A first approach on morphometrics studies of *Salvia fruticosa* found in Greece

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Abstract

***Salvia fruticosa* Mill. is a member of *Lamiaceae*, a widespread family of high importance for essential oils and pharmaceutical as well as ornamental use. The present study was conducted to test quantitative and qualitative morphological traits in order to find a set of suitable descriptors for distinguishing individuals with special ornamental characteristics that could be the initial plant material for breeding programs and clonal propagation of plants to be introduced to the floricultural industry. Morphological characteristics were used to study individuals of four different accessions (SfH, SfH1L, SfH2K, SfH3T) that were collected in their natural habitat in mount Ymittos, prefecture of Attiki, Greece. A total of 12 qualitative and six quantitative characters were selected and used based on descriptors for other plant species and their variability in different accessions. The first group of characters involved characters of vegetation (leaves and stem), flowers and fragrance. The second group involved leaf morphometrics that counted by a portable leaf scanner (length, width, perimeter, area, ratio and shape factors. NTSYS-pc v2.11f was used for statistical analysis, cluster analysis was conducted with the Unweighted Pair Group Method based on Arithmetic Average (UPGMA), using the Square Euclidian Distance coefficient, and the dendrograms were generated based on the genetic distance matrix. One-way ANOVA was used for determination of the differences between the mean values of the leaf traits. Cluster analysis separated the individuals according to their morphological characteristics in two branches. SfH(2)K were found to be distinct from other three individuals. SfH(3)T was found to be closer to SfH(1)L than SfH. The present study revealed morphological traits to differentiate *S. fruticosa* individuals from different accessions aiming to facilitate their clonal propagation and exploitation for ornamental or pharmaceutical use. The applied characteristics could be a basis for the development of a complete list of discriminating characteristics for *S. fruticosa*.**

Keywords: dendrogram, descriptor, leaf trait, morphological trait, qualitative character, quantitative character

INTRODUCTION

It is of high importance in the floriculture industry introducing new native plant species or clones for cultivation. Exploitation and preservation of the Greek flora could reveal its importance as a bank of genetic material. *Salvia* species native in Greece are an unexploited source of high potential value for Greek floriculture industry.

Salvia is one the largest genera of flowering plants, including 2.211 scientific plant names: of these 986 are accepted species names (The Plant List, 2013; Hu et al., 2018). The name of the genus is derived from the Latin verb “salvere” means ‘be well’, because of the curative properties of the plant. This name was paraphrased to sauja and sauge (in French), which has become the name of sage (Grieve, 1984). The genus is found throughout the Old World and the Americas, with three distinct regions of diversity, i.e., Central and South America, Eastern Asia and Central Asia and the Mediterranean (Walker et al., 2004). Many species of this genus are used or suggested for use as ornamental plants and for their essential

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oils found in the leaves in the food, medicine and perfumery industry, as well as culinary plants, and in herbal medicine (Goren et al., 2006; Abdollahi et al., 2012).

In Greece are found 30 taxa (species and subspecies) of the genus *Salvia*; the *Salvia fruticosa* Mill. having the widest distribution, as in all the Mediterranean region (Karousou et al., 2000). The plant is strongly aromatic, growing usually at altitudes up to 1000 m in the zone of xeric Mediterranean Phrygana and Grasslands (Flora of Greece, 2019), while in Crete, it occurs up to 1350 m (Jahn and Schönfelder, 1995). It is up to 1.00 m in height and very attractive during flowering bearing pink inflorescences of many flowering verticillate clusters (Harley et al., 2004). It is known under the common name 'faskomilo' and used in local medicine as a culinary herb (Fragaki, 1969). There is a continuing, great interest about the polyphenolic composition of the species since times of Ancient Greece to nowadays (Kintzios, 2000; Kavoura et al., 2019).

Morphological traits are widely used for diversity studies (Sarıkamıs et al., 2010; Lopes et al., 2012; Bertsouklis and Papafotiou, 2016) and assessing variability in plant species (Khurshid et al., 2004; Furat and Uzun, 2010). The morphological analysis is often an effective tool for both characterizing and distinguishing cultivars and hybrids and studying their relationships (Bertsouklis and Papafotiou, 2016; Blazakis et al., 2017). Morphological traits such as leaf size and flower characters have been used as descriptors of a number of *Salvia* species (D'Antunono et al., 2002; Celep et al., 2011).

There are reports about high morphological variability within *S. fruticosa* (Karousou and Kokkini, 1997; Reales et al., 2004; Leontaritou et al., 2020); hence the objective of the present study was to test quantitative and qualitative morphological traits aiming to find a set of suitable descriptors for distinguishing individual genotypes with special ornamental characteristics, which could be the initial plant material for breeding programs and clonal propagation of plants to be introduced to the floricultural industry.

MATERIALS AND METHODS

Four individual genotypes (individuals) were sampled for identification in 2019, at Mount Hymettus, prefecture of Attica at 460 m altitude (37°57'15.3N and 23°49'51.7E). The identification of the sampled individuals was followed by the classification in four different classes including one individual with typical morphological characteristics of *S. fruticosa* (SfH) and three more with varying phenotype, i.e., SfH1(L), SfH2(K) and SfH3(T). A total of 18 morphological traits (descriptors) of stem and flower were recorded for each individual; 12 qualitative (Table 1) and six quantitative: leaf length (l), width (w), perimeter (p), area (s), ratio (r) and shape factor (f) (Figure 1). Some of these descriptors had been used in a previous study on distribution and clinal variation of *S. fruticosa* on the island of Crete (Greece) (Karousou and Kokkini, 1997). The ratio factor is the ratio of the leaf length to its maximum width. The shape factor is the ratio of the leaf area to the leaf perimeter, corrected so that the shape factor of a circle is equal to 1 ($f = 4 \pi s/p^2$).

The matrix of average taxonomic distance for individuals was computed using the Euclidean distance coefficient. Cluster analysis was conducted on the taxonomic distance matrix with the Unweighted Pair Group Method based on Arithmetic Average (UPGMA) and the dendrograms were generated based on Euclidean distance of morphological character analysis (Greenacre and Underhill, 1982). One-way ANOVA was used for determination of the differences between the mean values of the leaf traits. Principal coordinate analysis (PCA) was used in order to verify cluster analysis and to assist in visualizing the data, and statistical analysis of morphological markers was conducted by the software NTSYS-pc version 2.11f (Rohlf, 1992).

RESULTS AND DISCUSSION

The distinguish of different individuals was mainly based on the morphology of stem, calyx and petals color. SfH1(K) and SfH2(T) had high density of dark, linear zones on stem, being a very attractive morphological character. The high inflorescence density of SfH1(L) was a very attractive character for this individual. Two more special characteristics of SfH1(K) were the dark green color of calyx and petals. SfH1(L) and SfH2(K) had higher strength of leaf

and flower fragrance. As regards leaf morphology, there were differences between the different individuals in terms of pubescent, texture and shape.

Table 1. Morphological traits measured in four *Salvia fruticosa* individuals used as descriptors. There is a score code for each one depending on its state among different individuals tested.

Code	Morphological trait (descriptor)	Score code – descriptor state		
1	Existence of dark/linear zone on stem	1: Yes	2: No	
2	Intensity of dark/linear zone	1: Low	2: Medium	3: High
3	Stem pubescent	1: Yes	2: No	
4	Leaf pubescent	1: Low	2: Medium	3: High
5	Leaf thickness	1: Thin	2: Medium	3: Thick
6	Leaf texture	1: Leathery-elastic	2: Membranaceous-tough	
7	Leaf shape	1: Elliptical to lanceolate	2: Elliptical	
8	Leaf color of upper side	1: Light green	2: Green	3: Dark green
9	Inflorescence density	1: Low	2: Medium	3: High
10	Color of calyx	1: Light green	2: Green	3: Dark green
11	Color of petals	1: Light puple-lilac	2: Purple-lilac	3: Dark purple-lilac
12	Strength of leaf and flower fragrance	1: Low	2: Medium	3: High

Figure 1. Flowering *Salvia fruticosa* plant (a) and morphological characters of vegetation used in analysis (b).

As regards the quantitative characteristics, analysis revealed that there were differences in leaf length and area, and in shape factor. SfH, SfH1(L) and SfH2(K) had longer leaves compared to SfH3(T), which had smaller leaf area compared to SfH and SfH1(L) but larger than that of SfH2(K) (Table 2). SfH had the largest shape factor with no statistically significant difference from SfH1(L).

Table 2. Leaf traits of four *Salvia fruticosa* individuals selected from mount Hymettus.

Code	Length (cm)	Width (cm)	Perimeter (cm)	Area (cm^2)	Ratio factor	Shape factor
SfH	6.3±0.3 a	2.1±0.1 a	14.2±0.6 a	7.5±0.3 a	3.0±0.2 a	0.50±0.03 a
SfH1(L)	6.3±0.3 a	2.2±0.1 a	14.8±0.6 a	7.3±0.4 a	2.9±0.2 a	0.40±0.02 ab
SfH2(K)	5.3±0.2 a	3.0±0.8 a	13.0±0.5 a	4.6±0.2 c	2.5±0.3 a	0.30±0.03 b
SfH3(T)	6.0±0.2 b	1.9±0.1 a	14.0±0.5 a	5.8±0.4 b	3.2±0.1 a	0.30±0.03 b
F	*	NS	NS	*	NS	*

SfH, SfH1(L), SfH2(K), SfH3(T): individuals of *S. fruticosa*.

*$p<0.05$, NS: $p>0.05$. Values followed by different lowercase letter within each trait are significantly different at the 5% level, determined by the one-way Anova.

Cluster analysis separated the individuals according to their morphological characteristics in two main branches (Figure 2). SfH(2)K was found to be distinct from the other three individuals. SfH(3)T was found to be closer to SfH(1)L than SfH (Figure 3). PCA analysis confirmed cluster analysis and six components have been arranged in decline series according to their importance, explaining the 89.26% of the total variability among the different individuals. All descriptors grouped in the same principal component have strong correlation and each component is strongly correlated with a group of the used descriptors so it could be estimated their contribution to variability (Table 3, Figure 2).

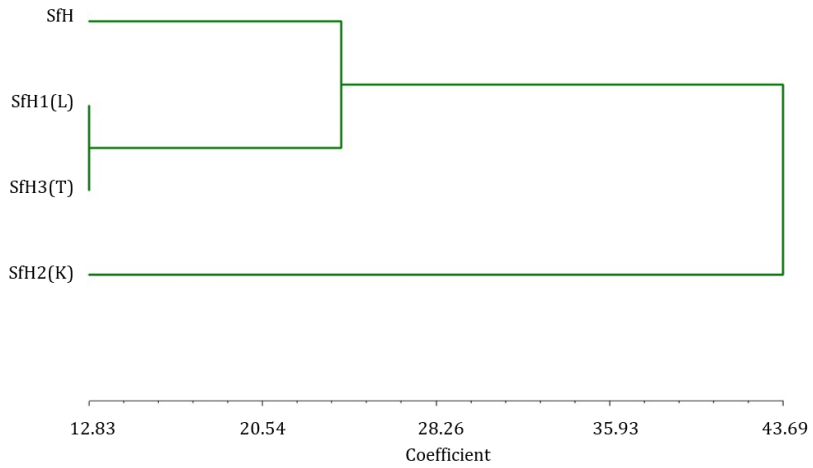

Figure 2. UPGMA dendrogram of four *Salvia fruticosa* individuals based on Euclidean distance of morphological character analysis.

Table 3. Evaluation of the descriptors and their contribution to the variability of the individuals studied.

Principal components					
1	**2**	**3**	**4**	**5**	**6**
% Contribution of variability					
33.55	23.44	10.57	9.96	6.19	5.55
Related descriptors					
IDLZ	LTe	SF	L	W	DLZ
LT	LCu	RF	A	LS	
CC	ID		P	CP	
	SF				

Intensity of dark/linear zone (IDLZ), leaf thickness (LT), color of calyx (CC), leaf texture (LTe), leaf color of upper side (LCu), linflorescence density (ID), strength of leaf and flower fragrance (SF), shape factor (SFa), ratio factor (RF), length (L), area (A), perimeter (P), width (W), leaf shape (LS), color of petals (CP), existence of dark linear zone (DLZ).

Thus, variation of morphological characters within populations of *S. fruticosa* in Mount Hymettus, Attica, was observed, as has been shown previously for populations in Crete and Peloponnese (Karousou and Kokkini, 1997; Leontaritou et al., 2020). The development of new clones for horticultural use presupposes their election, identification and then their multiplication, evaluation and promotion. The present work provides morphological traits to differentiate individuals of *S. fruticosa* from different accessions serving the aim to distinguishing individuals with special ornamental characteristics, which could be the initial plant material for breeding programs and clonal propagation of plants to be introduced to the floricultural industry. The applied morphological characteristics could be a basis for the development of a complete list of discriminating characteristics for *S. fruticosa*.

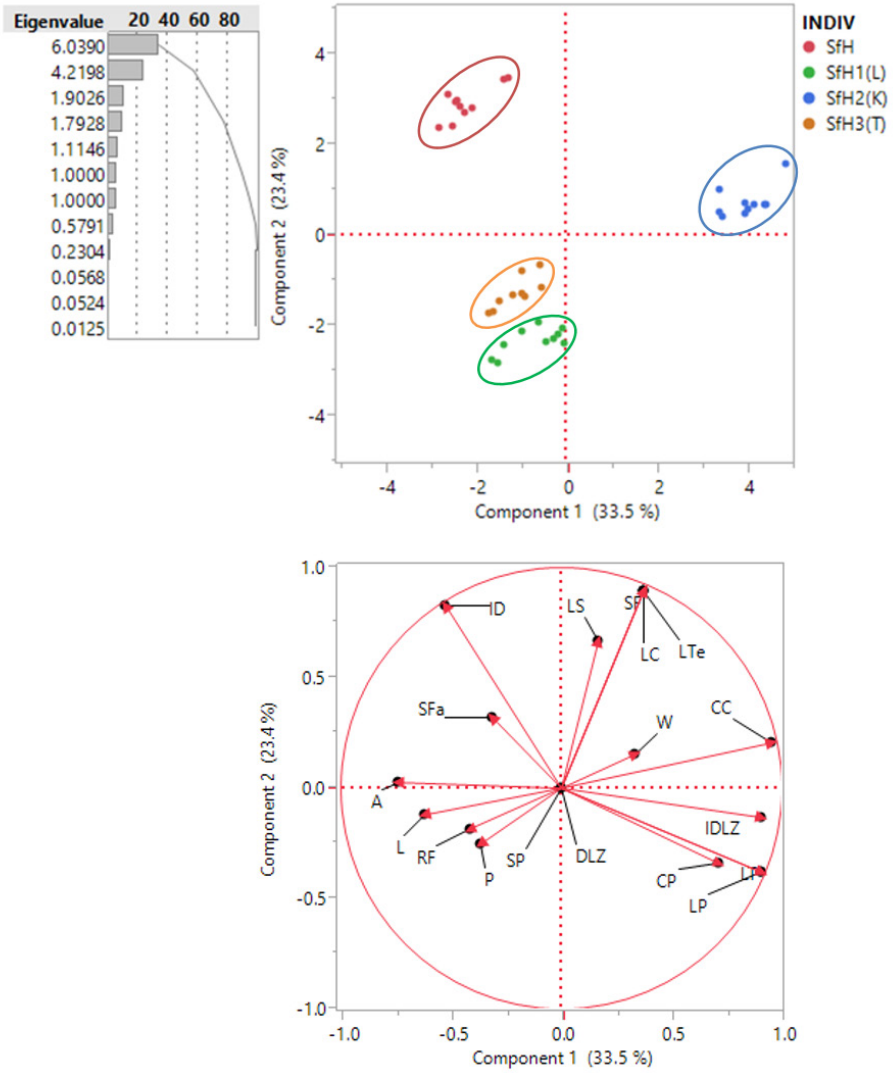

Figure 3. Evaluation of the descriptors and their contribution to the variability of the individual studied.

ACKNOWLEDGEMENTS

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A first approach on morphometrics studies of *Salvia fruticosa* found in Greece

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https://www.salvia-breed-gr.com/en/

INTRODUCTION

- *Salvia fruticosa* Miller has a wide distribution in the Mediterranean area.
- It is very attractive during flowering bearing pink inflorescences of many flowered verticillate clusters (Picture 1).
- **There is a high morphological variability within *S. fruticosa*, and hence the objective of the present study was to teat quantitative and qualitative morphological traits aiming to find out a set of proper descriptors to distinguish individuals with particular ornamental characteristics.**

Picture 1. *S. fruticosa* grows along coastal areas, from sea level to 1000 m, in central Greece, the Peloponnese and the islands.

Table 1. Morphological traits of *Salvia fruticosa* individuals

Code	Morphological Trait (descriptor)	Score code- descriptor state		
1	Existence of dark/linear zone on stem	1: Yes	2: No	
2	Intensity of dark/linear zone	1: Low	2: Medium	3: High
3	Stem pubescent	1: Yes	2: No	
4	Leaf pubescent	1: Low	2: Medium	3: High
5	Leaf thickness	1: Thin	2: Medium	3: Thick
6	Leaf texture	1: Leathery-elastic	2: Membranaceous-tough	
7	Leaf shape	1: Elliptical to lanceolate	2: Elliptical	
8	Leaf colour of upper side	1: Light green	2: Green	3: Dark green
9	Inflorescence density	1: Low	2: Medium	3: High
10	Colour of calyx	1: Light green	2: Green	3: Dark green
11	Colour of petals	1: Light puple-lilac	2: Purple-lilac	3: Dark purple-lilac
12	Strength of leaf and flower fragrance	1: Low	2: Medium	3: High

Table 2. Leaf traits of four *Salvia fruticosa* individuals selected from mount Hymettus

Code	Length (cm)	Width (cm)	Perimeter (cm)	Area (cm^2)	Ratio factor	Shape factor
SfH	6.3 ± 0.3 a	2.1 ± 0.1 a	14.2 ± 0.6 a	7.5 ± 0.3 a	3.0 ± 0.2 a	0.50 ± 0.03 a
SfH1(L)	6.3 ± 0.3 a	2.2 ± 0.1 a	14.8 ± 0.6 a	7.3 ± 0.4 a	2.9 ± 0.2 a	0.40 ± 0.02 ab
SfH2(K)	5.3 ± 0.2 a	3.0 ± 0.8 a	13.0 ± 0.5 a	4.6 ± 0.2 c	2.5 ± 0.3 a	0.30 ± 0.03 b
SfH3(T)	6.0 ± 0.2 b	1.9 ± 0.1 a	14.0 ± 0.5 a	5.8 ± 0.4 b	3.2 ± 0.1 a	0.30 ± 0.03 b
F	*	NS	NS	*	NS	*

MATERIALS AND METHODS

- A total of 18 morphological traits (descriptors) of stems and flower were recorded for each individual; 12 qualitative (Table 1) and six quantitative; leaf length (l), width (w), perimeter (p), area (s), ratio and shape factor (f). The ratio factor (r) is the ratio of the leaf length (l) to its maximum width (w).
- Cluster analysis was conducted on the taxonomic distance matrix with the Unweighted Pair Group Method based on Arithmetic Average (UPGMA) and the dendrograms were generated based on Euclidean distance of morphological character analysis.
- One-way ANOVA was used for determination of the differences between the mean values of the leaf traits.
- Principal coordinate analysis (PCA) was used in order to verify cluster analysis.

RESULTS AND DISCUSSION

- Variation of morphological characters was revealed
- The distinguish of different individuals was based on the morphology of stem, calyx and petals colour.
- SfH1(K) and SfH2(T) had high density of dark, linear zones on stem, being a very attractive morphological character. The high inflorescence density of SfH1(L) was a very attractive character for this individual. Two more special characteristics of SfH1(K) were the dark green colour of calyx and petals. SfH1(L) and SfH2(K) were found to have higher strength of leaf and flower fragrance.
- Quantitative characteristics; analysis revealed that there were differences in leaf length and area, and in shape factor (Table 2.)
- Cluster analysis separated the individuals in two main branches (Figure 1).
- PCA analysis confirmed cluster analysis (Figure 2).

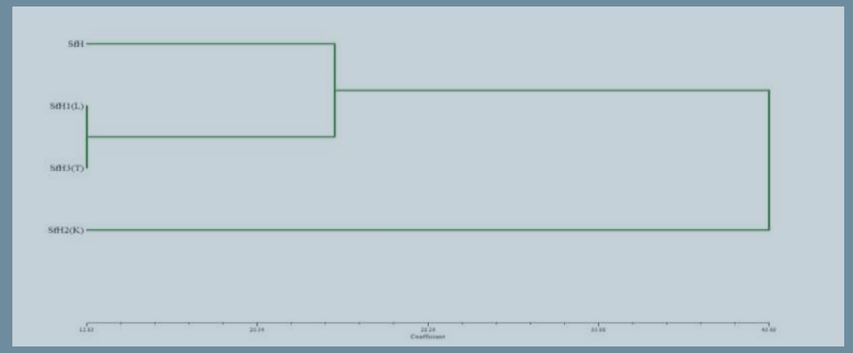
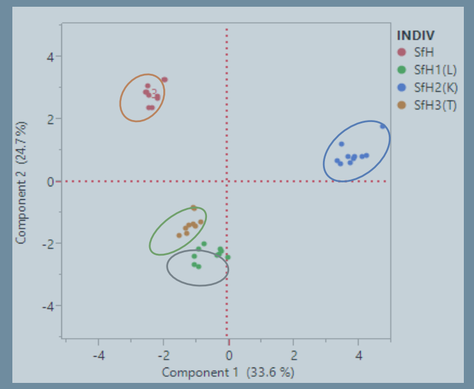


Figure 1. UPGMA dendrogram of four *Salvia fruticosa* individuals based on Euclidean distance of morphological character analysis.



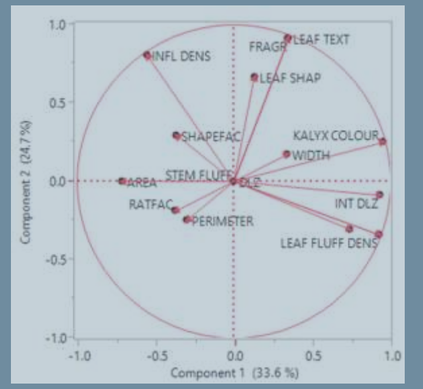


Figure 2. Evaluation of the descriptors and their contribution to the variability of the individual studied.

CONCLUSIONS

The present study provides morphological characteristics traits to differentiate individuals from different accessions aiming to facilitate their clonal propagation and exploitation for ornamental or pharmaceutical use. The applied morphological characteristics could be a basis for the development of a complete list of discriminating characteristics for *Salvia fruticosa*.

ACKNOWLEDGEMENTS

This research has been co-financed by the European Regional Development Fund of the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH – CREATE – INNOVATE (Project code: T1EDK-04923, Project: SALVIA-BREED-GR).