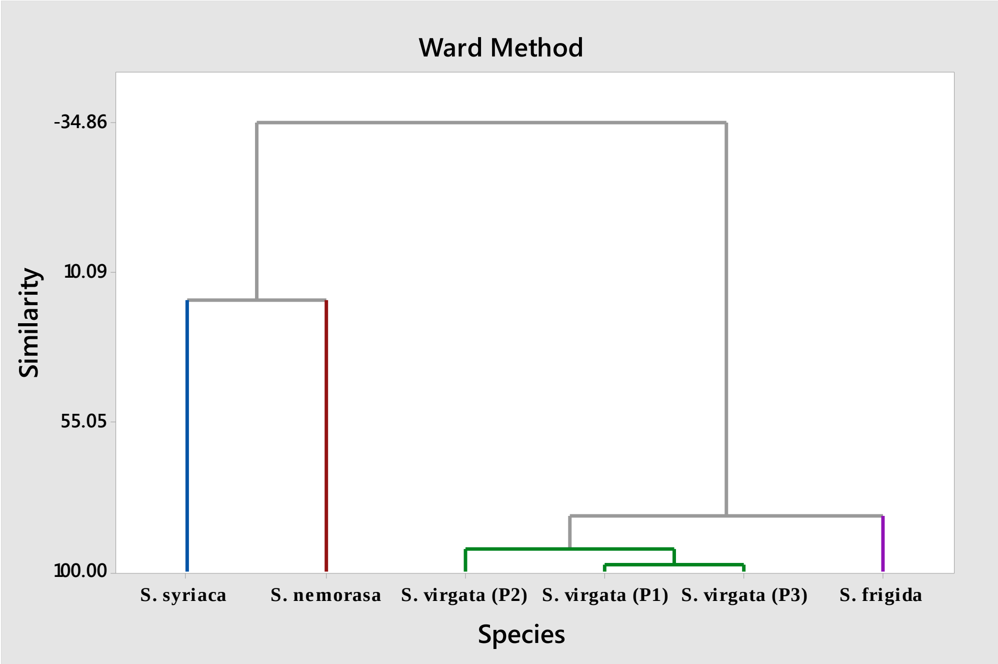

Fig. 1. Cluster analysis dendrogram of *Salvia* genotypes based on stamen and pistil length and distance between stamen tip and stigma surfaces (Ward method).

Table 3. Macro-morphological characteristics of different *Salvia* species planted at the same location in Mashhad*.

Species	Petiole color	PL1	LL	LW	LA	IL	PH	No. stem per plant	MIL	LIL	MFN	LFN	SF
S. nemorosa	Green	3.2^{b}	9.3^{a}	3.2^{a}	1836.3^{a}	8.3^{abc}	62.3^{b}	8.3^{a}	21.0^{ab}	16.3^{ab}	75.0^{a}	43.3^{ab}	22 April
S. syriaca	Green	4.5^{ab}	8.2^{a}	3.4^{a}	348.0^{c}	6.3^{bc}	37.0^{c}	1^{d}	16.0^{b}	10.7^{b}	24.0^{b}	15.0^{b}	26 April
S. frigida	Violet	7.0^{ab}	11.3^{a}	5.7^{a}	1073.3^{b}	9.7^{a}	70.0^{ab}	2.0^{cd}	21.0^{ab}	13.3^{ab}	47.3^{ab}	37.0^{ab}	8 May
S. virgata (A_1)	Green	6.3^{ab}	11.7^{a}	4.7^{a}	814.3^{bc}	8.7^{ab}	88.3^{a}	6.7^{ab}	21.3^{ab}	16.0^{ab}	68.7^{a}	60.0^{a}	30 April
S. virgata (A_2)	Violet	8.2^{a}	12.7^{a}	7.2^{a}	583.3^{bc}	9.3^{ab}	70.0^{ab}	4.7^{bc}	33.3^{a}	22.3^{a}	51.3^{ab}	37.7^{ab}	10 May
S. virgata (A_3)	Green	5.0^{ab}	9.8^{a}	4.3^{a}	1049.8^{b}	5.5^{c}	71.3^{ab}	5.0^{bc}	27.0^{ab}	19.3^{ab}	66.7^{a}	66.7^{a}	16 May

Species	SF	FD	PL2	SL1	DB-P & S	SL2	SW	Seed color	Seed shape	RA
S. nemorosa	22 April	35.0^{ab}	24.9^{a}	9.1^{d}	1.1^{c}	1.9^{b}	1.2^{c}	Bold Brown	Ovoid - elongated	83.3ab
S. syriaca	26 April	35.3^{ab}	20.9^{b}	9.0^{d}	5.2^{a}	2.3^{a}	2.1^{a}	Maize	Ovoid - spherical	25.0e
S. frigida	8 May	32.7^{bc}	16.0^{d}	10.0^{c}	2.0^{b}	2.6^{a}	2.2^{a}	Brown	Ovoid	51.7d
S. virgata (A_1)	30 April	40.7^{a}	16.1^{d}	11.1^{b}	2.5^{b}	1.9^{b}	1.4^{bc}	Black	Ovoid	66.0cd
S. virgata (A_2)	10 May	26.0^{c}	17.2^{cd}	12.0^{a}	2.1^{b}	1.8^{b}	1.5^{b}	Black	Ovoid	74.7bc
S. virgata (A_3)	16 May	30.7^{bc}	18.0^{c}	11.1^{b}	2.4^{b}	1.7^{b}	1.2^{c}	Black	Ovoid	91.0a

*Different letters mean significant difference at 95% (Tukey test - $p < 0.05$), PL1: Petiole length (cm), LL: Leaf length (cm), LW: Leaf width (cm), LA: Leaf area (cm^2), IL: Internode length (cm), PH, Plant height (cm), MIL: Main inflorescence length (cm), LIL: Lateral inflorescence length (cm), MFN: Main florets number, LFN: Lateral florets number, SF: Start flowering, FD: Flowering duration (day), PL2: Pistil length (mm), SL1: Stamen length (mm), DB-P & S: Distance between stigma surface and stamen tip (mm), SL2: Seed length (mm), SW: Seed width (mm), RA: Ratio of adaptability after 2 years (%).

multicellular NGTs were scattered over the adaxial and abaxial surfaces of the leaves. The results of the statistical analysis showed a significant difference ($P < 0.05$) in GTs morphology and density in the calyx (Table 4). The GTs and NGTs densities of leaves and flowers were variable in different species. In some cases, these trichomes were more scattered, but in other cases, they formed a dense covering and obscuring the epidermal surfaces. GTs were circular and had a short stalk which one basal epidermal cell. In all species, GTs were single, but not in some species such as *S. nemorosa* calyx (Fig 1. A), *S. syriaca* (Fig 2-A), *S. virgata* P2 (Fig 3-C) and P3 (Fig 3-H). *Salvia virgata* (P_2) had the highest trichomes number per area (29.9 per mm^2) and 18.45% of leaf surface was covered with GTs (Table 4). NGTs had a high frequency in the adaxial surface of the leaf (Fig 1-D, Fig 2-C, Fig 3-E). The structural diversity of peltate trichomes in different species was not observed. The NGTs appeared to be solitary, uniseriate and multicellular in various shapes.

Concerning the size, GTs were several times bigger than epidermal and stomatal cells. Statistical analyses of calyx GTs diameter (µm) and area (μm^2) in Salvias are shown in Table 4. Different accessions of *S. virgata* had the largest trichome diameter (91 µm) and area (6331 μm^2), while *S. syriaca* showed the smallest diameter (64 µm) and area (3925 μm^2). The GTs may be further subdivided into unicellular and multicellular categories. In the current study, multicellular GTs were observed in *S. nemorosa* (Fig 1-C) and *S. virgata* P_1 (Fig 3-D) and P_2 (Fig 3-G). The multicellular NGTs were observed in some species such as *S. nemorosa* (Fig 1-C), *S. syriaca* (Fig 2-B), *S. frigida* (Fig 2-E), and *S. virgata* P_1 (Fig 3-B). Three populations of *S. virgata* collected from different altitudes revealed variation in trichome density, diameter, and area (Fig 3 and Table 4). Even though three populations were cultivated in the same area, on both leaf surface and flowers, the frequency of trichome density in P_2 (2557 m) and P_3 (2559 m) was greater than P_1 (1239 m).

Table 4. Glandular trichome characteristics on the calyx in *Salvia* species

Species	Mean trichome diameter µm	Mean trichome area µm^2	Mean calyx trichome density per mm^2	Coverage Percentage
S. nemorosa	73.66^{c}	4374.33^{c}	19.47^{bc}	8.45^{d}
S. syriaca	68.41^{c}	3925.70^{d}	11.27^{de}	4.47^{e}
S. frigida	75.78^{c}	4222.13^{c}	9.06^{e}	3.85^{e}
S. virgata (A_1)	97.26^{a}	6915.03^{a}	15.33^{cd}	10.57^{c}
S. virgata (A_2)	90.29^{ab}	6165.63^{b}	30.58^{a}	18.45^{a}
S. virgata (A_3)	87.13^{b}	5914.70^{b}	24.16^{b}	14.31^{b}

*Different letters mean significant difference at 95% (Tukey test - $p < 0.05$)

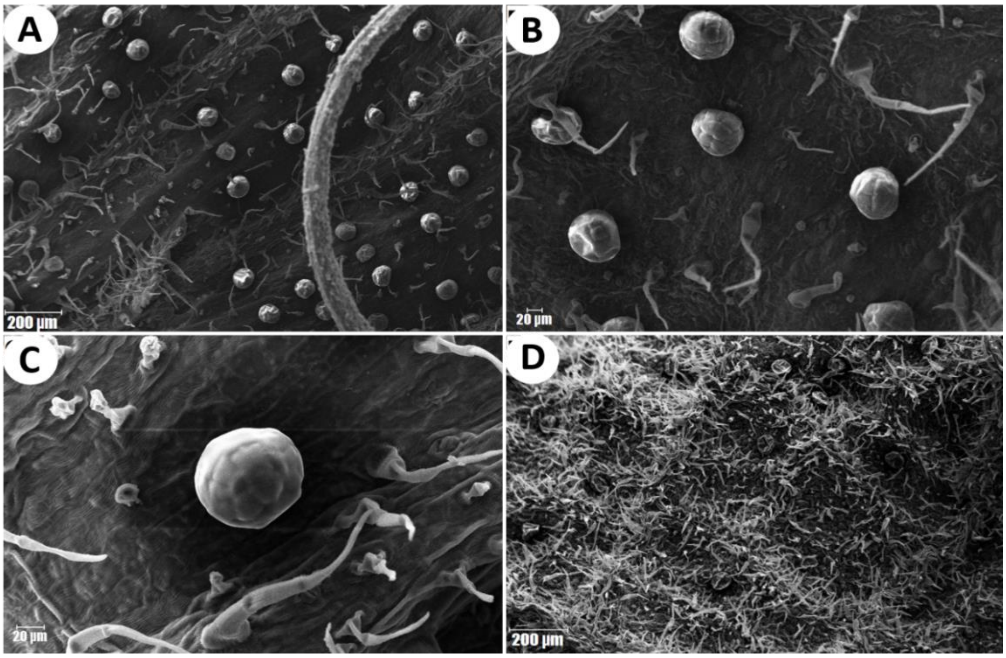

Fig. 2. Scanning electron micrographs of *S. nemorosa* trichomes. A, Density of GTs of flower. Bar= 200 µm. B and C, Peltate GTs and multicellular NGTs. Bar= 20 µm. D, High density of NGTs in adaxial surface of the leaf. Bar= 200 µm.

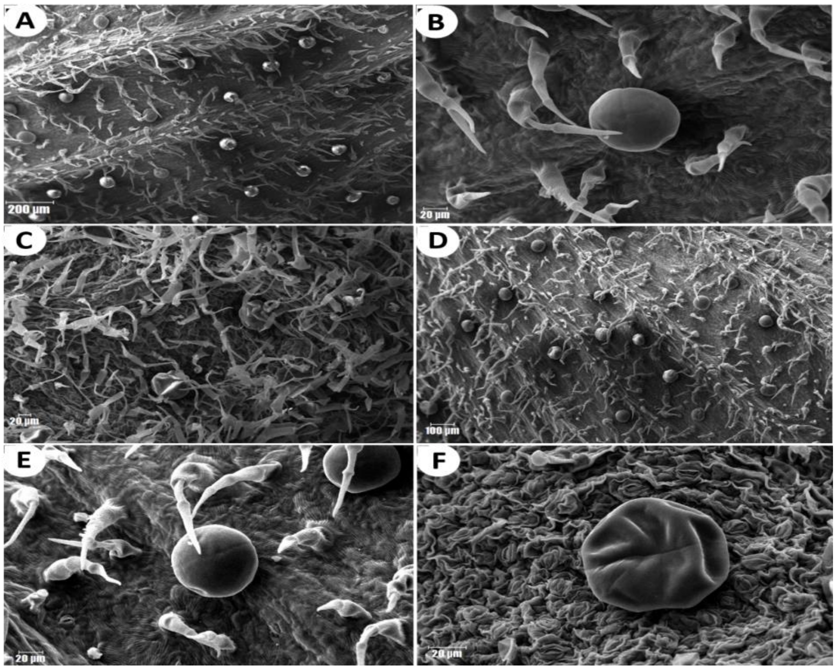

Fig. 3. Scanning electron micrographs of *S. syriaca* and *S. frigida* trichomes. A, Density of GTs and NGTs of the flower. Bar= 200 µm. B, Peltate GTs and multicellular NGTs of flower. Bar= 20 µm. C, High density of NGTs in adaxial surface of the leaf. Bar= 20 µm. E, Density of GTs and NGTs of the flower. Bar= 100 µm. E, GTs and multicellular NGTs of flower. Bar= 20µm. R, F, GTs of leaf. Bar= 20 µm.

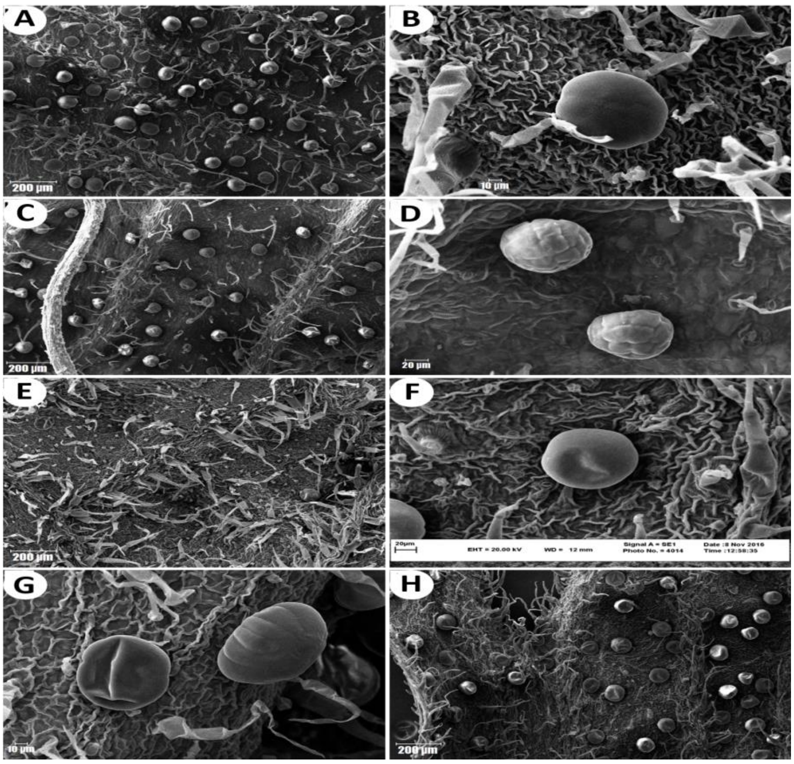

Fig 4. Scanning electron micrographs of three accessions of *S. virgata*. A, Density of GTs in the flower of *S. virgata* (A1) flower. Bar= 200 µm. B, GTs and multicellular NGTs of *S. virgata* (A1) flower. Bar= 10 µm. C, Density of GTs in the flower of *S. virgata* (A2). Bar= 200 µm. D, Multicellular GTs of *S. virgata* (A2) flower. Bar= 20 µm. E, NGTs of *S. virgata* (A2) leaf. Bar= 200 µm. F, GTs of *S. virgata* (A3) flower. Bar= 20. G, Multicellular GTs of *S. virgata* (A3) flower. Bar= 10 µm. H, Density of GTs of *S. virgata* (A3) flower. Bar= 200 µm.

Discussion

Morphological features such as leaf size and shape, flower parts and seed shape and color are taxonomically essential to identify different species in the same genus (Kahraman et al., 2009) and plant morphology is one of the knowledge for diversity studies (Wester and Claben-Bockhoff, 2007). Furthermore, some other morphological traits such as plant high (cm), number of stem per plant, the height of inflorescence (cm) and flowering period are important for agricultural practices and the right choice of commercial cultivation in breeding programs (Mossi et al., 2011). For example, plants with high height are suitable for mechanical harvesting. The results showed some morphological properties such as pistil and stigma length are useful for distinguishing *Salvia* species. Jamzad (2013) classified Iranian *Salvia* species according to flower features.

In the current study, the flowering period in all species was more than one month. Long flowering duration led to successful pollination on most flowers in a plant and enhanced variation. Also, it is a momentous index in ornamental plants. *Salvia* species are used as ornamental special in Europe and in the United States (Karabacak et al. 2009). *Salvia nemorosa* is an attractive plant that can be grown and propagated conveniently, which explains the reason for its popularity with its growers for many years. A small variation in *S. virgata* accessions was observed due to their different origins (Gales et al., 2010; Mossi et al., 2011). In the previous study it has been also reported that high variation in the growing period and environmental effects on flowering of different *Salvia* species implying the interaction between ecological parameters and plant species (Ayerza and Coates, 2009; Mossi et al., 2011).

Although, there was a distance between pistil surface and stamen tips in all genotypes, only *S. syriaca* had long-style flowers (Pin). In other species, the distance between pistil and stamen tips is due to the reason that stamens mature first and then release pollen grains before the gynoecium reaches its maximum maturation and elongation. Heterostyly flowers led to promote outcrossing in the *Salvia* genus (Takano, 2013). Most of *Salvia* species are characterized by a staminal lever as a unique mechanism in pollination. In this mechanism, pistil develops out of the corolla and touch pollinator's body and transfer pollen to the other flowers (Claßen-Bockhoff et al., 2004).

High variation was showed in seed morphology (Table 3). Seed morphology has significant importance on species identification. Understanding morphological properties are essential for the selection of best species and population for cultivation and breeding programs (Castro et al., 2008).

The high density of GTs and NGTs were observed in the calyx and adaxial surfaces of leaves, respectively. Similar results have been reported in other Lamiaceae genera (Karabourniotis et al., 1995; Bisio et al., 1999; Bhatt et al., 2010). Generally, there were abundant GTs on the inflorescence axis and calyx, but NGTs ones were mainly situated on the leaf and stem in Lamiaceae (Kahraman et al., 2010c). For this reason, the best harvesting time of Lamiaceae for essential oils is flowering stage. Generally, in the Lamiaceae family, the production of essential oils occurs in peltate GTs and the highest density of peltate GTs is in the inflorescence (Tissier, 2012; Najar et al., 2018). In addition to the role of GTs in the production and storage of the secondary metabolites, they inhibit transpiration in different ways, depending on the number, form, and position of trichomes (xeromorphic) (Dunkic, 2001), while NGTs have a protective role against UV radiation and other stresses. High NGTs leads to the reduction of air circulation on the leaf surface and creates a zone of stale air filled with water vapor, which changes the microclimatic conditions surrounding the leaf (Dunkic, 2001).

Based on the GTs structure, they are generally classified as either peltate or capitate. Peltate GTs were identified in Salvias SEM micrograph and multicellular GTS were observed in *S. nemorosa* and *S. virgata*. Kaya et al. (2003) reported two types of trichomes in *Salvia glutinosa*: simple and multicellular NGTs. Peltate trichomes matured of the Lamiaceae often contain several secretory cells, and in the literature, four (Kahraman et al., 2010c), eight (Tissier, 2012) to up to sixteen head cells (Kamatou et al., 2007) have been reported. The capitate GTs were not observed in this investigation, though in some *Salvia* species such as *S. sclarea* (Tissier, 2012), *S. macrochlamys* (Kahraman et al., 2010a), and *S. officinalis* (Avato et al., 2005), capitate hairs have been reported. Giuliani et al. (2018) reported capitate and peltate GTs in *Salvia verticillata*, both responsible for synthesis of terpenoid compounds.

Significant variations in the size and shape of trichomes have been reported in the Lamiaceae family (Bhatt et al., 2010). The trichome types and density have been successfully used to classify genera and even species in certain families and recognizing interspecific hybrids. The distribution of trichomes on the calyces is particularly crucial since the calyx features are often essential for taxonomic determination in Lamiaceae (Kaya et al., 2003; Krstic et al., 2006; Kahraman et al., 2010b). The high trichome density of *S. virgata* A_2 and A_3 in comparison to A1 accessions caused by different elevations (Table 1) and protection against UV radiation. Thus, it can be concluded that trichome properties are controlled by plant genome and geographical conditions (Mossi et al., 2011). Talebi et al. (2018) observed trichomes with various morphology and density in various accessions of *S. nemorosa* collected from different regions. This is consistent with the results reported by Gales et al. (2010) on *Origanum vulgare* and Kjær et al. (2012) on *Artemisia annua*.

Conclusion

Morphological evaluations carried out in this study made it possible to observe the occurrence of statistical differences among species and to identify the behavior of such species when cultivated in other regions. Different types of GTs and NGTs were reported in the Lamiaceae family. It should be noted that flowers of different species demonstrated little GTs diversity. Only peltate hairs were seen, and the greatest amount of GTs was found in the fertile bracts and sepals. Two types of trichomes were observed in the flowers and the leaves of *Salvia* species: peltate GTs and filiform NGTs. Studying trichome is important not only as a structure responsible for the synthesis of terpenes but also provide useful information for the recognition of species.

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