Sjournals
Scientific Journals
Scientific Journal of Biological Sciences (2012) 1(6) 126-137
ISSN 2322-1968

Contents lists available at Sjournals
Scientific Journal of
Biological Sciences
Journal homepage: www.Sjournals.com

Original article

Morphometric study of some *Salvia* L. (Lamiaceae) species in Iran

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ARTICLE INFO

Article history:
Received 25 November 2012
Accepted 12 December 2012
Available online 31 December 2012

Keywords:
Iran
Lamiaceae
Morphology
Salvia
Variability

ABSTRACT

This study concerns the morphological studies in some *Salvia* L. (Lamiaceae) species in Iran. In order to elucidate the taxonomic status and morphological studies, 53 accessions of 12 *Salvia* species were collected from their natural habitats in Iran. A total of 17 quantitative and 13 qualitative morphological characters were selected as diagnostic characters in *Salvia* species through the use of numerical methods. The statistical analysis consisted of cluster analysis with Euclidean distance coefficient, factor analysis, descriptive analysis and variance analysis using SPSS V.20.0 software. The clustering results of quantitative and qualitative morphological characters showed five groups. Despite the high morphological similarity between *S. nemorosa* L. and *S. virgata* Jaq., these species were separated using qualitative and quantitative characters and factor analysis. Regarding cluster and factor analyses, *S. spinosa* L. and *S. atropatana* Bunge accessions displayed high morphological diversity. Based on these findings, morphological characteristics such as the features of the leaf, calyx, corolla, bract, stamen and style were considered to be the appropriate diagnostic characters in the taxonomy of the *Salvia* species studied.

1. Introduction

Salvia L., with nearly 1000 species worldwide and 55 species in Iran, is one of the largest genus of Lamiaceae. It represents an enormous and cosmopolitan distribution and displays a remarkable range of variation (Walker *et al.*, 2004). The main speciation centers of this taxon are considered to be the eastern Mediterranean regions, south- west, western, eastern and central regions of Asia, South Africa, and Central and South America (Hedge, 1990; Kahraman and Dogan, 2010; Walker *et al*., 2004). It is distributed in subtropical, temperate, sub- arctic and arctic areas as well as the tropical regions of Iran (Hedge, 1982a; Walker et al., 2004). Some species are perennial, herbaceous, suffruticose, fruticose and sub-shrubby (Hedge, 1982a; Khan et al., 2002).

Having such morphological variability throughout the world, the genus has a significant taxonomic position among the plant taxonomists (Baikova, 1996). In addition, owing to greater similarity in morphological characters and the prevailing hybridization among *Salvia* species, high diversity in polyploid levels, the presence of heterozygous individuals and the taxonomical, ecological and genomic complexity, the species boundaries have become blurred (Haque, 1983; Hedge, 1982a). So far, a comprehensive numerical taxonomic study has not been sufficiently performed on *Salvia* species and it needs to be revised in terms of the systematic positions.

The stamen type and the leaf, calyx and corolla characters were useful features in taxonomic studies of the genus *Salvia* (Kahraman et al., 2010a; Ozdemir and Senel, 1999; Ozkan and Soy, 2007). The taxonomy of *S. officinalis* L. and *S. fruticosa* Mill. belonging to the section *Salvia* (Benth.) Hedge has been studied using leaf form, inflorescence axis indumentums, petiole and calyx indumentums (Reals et al., 2004). These characters have been mentioned for the taxonomic insights. In addition, using the stamen properties Walker and Sytsma (2006) determined the evolutionary interpretations which are related to the polyphyletic origin of *Salvia*. Through the morphological characters such as leaf and stem indumentums, bract form, bract color, bract margin, bract indumentums, calyx indumentums, filament appendage, calyx length, corolla length, bract length/bract width and corolla length/stamen length, Torke (2000) studied the phenetic analysis among the species belonging to the section *Ekmania* Epling. Corsi and Bottega (2002) and Kandemir (2003) also mentioned that the bract characteristics and the type of glandular hairs served to identify the *Salvia* species.

Based on the diversity properties, phenotypic variability in foliar characters among *Salvia* species has been provided and most of the characters showed highly significant inter- and intra- specific variations (Haque, 1983). Also, the morphological diversity and genetic variability among some of the *Salvia* species and accessions were reported using RAPD, AFLP and ISSR markers (Darmono and Okada, 2008; Saeidnia et al., 2009; Song et al., 2010; Zhand et al., 2009). Moreover, in our research Sajadi et al. (2010) reported the genetic diversity among 12 *Salvia* species using AFLP molecular markers.

Consequently, this study aims to identify the taxonomic position of 12 *Salvia* species in Iran via numerical analyses, evaluate the extent of the variations in the morphological characteristics, define the variability of *Salvia* accessions and introduce the diagnostic characters useful in separation of the species.

2. Materials and methods

2.1. Morphological studies

In this section, 53 accessions of 12 *Salvia* species were collected from their natural habitats in Iran (Tab. 1) and selected as OTUs. The voucher specimens were deposited in the Herbarium of Shahrekord University. A total of 17 quantitative and 13 qualitative characters were assessed as diagnostic characters in *Salvia* species and were used to study the taxonomic status and morphometric analysis using a stereo-microscope (Reals et al., 2004). The measured characters are presented in Tables 2, 3 and 4. These characters were selected according to two criteria: 1) the common use for taxonomic identification based on floras and our own observations and 2) the variability among different taxa. The taxa studied were as follows: *S. macrosiphon* Boiss., *S. spinosa* L., *S. sharifii* Rech. f. & Esfand., *S. nemorosa* L., *S. virgata* Jaq., *S. syriaca* L., *S. sclarea* L., *S. ceratophylla* L., *S. limbata* C.A. Mey., *S. atropatana* Bunge, *S. multicaulis* Vahl. and *S. hydrangea* DC. ex Hedge.

2.2. Statistical analysis

Statistical methods such as cluster analysis with Euclidean distance coefficient and Ward method, factor analysis (PCA), variance analysis test (ANOVA) and descriptive analysis as Coefficient of Variation (C.V.) using SPSS

V. 20.0 software were applied and the variability of *Salvia* accessions, the taxonomic positions of *Salvia* species and the variability of morphological characters were determined.

Table 1
The locality of *Salvia* species studied in Iran

Species	Locality	Height
1- *S. atropatana* 41	Guilan- Kelardasht	2400
2- *S. atropatana* 91	Chahrmahal va Bakhtiari - Tangeh sayad, dashteh chah	2850
3- *S. atropatana* 4	Mazandaran- pol-e Zangoule	2280
4- *S. ceratophylla* 15	Kermanshah- Sanandaj	1360
5- *S. ceratophylla* 13	Chahrmahal va Bakhtiari- Tangeh sayad, Sefid dasht, Bostan Shir	2120
6- *S. ceratophylla* 4	Fars- Abadeh	1500
7- *S. ceratophylla* 16	Tehran- Karaj	1489
8- *S. limbata* 106	Chahrmahal va Bakhtiari– Saman, Horeh, Savadjan	2070
9- *S. limbata* 107	Chahrmahal va Bakhtiari– Saman, Horeh	2200
10- *S. spinosa* 1	Hamadan- ganjnameh	1800
11- *S. spinosa* 8	Chahrmahal va Bakhtiari- Rousta-e kaj	1600
12- *S. spinosa* 9	Chahrmahal va Bakhtiari- North west of Ghaleh Darvish	1720
13- *S. spinosa* 10	Lurestan- Khoramabad	1850
14- *S. spinosa* 11	Chahrmahal va Bakhtiari- Saman- poleh zaman khan, shate Aliabad	2040
15- *S. spinosa* 12	Chahrmahal va Bakhtiari- Saman, Horeh, Savadjan	2070
16- *S. spinosa* 17	Qazvin-manjil	450
17- *S. spinosa* 18	Isfahan- sadeh Zayandeh rood	2500
18- *S. spinosa* 42	Tehran-Delijan	2950
19- *S. sharifii* 5	Isfahan-Kolahghazi	1670
20- *S. sharifii* 8	Isfahan-Kolahghazi	1690
21- *S. sharifii* 4	Isfahan-Kolahghazi	1700
22- *S. sclarea* 22	Chaharmahal va Bakhtiari- Chartagh, Sabzeh kooh	2150
23- *S. sclarea* 23	West of Azerbayjan-Moghan	700
24- *S. sclarea* 24	Chahrmahal va Bakhtiari- 10 Km Baba Heydar, Chelgerd	2320
25- *S. sclarea* 25	Kohkilouy-e va Boyerahmad- Sisakht	2110
26- S. macrosiphon 6	Isfahan-koh-e Sopheh	2300
27- S. macrosiphon 7	Fars- abadeh, Tashak,	1850
28- S. macrosiphon 63	Isfahan-Kolahghazi	1700
29- S. macrosiphon 64	Isfahan-Kolahghazi	1710
30- S. macrosiphon 5	Kermanshah- Karand	1730
31- S. macrosiphon 66	Chahrmahal va Bakhtiari- Saman,Ilbagi	2070
32- S. macrosiphon 68	Lurestan-Khoramabad	1200
33- S. syriaca 28	Chahrmahal va Bakhtiari- Boldaji, Hamzeh Ali	2180
34- S. syriaca 29	West of Azerbayjan-Ardabil	1250
35- S. syriaca 80	Chahrmahal va Bakhtiari- Rousta-e Hosein abad, Ghaleh Darvish	1720
36- S. syriaca 82	Hamedan- Ganjnameh	1900
37- S. syriaca 83	Guilan- Masouleh	2070
38- S. syriaca 84	Isfahan- Dalan kouh	1970
39- S. syriaca 8	Kokkilouye va boyer Ahmad-sisakht	2050
40- S. syriaca 2	Kokkilouye va boyer Ahmad- Yasouj	1980
41- S. multicaulis 43	Chahrmahal va Bakhtiari- Tangeh sayad, Sefid dasht, Bostan Shir	2120
42- S. multicaulis 44	Mazandaran-Siyah bisheh	2000
43- S. multicaulis 45	Kermanshah- Harsin	2335
44- S. multicaulis 47	Chahrmahal va Bakhtiari- Tangeh sayad, koh-e Kondeh Rahim	2550
45- S. multicaulis 48	Kordestan- Bijar	2335
46- S. multicaulis 49	Lurestan-Oshtoran kuh	1750
47- S. multicaulis 50	West of Azerbayjan- Miyaneh	1200

48- S. multicaulis 51	Fars- Kazeroun	2250
49- S. multicaulis 52	Isfahan- Semirom, Cheshme naz	2270
50- S. nemorosa 19	Ahvaz- Izeh	2000
51- S. virgata 20	Gorgan- Gonbad-e Kavous	1085
52- S. virgata 21	Chahrmahal va Bakhtiari- Dastgerd	2000
53- S. hydrangea 26	Chahrmahal va Bakhtiari- Tangeh sayad- koh-e Shor shor	2400

Table 2
The quantitative morphological characters studied in *Salvia* species.

1- calyx length (mm)	2- calyx spin length (mm)
3- bract length (mm)	4- bract width (mm)
5- calyx length/bract length	6- corolla length (mm)
7- bract length/ corolla length	8- theca length (mm)
9- style length (mm)	10- filament length/theca length
11- nutlet length (mm)	12- anther length (mm)
13- filament length/corolla length	14- nutlet width (mm)
15- filament length/style length	16- style length/corolla length
17- corolla tube length (mm)	

Table 3
The qualitative morphological characters studied in *Salvia* species.

1- leaf form: pinnatisect, pinnatifid, oblong, linear-oblong, oblong-lanceolate, ovate, broadly ovate, ovate-oblong, ovate-lanceolate, elliptic, broadly elliptic, sub-orbicular, linear, linear-lanceolate	8- calyx form: campanulate, campanulate-infundibular, tubular, tubular-campanulate, broadly tubular, ovate-campanulate, broadly campanulate
2- leaf base form: cordate, sub-cordate, cuneate, narrowed, oblique, rounded	9- corolla tube form: invaginate and squamulate, non-invaginate and non-squamulate
3- leaf margin form: entire, sub-entire, crenulate, serrate, serrate-erose, serrulate, erose, crenate, lobate, sub-lobate, dentate	10- style apex form: simple, thin dichotomous, broad dichotomous
4- leaf apex form: acute, obtuse, sub-obtuse, rounded	11- stamen type: type A, type B
5- bract form: ovate, broadly ovate	12- nutlet form: spherical, sub-spherical, rounded-trigonous, ovoid, broad ovoid
6- bract apex form: acuminate-spinulose, acuminate, cuspidate	13- nutlet color: light brown, dark brown, yellow, light gray, black
7- bract color: green, green-yellow, green-white, pink, violet	

3. Results

As the results of this study show, the quantitative morphological characters such as the calyx length, bract length, bract length/ corolla length, calyx length/bract length, corolla length, corolla tube length, filament length, filament length/theca length, filament length/style length, style length and style length/corolla length are the most appropriate taxonomical characters. Among the qualitative morphological characters we found the best to be; leaf form, margin leaf form, bract form, bract colour and bract apex form.

Based on ANOVA analysis, bracteole length (sig. = 0.003), corolla length (sig. =0.00), bract length/ corolla length (sig. =0.002), filament length (sig. =0.001), filament length/theca length (sig. =0.047), filament length/style length (sig. =0.039), style length (sig. = 0.018) and style length/corolla length (sig. = 0.025) display significant differences ($P< 0.05$).

The highest morphological variability was found in theca length (C.V. = 81.4) and the lowest was found in filament length/ theca length (C.V. = 10.8) (Tab. 5). The morphological variability among the *Salvia* accessions showed that the highest variations were related to the theca length (C.V. = 98.3, 90.2; *S. atropatana, S. ceratophylla*, respectively), anther length (C.V. = 97.7; *S. nemorosa*), filament length/corolla length (C.V. = 92.8, 92.3, 71.4; *S. sclarea, S. macrosiphon, S. spinosa*, respectively), filament length/theca length (C.V.= 92.1; *S. syriaca*), nutlet length (C.V. = 91.6; *S. sharifii*), calyx length/bract length (C.V.= 90.6; *S. virgata*), corolla length (C.V. = 83.8, 80; *S. multicaulis*, *S. hydrangea* respectively) and calyx spin length (C.V. = 51.4; *S. limbata*).

Table 4
The data matrix of qualitative morphological characters studied in *Salvia* species.

Ch./sp.[1]	1	2	3	4	5	6	7	8	9	10	11	12
leaf form	Ovate-oblong, elliptic, ovate	Ovate, broadly elliptic, ovate-oblong, broadly ovate	Ovate	Ovate-oblong, broadly ovate, oblong	Oblong-lanceolate	Ovate, ovate-oblong, ovate-lanceolate	Linear, linear-oblong, elliptic, linear-lanceolate	Pinnatifid	Elliptic, sub-orbicular	Pinnatisect	Broadly ovate	Ovate, ovate-oblong, broadly ovate
leaf base form	Rounded, cordate, sub-cordate	Sub-cordate, cuneate, rounded, cordate	Rounded	Cordate, sub-cordate	Cordate, sub-cordate	Cordate	Oblique, cuneate	Narrowed	Oblique, rounded	Narrowed	Cordate	Cordate,sub-cordate rounded
leaf margin form	Sub-entire, serrate, lobate	Entire, erose, sub-entire, dentate	Sub-entire, serrate	Erose, sub-entire, crenulate, serrate	Crenate, serrulate	Erose, serrulate	Erose, lobate, sub-entire, crenate, serrate	Entire, sublobate	Crenulate	Entire	Erose, sub-lobate	Crenate, erose, serrate-erose
leaf apex form	Obtuse, acute, rounded	Acute, obtuse	Acute, obtuse	Acute, obtuse, rounded	Acute	Acute, obtuse	Obtuse	Obtuse	Obtuse	Acute	Acute, obtuse	Obtuse sub-obtuse, acute
bract form	Broadly ovate	Broadly ovate	Ovate	Ovate	Ovate	Ovate	Broadly ovate, ovate	Broadly ovate, ovate	Ovate, broadly ovate	Ovate	Ovate	Broadly ovate, ovate
bract apex form	Acuminate	Acuminate-spinulose	Acuminate	Acuminate	Acuminate	Acuminate	Acuminate	Cuspidate	Acuminate	Acuminate	Acuminate	Acuminate, cuspidate
bract color	Green-yellow, pink	Green-yellow, pink, green	Green	Green	Violet	Green-white	Green, pink	Green	Green, pink	Green	Green	Pink, green-white
calyx form	Tubular	Broadly tubular	Tubular	Tubular-campanulate	Tubular-campanulate	Tubular	Campanulate-infundibular	Ovate-campanulate	Broadly campanulate	Campanulate-infundibular	Campanulate	Ovate-campanulate
corolla tube form	Non-invaginate and non-squamulate	Non-invaginate and non-squamulate	Non-invaginate and non-squamulate	Non-invaginate and non-squamulate	Non-invaginate and non-squamulate	Non-invaginate and non-squamulate	Invaginate and squamulate	Invaginate and squamulate	Non-invaginate and non-squamulate	Non-invaginate and non-squamulate	Invaginate and squamulate	Invaginate and squamulate
style apex form	Broad dichotomous	Simple, broad dichotomous	Broad dichotomous	Thin dichotomous	Thin dichotomous	Broad dichotomous	Broad dichotomous	Broad dichotomous, thin dichotomous	Broad dichotomous, simple	Thin dichotomous	Broad dichotomous	Thin dichotomous
stamen type	Type B	Type B	Type B	Type B	Type B	Type B	Type B	Type B	Type A	Type A	Type B	Type B
nutlet form	Broad ovoid	Rounded-trigonous, spherical	Ovoid	Ovoid	Ovoid	Rounded-trigonous	Ovoid	Spherical	Ovoid, rounded-trigonous	Sub-spherical, rounded-trigonous	Ovoid	Rounded-trigonous, spherical
nutlet color	Light brown	Light brown, dark brown, light gray	Light brown	Black, dark brown	Black, dark brown	Yellow	Yellow, light brown	Black	Light brown, dark brown	Light brown	Light brown	Light brown

The lowest variations were found in calyx length (C.V. = 11.6, 8.9, 7.26, 5.98, 5.3, 4.5, 3.12; *S. spinosa, S. syriaca, S. macrosiphon, S. sclarea, S. ceratophylla, S. sharifii*, *S. hydrangea* respectively), style length (C.V. = 10.4; *S. virgata*), nutlet length (C.V. = 9; *S. multicaulis*), nutlet width (C.V. = 5.3; *S. ceratophylla*), corolla tube length (C.V. = 1.27; *S. limbata*), calyx spin length (C.V. = 1.2; *S. atropatana*) and bract length/ corolla length (C.V. = 1.6; *S. nemorosa*) (Tab. 6).

We obtained the dendrogram shown in figure 1. The distribution of the morphological characters show how these are used for the taxonomic differentiation of the groups studied. In cluster analysis using the quantitative and qualitative morphological characters, we distinguish five clusters including: group 1) *S. sharifii, S. macrosiphon* and *S. spinosa,* group 2) *S. limbata* and *S. atropatana*, group 3) *S. multicaulis, S. sclarea*, *S. ceratophylla*, *S. hydrangea* and *S. atropatana* group 4) *S. nemorosa*, *S. virgata,* and *S. syriaca* and group 5) *S. spinosa* (Fig.1). We found that *S. multicaulis* accessions comprised four groups in cluster 3, which clustered with *S. hydrangea*, *S. sclarea* and *S. ceratophylla*. Moreover, *S. nemorosa* and *S. virgata* were mainly grouped. *S. syriaca*, *S. ceratophylla, S. sclarea* and *S. macrosiphon* contained two groups in clusters 4, 3, 3 and 1 respectively, *S. sharifii*, *S. atropatana* and *S. spinosa* comprised two groups in clusters c1, c2-c4 and c1-c5 respectively which displayed high diversity among their accessions (Fig. 1).

Using quantitative and qualitative morphological characters and 12 *Salvia* species and 52 accessions, the factor analysis (PCA) confirmed the cluster analysis results. In this case five groups comprised. *S. limbata* and *S. atropatana* were definitely distinct from *S. sclarea* and *S. ceratophylla*. Despite the high similarity between *S. nemorosa* and *S. virgata*, these species are definitely distinct. Moreover, *S. spinosa* and *S. macrosiphon* were closely grouped but definitely distinct (Fig. 2).

Table 5
Mean, standard deviation and coefficient of variation in quantitative characters studied in *Salvia* species.

characters	Mean	Standard Deviation	C.V.
1- calyx length (mm)	16.27	5.11	31.40
2- calyx spin length (mm)	0.82	0.59	71.9
3- bract length (mm)	17.9	10.26	57.3
4- bract width (mm)	13.1	8.4	64.1
5- calyx length/bract length	1.11	0.58	52.2
6- corolla length (mm)	4.42	3.2	72.3
7- bract length/ corolla length	4.2	3.2	76.1
8- theca length (mm)	7	5.7	81.4
9- filament length/theca length (mm)	4.8	0.52	10.8
10- style length (mm)	20.9	12.46	59.6
11- anther length (mm)	1.83	1.18	64.4
12- nutlet length (mm)	2.29	1.22	53.2
13- nutlet width (mm)	1.96	1.03	52.5
14- filament length/corolla length (mm)	1.9	0.3	15.7
15- style length/corolla length (mm)	1.27	0.71	55.9
16- filament length/style length (mm)	1.2	0.15	12.5
17- corolla tube length	15.17	9.59	63.2

Table 6
The variability of quantitative morphological characters (C.V.) among the accessions of *Salvia* species.

characters	**mac.**	**spin.**	**sha.**	**virg.**	**nem.**	**syr.**
1- Calyx length (mm)	7.26	11.6	4.5	21.9	9.8	8.9
2- calyx spin length (mm)	47	27	13.9	75.4	62.3	83.3
3- bract length (mm)	22.8	27.5	27.2	29.6	32.4	66.1
4- bract width (mm)	32.3	16.8	47.2	15.2	37.9	68.6
5- calyx length/bract length	25	43.7	35.7	90.6	30.6	66.6
6- corolla length (mm)	23	64	57.5	32	36.2	60
7- bract length/ corolla length	31.2	79.6	66.6	31	1.6	73.3
8- theca length (mm)	18.2	48.4	68.9	58.3	40.8	12.6
9- filament length/theca length (mm)	17.3	54.5	72.2	62.7	72.9	92.1
10- style length (mm)	16.1	46.6	39.8	10.4	11.5	60.4
11- anther length (mm)	21.4	57.1	86	70.7	97.7	73.1
12- nutlet length (mm)	50	45.8	91.6	68.4	37.6	66.6
13- nutlet width (mm)	51.8	50	90	63	56.4	67.1
14- filament length/corolla length (mm)	92.3	71.4	37.1	33.3	21.1	81.8
15- style length/corolla length (mm)	17.5	48.3	47.8	30.7	30.1	82
16- filament length/style length (mm)	80	50	50	33.3	20	62.5
17- corolla tube length	14.9	39.4	40.5	11	24.1	59.4

mac: S. macrosiphon, spin: S. spinosa, sha: S. sharifii, virg.: S. virgata, syr.: S. syriaca

Table 6: continue

characters	atr.	cer.	mult.	hyd.	lim.	scl.
1- Calyx length (mm)	3.27	5.3	13.5	3.12	20	5.98
2- calyx spin length (mm)	1.2	29	68.1	54.1	51.4	39
3- bract length (mm)	11.6	45.4	38.8	20.7	10.5	10.2
4- bract width (mm)	16.4	44	26.5	15.75	20.2	21.3
5- calyx length/bract length	15.5	13.1	36.6	17.5	30	71.4
6- corolla length (mm)	47.1	10	83.8	80	23.3	84.4
7- bract length/ corolla length	46.4	44.7	78.7	9.5	7.7	43.2
8- theca length (mm)	98.3	90.2	73	72	13	71
9- filament length/theca length (mm)	96.6	8.3	69.6	73	23	80
10- style length (mm)	31.9	88.1	75.1	77.4	6.1	81.5
11- anther length (mm)	43.3	89.4	66.1	60	8.42	11.7
12- nutlet length (mm)	14	6	9	6.49	11.7	10
13- nutlet width (mm)	14	5.3	15.1	8.5	11.8	62.2
14- filament length/corolla length (mm)	50	11	29.2	76.9	13.3	92.8
15- style length/corolla length (mm)	18.7	10.6	80	77.5	7.2	13.6
16- filament length/style length (mm)	5.6	10	72.7	78.5	20	71.4
17- corolla tube length	40	34.9	70.2	76.9	1.27	76.6

atr: S. atropatana, cer: S. ceratophylla, mult: S. multicaulis, lim: S. limbata, scl: S. sclarea.

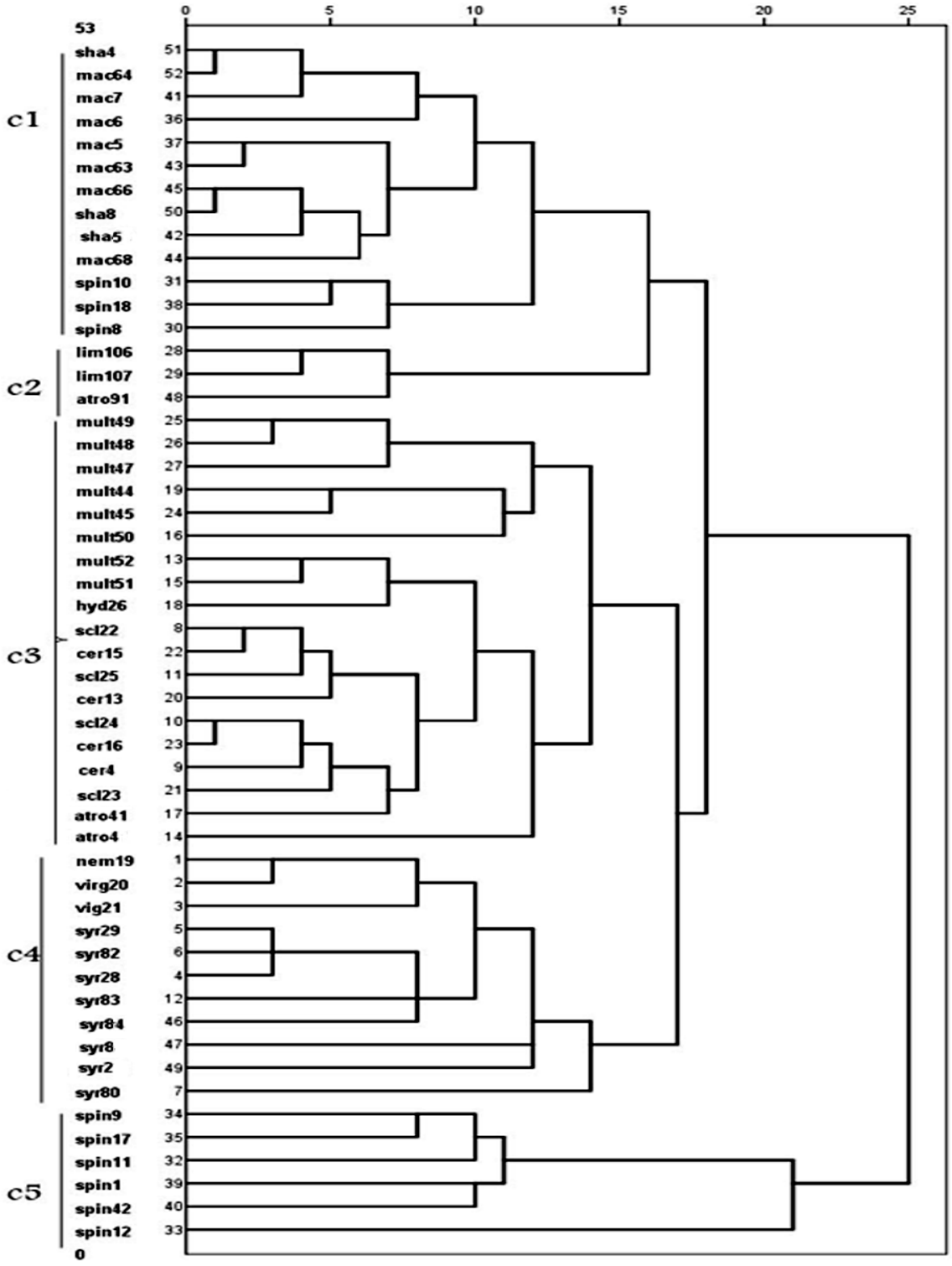

Fig.1. Dendrogram of cluster analysis using morphological characters and *Salvia* accessions.
mac: S. macrosiphon, spin: S. spinosa, sha: S. sharifii, virg.: S. virgata, syr.: S. syriaca, atr: S. atropatana, cer: S. ceratophylla, mult: S. multicaulis, hyd: S. hydrangea, limb: S. limbata, scl: S. sclarea. nem: S. nemorosa.

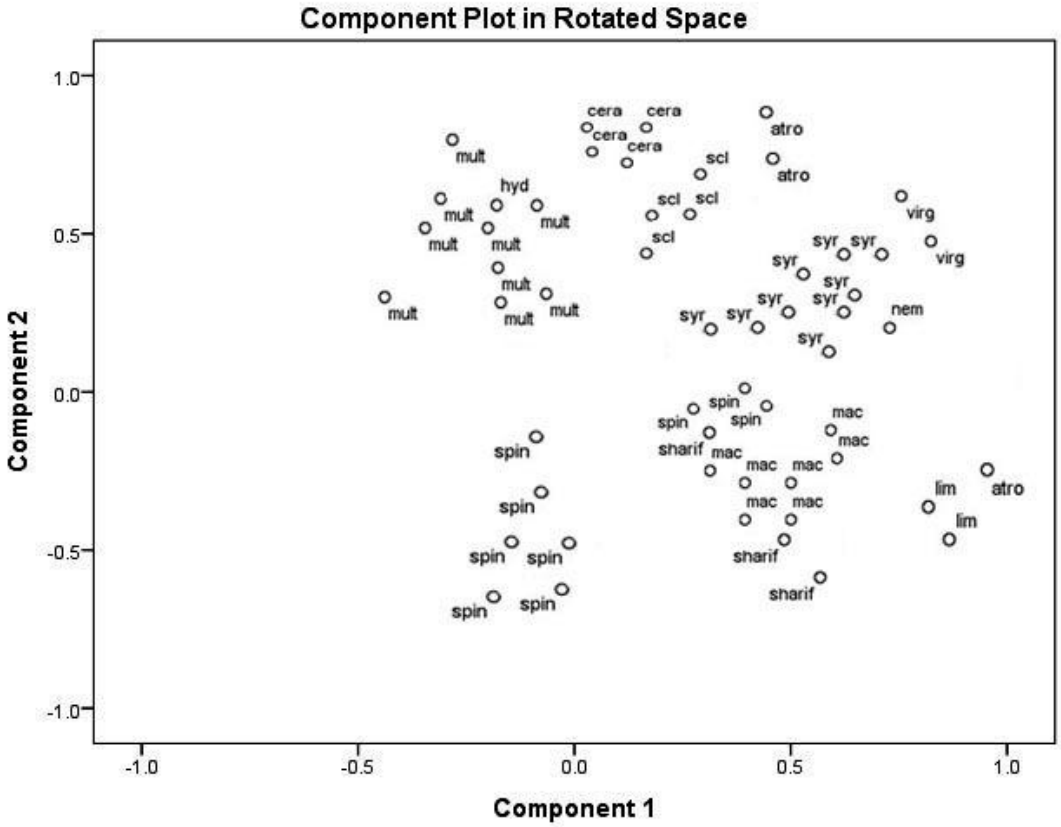

Fig. 2. Factor analysis in 52 *Salvia* accessions using morphological characters.

4. Discussion

As the results of the morphological studies demonstrate, most of the *Salvia* species studied have been discriminated by calyx form, calyx length, corolla length, corolla tube length, bract length, bract length/ corolla length, filament length and style length, which are in accordance with Kahraman and Dogan (2010), Kahraman et al. (2010b), Ozkan and Soy (2007) and Reals et al. (2004). *S. spinosa* is closely related to *S. macrosiphon*, but it differs in diagnostic characters such broader leaves and calyx, which is based on the Kharazian (2009) results. Moreover, in our results *S. nemorosa* and *S. virgata*, which have a high similarity in morphological characters, are diagnosed by bract colour, corolla length, filament and style length. As the results of statistical analysis show, the best morphological characters were selected to evaluate the taxonomic position. Thus, the characters relating to quality and quantity of calyx, corolla, bract, stamen and style are introduced as appropriate morphological characters and are used in the taxonomic status of this genus (Tab. 2 and 3). Moreover, Kahraman et al. (2010b), Kahraman et al. (2009) and Kaya et al. (2007) reported that the stem indumentums, corolla length, bract length and stamen type are of sufficient taxonomical significance to identify some *Salvia* species. Ryding (1994) proved that the length of hairs in the bract and bracteole margin can be applied to taxonomical studies. This evidence is not in agreement with our morphological studies in each *Salvia* species.

According to Hedge's classification (1982a), the *Salvia* species have been divided into five groups. *S. multicaulis, S. hydrangea, S. ceratophylla* were categorized in a group with simple, pinnatisect and pinnatifid leaf, biennial and perennial herbaceous, suffruticose or fruticose stem, large and non-convoluted leaf and corolla length 8-40 mm. Moreover, *S. multicaulis* was divided into group with fertile pollen, anther- like lower theca and calyx clearly expanded in fruit or scarcely expanded. *S. syriaca, S. nemorosa, S. virgata, S. macrosiphon, S. spinosa*, and *S. sharifii* were in a group including corolla tube not-invaginated and not- squamulate and inside of corolla tube not

perfectly or perfectly annulated. *S. sclarea, S. atropatana, S. ceratophylla* and *S. limbata* were in a group including invaginated and squamulate corolla tube, and inside of corolla tube glabrous (Hedge, 1982a).

In cluster and factor analyses, *S. multicaulis* and *S. hydrangea* were in accordance with Hedge's classification (1982a), *S. ceratophylla* accessions were clustered in different groups such as *S. multicaulis* and *S. sclarea* which was in agreement with Hedge's taxonomy (Fig. 1 and 2). Obviously, based on qualitative and quantitative characters, *S. multicaulis* comprised different groups which are nearly in concurrence with Hedge's treatment (1982a) (Fig. 1). Hedge (1982a) only reported the variation of leaf and stem indumentums in this species. In our research, these variations are generally connected to the indumentums of stem, leaf, petiole, calyx and inflorescence axis, leaf form, calyx apex, calyx color and bract form. It appears that morphological variations are closely related to the varieties, forms or the polymorphism characters for this species. In our research using AFLP molecular markers, we concluded that *S. multicualis* and *S. hydrangea* were mainly clustered. In addition, using this method *S. multicaulis* comprised one group (Sajadi et al., 2010), which has been partially confirmed by morphological characters and cluster analysis (Fig. 1; cluster c3).

As mentioned above, most of the species which were in groups with corolla tube not-invaginated and not-squamulate have been confirmed by the clustering and factor analysis of this study (Fig. 1 and 2). In cluster analysis, *S. macrosiphon* accessions were in two different groups in cluster c1 (Fig. 1). Owing to the hybridization and introgression of *S. macrosiphon* with other species, such as *S. moorcroftiana* Wall. Ex Benth. and *S. reuterana* Boiss, it can also be considered as variation patterns in this species (Hedge, 1990,1982a). In addition, this variability was observed in Sajadi et al. (2010). In this research, most of the variations were in leaf indumentums, leaf form, leaf base, leaf margin, bract indumentums, corolla color, corolla tube length, calyx length, calyx apex and inflorescence indumentums (Kharazian, 2008).

In the clustering results, *S. spinosa* and *S. macrosiphon* were closely grouped in cluster c1. As previously mentioned these two species are extremely similar but were distinguished using diagnostic characters (Kharazian, 2009). Moreover, the high variability of *S. spinosa* accessions (including two groups) seems to either show the high hybridization and introgression with *S. macrosiphon* or prove the variation of this species in Iran (Kharazian, 2009). Additionally, these results are in accordance with our molecular marker research (Sajadi et al., 2010). The morphological variations among the *S. spinosa* accessions were in leaf form, leaf margin form, leaf indumentums, stem indumentums, inflorescence indumentums, bract indumentums, bract dimension, bract color, calyx indumentums, calyx dimension, corolla indumentums and corolla length (Kharazian, 2009).

It has been noted that based on the quantitative and qualitative findings, *S. sharifii* accessions showed different groups, which is in agreement with Sajadi et al. (2010). This variation is related to the stem, leaf and bract indumentums. In most cases, *S. sharifii* was grouped with *S. macrosiphon* (Fig. 1) which is based on the findings of Sajadi et al. (2010). Some of the *S. sharifii* accessions belonging to south and south-east regions of Iran are related to *S. macrosiphon* (Hedge, 1982a), confirming our results (Fig. 1). In addition, *S. sharifii* and *S. macrosiphon* are morphologically closely related. It can be concluded that the morphological characters such as bract length and corolla length are diagnostic features among these two species.

In factor analysis, *S. nemorosa* was strongly separated from *S. virgata* (Fig. 2), which is in accordance with Sajadi et al. (2010), but in cluster analysis, were closely clustered (Fig. 1). Based on Hedge's classification (1982a), these two species were diagnosed using bract color and corolla length. As previously mentioned in our results, these are also separated through bract, calyx, filament and style features. According to Kaya et al. (2007), some of these characters are well-documented taxonomic characters in *Salvia* genus.

Furthermore, using cluster analysis *S. syriaca* was found to comprise of two groups in cluster c4 and mainly grouped with *S. nemorosa* and *S. virgata*, including the group with corolla tube not- invaginated and not-squamulate (Hedge, 1982a). The high morphological variability in *S. syriaca* is due to the stamen features (Fig. 1). According to Ozkan et al. (2009), stamen feature in *Salvia* species represents the morphological diversity. This diversity was also displayed in molecular markers (Sajadi et al., 2010).

In relation to the group with invaginated and squamulate corolla tubes, *S. atropatana* accessions were found to mainly consist of two different groups, which were related to the high polymorphism characters among the accessions (Kharazian, 2012). In cluster analysis, *S. atropatana* was clustered with *S. limbata*, which is in agreement with Hedge's classification (1982a) and in 240 another case, clustered with *S. sclarea*, which is in accordance with Sajadi et al. (2010) (Fig. 1). These variations are in the stem and leaf indumentums, leaf form and leaf margin form, inflorescence indumentums, bract indumentums, pedicel indumentums, corolla and calyx length, corolla and calyx indumentums, corolla tube indumentums and style length. Hedge (1982a) stated that this species displays

variability only in leaf features. In our AFLP molecular marker results, we observed one group of this species (Sajadi et al., 2010) which was grouped with *S. limbata*. Consequently, the AFLP marker partially confirmed these results. In this regard, Reals et al. (2004) found a relationship between the environment and some of the morphological characters within *Salvia* accessions.

In our results, *S. limbata* mainly showed one group and seems to be a different species, which is confirmed Sajadi et al. (2010). Using clustering and factor analyses, *S. limbata* was shown to differ from *S. ceratophylla* and *S. sclarea*, which is not confirmed by Hedge's classification (1982a) (Fig. 1 and 2). Furthermore, *S. sclarea* and *S. ceratophylla* are strongly grouped, which is also observed using AFLP molecular markers (Sajadi et al., 2010). Ozdemir and Senel (1999) also showed the morphological properties of *S. sclarea* in Turkey, which has some similarities and differences compared to other findings in taxonomic literature. In our results, some accessions of *S. sclarea* displayed different groups, which is probably due to the morphological variations or ecological adaptations. It seems that these taxonomic differentiations were due to polymorphism in the morphological characters, hybridization between species and geographical distribution (Hedge, 1982a). *S. sclarea* has variability in terms of the bract color, bract length, corolla upper lip color, leaf form and leaf margin. Based on our findings in AFLP results, *S. sclarea* accessions were did not display variability (Sajadi et al., 2010). In the same way, Kahraman et al. (2010b) and Kaya et al. (2007) revealed that the morphological features of Turkish *S. macroclamys* Boiss. & Kotschy and *S. halophila* Hedge such as petiole length, bract and flower dimension, stem indumentums, leaf length, calyx length, corolla length and nutlet dimension provide some additional data to that reported by Hedge (1982b).

5. Conclusion

As a final point, we conclude that the morphological character studied here appear to be the appropriate features in the taxonomy of *Salvia* genus. The qualitative characters and quantitative characters definitely separate the *Salvia* species. It can be assumed that the phenotypic variations observed in the *Salvia* species might have arisen due to segregation, recombination, reproductive system and adaptation against adverse environmental conditions (Aktas et al., 2009; Baran et al., 2008; Haque, 1983; Wang et al., 2007). In some way, the morphological characters seem to be appropriate features to display the accessions variability and taxonomic levels.

Acknowledgement

The author is thankful to Mr. H. Fatahi and Mrs. F. Hasanzadeh for kindly helping with this research. This study has been supported by the Research Deputy of Shahrekord University, Shahrekord, Iran with No. 1978.

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