MORPHOLOGICAL CHARACTERS OF NEW INTERSPECIFIC HYBRIDS OF SAGE ORIGINATED FROM *SALVIA OFFICINALIS, S. POMIFERA* SSP. *POMIFERA* AND *S. TOMENTOSA*

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Abstract

Native plant species or clones are an important source of new products for the floricultural industry. Greek flora is a bank of genetic material due to its biodiversity and includes *Salvia* spp. of high potential value, which have not been exploited like other commercial species. The present study aims to reveal the morphology of two new interspecific hybrids, between *S. officinalis* and *S. pomifera* ssp. *pomifera* or *S. tomentosa*, i.e *S. officinalis* × *S. pomifera* ssp. *pomifera* (OP) and *S. officinalis* × *S. tomentosa* (OT). A total of eight quantitative and 14 qualitative characters were selected and used based on descriptors for other plant species. The first group of characters involved leaf and flower morphometrics and the second characters of vegetation, flowers and fragrance. One way ANOVA was used for determination of the differences between the mean values of leaf and flower traits and a dendrogram was generated based on the genetic distance matrix. OP and OT hybrids found to be closer to *S. officinalis*. Both *S. officinalis* and *S. pomifera* ssp. *pomifera* are strongly aromatic plants and their hybrid has a pleasant distinctive aroma, which is slightly closer to the aroma of *S. pomifera*. OT is a compact plant with numerous lateral shoots and a lighter aroma than that of *S. officinalis*. The present study revealed morphological characteristics to differentiate the new hybrids.

Keywords: *Dendrogram, descriptors, flower morphometrics, Mediterranean sage, leaf morphometrics, qualitative and quantitative characters.*

Introduction

Salvia is one the largest genera of flowering plants and includes 2.100 scientific plant names, 1042 being accepted species names (WFO, 2022). There are three distinct regions of its diversity, i.e., Central and South America, Eastern Asia and Central Asia and the Mediterranean (Walker *et al.*, 2004). Greek flora is a bank of genetic material and Salvia species native in Greece have a high potential value for floriculture industry; 30 taxa (species and subspecies) of the genus Salvia can be found in Greece, (Karousou *et al*., 2000). *S. officinalis* is one of the most widely used species in traditional medicine (Llurba-Montesino and Schmidt, 2018) being with *S. fruticosa* one of the most researched European species (Karalija *et al,* 2022). *S. officinalis* is a perennial subshrub, native to the coastal regions of the southern Europe with a habitat reaching south into northwest Greece (di Pietro, 2011). *S. pomifera* spp. *pomifera* is endemic in dry, rocky places in Peloponnese and Crete being unexploited (Strid, 2016). *S. tomentosa* resembles to *S. officinalis* and can be found in areas of macchia vegetation and on limestone slopes in the North-

Eastern and Central Greece and the North-Eastern and Eastern Aegean Islands (Dimopoulos *et al.*, 2013).

Considering that the floriculture industry is seeking the introduction of new native plant species, hybrids or clones for use either as pot or landscape plants, the introduction of artificial hybrids between Salvia species found in Greece is a challenge. It must be emphasized that few instances of natural hybridization have been documanted between native Salvia species (Celep *et al.*, 2020).

Morphological analysis is an effective tool for both characterizing and distinguishing hybrids and studying their relationships (Santos et al., 2011). Morphological traits are widely used for studies in hybrids and assessing variability in plant species (Khurshid *et al.*, 2004; Arabaci *et al.*, 2021); leaf size and flower characters have been used as descriptors of a number of *Salvia* species (Celep *et al.*, 2011; Leontaritou *et al*., 2020; Bertsouklis *et al.*, 2021). In the present study, we aim to test quantitative and qualitative morphological traits that could be the base of the development of suitable descriptors for exploring the phylogenetic relations between new artificial Salvia hybrids and their parental species aiming to facilitate their use by the floricultural industry.

Materials and Methods

Two Salvia species *S. officinalis* (O) and *S. tomentosa* (T), one subspecies, *S. pomifera* ssp. *pomifera* (P) and two new hybrids *S. officinalis* × *S. tomentosa* (OP) and *S. officinalis* × *S. pomifera* ssp *pomifera* (PT) were sampled for identification in 2022, at Agricultural University of Athens (37°58′58.051′′N and 23°42′17.499′′E). The hybrids were products of the SALVIA-BREED-GR research project and both had *S. officinalis* as seed parent. A total of eight quantitative (leaf and flower/inflorescence morphometrics) and 14 qualitative characters (characters of vegetation, flowers and fragrance) were selected and used based on descriptors for other plant species (Table 1, Figure 1). Some of these descriptors had been used in a previous study of morphometrics of *S. fruticosa* in Greece (Bertsouklis *et al*., 2021). One-way ANOVA was used for determination of the differences between the mean values of leaf and flower traits and a dendrogram was generated based on the genetic distance matrix. 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-squared of morphological character analysis (Greenacre and Underhill, 1982). 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

Regarding the quantitative characteristics, analysis revealed that were differences in all morphological traits (Table 2). T had the longest and widest leaves, as well as the longest inflorescences (Table 2). The quantitative characteristics of the hybrids, revealed that OP had intermediate leaf length, width, length/width, while the inflorescence and flower length had no difference to that one of O and internode length had no difference to that one of P. OT had the smallest flower and calyx length compared to its parents with the smallest internode length of all species and hybrids analyzed being a compact plant with numerous lateral shoots. Cluster analysis separated the species and their hybrids according to their morphological characteristics

in three main branches (Figure 2). Both hybrids, OP and OT, were found to be closer to O (Figure 2). T and P were found to be distinct from the hybrids. O and P are strongly aromatic plants and their hybrid has a pleasant distinctive aroma, which is slightly closer to the aroma of P, while OT has a lighter aroma than that of O.

Table 1. Morphological traits measured in two Salvia species (O, T), one subspecies (P) and two interspecific hybrids (OP, OT) were used as descriptors. There is a scoring code for each one depending on its status among different individuals tested.

Code	Descriptor	Score code - descriptor state					
1	Leaf pubescent	1: Low	2: Medium	3: High			
2	Leaf texture	1: Leathery-elastic	2: Membranaceous-smooth	3: Membranaceous-tough			
3	Leaf shape	1: Elliptical to lanceolate	2: Elliptical				
4	Leaf colour of upper side	1: Light green	2: Green				
5	Leaf simple	1: Yes	2: No				
6	Leaf lobes	1: Yes	2: No				
7	Existence of dark/linear zone on stems	1: Yes	2: No				
8	Colour of petals	1: Pink	2: Light pink	3: Light pink-purple	3: Light purple	5: Purple	6: Dark purple
9	Inflorescence density	1: Low	2: Medium	3: High			
10	Existence of dark/linear zone on calyx	1: Yes	2: No				
11	Intensity of dark/linear zone on calyx	1: Low	2: Medium	3: High			
12	Calyx pubescent	1: Yes	2: Medium	3: High			
13	Colour of calyx	1: Light green	2: Green	3: Dark green			
14	Strength of leaf and flower fragrance	1: Low	2: Medium	3: High			

Cluster analysis was confirmed by PCA analysis and five components have been arranged in decline order according to their importance, explaining the 95.2% of the total variability among the different individuals. The suggested 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 3).

Morphological traits have been used to study the variability of *S. fruticosa* in a previous work (Bertsouklis *et al.*, 2021). The applied morphological characteristics could be a basis for the development of a complete list of discriminating characteristics for new Salvia hybrids serving the aim to distinguishing plants 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.

Figure 1. Leaves and inflorescences of *Salvia* spp and interspecific hybrids used in analysis

Table 2. Leaf, stem and inflorescence traits of two *Salvia* spp. (O, T), one subspecies (P) and two interspecific hybrids (OP, OT)

	Leaf				**Inflorescence**			**Stem**
Code	**Length (cm)**	**Width (cm)**	**Length/ Width**	**Thickness (mm)**	**Length (cm)**	**Flower length (cm)**	**Calyx Length (cm)**	**Internode length (cm)**
O	4.4 bc	1.4 d	3.2 a	0.7 c	32.3 b	2.3 c	1.2 c	1.0 b
P	4.4 bc	2.4 b	1.8 c	0.7 c	24.8 c	3.7 a	1.6 a	1.2 a
T	7.1 a	2.9 a	2.5 b	0.7 c	37.4 a	3.0 b	1.4 b	1.0 b
OP	4.9 b	2.0 c	2.6 b	0.9 b	30.4 b	2.3 c	1.0 d	1.2 a
OT	3.9 c	1.4 d	2.8 b	1.8 a	13.8 d	2.1 d	1.0 d	1.0 b
F	***	***	***	***	***	***	***	***

Mean separation in columns by Student's t test at $P \leq 0.05$, ***significant at $P \leq 0.001$, Values followed by different lowercase letter within each trait are significantly different

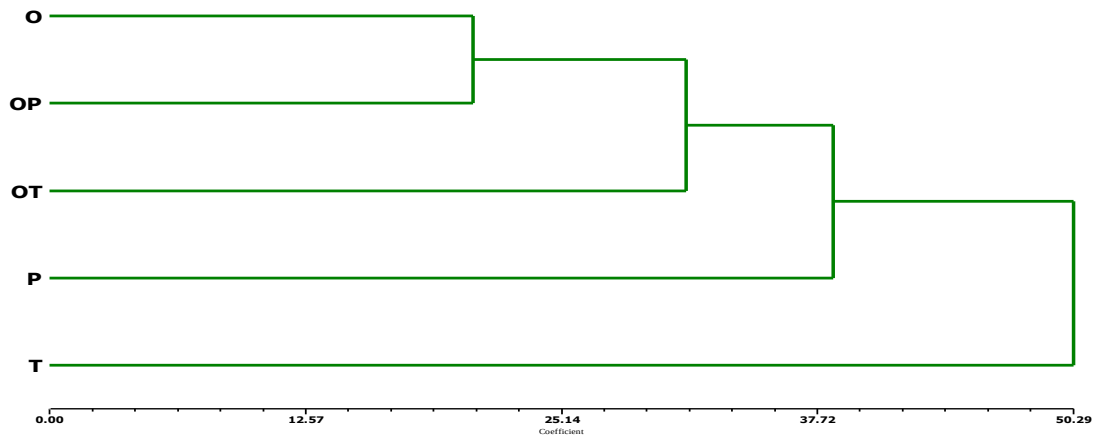

Figure 2. UPGMA dendrogram of two new interspecific hybrids (OP, OT) originated from two *Salvia* spp (O, T) and one subspecies (P) based on Euclidean distance-squared of morphological character analysis

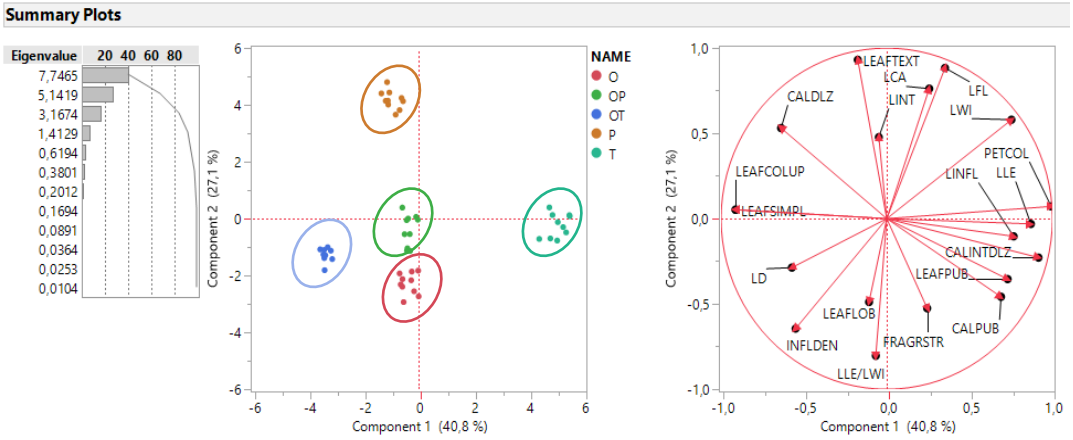

Figure 3. Evaluation of the descriptors and their contribution to the variability of the species and hybrids studied

Table 3. Results of principal components calculation

Principal Components				
1	2	3	4	5
% Contribution of variability				
40.77	27.06	16.67	7.43	3.26
Related descriptors				
CALINTDLZ	LFL	CALDLZ	CALPUB	LLE
LEAFPUB	LEAFTEXT	FRAGRSTR	LD	LLE/LWI
LWI		LEAFLOB	LCA	LINT
LINFL			INFLDEN	
LEAFC0LUP				

PETCOL
LEAFSIMPL

Leaf pubescent (LEAFPUB), Leaf texture (LEAFTEXT), Leaf shape (LEAFSHAPE), Leaf colour of upper side (LEAFCOLUP), Colour of petals (PETCOL), Inflorescence density (INFLDEN), Existence of dark/linear zone on calyx (CALINTTDLZ), Intensity of dark/linear zone on calyx (CALDLZ), Calyx pubescent (CALPUB) Colour of calyx (CALCOL), Strength of leaf/flower fragrance (FRAGRSTR), Leaf Length (LLE), Leaf Width (LWI), Leaf Width/Leaf Length (LLE/LWI), Leaf Thickness (LD), Inflorescence Length (LINFL), Flower length (LFL), Calyx Length (LCA), Internode length (LINT), Leaf simple (LEAFSIMPLE), Leaf lobes (LEAFLOB)

Conclusions

The present experimental procedure leads to the develpoment of suitable descriptors in order to explore the phylogenetic relations between new artificial sage hybrids and their parental species. The use of suitable descriptors may facilitate the needs for increased demand that floriculture or ornamental horticulture face and in producing new hybrids for commercial purposes.

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References

Arabaci T., Çelenk S., Özcan T., Martin E., Yazici T., Açar M., ... Dirmenci, T. (2021). Homoploid hybrids of Origanum (Lamiaceae) in Turkey: morphological and molecular evidence for a new hybrid. Plant Biosystems, 155(3), 470-482.

Bertsouklis K.F., Martini A.N., Vlachou G., Dariotis E. and Papafotiou M. (2021). A first approach on morphometrics studies of *Salvia fruticosa* found in Greece. Acta Hortic. 1327, 97-102.

Celep F., Kahraman A., and Doğan M. (2011). A new taxon of the genus Salvia L. (Lamiaceae) from Turkey. Pl. Ecol. Evol. 144, 111–11.

Celep F, Raders E. and Drew B. (2020). Two new hybrid species of *Salvia* (*S.* × *karamanensis* and *S.* × *doganii*) from Turkey: evidence from molecular and morphological studies, Turk. J. Bot. 44, 647-660.

di Pietro R. (2011). New dry grassland associations from the Ausoni-Aurunci mountains (Central Italy)-Syntaxonomical updating and discussion on the higher rank syntaxa. Hacquetia 10, 183-231.

Greenacre M.J. and Underhill L.G. (1982). Scaling a data matrix in a low-dimensional Euclidean space. In Topics in applied multivariate analysis. D.M. Hawkins, ed. (New York: Cambridge Univ. Press), pp.183–268.

Dimopoulos P.; Raus T.; Bergmeier E.; Constantinidis T.; Iatrou G.; Kokkini S.; Strid S.; Tzanoudakis D. (2013). Vascular Plants of Greece: An Annotated Checklist; Botanischer Garten und Botanisches Museum: Berlin, Germany.

Karalija E., Dahija S., Tarkowski P. and Ćavar Zeljkovic´ S. (2022) Influence of Climate-Related Environmental Stresses on Economically Important Essential Oils of Mediterranean Salvia sp. Front. Plant Sci. 13:864807.

Karousou R., Hanlidou E., and Kokkini S. (2000). The sage plant of Greece: distribution and intraspecific variation. In: Medicinal and Aromatic Plants-Industrial Profiles; Vol. 14, Sage, the genus Salvia; (Kintzios S.E, ed), Harwood Academic, United Kingdom 27-46.

Khurshid S., Ahmad I., and Anjum M.A. (2004). Genetic diversity in different morphological characteristics of Litchi (*Litchi chinensis* Sonn.). Int. J. Agric. Biol. 6(6), 1062–1065.
Leontaritou P., Lamari F. N., Papasotiropoulos V. and Iatrou G. (2020). Morphological, genetic and essential oil variation of Greek sage (*Salvia fruticosa* Mill.) populations from Greece. Ind. Crops Prod., 150, 112346.
Llurba-Montesino N. and Schmidt T.J. (2018). Salvia species as sources of natural products with antiprotozoal activity. Int. J. Mol. Sci. 19:264.
Rohlf F.J. (1992). NTSYS-pc. Numerical Taxonomy and Multivariate Analysis System. Release 2.11f (Setauket, NY: Exeter Software).
Santos E. A., Souza M.M., Viana, A.P., Almeida A.A.F., Freitas J.C.O., and Lawinscky P. R. (2011). Multivariate analysis of morphological characteristics of two species of passion flower with ornamental potential and of hybrids between them. Genetics and molecular research, 10(4), 2457-2471.
Strid, A. (2016). Atlas of the Aegean Flora. Part 1: Text and plates. Part 2: Maps. Berlin: Botanic Garden and Botanical Museum Berlin.
Walker J.B., Sytsma K.J., Treutlein J. and Wink M. (2004). Salvia (Lamiaceae) is not monophyletic: implications for the systematics, radiation, and ecological specializations of Salvia and tribe Mentheae. Am. J. Bot. 91 (7), 1115-25 http://doi:10.3732/ajb.91.7.1115
WFO (2022): World Flora Online. Published on the Internet; http://www.worldfloraonline.org. Accessed on: 06 Jun 2022.

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Introduction

Native plant species or clones are an important source of new products for the floricultural industry. Greek flora is a bank of genetic material due to its biodiversity and includes *Salvia* spp. of high potential value, which have not been exploited like other commercial species. The present study aims to reveal the morphology of two new interspecific hybrids, between *S. officinalis* and *S. pomifera* ssp. *pomifera* or *S. tomentosa,* i.e *S. officinalis* × *S. pomifera* ssp. *pomifera* (OP) and *S. officinalis* × *S. tomentosa* (OT).

Materials and Methods

Two *Salvia* species *S. officinalis* (O) and *S. tomentosa* (T), one subspecies, *S. pomifera* ssp. *pomifera* (P) and two new hybrids *S. officinalis* × *S. tomentosa* (OT) and *S. officinalis* × *S. pomifera* ssp *pomifera* (OP) were sampled for identification in 2022, at Agricultural University of Athens. The hybrids were products of the SALVIA-BREED-GR research project, and both had *S. officinalis* as seed parent. A total of eight quantitative (leaf and flower/inflorescence morphometrics) and 14 qualitative characters (characters of vegetation, flowers and fragrance) were selected and used based on descriptors for other plant species (Table 1, Figure 1).

One-way ANOVA was used for determination of the differences between the mean values of leaf and flower traits and a dendrogram was generated based on the genetic distance matrix. 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-squared of morphological character analysis (Greenacre and Underhill, 1982). 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.

Results

Regarding the quantitative characteristics, analysis revealed that were differences in all morphological traits (Table 2). T had the longest and widest leaves, as well as the longest inflorescences (Table 2). The quantitative characteristics of the hybrids, revealed that OP had intermediate leaf length, width, length/width, while the inflorescence and flower length had no difference to that one of O and internode length had no difference to that one of P. OT had the smallest flower and calyx length compared to its parents with the smallest internode length of all species and hybrids analyzed being a compact plant with numerous lateral shoots. Cluster analysis separated the species and their hybrids according to their morphological characteristics in three main branches (Figure 2). Both hybrids, OP and OT, were found to be closer to O (Figure 2). T and P were found to be distinct from the hybrids. O and P are strongly aromatic plants and their hybrid has a pleasant distinctive aroma, which is slightly closer to the aroma of P, while OT has a lighter aroma than that of O.

Cluster analysis was confirmed by PCA analysis, and five components have been arranged in decline order according to their importance, explaining the 95.2% of the total variability among the different individuals. The suggested 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 (Figure 3). The applied morphological characteristics could be a basis for the development of a complete list of discriminating characteristics for new *Salvia* hybrids serving the aim to distinguishing plants 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.

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5	Leaf simple	1: Yes	2: No				
6	Leaf lobes	1: Yes	2: No				
7	Existence of dark/linear zone on stems	1: Yes	2: No				
8	Colour of petals	1: Pink	2: Light pink	3: Light pink-purple	3: Light purple	5: Purple	6: Dark purple
9	Inflorescence density	1: Low	2: Medium	3: High			
10	Existence of dark/linear zone on calyx	1: Yes	2: No				
11	Intensity of dark/linear zone on calyx	1: Low	2: Medium	3: High			
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13	Colour of calyx	1: Light green	2: Green	3: Dark green			
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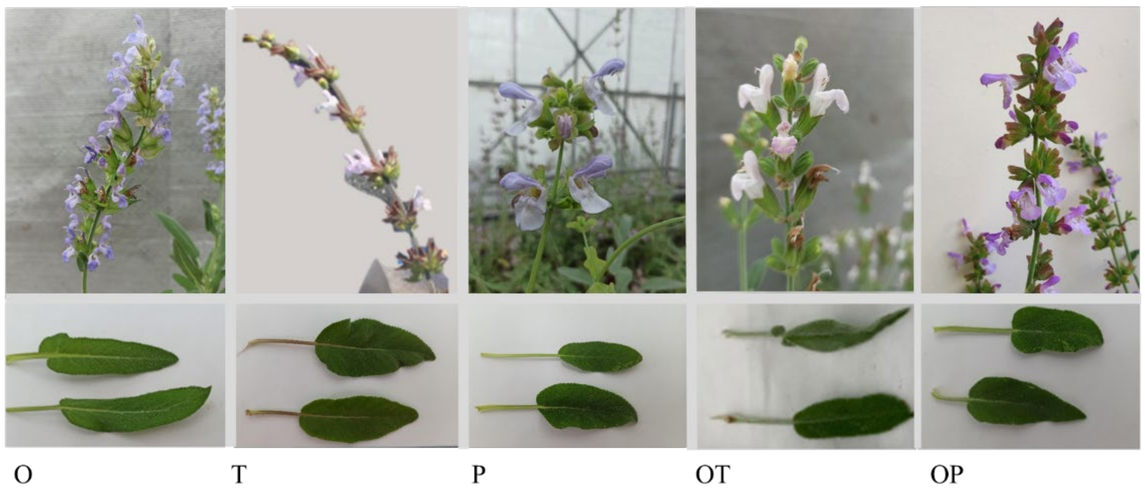

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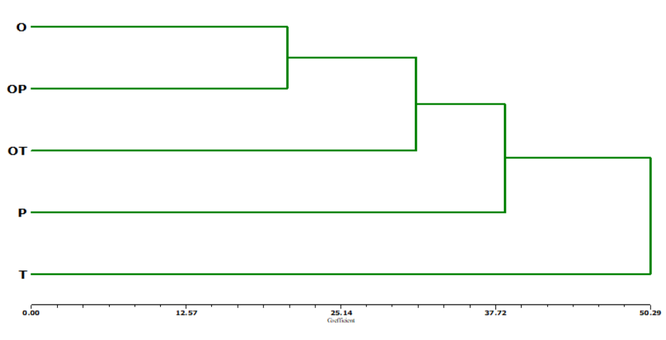

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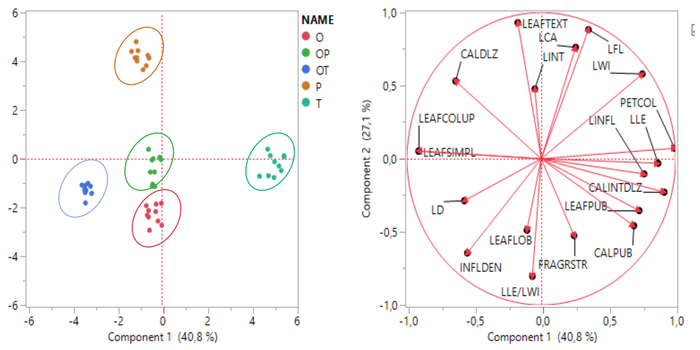

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Code	Leaf				Inflorescence			Stem
	Length (cm)	Width (cm)	Length/ Width	Thickness (mm)	Length (cm)	Flower length (cm)	Calyx Length (cm)	Internode length (cm)
O	4.4 bc	1.4 d	3.2 a	0.7 c	32.3 b	2.3 c	1.2 c	1.0 b
P	4.4 bc	2.4 b	1.8 c	0.7 c	24.8 c	3.7 a	1.6 a	1.2 a
T	7.1 a	2.9 a	2.5 b	0.7 c	37.4 a	3.0 b	1.4 b	1.0 b
OP	4.9 b	2.0 c	2.6 b	0.9 b	30.4 b	2.3 c	1.0 d	1.2 a
OT	3.9 c	1.4 d	2.8 b	1.8 a	13.8 d	2.1 d	1.0 d	1.0 b
$F_{\text{one-way ANOVA}}$	***	***	***	***	***	***	***	***

Mean separation in columns by Student's *t, P* ≤ 0.05. ***: significant at *P* ≤ 0.001, *n*=20

Conclusions

The present experimental procedure leads to the develpoment of suitable descriptors in order to explore the phylogenetic relations between new artificial sage hybrids and their parental species. The use of suitable descriptors may facilitate the needs for increased demand that floriculture or ornamental horticulture face and in producing new hybrids for commercial purposes.

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

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