Plant Syst Evol (2014) 300:925–935
DOI 10.1007/s00606-013-0932-5

ORIGINAL ARTICLE

Multivariate analysis of motherwort germplasm in Iran using morphological variables and essential oil content

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Received: 22 April 2013 / Accepted: 12 October 2013 / Published online: 5 November 2013

Abstract The morphological variation and essential oil content of 150 individuals of motherwort (*Leonurus cardiaca*) from six natural regions of Iran were examined biometrically based on multivariate analysis. The highest variation was observed in plant height, length of main stems, main inflorescence length, floral cycles of main stem, lateral branches length, basal leaf length, floral leaf length width, flower length, and calyx color. Results of simple correlation analysis showed the existence of significant positive and negative correlations among some important parameters. The highest correlation was observed between plant height and length of main stems and between lateral branch length and lower surface color of leaf. Populations were clustered in two groups representing subspecies *cardiaca* and *persicus*. The subspecies *cardiaca* populations were closely related with each other and differentiated from the subspecies *persicus* by pubescence stems and leaves, more number of floral cycles, higher compaction of floral cycles, lower number of lateral branches, shorter length of petioles and leaves and smaller floral leaves. Essential oil yields varied from 0.02 (for Taleghan population) to 0.053 mg/100 g dry mater (for Kerman population). The conservation of the highly diverse native populations of Iranian motherwort germplasm is recommended.

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Keywords *Leonurus cardiaca* · Genetic resources · Quantitative and qualitative characters · Essential oil · Correlation coefficients

Introduction

Genus *Leonurus* (Lamiaceae) comprises about 20 species distributed throughout the world. The genus is represented by one species, *L. cardiaca* L. (motherwort in English and Dome-Shir in Persian (Mozaffarian 1996) in flora of Iran. *Leonurus cardiaca* is a perennial species extending from Europe through southwest and central Asia to parts of China (Hedge 1990).

Motherwort is especially valuable in the treatment of female weaknesses and disorders, allaying nervous irritability, inducing quiet and passivity of the whole nervous system. It is also seen as a remedy for heart palpitations, it has a strengthening effect, especially on a weak heart. The antispasmodic and sedative effects promote relaxation rather than drowsiness. The leaves are antispasmodic, astringent, cardiac, diaphoretic, nervine, sedative, stomachic, tonic, and uterine stimulant (Muhammad et al. 2007; Popescu et al. 2009).

The taxonomy of *L. cardiaca* is controversial. There is as yet no overall review of this species; only accounts in local or regional Floras. In the past years, the complex taxonomy of *L. cardiaca* and related species was emphasized by Holub (1993). Especially stem indumentum (and to some extent also leaf shape) proved to be worthy characters to separate a rather distinct Asian taxon, *L. cardiaca* subsp. *villosus* (Desf. ex d'Urv.) Hyl. According to Rechinger (1982) in Flora Iranica, *L. cardiaca* is divided to four subspecies based on leaf and indumentum characters including *L. cardiaca* subsp. *nuristanicus* (Kitamura) Rech. F., subsp.

persicus (Boiss.) Rech. F., subsp. *cardiaca* and subsp. *turkestanicus* (V. Krecz, & Kuprian.) Rech. F., the three later are found in Iran. Mill (1982) in Flora of Turkey has given species rank to the mentioned taxa. The subspecies are distinguishable from each other by some morphological characteristics, subspecies *persicus* differs from the two others by smaller floral leaves and remote vericillasters, subspecies *cardiaca* differs from the *turkestanicus* by taller height, all leaves 3-lobed and subglabrous or sparsely pubescent stems. In spite of the description of taxa based on morphological characters, the morphological variation of *L. cardiaca* has not been studied intensively in Iran.

Traditional methods for characterization and identification of species and genotypes are based on phenotypic observations. Morphological traits are useful for preliminary evaluation because they facilitate fast and simple evaluation and can be used as a general approach for assessing genetic diversity among morphologically distinguishable individuals. Morphological characterization combined with multivariate statistical methods, such as principal component analysis (PCA), the most commonly applied, and cluster analysis, are useful tools for screening individuals for many plants such as almond (Chalak et al. 2007), *Cerasus* subgenus (Khadivi-Khub et al. 2008, 2012), pomegranate (Sarkhosh et al. 2009) and *Origanum vulgare* (Andi et al. 2011). Multivariate techniques can help to evaluate large data sets and resolve several phenotypic and genotypic measurements into fewer more interpretable and more easily visualized groups.

Information about the diversity of morphological characters within and among different subspecies and populations of *L. cardiaca* may be used in delimitation of subspecies and also in planning breeding programs and conservation of this valuable plant. This paper describes morphological variable and essential oil analyses at the population level within *L. cardiaca* which also could be useful in understanding the taxonomy of this species.

Materials and methods

Plant materials

A total of 150 individuals of motherwort were studied in their natural habitats from six regions of six provinces in Iran (Fig. 1). Twenty-five plants were sampled randomly from each population. Sampling locations and their geographic coordinates are shown in Table 1. The interval between samples was 300–500 m, whereas the pairwise distance between main regions was 300–600 km. The sampled stands were chosen in order to provide maximum representation of the ecological conditions of the area (Fig. 2).

Morphological and essential oil analysis

Morphological study was conducted during full flowering stage in spring and summer of 2011 and 2012. Thirty-four

Fig. 1 Geographic location of collection sites of *Leonurus cardiaca* populations (*black dots*)

Table 1 Locations and number of individuals of studied *Leonurus cardiaca* populations

No.	Population	Number of individual	Province	Altitude (m)	Longitude (E)	Latitude (N)
1	Kerman	1–25	Kerman	2,600	56°50′31″	29°18′36″
2	Dargaz	26–50	South-Khorasan	2,194	58°42′1″	37°34′42″
3	Taleghan	51–75	Tehran	1,850	50°45′34″	36°10′27″
4	Khansar	76–100	Esfahan	2,210	50°17′57″	33°15′50″
5	Sarab	101–125	Ardabil	1,687	47°31′40″	37°55′59″
6	Sari	126–150	Mazandaran	2,170	53°12′3″	36°3′37″

Fig. 2 Pictures of *Leonurus cardiaca* collected from different regions of Iran

morphological traits and the important attributes influencing the amount and type of active compounds (28 quantitative and 6 qualitative) were evaluated in natural ecosystems (Table 2). Some of morphological characters were measured at the sampling location, and then the samples were transported to the laboratory for further study evaluation. Furthermore, to determine the essential oil content of studied populations as one the index factor in selecting of favorable populations, flower and aerial parts of plants were collected and dried in shade, then essential oils were extracted by water distillation method for 4 h based on Britain pharmacopeia.

Morphological data were analyzed by SAS program for analysis of variance. Mean of values were compared using

Table 2 Descriptive statistics for 34 morphological traits among studied *Leonurus cardiaca* populations

No.	Character	Abbreviation	Unit	Min.	Max.	Mean	CV%
1	Plant height	PH	cm	47	167	93.79	16.92
2	Plant width	PW	cm	10	86	36.97	32.37
3	No. of leaves	NL	No.	22	224	78.19	40.05
4	No. of main stems	NMS	No.	1	35	7.91	52.18
5	Length of main stems	LMS	cm	46	155	88.52	17.87
6	Main inflorescence length	MIL	cm	5.6	76	27.94	34.46
7	No. of floral cycles in main stem	NFCMS	No.	8	40	16.98	25.47
8	Floral cycles of main stem	NFFCMS	No.	13	32	20.93	39.60
9	Stem color	SC	Code	3	7	–	–
10	Congestion of upper floral cycles	CUFC	Code	3	5	–	–
11	No. of lateral branches	NLB	No.	0	13	4.65	50.39
12	Lateral branches length	LBL	cm	0	62.33	17.49	55.99
13	Lateral inflorescences length	LIL	cm	0	46.5	9.31	82.47
14	No. of floral cycles lateral inflorescence	NFCLI	No.	0	24.67	6.43	64.47
15	No. of flowers/floral cycles of lateral branches	NF/FCL	No.	0	24	13.96	30.77
16	Basal leaf length	BLL	cm	4.13	12.33	7.51	15.28
17	Basal leaf width	BLW	cm	3	11.47	5.98	19.75
18	Basal leaf length/width	BLL/W	cm	0.76	2.30	1.30	14.98
19	Petiole length of basal leaves	PLBL	cm	1	6.97	2.85	26.25
20	Length of basal internodes	LBI	cm	4.67	20.33	10.56	20.70
21	Floral leaf length	FLL	cm	2.43	41.33	6.38	45.98
22	Floral leaf width	FLW	cm	1	6.77	3.06	26.32
23	Floral leaf length/width	FLL/W	cm	1.27	9.92	2.13	34.06
24	Petiole length of floral leaf	PLFL	cm	0.63	4	1.99	20.96
25	Interval between two floral cycles	IBFC	cm	0.63	13.67	3.29	39.78
26	Leaf thickness	LT	mm	0.14	0.56	0.30	20.66
27	Petiole diameter	PD	mm	0.29	1.33	0.79	20.50
28	Upper surface color of leaf	USCL	Code	5	7	–	–
29	Lower surface color of leaf	LSCL	Code	3	5	–	–
30	Leaf area	LA	mm^2	400.27	3,890.78	1,570.91	31.35
31	Crown diameter	CD	mm	2.49	10.11	6.10	18.50
32	Flower length	FL	mm	7.47	12.82	10.18	8.35
33	Corolla color	CoC	Code	3	7	–	–
34	Calyx color	CaC	Code	3	7	–	–

analysis of variance and Dunkan's procedure. The simple correlation coefficient was calculated to determine the relationships between the studied traits and PCA was performed using the SPSS software. Morphological variables were used to calculate the Euclidean distance for all pairs of individuals using the Simgend module of NTSYS-pc software version 2.01 (Rohlf 2000). These distance coefficients were used to construct a dendrogram using the unweighted pair group method with arithmetic averages (UPGMA) employing the sequential, agglomerative, hierarchical, and nested clustering algorithm. Also, scatter plot of the first two PCs was created by PAST statistics software.

Results

Characteristics of individuals

The variance analysis on 34 morphological characters showed that the differences between populations were statistically significant. Due to high diversity in the measure traits, it is more probable that we can use those from the future breeding programs in order to obtaining superior genotype. *Leonurus cardiaca* as described by other authors and found in this study is a very variable species. Opinions about the dividing the species into subspecies are very controversial. In the plant list database, all the varieties and

subspecies were considered as synonyms and *L. cardiaca* is the only accepted name. In this study, we followed Rechinger's classification (1982), which divided the species into four subspecies. During specimens collection, we found only two subspecies including subsp. *cardiaca* and subsp. *persicus*. Although subspecies *turkestanicus* has been described from Kopea-Dagh by Rechinger in (1982), we could not find any specimens of this subspecies in mentioned area, specimens collected from Tandoure-Park in Dargaz, a part of Kopea-Dagh mountain, were identified as subspecies *persicus*.

Descriptive statistics for each of studied characters were presented in Table 2 (minimum, maximum, mean and coefficient of variation). Mean values of the studied morphological characteristics showed considerable variations between individuals for all of characters. In overall, variability of the morphological characters in examined populations was summarized as follows: plants were 47–167 cm tall, with number of 1–35 main stems covered with smooth hairs. Specimens from Khansar, Sarab and Taleghan populations were conspicuously taller (96–115 cm) than Kerman, Dargaz and Sari (55–77 cm). Plants from Taleghan have more main stems than other populations. Leaves were ovate in outline, lamina 4–12 × 3–11 cm, 3–7 lobed, pilose to densely pubescent with many small sessile oil glands below. Petioles were 1–7 cm in length. Kerman and Dargaz populations were distinguished from other studied populations by lower degree of leaves density and also leaf length (5–7 cm). Sari and Dargaz compare to other populations showed densely pubescent leaves.

Inflorescences were 5.60–76 cm in length, including floral cycles in axes of floral leaves, distant below and approximating somewhat above, possess 8–40 floral cycles in each inflorescence. Based on length of inflorescence and number of flowers in each cycle two distinct groups of populations recognized: the first, populations with inflorescence length of 14–30 cm and 12–14 flowers in a cycle including Kerman, Dargaz and Sari and the second, populations with 35–76 cm long of inflorescence and 13–32 flowers in a cycle including Taleghan, Khansar and Sarab. Flowers were 7.47–12.82 mm in length, sessile, calyx 2–3 mm long. Lateral inflorescence length expressed the highest variability (CV = 82.47 %) and was followed by number of floral cycles in lateral branches, lateral branch length, number of branches, number of lateral branches and floral leaf length.

Simple correlations

Table 3 indicates that some studied characters are significantly correlated. The highest correlation was observed between plant height and length of main stems and between lateral branch length and lower surface color of leaf. These correlations indicated probably that two linked genetic factors are controlling both characters. As expected, there were significant correlation between basal leaf width and floral leaf width, basal leaf length and floral leaf length at 1 % level. Number of leaves also was correlated to lateral inflorescence length at 1 % level, to number of flower in each floral cycle of main stem and number of floral cycles in lateral stem at 5 % level. Main stem length was correlated with some traits such as crown diameter and length of basal internodes at 1 % level and with main and lateral inflorescence length and number of floral cycles in main stem at 5 % level. Main and lateral inflorescences length showed significantly high correlation with the number of floral cycles of main stem and lateral inflorescence length at 5 % level and with number of floral cycles in lateral stem at 1 % level. High degree of correlation also was observed between leaf area with leaf length and petiole length. In other word, with increase in leaf length and petiole length, leaf area as the main place to trap sunlight for photosynthesis, is increased and consequently it provides the condition to produce secondary metabolites by increasing primary metabolites. Taleghan and Khansar populations possess more leaf area than others. Basal internodes length showed positive correlation with main stem length and plant height. Plant height which is an important character in medicinal plant breeding for mechanical harvest, showed significant positive correlation with number and length of main stem, basal internodes length, number of floral cycles and number of flowers in each main stem.

Factor analysis

Factor analysis was used based on principal components to provide a reduced dimension model, indicating differences measured among groups. This analysis has been widely used as a data reduction method to analysis multiple data categories of morphological characteristics and it has proven to be a valuable tool to understand relationships between characteristics as well as between populations. For each factor, loading values above 0.60 were considered as significant. Thus, the studied variables were placed in five components with explaining 98.83 % of the total variance. The first three components explained 82.31 % of the total variability observed (Table 4). PCA results indicated that the first component (PC1) related to 19 characters such as plant height, length of main stems, main inflorescence length, floral cycles of main stem, lateral branches length, basal leaf length, floral leaf length width, flower length and calyx color, accounted for 40.60 % of the total variation, indicated that those are the most important traits and therefore found to be most useful for studying the variability of *L. cardiaca* populations. The second component (PC2) which explained 27.25 % of the total variation, was

Table 3 Correlation coefficient between studied traits in *Leonurus cardiaca*

	PH	PW	NL	BLL	FLL	BLW	FLW	BLL/W	FLL/W	PLBL	PLFL
PH	1.00										
PW	−0.31	1.00									
NL	0.60	0.31	1.00								
BLL	0.71	−0.14	0.77	1.00							
FLL	0.71	−0.14	0.77	0.98**	1.00						
BLW	0.43	−0.66	−0.03	0.60	0.60	1.00					
FLW	0.43	−0.66	−0.03	0.60	0.60	1.00**	1.00				
BLL/W	0.03	0.67	0.55	−0.06	−0.06	−0.70	−0.70	1.00			
FLL/W	0.26	0.31	0.89*	0.71	0.71	−0.03	−0.03	0.41	1.00		
PLBL	0.66	−0.66	0.37	0.83*	0.82*	0.81*	0.81*	−0.49	0.37	1.00	
PLFL	0.77	−0.43	0.66	0.94**	0.94**	0.66	0.66	−0.20	0.60	0.94**	1.00
LT	0.26	−0.26	0.14	−0.09	−0.09	−0.37	−0.37	0.06	0.09	0.09	0.14
PD	0.31	−0.70	−0.31	0.14	0.14	0.66	0.66	−0.67	−0.31	0.66	0.43
USCL	−0.15	0.15	−0.32	−0.23	−0.23	0.17	0.17	0.12	−0.46	−0.32	−0.38
LSCL	−0.52	−0.33	−0.52	−0.09	−0.09	0.52	0.52	−0.52	−0.21	0.15	−0.09
LA	0.77	−0.43	0.66	0.94**	0.94**	0.66	0.66	−0.20	0.60	0.94**	0.98**
NMS	−0.37	0.49	−0.43	−0.37	−0.37	−0.14	−0.14	−0.23	−0.43	−0.49	−0.54
LMS	0.99**	−0.31	0.60	0.71	0.71	0.43	0.43	0.03	0.26	0.66	0.77
CD	0.99**	−0.35	0.64	0.75	0.75	0.46	0.46	0.07	0.32	0.70	0.81*
LBI	0.94**	−0.37	0.37	0.54	0.54	0.49	0.49	−0.03	−0.03	0.54	0.60
IBFC	0.37	0.43	0.26	−0.03	−0.03	−0.43	−0.43	0.26	−0.03	−0.26	−0.09
NLB	−0.09	0.77	0.71	0.26	0.26	−0.49	−0.49	0.81*	0.77	−0.26	0.03
LBL	0.66	0.37	0.71	0.31	0.31	−0.37	−0.37	0.64	0.37	−0.03	0.26
SC	−0.46	0.64	0.00	−0.52	−0.52	−0.73	−0.73	0.80	−0.03	0.81*	−0.67
MIL	0.83*	−0.03	0.77	0.54	0.54	−0.09	−0.09	0.41	0.49	0.37	0.60
NFCMS	0.83*	−0.26	0.77	0.71	0.71	0.26	0.26	0.35	0.54	0.60	0.77
NFFCMS	0.60	−0.03	0.89*	0.94**	0.95**	0.37	0.37	0.17	0.89*	0.71	0.88*
CUFC	0.09	0.49	0.37	0.09	0.09	−0.49	−0.49	0.17	0.37	−0.14	0.03
LIL	0.71	0.20	0/95**	0.66	0.66	−0.09	−0.09	0.64	0.71	0.31	0.60
NFC.LI	0.54	0.26	0.82*	0.43	0.43	−0.26	−0.26	0.81*	0.60	0.09	0.37
NFFCLB	−0.14	0.26	0.60	0.31	0.31	−0.26	−0.26	0.52	0.83*	0.09	0.26
FL	−0.54	−0.03	−0.54	−0.37	−0.37	−0.09	−0.09	−0.61	−0.26	−0.14	−0.31
CoC	−0.06	0.00	0.06	−0.32	−0.32	−0.41	−0.41	0.66	−0.06	−0.35	−0.26
CaC	−0.41	0.23	−0.52	−0.26	−0.26	0.12	0.12	−0.54	−0.41	−0.23	−0.38

	LT	PD	USCL	LSCL	LA	NMS	LMS	CD	LBI	IBFC	NLB
PH											
PW											
NL											
BLL											
FLL											
BLW											
FLW											
BLL/W											
FLL/W											
PLBL											
PLFL											
LT	1.00										

Table 3 continued

	LT	PD	USCL	LSCL	LA	NMS	LMS	CD	LBI	IBFC	NLB
PD	0.26	1.00									
USCL	0.81*	−0.15	1.00								
LSCL	−0.64	0.33	0.40	1.00							
LA	0.14	0.43	−0.38	−0.09	1.00						
NMS	−0.37	−0.49	0.29	0.03	−0.54	−0.54	−0.37				
LMS	0.26	0.31	−0.15	−0.52	0.77	0.77	0.71				
CD	0.20	0.35	−0.10	−0.43	0.81*	0.83*	0.75	1.00			
LBI	0.09	0.37	0.15	−0.40	0.60	0.60	0.54	0.92**	1.00		
IBFC	0.43	−0.43	−0.26	0.89*	−0.09	−0.09	−0.03	0.23	0.31	1.00	
NLB	−0.14	−0.77	−0.06	−0.27	0.03	0.03	0.26	−0.03	−0.26	0.09	1.00
LBL	0.43	−0.37	−0.23	0.94**	0.26	0.26	0.31	0.61	0.54	0.77	0.43
SC	−0.23	−0.64	0.46	−0.09	−0.67	−0.67	−0.52	−0.41	−0.38	−0.03	0.58
MIL	0.60	0.03	−0.46	0.98**	0.60	0.60	0.54	0.81*	0.66	0.54	0.26
NFCMS	0.26	0.26	−0.17	−0.40	0.77	0.77	0.71	0.90*	0.71	0.03	0.26
NFFCMS	0.03	0.03	−0.35	−0.15	0.88*	0.86*	0.95**	0.67	0.37	−0.09	0.49
CUFC	0.54	−0.49	−0.70	−0.70	0.03	0.03	0.09	−0.03	−0.14	0.77	0.31
LIL	0.26	−0.20	−0.23	−0.64	0.60	0.60	0.66	0.75	0.54	0.31	0.60
NFC.LI	0.20	−0.26	−0.06	−0.58	0.37	0.37	0.43	0.61	0.43	0.20	0.66
NFFCLB	0.09	−0.26	−0.35	0.03	0.26	0.26	0.31	−0.03	−0.37	−0.37	0.77
FL	0.20	0.03	−0.44	0.21	−0.31	−0.31	−0.37	−0.64	−0.60	0.09	−0.37
CoC	0.09	0.00	0.31	−0.09	−0.26	−0.26	−0.32	0.04	0.03	−0.26	0.26
CaC	−0.38	−0.23	0.16	0.28	−0.38	−0.38	−0.26	−0.52	−0.32	0.20	−0.32

	LBL	SC	MIL	NFCMS	NFFCMS	CUFC	LIL	NFCLI	NFFCLB	FL	CoC	CaC
PH												
PW												
NL												
BLL												
FLL												
BLW												
FLW												
BLL/W												
FLL/W												
PLBL												
PLFL												
LT												
PD												
USCL												
LSCL												
LA												
NMS												
LMS												
CD												
LBI												
IBFC												
NLB												
LBL	1.00											
SC	0.15	1.00										

Table 3 continued

	LBL	SC	MIL	NFCMS	NFFCMS	CUFC	LIL	NFCLI	NFFCLB	FL	CoC	CaC
MIL	0.88*	−0.17	1.00									
NFCMS	0.60	−0.15	0.83*	1.00								
NFFCMS	0.37	−0.32	0.60	0.77	1.00							
CUFC	0.54	−0.15	0.43	−0.09	0.14	1.00						
LIL	0.82*	0.09	0.87*	0.85*	0.77	0.26	1.00					
NFC.LI	0.77	0.38	0.77	0.83*	0.60	0.09	0.94**	1.00				
NFFCLB	0.09	0.32	0.20	0.37	0.60	0.09	0.49	0.54	1.00			
FL	−0.49	−0.38	−0.49	−0.77	−0.43	0.43	−0.71	0.82*	−0.26	1.00		
CoC	0.17	0.72	0.15	0.35	−0.12	−0.46	0.29	0.58	0.35	−0.67	1.00	
CaC	−0.41	−0.25	−0.61	−0.812*	−0.46	0.23	−0.70	0.82*	−0.58	0.73	−0.77	1.00

dominated by eight characteristics such as basal leaf width, floral leaf width, petiole diameter and leaf area. Furthermore, the characteristics related to number of main stems, stem color and corolla color performed as the third main factor (PC3) and explained 14.46 % of the total variance. In conclusion, according to this study, all plant height, leaf and flower characteristics showed the highest discriminating values and can be used for evaluation of phenotypic diversity in *L. cardiaca*.

Cluster and scatter plot analysis

The UPGMA dendrogram of the entire data set based on all the studied morphological characters generated two main clusters for 150 individuals of *L. cardiaca* with high variability within and among populations (not shown), so that, a high variation was observed between individuals of a population and they were grouped with individuals of other populations in some cases. For instance, individuals of Kerman population were placed in several subgroups with high dissimilarities. Phenotypic relationship among studied individuals was also visualized by creating scatter plot according to the PC1 and PC2. Results of scatter plot supported the results of cluster analysis and individuals were distributed in four sides of scatter plot (Fig. 3) and revealed high inter- and intra-population relationships in studied germplasm.

In the UPGMA dendrogram based on population analysis (Fig. 4), the studied six populations were placed in two main clusters. Four populations of subsp. *cardiaca* including Khansar, Sari, Sarab and Kerman were grouped together in the first cluster and separated from the other populations by pubescence of stems and leaves, more number of floral cycles, higher congestion of floral cycles, lower number of lateral branches, shorter length of petioles and leaves and shorter floral leaves.

Dargaz and Taleghan populations identified as subsp. *pesicus* and *cardiaca*, respectively, were placed in second clusters due to high morphometric variability, number. Within the *cardiaca* subspecies populations in first cluster, Kerman and Sari populations formed a separate group, distinct from the two other populations of the group by differences in lengths of main stems, main inflorescence, lateral inflorescences, floral leaf, basal internodes, in, number of floral cycles in main stem, number of flowers in each cycle and leaf area.

Essential oil content

Essential oil yields varied from 0.02 (for Taleghan population) to 0.053 mg/100 g dry mater (for Kerman population). Sarab, Sari, Dargaz and Khansar populations had 0.041, 0.029, 0.027 and 0.026 mg/100 g dry mater essential oil, respectively. The essential oil contents of the species can be affected by climate or genetic parameters or interaction between them.

Discussion

Morphological analysis has been employed in the genotypic identification and phenotypic characterization of selected *L. cardiaca* populations in Iran. Based on the morphological characters, Kerman and Sari were the most related, while Dargaz and Taleghan were the most isolated populations. Morphological variation was high and high enough to support the conclusion that the selected material for the *ex situ* gene conservation plantation is genetically diverse, especially when compared with values reported for *L. cardiaca* collections. This result is also important for *L. cardiaca* breeding as the success of a breeding program largely depends on the availability of a wide genetic base. Also, the maintenance of genetic variation is one of the major objectives in conserving endangered and threatened species (Hamrick and Godt 1989). Knowledge of genetic variation within and among populations provides

Table 4 Eigenvectors of the first principal component axes from PCA analysis of studied *Leonurus cardiaca* populations

Character	Component		
	PC1	PC2	PC3
Plant height	0.86**	0.29	0.28
Plant width	−0.13	−0.70**	0.31
No. of leaves	0.81**	−0.51	−0.06
No. of main stems	−0.58	−0.43	0.69**
Length of main stems	0.86**	0.22	0.29
Main inflorescence length	0.70**	0.06	0.40
No. of floral cycles in main stem	0.89**	−0.03	−0.04
floral cycles of main stem	0.84**	0.02	−0.38
Stem color	0.19	−0.44	0.76**
Congestion of upper floral cycles	−0.04	−0.67	−0.69**
No. of lateral branches	0.44	−0.63**	−0.28
Lateral branches length	0.72**	−0.48	0.40
Lateral inflorescences length	0.80**	−0.47	0.02
No. of floral cycles/lateral inflorescence	0.78**	−0.44	−0.31
No. of flowers/floral cycles of lateral branches	0.78**	−0.29	−0.39
Basal leaf length	0.80**	0.54	0.01
Basal leaf width	0.28	0.95**	0.01
Basal leaf length\ width	0.55	−0.82**	−0.09
Petiole length of basal leaves	0.44	0.82**	0.02
Length of basal internodes	0.66**	0.35	0.38
Floral leaf length	0.68**	0.56	−0.07
Floral leaf width	0.35	0.87**	−0.10
Floral leaf length/width	0.76**	−0.37	−0.15
Petiole length of floral leaf	0.69**	0.54	−0.21
Interval between two floral cycles	0.38	0.01	0.82**
Leaf thickness	0.40	−0.22	0.56
Petiole diameter	0.19	0.87**	0.04
Upper surface color of leaf	−0.35	0.22	−0.55
Lower surface color of leaf	−0.61**	0.58	−0.49
Leaf area	0.58	0.70**	0.01
Crown diameter	0.93**	0.19	0.12
Flower length	−0.69**	0.26	0.29
Corolla color	0.39	−0.45	−0.60**
Calyx color	−0.82**	0.24	0.43
Eigenvalue	13.80	9.26	4.92
% Variance	40.60	27.25	14.46
% Cumulative	40.60	67.85	82.31

** Eigen values significant ≥ 0.60

information essential in the formulation of appropriate management strategies for conservation (Milligan et al. 1994; Francisco-Ortega et al. 2000). First, it must be prohibited to maintain effective population sizes because population size is an important restoration consideration in endangered and rare species (Allendorf 1986). Cruse-Sanders et al. (2005) suggested that conserving a proportion of the mature individuals in populations is important to the protection of reproductive fitness and the evolutionary potential of the species. Second, the construction of an in situ conservation area is an ideal way to protect wild *L. cardiaca* genetic resources. It will result in effective conservation of their genetic resources and the evolution of the resources under natural environments. Third, it is necessary to protect existing natural populations in order to preserve as much genetic variety as possible. Forth, *ex situ* conservation based on seed harvest from multiple sources must be carried out to capture most of the genetic variability existed among populations. As an important traditional medicinal plant, promoting domestication and cultivation of this wild

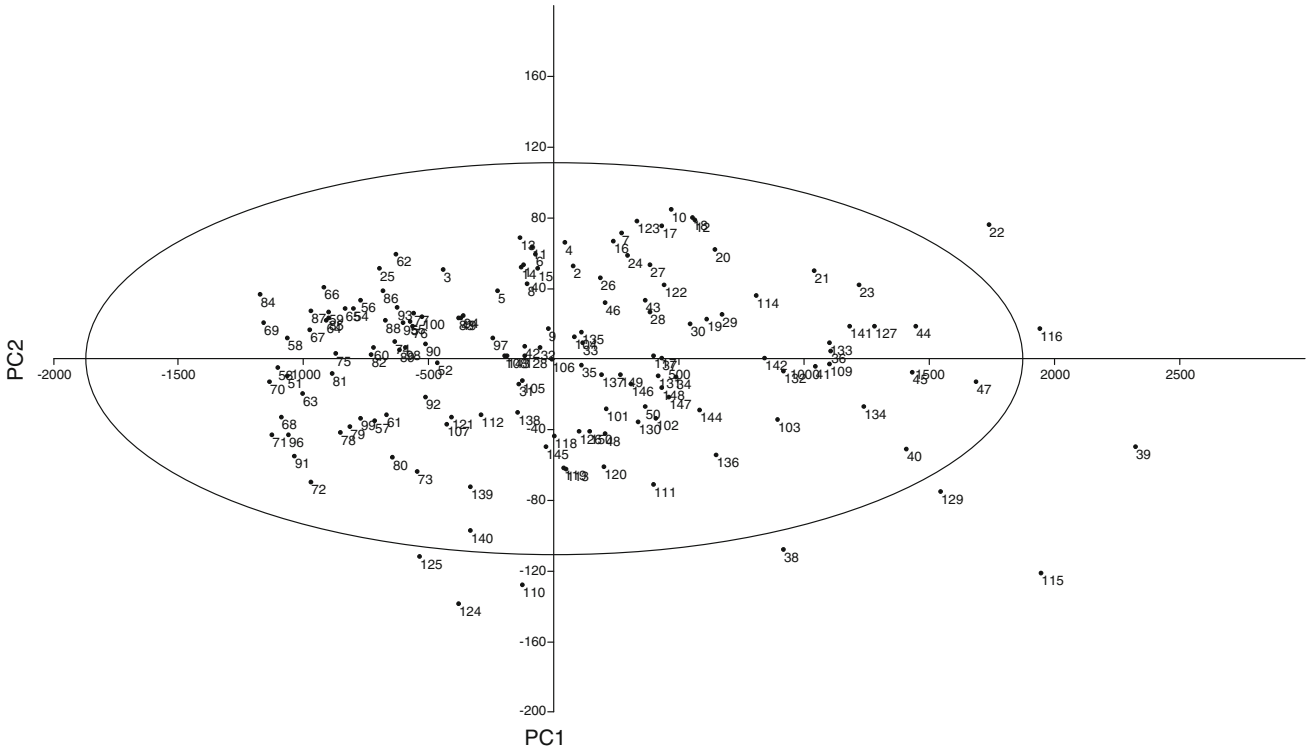

Fig. 3 Scatter plot for the first two principal components (PCs) for studied individuals of *Leonurus cardiaca* based on morphological characters (for explanation of individual numbers, see Table 1)

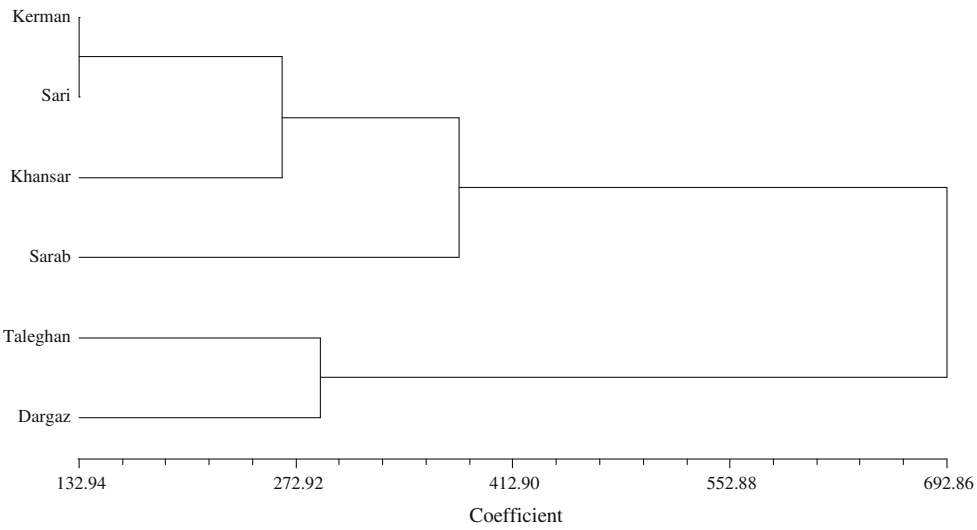

Fig. 4 UPGMA dendrogram of population analysis for *Leonurus cardiaca* using Euclidean distance

resource are necessary both to satisfy market demand and protect the wild resource. Successfully cultivation may decrease the harvest of wild populations of *L. cardiaca*, and contribute to the protection of this important medical plant.

Essential oils of plants and their other products from secondary metabolism have a great usage in folk medicine, food flavoring, fragrance, and pharmaceutical industries (Alma et al. 2004). Studied populations showed suitable essential oil content and could be used as an candid for this product. Finally, the identification and selection of populations that are genetically far distance from others can be used for performing breeding work, so that Dargaz population crosses with other populations can be applied as an excellent option for producing hybrids with desired functionality. Collecting seeds and transferring seedlings from different populations to the suitable habitats for *L. cardiaca* and artificially increase the gene flow among populations.

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