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ORIGINAL ARTICLE

Do we have infraspecific taxa of *Salvia multicaulis* Vahl. (Lamiaceae) in Iran?

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Salvia multicaulis is a widespread species of Lamiaceae family in Iran. There are many discussions about its infraspecific variations. Although some varieties were definite for this species in various parts of the world, no infraspecific taxon was reported in Iran and all samples of this species were named as *S. multicaulis.* In this study, morphological characteristics of *S. multicaulis* populations, naturally growing in Iran, was examined. Twenty-two traits were examined in 94 individuals of this species to identify their phenotypic difference. Most of the investigated features were showing a high degree of variability, but it was highly pronounced for some characteristics such as basal leaf shape, basal leaf width, basal leaf length/ width ratio and basal petiole length. Significant positive/negative correlations were observed between some morphological variables. Furthermore, significant negative correlations were found between the eastern distribution of populations with basal leaf petiole length and basal leaf length/ width ratio. Based on the UPGMA cluster analysis, populations were divided into two main branches. The first branch contained four populations, while the second branch was bigger and clustered in two sub-branches. In one of them, three populations and in another one the rest populations arranged in two groups. CA joined plot confirmed that each of studied populations or group of populations had distinct morphological trait(s), which were useful in identification of them. Our findings supported population no. 13 had unique morphological traits such as the largest bracts and basal leaf petiole, highest flower number of each inflorescence cycle, widest and largest calyx. The conservation of the highly diverse populations of Iranian *S. multicaulis* is recommended.
Key words: morphology; population; *Salvia multicaulis;* infraspecific

Introduction

Salvia L. is the largest genus of Lamiaceae that comprises of more than 900 taxa in the world (Davis 1982). This genus has 62 species in Iran (Jamzad, 2012). Different species of the genus have been used in folk medicine for the treatment of many disorders such as diabetes (Jimenez et al. 1986) and skin diseases like psoriasis and eczema (Topcu et al. 2007).
Salvia multicaulis Vahl (Lamiaceae) is an aromatic plant of the genus that widely distributed in different parts of Iran (Jamzad, 2012). This species is also used in folk medicine to treat various diseases, such as colds (Çakılcıoğlu et al. 2010), the flu and tonsillitis (Tetik et al. 2013).
Ulubelen et al. (1997) reported seven new aromatic secondary compounds from the roots of this species. They were: norditerpenoids, multicaulin, 12-demethylmulticauline, multiorthoquinone, 12-demethylmultiorthoquinone, 12-methyl-5-dehydrohorminone, 12-methyl-5-dehydroacetylhorminone and salvipimarone. Moreover, they observed the antituberculous activity of these compounds against *Mycobacterium tuberculosis* strain H37Rv.
Investigations of infraspecific morphological diversity have been performed for different species of Labiatae family (e.g., Talebi, 2014, Talebi, 2015). However, despite many reports about essential oil composition of *S. multicaulis*, we still do not have comprehensive information about the morphological variations of this species. Therefore, the main objective of this investigation was to identify the variability in 94 accessions of 14 populations of this species growing wild in Iran, to determine the possible correlation between the characteristics, to identify the most useful features for discrimination between the populations and to detect relationships between them.

Materials and method

Plant material

Six qualitative and sixteen quantitative morphological traits of fourteen populations of *S. multicaulis* were examined (Table 1). These samples were elected from natural populations of the species during spring 2016 and identified based on the descriptions provided in valuable references like Flora Iranica (Rechinger, 1982) and Flora of Iran (Jamzad, 2012). On the basis of population size, up to eight individuals were selected for each population. The mean and also standard deviation was determined for quantitative features (Table 2). The studied variables were: stem length, shapes of basal and floral leaves margin, blade and their length, width, length/wide ratio and petiole length, calyx and corolla color, length, width, their length/ width ratio and pedicle length.

Statistical analysis

Analysis of variance was performed for morphological traits using SPSS software. The mean and standard deviation and simple correlation coefficient were calculated to determine the relationships between the studied morphological features using the Pearson correlation coefficient by SPSS.

Relationships among the populations were studied by principal component analysis (PCA). The morphological similarity coefficients according to Manhattan method were calculated using the MVSP ver. 3.2 program of the numerical taxonomy multivariate analysis and the dendrogram were constructed using the unweighted pair group method with arithmetic means (UPGMA). PCA and PCO scatter plots were constructed for representing morphological similarity among the studied populations. The CA-Joined plot and PCA-biplot representing morphological similarity relationships among populations, linking the morphometric traits (Podani, 2000).

Table 1. Localities address and coding of studied *S. multicaulis* populations.

Population cods	Habitat
1	Markazi province, Zarandiyeh, Noshveh, 1814 m.
2	Markazi province, Zarandiyeh, Vidar, 1771 m.
3	Kurdistan province, Sanandaj, Husain Abad 1810 m.
4	Hamedan province, Hamehkasi, 2201m.
5	Kurdistan province, Marivan-Saqqez, 1585 m.
6	Kurdistan province, Marivan, 1363m.
7	Hamedan province, Gol-e-e khandan, 1734 m.
8	Markazi province, Zarandiyeh, Vardeh 1810 m.
9	Tehran province, Tehran, Lashkarak , 2225 m.
10	West Azerbaijan, Tekab, 2047 m.
11	Markazi province, Saveh, Chamran, 1775m.
12	Markazi province, Arak, Sefidkhani, 2180m
13	Markazi province, Saveh-boen Zahra road 1931m.
14	West Azerbaijan, Urmia, Sero, 1680 m.

Results

ANOVA test showed significant differences ($p \leq 0.05$) for all of the quantitative studied characteristics with except basal leaf width, floral leaf length/width ratio and calyx leaf length/width ratio (Table 3). Quantitative features such as floral and basal leaf blade shape and their marginal shape differed between the populations. They were oblong, ovate, obovate, lanceolate, oblanceolate, linear and linear-lanceolate. Moreover, the color of calyx was purple or violet in all of the studied populations, with except population number 13, which had lavender calyx. It means that most of the morphological traits varied significantly among the populations. PCA analyses proved that seven characteristics: stem length, basal leaf shape, basal leaf width, basal leaf length/ width ratio and basal petiole length comprises 72.47% of variations. Therefore these traits were more variable among the examined characters (Table 4).

Significant positive/negative correlations were seen between some morphological traits. For example, corolla length had significant negative correlations ($P \leq 0.05$) with floral leaf length and width, calyx width, flower number of each inflorescence cycle. Significant positive correlations ($P \leq 0.01$) were found between calyx length and width with floral leaf length, pedicle length, and flower number per each inflorescence cycle.

Basal leaf length and width had significant positive correlations ($P \leq 0.01$) with stem length, floral leaf length and width and pedicle length.

The studied populations separated from each other in UPGMA dendrogam (fig 1); in addition, PCA, as well as PCO plots (figs 2, 3) created similar results. So population's arrangement in the dendrogam was discussed here: it is composed of two branches (A, B). The smallest one (A) had two sub-branches. Populations number 5, 7, and 10 were grouped in the bigger sub-branch, while population no. 13 was found in the smaller one far from others. The largest branch (B) divided into two sub-branches; one of them was small (C) and composed of three populations number 4, 8, and 14. In the bigger sub-branch (D), populations constructed two groups. Populations number 1, 2, and 3 arranged in one group (E) and the rest populations were clustered in the other group (F), so that populations number 6 and 11 placed closely and the population no. 12 were far from the others.

Table 3. Results on the ANOVA analysis to assess for differences in morphological traits of studied *S. multicaulis* populations. d.f.: degrees of freedom; F: F-statistic; P: probability.

		Sum of Squares	df	Mean Square	F	P
Stem length	Between Groups	3238.748	13	249.134	8.569	.000
	Within Groups	2326.028	80	29.075		
	Total	5564.777	93			
Basal leaf length	Between Groups	18.052	13	1.389	2.817	.002
	Within Groups	39.433	80	.493		
	Total	57.486	93			
Basal leaf petiole length	Between Groups	38.988	13	2.999	1.885	.044
	Within Groups	127.284	80	1.591		
	Total	166.272	93			
Basal leaf width	Between Groups	2.146	13	.165	.916	.540
	Within Groups	14.413	80	.180		
	Total	16.559	93			
Basal leaf length/ width ratio	Between Groups	9.584	13	.737	2.871	.002
	Within Groups	20.545	80	.257		
	Total	30.130	93			
Floral leaf length	Between Groups	41.700	13	3.208	3.590	.000
	Within Groups	71.488	80	.894		
	Total	113.188	93			
Floral leaf width	Between Groups	2.073	13	.159	2.353	.010
	Within Groups	5.421	80	.068		
	Total	7.494	93			
floral leaf length/ width ratio	Between Groups	38.803	13	2.985	1.492	.139
	Within Groups	160.033	80	2.000		
	Total	198.837	93			
Corolla length	Between Groups	3.397	13	.261	4.550	.000
	Within Groups	4.595	80	.057		
	Total	7.992	93			
Calyx length	Between Groups	6.484	13	.499	14.197	.000
	Within Groups	2.810	80	.035		
	Total	9.294	93			
Pedicle length	Between Groups	.546	13	.042	4.148	.000
	Within Groups	.810	80	.010		
	Total	1.357	93			
Calyx width	Between Groups	20.063	13	1.543	15.372	.000
	Within Groups	8.032	80	.100		
	Total	28.095	93			
Calyx length/width ratio	Between Groups	2.623	13	.202	1.357	.199
	Within Groups	11.890	80	.149		
	Total	14.513	93			
Flower number of each cycle	Between Groups	471.645	13	36.280	4.892	.000
	Within Groups	593.259	80	7.416		
	Total	1064.904	93			
Bract length	Between Groups	4.565	13	.351	3.482	.000
	Within Groups	8.068	80	.101		
	Total	12.632	93			

C.A joined plot showed that each of studied populations or identified group of populations had the distinct morphological variable(s), which were useful in identification of them (fig. 4). For example, floral leaf shape, the ratio of basal leaf blade length/ petiole length and calyx length/ pedicle length ratio were valuable traits for populations no. 8, 14 and 4, respectively. Furthermore, petal color putts population no. 14 far from populations no. 8 and 4. Stem length was a distinguishing character for branch A. Then, each of these populations had distinct features and separated from each other. Floral leaf length was a specific trait for populations no. 5 and 10. Pedicle length, calyx length and width and flower number of each inflorescence cycle separated population no. 13.

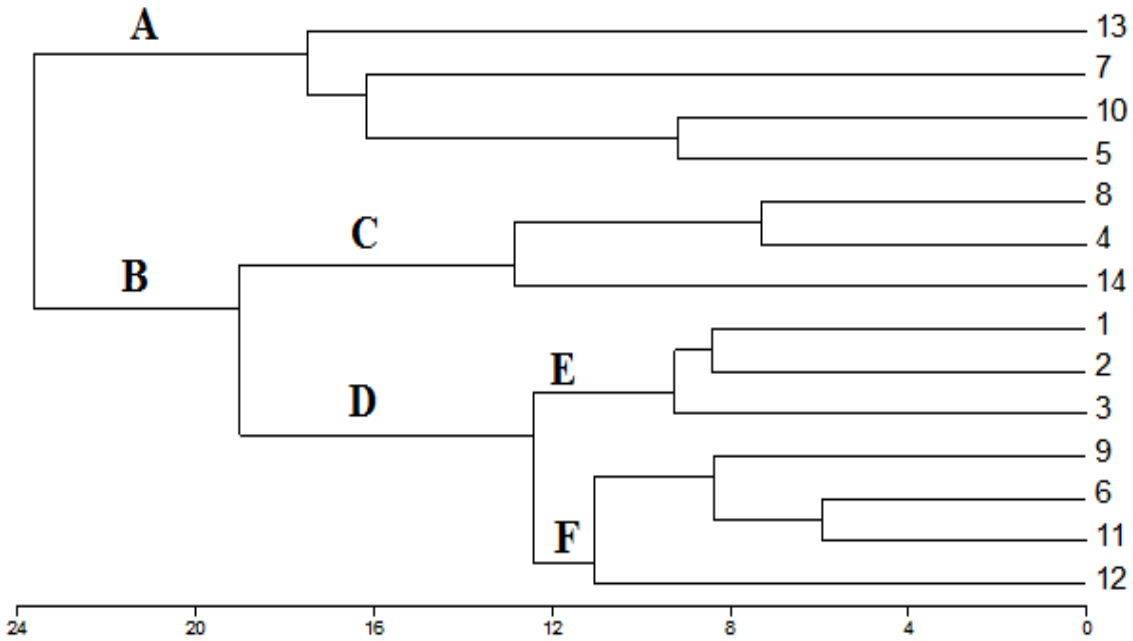

Fig. 1. UPGMA dendrogam of the studied populations based on the morphometric data. Numbers indicate population's code 1-14 as in Table 1.

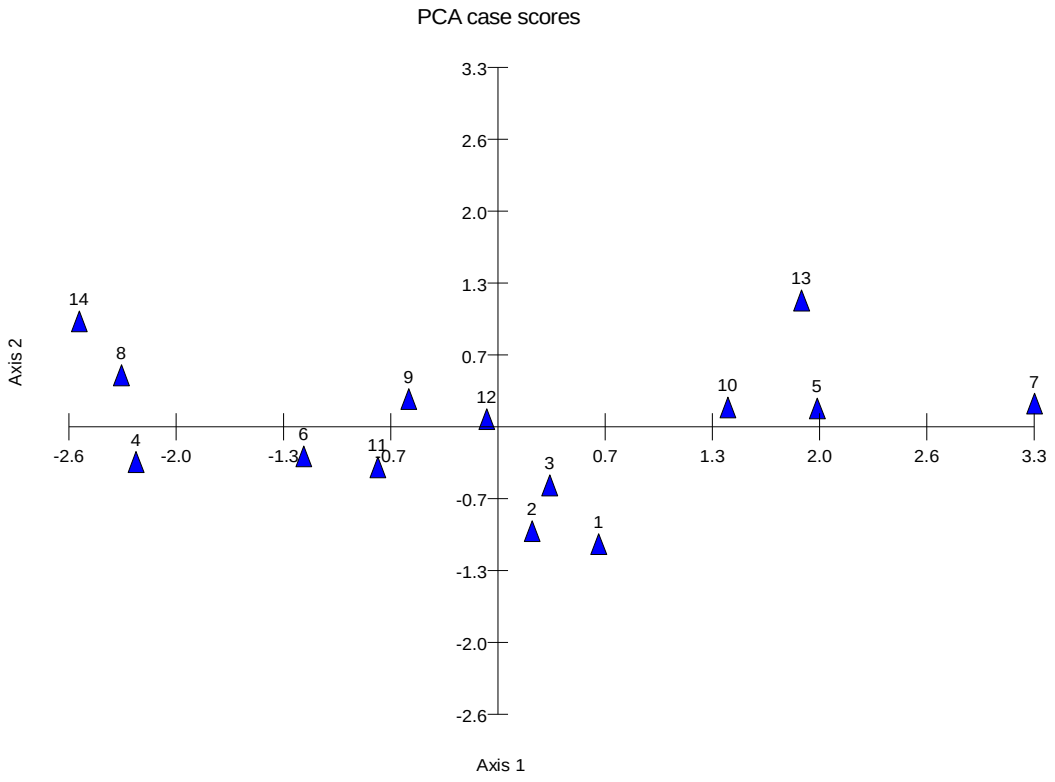

Fig. 2. PCA Scatter plot for the studied *S. multicaulis* populations based on morphological characters. Numbers indicate population's code 1-14 as in Table 1.

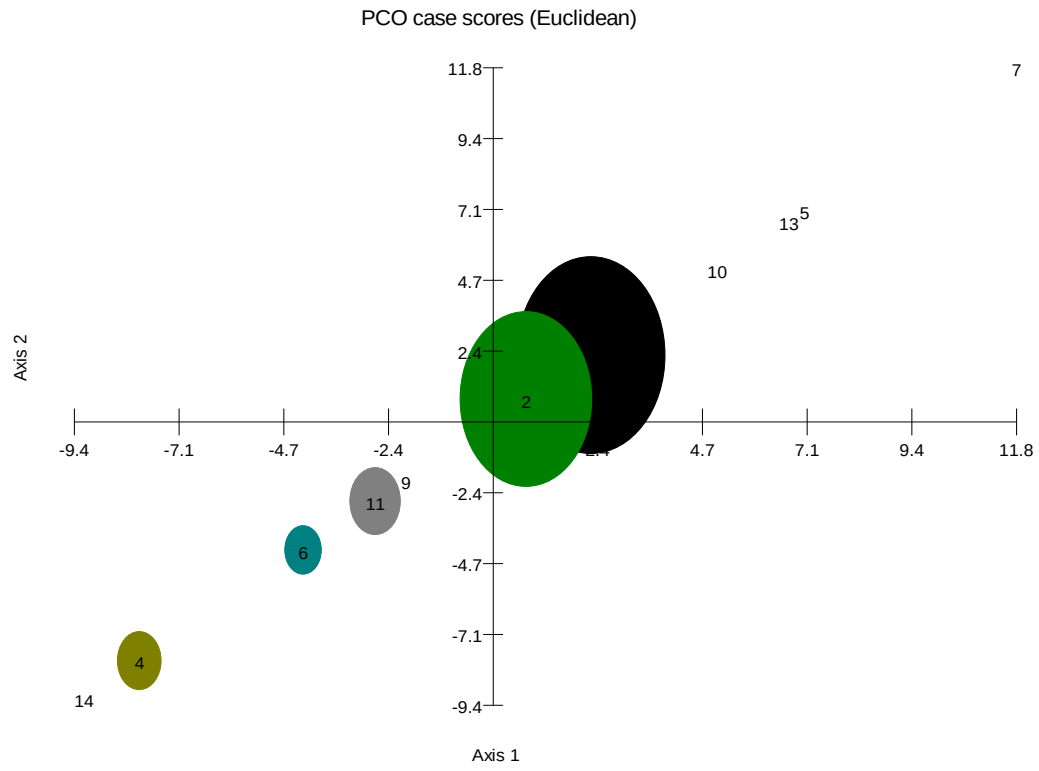

Fig. 3. PCO plot of the studied populations on the bases of morphological traits. Numbers indicate population's code 1-14 as in Table 1.

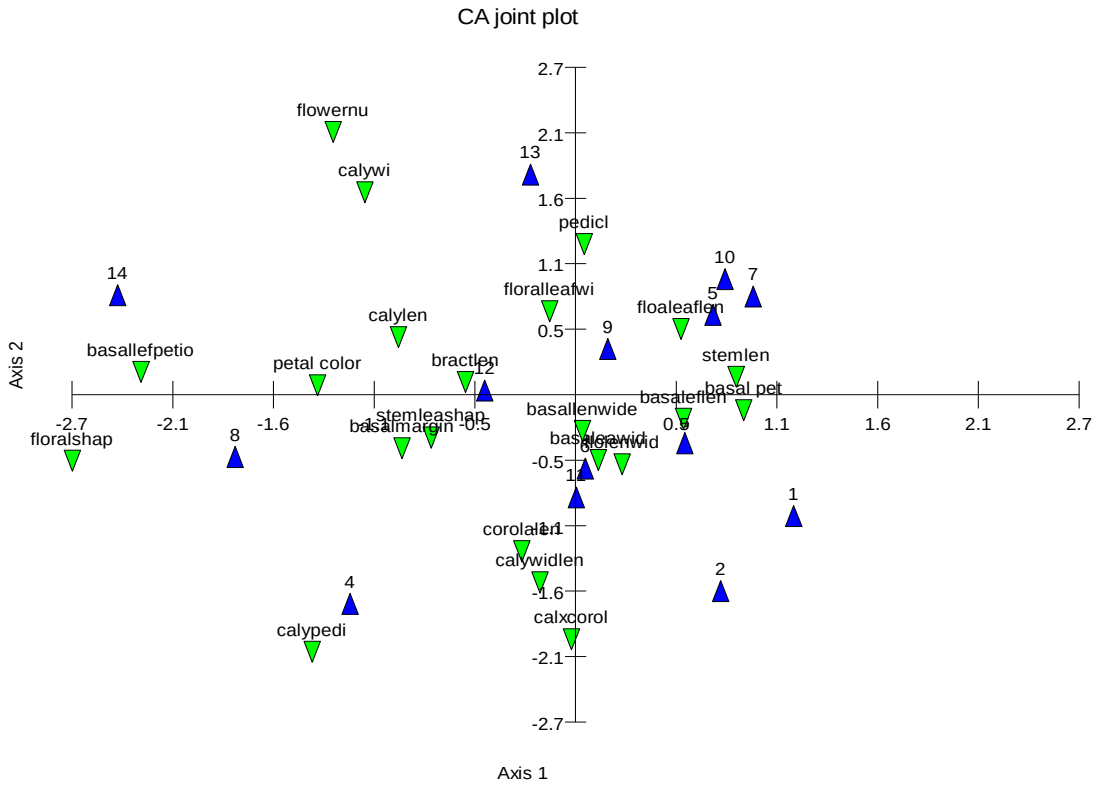

Fig. 4. CA joined plot representing morphological similarity relationships among populations of *S. multicaulis*, linking the morphometric traits. Numbers indicate population's code 1-14 as in Table 1. Abbrivations: stemlen: Stem length, basalelen: Basal leaf length, basal pet: Basal leaf petiole length, basallewid: Basal leaf width, basallenwide: Basal leaf length/ width ratio, floralshap: floral leaf shape, floraleaflen: Floral leaf length, floralleafwi: Floral leaf width, floral leaf length/ width ratio, corolalen: Corolla length, calylen: Calyx length, pedid: Pedicle length, calywi: Calyx width, calywidlen: Calyx length/width ratio, flowerno: Flower number of each cycle, bractlen: Bract length, calypedi: calyx/pedicle length ratio.

Table 4. PCA analyses of the studied morphological variables between the studied populations.

	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
Component	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
Stem length	5.249	23.860	23.860	5.249	23.860	23.860	3.622	16.461	16.461
Basal leaf shape	3.128	14.220	38.080	3.128	14.220	38.080	3.339	15.177	31.638
Basal leaf length	2.139	9.724	47.804	2.139	9.724	47.804	1.931	8.779	40.417
Basal leaf petiole length	1.593	7.240	55.044	1.593	7.240	55.044	1.892	8.598	49.015
Blade length petiole length ratio	1.416	6.435	61.478	1.416	6.435	61.478	1.713	7.785	56.800
Basal leaf width	1.278	5.809	67.288	1.278	5.809	67.288	1.637	7.442	64.243
Basal leaf length/ width ratio	1.142	5.189	72.477	1.142	5.189	72.477	1.469	6.676	70.918
Basal margin shape	1.071	4.870	77.347	1.071	4.870	77.347	1.414	6.429	77.347
Floral leaf length	.918	4.174	81.521						
Floral leaf width	.814	3.701	85.222						
floral leaf length/ width ratio	.708	3.220	88.442						
floral leaf shape	.579	2.633	91.075						
Corolla length	.505	2.294	93.370						
Calyx length	.417	1.894	95.263						
Corolla/ calyx length ratio	.326	1.480	96.744						
Pedicle length	.283	1.285	98.029						
Calyx/pedicle length ratio	.174	.790	98.818						
Calyx width	.109	.496	99.314						
Calyx length/width ratio	.063	.288	99.603						
Flower number of each cycle	.049	.221	99.823						
Bract length	.024	.111	99.934						
Petal color	.014	.066	100.000						

Discussion

S. multicaulis has several populations in various parts of Iran, which growth under different ecological conditions. Therefore, this species can definite as an ecological generalist plant species. These plants have highly plastic genotypes and phenotypes (Sultan, 1995), but taxa whose constituent individuals express limited adaptive plasticity might be restricted to narrower, 'specialist' ecological ranges. Williams et al. (1995) stated that plasticity might facilitate the rapid distribution of introduced and also native species into new habitats without the evolutionary lag time needed to adapt to new habitats through natural selection.

Our results revealed high morphological diversity between different populations of this species. ANOVA and PCA tests showed significant differences for most of the studied morphological traits between the studied populations and different shapes and amounts of qualitative as well as qualitative characteristics were observed between the populations. For this reason, high degree of phenotypic plasticity is seen in this species. Sultan (2000) stated that a single genotype can create various phenotypes in different ecological conditions. This phenomenon is definite as phenotypic plasticity. More investigations have proved that plants are plastic for a remarkable array of environmental important factors. Comparative, quantitative genetics and molecular approaches are leading to new insights into the adaptive nature of plasticity, its underlying mechanisms and its role in the ecological distribution and evolutionary diversification of plants (Sultan, 2000).

Previous studies showed high infraspecific variations in essential oil compositions of this species in Iran. Ahmadi and Mirza (1999) reported bornyl acetate (18.1%), β-caryophyllene (16.5%), α-pinene (15.6%), 1,8-cineole (8.3%) and limonene (8.3%) as

major constituents of essential oil compositions of this plant, while results of Morteza-Semnani et al (2005) studies showed that the major constituents of the essential oil of *S. multicaulis* are camphor (11.0%), 1,8-cineole (10.7%), borneol (8.6%) and α-pinene (7.5%).

On the basses of mentioned essential oil researches on this species, it seems that a phenomenon beyond the phenotypic plasticity creates such morphological variations between the populations of *S. multicaulis.* Although, no infraspecific taxon definite for this species in Flora Iranica (Rechinger 1982), Flora of Iran (Jamzad 2012) and Flora of Turkey (Davis, 1982), in Flora of Lebanon one variety of this species, *Salvia multicaulis* Vahl. var. *simplicifolia* Boiss, is present (Senatore et al 2004).

The population no. 13 had special morphological characteristics. When morphological traits of it were compared with morphological descriptions of *S. multicaulis* in Flora Iranica (Rechinger, 1982), Flora of Iran (Jamzad, 2012) and Flora of Turkey (Davis, 1982), it was cleared that some main morphological traits of this population that were useful in identification of this species were different from *S. multicaulis*. For example, the color of calyx and corolla length of this population was not similar to *S. multicaulis* description. So these characteristics are used in identification key of this species, therefore we predict that this population was a new variety or subspecies of this species in Iran.

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Table 2. Mean and standard deviation of some examined morphological characteristics between the studied populations (all values are in cm). Numbers indicate population's code 1-14 as in Table 1.

populations		Stem length	Basal leaf shape	Basal leaf length	Basal petiole length	Basal leaf width	Basal leaf margin	Floral leaf length	Floral leaf length/width	Floral leaf width	Floral leaf shape	Corolla length	Calyx length	Pedicle length	Calyx width	Calyx length/width	Flower number of each cycle	Bract length	Petal color
1	Mean	28.8		2.45	3.26	1.37		2.42	0.71	3.63	1.38	1.52	0.95	0.27	0.93	1.14	2.50	1.06	
	N	8	elliptic	8	8	8	serralate	8	8	8	8	8	8	8	8	8	8	8	violet
	Std. Dev	5.79		1.11	1.62	0.52		0.92	0.15	1.74	0.79	0.20	0.10	0.12	0.34	0.47	0.53	0.35	
2	Mean	27.50		2.05	3.51	1.11		1.93	0.45	4.13	0.78	1.66	0.98	0.16	0.93	1.1838	3.25	0.73	
	N	8	lanceolate	8	8	8	serralate	8	8	8	8	8	8	8	8	8	8	8	violet
	Std. Dev	3.38		0.83	1.21	0.35		0.96	0.07	1.29	0.24	0.23	0.20	0.05	0.23	0.68	0.88	0.20	
3	Mean	27.60		2.14	3.42	1.18		1.86	0.52	1.99	1.95	2.10	1.20	0.36	1.08	1.16	4.20	0.78	
	N	5	ovate	5	5	5	serrate	5	5	5	5	5	5	5	5	5	5	5	purple
	Std. Dev.	4.39		0.83	0.89	0.24		2.13	0.50	2.08	1.41	0.33	0.44	0.08	0.40	0.33	1.48	0.16	
4	Mean	18.80		1.58	2.04	1.06		1.36	0.36	3.12	2.45	1.40	0.84	0.16	0.68	1.22	4.20	0.98	
	N	5	elliptic	5	5	5	serralate	5	5	5	5	5	5	5	5	5	5	5	purple
	Std. Dev.	9.33		0.25	0.28	0.33		0.78	0.21	1.88	1.44	0.14	0.15	0.05	0.04	0.18	1.92	0.39	
5	Mean	33.00		3.18	3.55	1.23		3.80	0.86	4.56	1.12	1.46	1.01	0.32	1.07	1.08	7.62	0.66	
	N	8	Linear-lanceolate	8	8	8	serrate	8	8	8	8	8	8	8	8	8	8	8	purple
	Std. Dev.	4.27		0.78	1.53	0.42		0.56	0.18	1.11	1.02	0.32	0.24	0.19	0.53	0.41	3.20	0.19	
6	Mean	22.12		1.98	2.67	1.13		1.88	0.60	3.29	1.37	1.60	0.86	0.22	0.76	1.16	4.75	0.66	
	N	8	elliptic	8	8	8	serrate	8	8	8	8	8	8	8	8	8	8	8	purple
	Std. Dev.	3.64		0.49	0.80	0.30		0.50	0.20	0.77	1.32	0.29	0.15	0.04	0.19	0.22	1.16	0.14	
7	Mean	38.00		1.77	3.68	1.20		2.47	0.82	2.98	1.53	1.37	0.83	0.25	0.67	1.43	8.25	1.03	
	N	8	oblanceolate	8	8	8	serralate	8	8	8	8	8	8	8	8	8	8	8	purple
	Std. Dev.	7.91		0.70	1.77	0.43		1.31	0.34	1.04	0.98	0.18	0.11	0.07	0.31	0.52	3.32	0.25	
8	Mean	18.11		1.75	2.22	1.13		1.58	0.61	2.90	3.22	1.58	0.86	0.22	0.82	1.10	6.88	0.68	
	N	9	ovate	9	9	9	serralate	9	9	9	9	9	9	9	9	9	9	9	purple
	Std. Dev.	3.14		0.42	1.07	0.30		0.26	0.21	1.08	0.66	0.25	0.12	0.13	0.16	0.32	5.06	0.20	
9	Mean	24.00		2.00	3.75	1.25		2.48	0.70	3.90	1.59	1.63	0.91	0.31	0.83	1.15	6.87	0.63	
	N	8	ovate	8	8	8	serrate	8	8	8	8	8	8	8	8	8	8	8	purple
	Std. Dev.	5.31		0.70	1.74	0.43		0.78	0.28	1.27	1.26	0.24	0.09	0.08	0.23	0.32	2.23	0.23	
10	Mean	31.00	elliptic	2.50	3.37	1.10	serrate	3.12	0.70	4.54	0.87	1.45	0.92	0.37	1.15	0.80	7.25	0.95	
	N	4		4	4	4		4	4	4	4	4	4	4	4	4	4	4	purple
	Std. Dev.	3.16		0.49	1.10	0.60		1.35	0.21	1.80	0.14	0.17	0.15	0.09	0.12	0.15	1.70	0.70	
11	Mean	23.87		1.90	3.18	1.01		1.48	0.51	3.47	1.71	1.58	0.95	0.22	0.88	1.14	4.62	0.87	
	N	8	ovate	8	8	8	serrate	8	8	8	8	8	8	8	8	8	8	8	purple
	Std. Dev.	7.01		0.55	1.09	0.40		0.39	0.24	1.80	1.20	0.19	0.10	0.07	0.24	0.37	1.84	.26	
12	Mean	26.20		2.14	2.06	1.18		2.16	.60	3.54	2.14	1.70	1.36	0.24	1.42	1.02	6.20	0.92	
	N	5	ovate	5	5	5	serralate	5	5	5	5	5	5	5	5	5	5	5	Medium purple
	Std. Dev.	5.93		0.25	0.94	0.24		0.91	0.14	0.99	1.38	0.33	0.15	0.05	0.46	0.28	1.92	0.42	
13	Mean	32.42		2.5000	3.88	1.51		2.82	0.87	3.64	2.60	1.17	1.81	0.44	2.51	0.72	10.71	1.44	
	N	7	ovate	7	7	7	serrate	7	7	7	7	7	7	7	7	7	7	7	lavender
	Std. Dev.	4.23		0.87	0.94	0.70		0.86	0.30	1.61	1.19	0.11	0.21	0.07	0.39	0.08	3.72	0.51	
14	Mean	17.00		1.26	1.80	0.73		1.73	0.76	2.55	2.16	1.36	1.33	0.26	1.50	0.90	8.33	1.0000	
	N	3	ovate	3	3	3	serrate	3	3	3	3	3	3	3	3	3	3	3	purple
	Std. Dev.	3.60		0.49	0.43	0.25		1.16	0.51	0.92	1.25	0.05	0.28	.05774	0.43	0.08	4.04	0.40	