MORPHOLOGICAL ANALYSIS IN NEW INTERSPECIFIC HYBRIDS OF *SALVIA* SPP. ORIGINATED FROM *S. FRUTICOSA, S. OFFICINALIS, S. POMIFERA* SSP. *POMIFERA* AND *S. RINGENS*

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Abstract

The introduction of new hybrids and clones suitable for xeriscaping is a challenge for modern floriculture. The Mediterranean sages *Salvia ringens* and *S. pomifera* ssp. *pomifera* are unexploited versus *S. officinalis* and *S. fruticosa* that are widely used in floriculture and medicinal industry. The present study was conducted to test quantitative and qualitative morphological traits to find suitable descriptors for the discrimination of new *Salvia* hybrids. Morphological characteristics were used to study new hybrids, i.e., OR (*S. officinalis* × *S. ringens*), FR (*S. fruticosa* × *S. ringens*), PR (*S. pomifera* ssp. *pomifera* × *S. ringens*). 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. One way ANOVA was used for determination of the differences between the mean values of leaf, stem and flower traits and a dendrogram was generated based on the genetic distance matrix. *S. ringens* inherited its segmented leaves and their light aroma to all its hybrids. PR and FR hybrids formed leaves and inflorescences with intermediate length between their parents, while the inflorescence length of OR had no difference with *S. officinalis*. The present study suggests morphological characteristics to differentiate the new hybrids from their parents in order to enhance their introduction to the floricultural industry.

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

Introduction

The Greek flora is a pool of genetic material and Salvia species native in Greece have a high potential value for floriculture industry. Salvia includes 2.100 scientific plant names; 1042 are accepted species names being one the largest genera of flowering plants, (WFO, 2022). The genus has three distinct regions of diversity, i.e., Central and South America, Eastern Asia and Central Asia and the Mediterranean (Walker *et al.*, 2004). 30 taxa (species and subspecies) of the genus *Salvia* can be found in Greece, the *Salvia fruticosa* Mill. having the widest distribution, as in all the Mediterranean basin (Karousou *et al.*, 2000). *S. officinalis* is one of the most widely used species in traditional medicine (Llurba-Montesino and Schmidt, 2018) and 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* occurs in dry, rocky places in Crete and Peloponnese being unexploited (Strid, 2016). *S. ringens,* is a hardy herbaceous perennial

herb, up to 30 cm (60 cm with inflorescences) that inhabits dry stony and grass-covered places of South and Eastern parts of Balkan Peninsula, being drought tolerant and long lived (Hedge, 1972). The floriculture industry is looking for introducing new native plant, species, hybrids or clones for cultivation. Therefore, artificial hybrids between *Salvia* species found in Greece could be introduced for exploitation providing new ornamental plants for use either as pot or landscape plants. Taking into account that few instances of natural hybridization has been documanted between native Salvia species (Celep *et al.,* 2020) it would be a challenge to point out the morphological characteristics of new, artificial hybrids. Morphological analysis has proved an effective tool for both characterizing and distinguishing hybrids and studying their relationships (Bertsouklis and Papafotiou, 2016; Bertsouklis *et al,* 2021). Morphological traits are widely used for diversity studies (Lopes *et al.,* 2012) and assessing variability in plant species (Khurshid *et al.,* 2004); leaf size and flower characters have been used as descriptors of a number of *Salvia* species (Celep *et al.,* 2011; Bertsouklis *et al.,* 2021). The objective of the present study was to test quantitative and qualitative morphological traits aiming to find a set of suitable descriptors for exploring the phylogenetic relations between new artificial hybrids and their parental species aiming to enhance their introduction to the floricultural industry.

Materials and Methods

Three *Salvia* species *S. fruticosa* (F), *S. officinalis* (O), *S. ringens* (R), one subspecies, *S. pomifera* ssp *pomifera* (P), and three new hybrids *S. fruticosa* × *S. ringens* (FR), *S. officinalis* × *S. ringens* (OR), *S. pomifera* ssp *pomifera* × *S. ringens* (PR), 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. The pollen parent of all hybrids was *S. ringens*. Three-year-old plants derived from stem cuttings of plants grown in a glasshouse. 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

As regards the quantitative characteristics, analysis revealed that were differences in all morphological traits (Table 2). R had the longest and widest leaves, as well as the longest inflorescences, and internodes (Table 2). As regards the quantitative characteristics of the hybrids, FR had intermediate leaf length, width, length/width, inflorescence length, flower and calyx length compared to its parents having the smallest internode length of all species and hybrids analyzed; OR and PR had intermediate leaf length, width, inflorescence length, and internode compared to their parents (Table 2).

Table 1. Morphological traits were measured in three Salvia species (F, O, R), one subspecies (P) and three interspecific hybrids (FR, OR, PR) and 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 separated the species and their hybrids according to their morphological characteristics in two main branches (Figure 2). R and PR hybrid were found to be distinct from the other two species and their hybrids and R inherited its segmented leaves and light aroma of the leaves to all its hybrids. OR was found to be closer to PR and FR closer to F than R (Figure 2). PCA analysis confirmed cluster analysis and five components have been arranged in decline order according to their importance, explaining the 93.48% 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 3). Morphological traits have

been proved useful to study the variability of *S. fruticosa* in a previous work (Bertsouklis *et al*., 2021) The present study provides morphological traits to differentiate new interspecific hybrids originated from *S. fruticosa, S. officinalis, S. pomifera* spp. *pomifera* and *S. ringens* 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. The applied morphological characteristics could be a basis for the development of a complete list of discriminating characteristics for new Salvia hybrids.

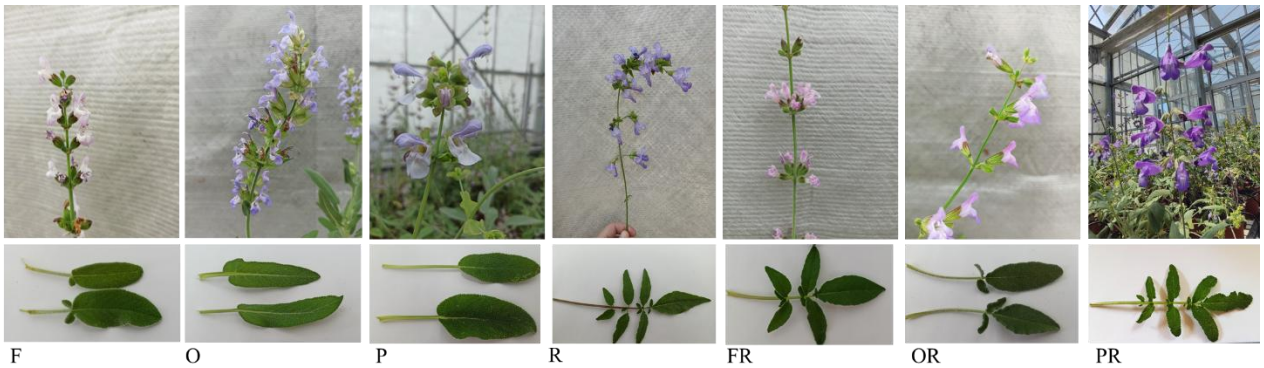

Figure 1. Leaves and inflorescences of *Salvia* spp and interspecific hybrids used in analysis

Conclusions

Evaluating data of the present study and taking in account that the commercial production and use in the landscape of Salvia *spp* plants will be affected by the changing climate, the production of new hybrids could be a key point to face the problem. The present study revealed morphological characteristics to differentiate the new hybrids from their parents so that they could facilitate their clonal propagation and exploitation for ornamental and pharmaceutical use.

Table 2. Leaf, stem and inflorescence traits of three *Salvia* spp (F, O, R), one subspecies (P) and three interspecific hybrids (FR, OR, PR)

	Leaf				**Inflorescence**			**Stem**
Code	**Length (cm)**	**Width (cm)**	**Length/ Width**	**Thickness (mm)**	**Length (cm)**	**Flower length (cm)**	**Calyx Length (cm)**	**Internode length (cm)**
F	4.4 d	2.2 e	2.0 b	0.8 a	20.5 d	1.8 e	0.8 f	1.6 ab
O	4.4 d	1.4 f	3.2 a	0.7 b	32.3 c	2.3 d	1.2 d	1.0 d
P	4.4 d	2.4 e	1.8 bc	0.7 b	24.8 d	3.7 b	1.6 a	1.2 c
R	11.0 a	9.5 a	1.2 d	0.8 a	74.5 a	4.0 a	1.3 c	1.7 a
FR	5.2 c	3.5 d	1.5 c	0.8 a	45.0 b	2.2 d	1.0 e	0.9 f
OR	6.4 b	4.2 c	1.5 c	0.7 b	34.5 c	2.8 c	1.1 d	1.3 c
PR	6.8 b	6.2 b	1.1 d	0.8 a	47.8 b	3.7 b	1.5 a	1.5 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

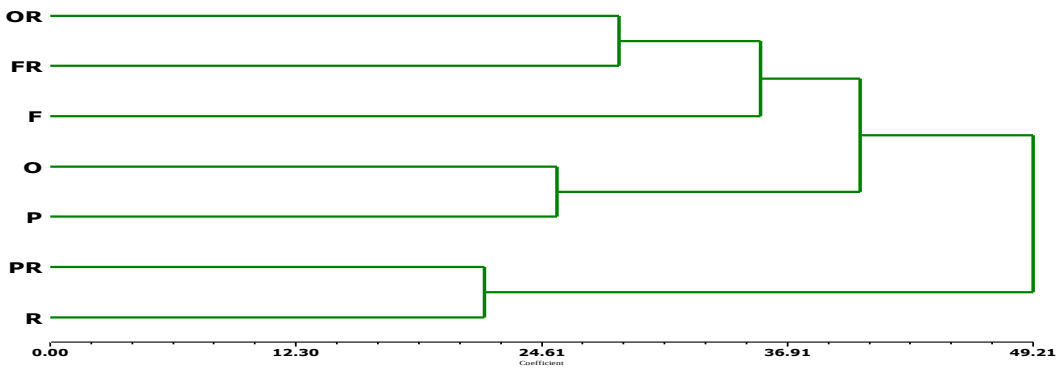

Figure 2. UPGMA dendrogram of three new interspecific hybrids (FR, OR, PR) originated from three *Salvia* spp (F, O, R) and one subspecies (P) based on Euclidean distance-squared of morphological character analysis

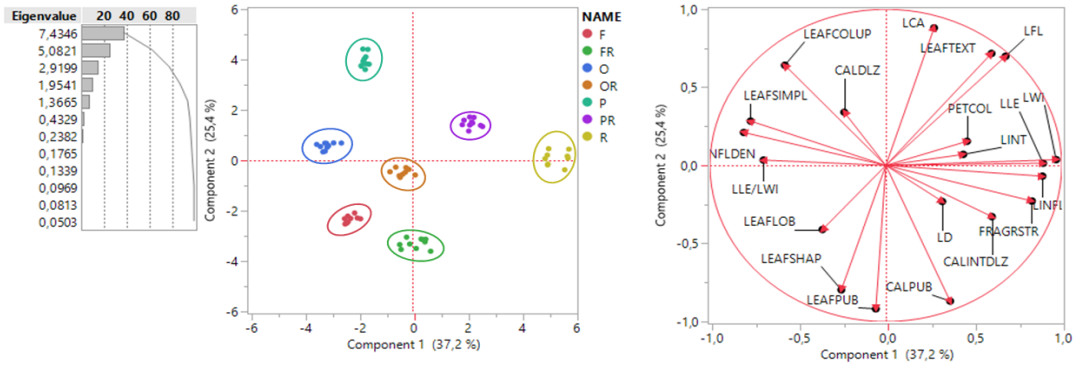

Figure 3. Evaluation of the descriptors and their contribution to the variability of the species and hybrids studied

Table 3. Results of principal components calculation

Principal Components				
1	2	3	4	5
% Contribution of variability				
37.17	25.41	14.59	9.77	6.8
Related descriptors				
LLE	CALPUB	CALDLZ	LLE/LWI	LD
LWI	LEAFPUB	LINT	PETCOL	INFLDEN
LINFL	LCA	LEAFLOB	CALINTTDLZ	LEAFSHAPE
FRAGRSTR	LFL		LEAFSIMPLE	
	LEAFTEXT		LEAFCOLUP	

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)

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References

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.

Bertsouklis K.F., and Papafotiou M. (2016). Morphometric and Molecular Analysis of the Three Arbutus Species of Greece. Not Bot Horti Agrobo, 44(2), 423-430.

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.

Hedge I.C. (1972). Salvia L. In: Tutin, T.G., Heywood V.H., Burges N.A., Valentine D.H., Walters S.M., Webb, D.A. (Eds.), Flora Europaea, vol. 3. Cambridge University Press, Cambridge, pp. 188–192.

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.

Lopes L., Sá O., Pereira J.A. and Baptista P. (2012). Genetic diversity of Portuguese *Arbutus unedo* L. populations using leaf traits and molecular markers: An approach for conservation purposes. Sci. Hortic. 142, 57-67.

Rohlf F.J. (1992). NTSYS-pc. Numerical Taxonomy and Multivariate Analysis System. Release 2.11f (Setauket, NY: Exeter Software).

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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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. 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

As regards the quantitative characteristics, analysis revealed that were differences in all morphological traits, R having the longest and widest leaves, as well as the longest inflorescences, and internodes (Table 2). As regards the quantitative characteristics of the hybrids, FR had intermediate leaf length, width, length/width, inflorescence length, flower and calyx length compared to its parents having the smallest internode length of all species and hybrids analyzed; OR and PR had intermediate leaf length, width, inflorescence length, and internode compared to their parents (Table 2).

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5	Leaf simple	1: Yes	2: No				
6	Leaf lobes	1: Yes	2: No				
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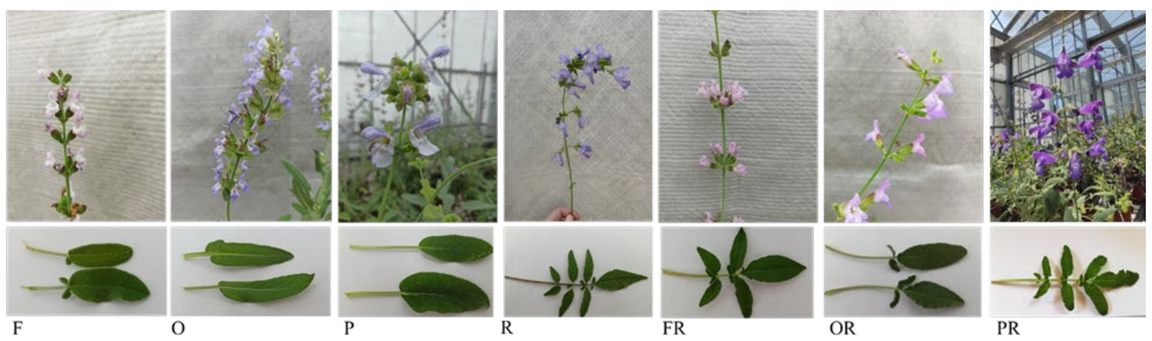

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Code	Leaf				Inflorescence			Stem
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O	4.4 d	1.4 f	3.2 a	0.7 b	32.3 c	2.3 d	1.2 d	1.0 d
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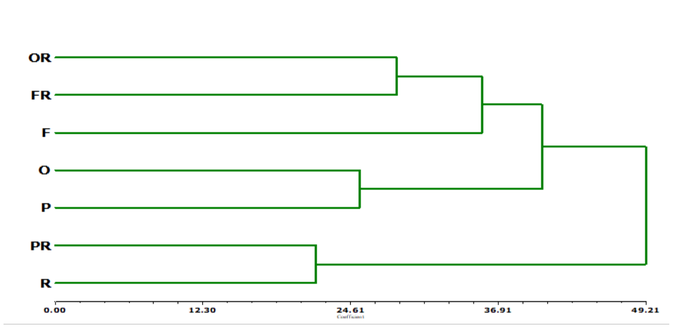
R	11.0 a	9.5 a	1.2 d	0.8 a	74.5 a	4.0 a	1.3 c	1.7 a
FR	5.2 c	3.5 d	1.5 c	0.8 a	45.0 b	2.2 d	1.0 e	0.9 f
OR	6.4 b	4.2 c	1.5 c	0.7 b	34.5 c	2.8 c	1.1 d	1.3 c
PR	6.8 b	6.2 b	1.1 d	0.8 a	47.8 b	3.7 b	1.5 a	1.5 b
$F_{\text{one-way ANOVA}}$	***	***	***	***	***	***	***	***

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



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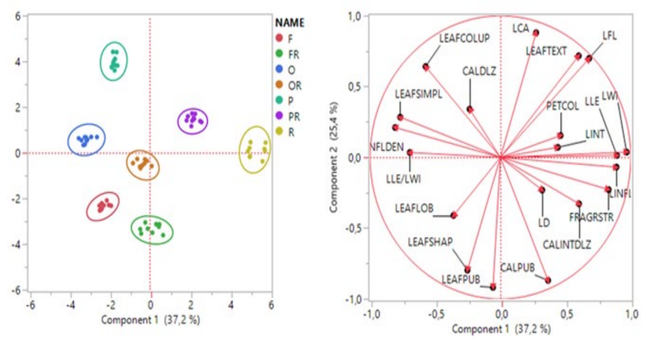

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https://www.salvia-breed-gr.com/el/