African Journal of Biotechnology Vol. 11(48), pp. 10859-10867, 14 June, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB12.513
ISSN 1684–5315 © 2012 Academic Journals

Full Length Research Paper

Inter-populations genetic and morphological diversity in three *Silene* (Caryophyllaceae) species

Masoud Sheidai[1*], Roza Eftekharian[1], Zahra Noormohammadi[2] and Abbas Gholipoor[3]

[1]Shahid Beheshti University, GC, Faculty of Biological Sciences, Tehran, Iran
[2]Biology Department, School of Basic Sciences, Science and Research Branch, Islamic Azad University (SRBIAU), Poonak, Tehran, Iran.
[3]Department of Biology, Faculty of Science, Payame Noor University, P. O. Box 19395-3697 Tehran, Iran.

Accepted 30 March, 2012

The inter-populations morphological and genetic variations were studied in three species of *Silene* (*Silene indeprensa, Silene gynodioica* and *Silene crispans*) of the section *Auriculatae*, which grow and form several populations in different regions of Iran. Morphological analysis of variance (ANOVA) and analysis of molecular (AMOVA) analyses showed the species distinctness. Unweighted paired group with arithmetic average (UPGMA) clustering of morphological characters and neighbor-joining (NJ) tree of molecular features almost separated the species from each other. In *S. gynodioica*, Soltaneiyes and Gheydar populations differed from the other populations in both morphological and molecular features, and in case of *S. indeprensa*, Ghorkhood and Hezarmasjed populations differed significantly from the other populations in both morphological and molecular features. Therefore, these populations are considered as a new subspecies in these two species. Some of the populations showed the presence of specific inter-simple sequence repeat (ISSR) band/locus, indicting genetic divergence of the populations possibly either due to local adaptations or genetic drift. Mantel test performed did not show significant correlation between morphological/molecular distance and geographical distance of the populations studied.

Key words: Diversity, ISSR, morphometry, *Silene*.

INTRODUCTION

The genus *Silene* L. (Caryophyllaceae) has about 700 species with world-wide distribution. These species mainly grow in the northern hemisphere, Europe, Asia and northern Africa (Greuter, 1995) and are annual, biennial or perennial herbs. Several important weedy species as well as horticultural plants are included in this genus. About 110 *Silene* species grow in Iran out of which about 35 species are endemic with very limited geographical distribution (Melzheimer, 1988). Three species of *S. indeprensa, S. gynodioica* and *S. crispans* of the sect *Auriculatae* grow in different regions of Iran having several geographical populations. Our field observations and previous cytological study in these 3 species, revealed variations at the population level (Sheidai et al., 2011), including the polyploidy level (2*n* = 2*x* = 24, 2*n* = 4*x* = 48 and 2*n* = 8*x* = 96), the size of chromosomes, occurrence of structural changes in the chromosomes and genetic potential of some populations to produce unreduced (2n) pollen grains (Sheidai et al., 2011). Therefore, we decided to investigate the inter-population morphological and molecular variations in different populations of these three species and find out if such variations are correlated to the geographical distance of the populations studied.

A native species varies genetically in its adaptation to the particular localities and environmental conditions under which it grows. This results in a number of ecotypes of the same species or gradations (clines) between populations. Genetically based variations have been correlated with various environmental factors including

*Coresponding author E-mail: msheidai@yahoo.com

Table 1. The species, populations and their localities.

S/N	Taxon	Locality
1	*S. gynodioica* (gyn1)	Kuhgyloieh va Boyer Ahmad, Yasuj, Dena, 30 53 245 N, 51 30 225, 3200 m, 1386/03/26, 880017, Abbas gholipour
2	*S. gynodioica* (gyn2)	Chaharmahal-o Bakhtiari, Farsan, Kuhrang, Zard kuh, 32 18 704 N 50 08 574 E, 3300-3400 m, 1387/05/08, 88007, Abbas Gholipour
3	*S. gynodioica* (gyn3)	Esfahan, Samirem to Shahreza, Mehrgerd, Vardasht, 31 34 623 N 51 32 169 E,2430m, 1386/3/28,880021, Abbas gholipour
4	*S. gynodioica*(gyn4)	Kuhgyloieh va Boyer Ahmad, Yasuj, Cheshme mishi, 30 51 42.90 N 51 30 13.6 E, 2789 m, 1389/3/16, 880022, Abbas Gholipour.
5	*S. gynodioica* (gyn5)	Kashan,Qohroud, 33 37 751 N 51 23 579 E, 2700m, 1386/03/24, 880016, Abbas Gholipour.
6	*S. gynodioica* (gyn6)	Fars, Saadatshahr, ghavam abad, 29 34 41/76 N 53 20 16/64 E, 1387/05/04,88009, Abbas Gholipour.
7	*S. gynodioica* (gyn7)	Ardebil, Miane to Khalkhal, 37 37 3.92 N 48 31 35.57 E 1900m,1385/3/18,880023, Abbas Gholipour.
8	*S. gynodioica* (gyn8)	Kuhgyloieh va Boyer Ahmad, Yasuj, Sisakht, 30 52 01.36 N 51 26 59.58 E, 2789 m, 1389/3/16,880024, Abbas Gholipour.
9	*S. gynodioica* (gyn9)	Esfahan, Padena,30 52 25.39 N 51 30 17.94 E, 2700 m, 1387/05/04,880025, Abbas Gholipour.
10	*S. gynodioica* (gyn10)	Kashan, ghamsar,33 44 48.89 N 51 25 49.92 E, 1386/03/24, 880026, Abbas Gholipour.
11	*S. gynodioica* (gyn11)	Esfahan, Samirem to Shahreza, Mehrgerd, Vardasht to Shahrekord, 31 32 901 N, 51 27 783 E, 2900m, 1386/3/28,880027, Abbas Gholipour.
12	*S. gynodioica* (gyn12)	Fars, Eqlid, Bel mountain, 30 49 N 52 43 E, 2700-2900 m, 1387/02/13,880010, Abbas Gholipour
13	*S. gynodioica* (gyn13)	Zanjan, Soltaniieh, Salvar village, 36 21 969 N 48 43557 E, 2064 m, 1386/03/17, 880014, Abbas gholipour
14	*S. gynodioica* (gyn14)	Zanjan, Soltaniieh, Qidar (Kuh-e ghalleh bayer) 36 09 014 N 48 31 351 E, 2182 m, 1386/03/17, 880015, Abbas Gholipour.
15	*S. gynodioica* (gyn15)	Esfahan, Golpayegan to Khansar, Vaneshan village, 33 20 N 50 21 E, 1990m,1387/2/12,880028, Abbas Gholipour.
16	*S. gynodioica* (gyn16)	Kuhgyloiieh- va- Boyer ahmad, Ardakan, to Yasuj, Kakan,30 37 14.24 N 51 47 12.95, 2700 m, 1387/05/04,880011, Abbas Gholipour.
17	*S. crispans* (cris1)	North Khorasan, Ghochan to Daregaz, 37 23 5.2 N 58 31 33.5 E, 1779 m, 1389/3/17,88005, Abbas Gholipour.
18	*S. crispans* (cris2)	Mashhad, ferdos, ardak, talghur, 36 47 457 N, 59 25 207 E, 1420m, 1386/3/5,880020, Abbas gholipour
19	*S. indeprensa* (indep1)	North Khorasan, Chamanbid, Ghorkhod protected area, Zard village, 37 30 52.9 N 56 28 56.7 E, 1525-60 m, 1389/3/19,88003, Abbas Gholipour.
20	*S. indeprensa* (indep2)	Khorasan, Chenaran, Boghmadj, Hezar masjed mountains, 37 00 25.2 N 59 09 19.6 E, 2789 m, 1389/03/16,880019, Abbas Gholipour.
21	*S. indeprensa*(indep3)	North Khorasan, between Ashkhaneh and Robat gharbil, Jozak, 37 25 17.7 N 56 40 14.2 E, 1289m, 1389/03/15,88001, Abbas gholipour
22	*S. indeprensa*(indep4)	Mashhad to kalat, sandoghshekan pass, 36 38 13.8 N, 59 52 31.7 E, 1880m, 1389/03/15, 88002, Abbas Gholipour.
23	*S. indeprensa*(indep5)	North Khorasan, Ghochan to Daregaz, 37 23 5.2 N 58 31 33.5 E, 1779 m, 1389/3/17,88004, Abbas Gholipour.

light, water, temperature, rain, etc. (Rapson and Wilson, 1992). A few molecular studies have been performed in the genus *Silene* to study the intra-specific diversity. These studies considered the occurrence of mitochon-drial heteroplasmy in the natural populations of *S. gynodioecious* and *S. vulgaris* (Welch et al., 2006), genetic differen-tiation and demographic adaptation of the *S. latifolia* (Richards et al., 2003; Jolivet and Bernasconi, 2007) and *S. tatarica* populations (Tero, 2003) and studied the genetic structure of hybrid zones formed bet-ween *S. latifolia* and *S. dioica* (Minder et al., 2007).

In the present study, the inter-population morphological and molecular diversity are consi-dered together at both the population and at the species level in three *Silene* species growing in Iran with the aim to investigate if these variations are related to geographical distance among populations and if such changes have led to the formation of new taxonomic forms in each species.

MATERIALS AND METHODS

Morphological and molecular studies were performed in 23 populations (Table 1), including 16 populations of *Silene gynodioica* Ghazanfar, two populations of *S. crispans* Litw. and 5 populations of *S. indeprensa* Schischk. The vouchers specimens are deposited in the Herbarium of Shahid Beheshti University (HSBU), Iran (Table 1).

Morphological study

In total, 37 morphological characters (quantitative and

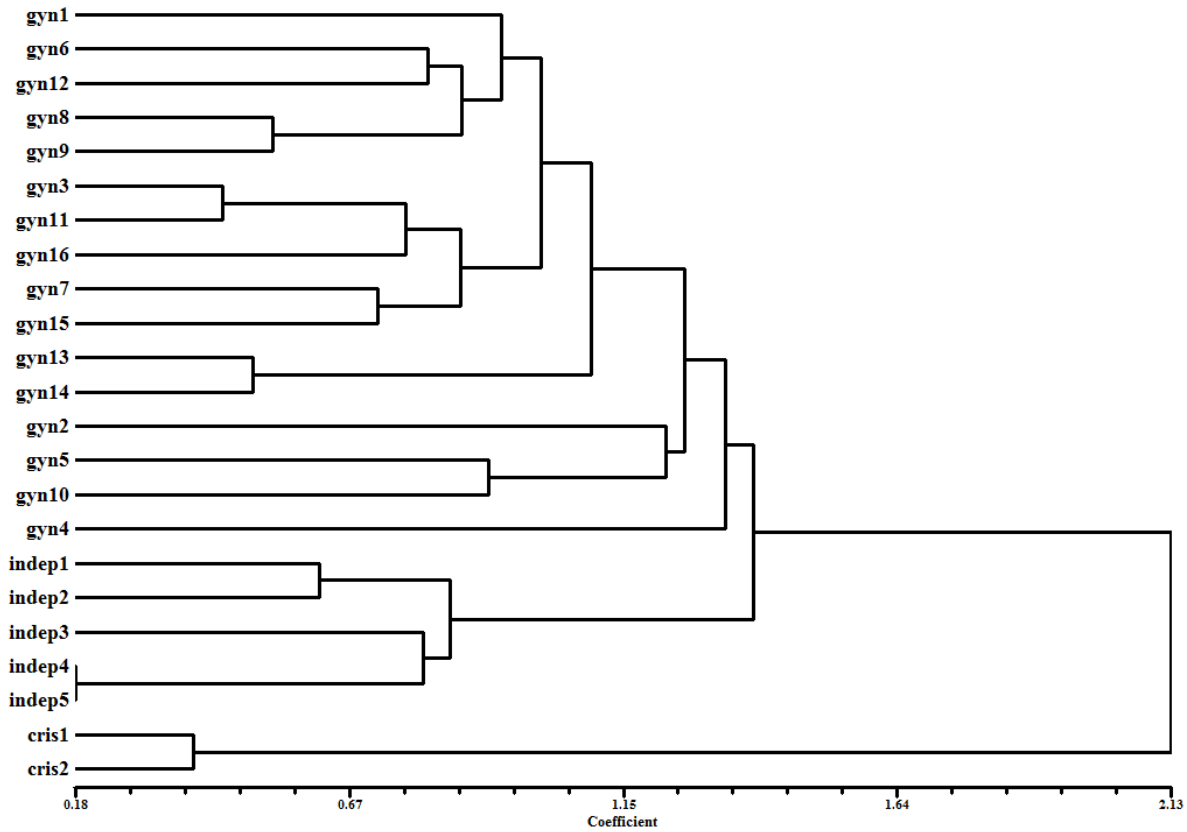

Figure 1. UPGMA tree of morphological characters by using Manhatan distance. Species code: gyn = *S. gynodioica*, indep = *S. indeprensa* and cris = *S. crispans*.

qualitative) were studied (Table 2). Analysis of variance test (ANOVA) was performed to show significant difference in quantitative morphological characters among the species. For multivariate analyses the mean of quantitative characters were used, while qualitative characters were coded as binary/multistate characters. Standardized variables (mean = 0, variance = 1) were used for statistical analyses. The average taxonomic distance and Manhattan distance were used as dissimilarity coefficients independently in cluster analysis of morphological data (Podani 2000). Principal Components Analysis (PCA) was performed to identify the most variable morphological characters and plot of the first and second component were used to investigate the species grouping (Podani, 2000).

ISSR assay

Total genomic DNA was extracted from fresh leaves using the cyltrimethylammonium bromide (CTAB) method by Murray and Thompson (1980) with the modification described by De la Rosa et al. (2002). Eight inter-simple sequence repeat (ISSR) primers (UBC807, UBC810, UBC811, UBC823, UBC849, $(GA)_9A$, $(GA)_9C$ and $(GA)_9T$) commercialized by UBC (the University of British Columbia) were used. Polymerase chain reaction (PCR) reactions were performed in a 25 µL volume containing 10 mM Tris–HCl buffer at pH 8, 50 mM KCl, 1.5 mM MgCl2, 0.2 mM of each dNTP, 0.2 µM of a single primer, 20 ng genomic DNA and 3 unit of Taq DNA polymerase (Bioron, Germany). Amplifications reactions were performed in Techne thermocycler (Germany) with the following program: 5 min initial denaturation step 94°C, 30 s at 94°C; 1 min at 50°C and 1 min at 72°C. The reaction was completed by a final extension step of 7 min at 72°C. Amplification products were visualized by running on 2% agarose gel, following ethidium bromide staining.

The ISSR bands obtained were treated as binary characters and coded accordingly (presence =1, absence = 0). Jaccard similarity as well as Nei's genetic distance (Nei, 1973) was calculated among populations. Unweighted Paired Group with Arithmetic Average (UPGMA), NJ (Neighbor Joining) algorithms and ordination based on principal coordinate analysis (PCO) and PCA (Podani 2000) were used to analyze population clustering. NTSYS Ver. 2.02 (1998) and DARwin ver. 5 (2008) were used for clustering and PCO analyses.

In order to determine molecular difference between populations, analysis of molecular variance (AMOVA) test was performed. The Mantel test with 999 random permutations was used to check the agreement between morphological and ISSR trees and also to test if correlation existed between the geographical, morphological and molecular distances. Consensus tree was built to combine both morphological and molecular trees obtained (Podani, 2000). The GENALEX 6 (Peakall and Smouse, 2006) was used for statistical analyses.

RESULTS

Morphometry

ANOVA test showed significant difference for almost all

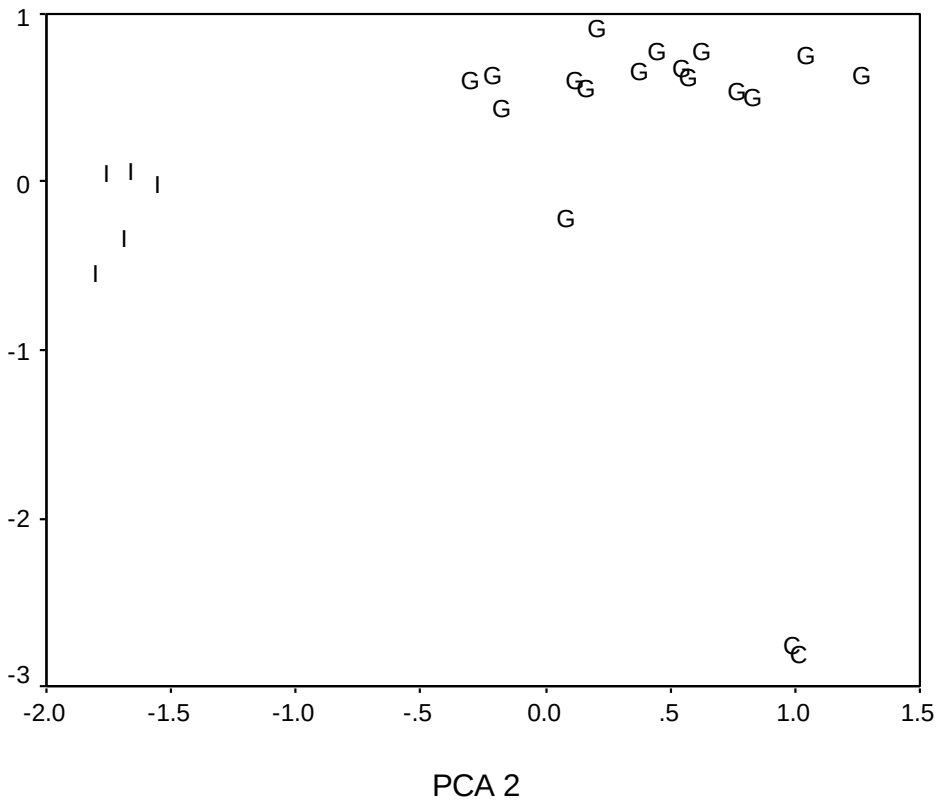

Figure 2. PCA plot of morphological data. Species code: G = *S. gynodioica*, I = *S. indeprensa* and C = *S. crispans*.

quantitative morphological characters studied except for basal leaf length, basal leaf width and stem width. UPGMA and NJ trees of morphological characters clearly separated the three species, a behavior (since both trees produced similar results, only UPGMA is given; Figure 1) also supported by PCA plot (Figure 2). However, almost within each species cluster, the populations differed somewhat from each other and were joined together with different distances. PCA analysis of morphological data revealed that the first 4 components comprise about 72% of total variance (data not shown). In the first component with about 31% of total variance, morphological characters including petal claw length, seed width, ratio of seed length/width and basal leaf form showed the highest positive correlation (>0.90), while characters ratio of basal leaf width/length and length of calyx tooth had the highest negative correlation (-0.80). In the second component with about 20% of total variance, calyx vein had the highest positive correlation (>0.90) while characters like the seed length and cauline leaf form had the highest negative correlation (-0.90). Therefore, these are the most variable morphological characters among the species studied.

Both UPGMA tree and PCA plot showed morphological variations among populations of each species. For example, Cheshmemishi, Zardkooh, Ghohrood and Absharemargoon populations (gyn4, 2, 5 and 10 in *S. gynodioica* respectively) differed from the other populations. Similarly, Forkhood and Hezarmasjed populations (indep1 and indep2, respectively) in *S. indeprensa*, differ from the other populations studied in this species and stood far from them.

ISSR analysis

The eight ISSR primers used in this experiment produced 143 reproducible bands (Figure 3), out of which 140 bands were polymorphic and 3 bands were monomerphic. The highest number of polymorphic bands (20 bands) was obtained for the ISSR primer $(GA)_9C$, while ISSR primers $(GA)_9A$ produced only 9 polymorphic bands. Two populations of Vardasht and Bel mountain in *S. gynodioica*, had the highest number of ISSR bands (38), while Dargaz population of *S. crispans* and Hezarmasjed in *S. indeprensa*, had the lowest number of bands (18). Some of the populations showed the presence of specific ISSR band, for example, Ghahrood population in *S. gynodioica* and Ashkhaneh population in *S. indeprensa* showed the highest number of specific bands (6). Moreover, Dena population of *S. indeprensa* was the only population showing ISSR band (910 bp) of

Table 2. Morphological characters and their code.

Character	Code				
Plant height	x<20	20≤x≤35	x>35		
Basal leaf length	x<35	35≤x≤55	x>55		
Basal leaf width	x<2.5	2.5≤x≤5	x>5		
Length/width	x≤0.5	x>0.5			
Basal	x<25	25≤x<35	x>35		
Cauline leaf length	x2.5>	2.5≤x≤5	x>5		
Cauline leaf width	x<0.8	0.8≤x≤0.11	x>0.11		
L cauline leaf width/length	x<2	2≤x≤5	5≤x≤10	x>10	
Alar pedicel length	x<15	15≤x≤20	21≤x≤32	x>32	
Calyx length	x<2.5	2.5≤x≤5	x>5		
Calyx tooth length	x<10	10≤x≤15	x>15		
Petal claw length	x<5	5≤x≤7	x>7		
Petal limb length	x<1.3	x>1.3			
Capsule length	x<7	7≤x≤10	x>10		
Antophore length	x<5	5≤x≤10	11≤x≤15	x>15	
Seed length	x<1.75	x>1.75			
Seed width	x<1.75	x>1.75			
Seed width/length	x<1.75	x>1.75			
Habit	Sufferutescent	Cespitose-sufferutescent	Cushine shape	Rhizomate	
Basal leaf form	Oblanceolate	Spatulate	Linear	Linear	
Cauline leaf form	Ovate	Cordate	Oblanceolate	Oblanceolate	Linear
Cauline leaf indumentum	Glandular	Absent			
Inflorescence type	Compound dichasium	Tyroside			
Calyx form	Campanulate-inflate				
Calyx outside indumentum	Glandular	Absent			
Calyx inside indumentum	Present				
Calyx veins	Parallel				
Capsule situation to calyx	Exerted from calyx				
Claw situation to calyx	Exerted from calyx				
Petal limb division length	Shorter than 1/2 limb	Longer than 1/2 limb			
Auricle size	Inconspicuous				
Alternate filament length	Shorter than epipetal	As long as epipetal			
Filament indumentum	Absent				
Style indumentum	Absent				
Capsule form	Oblong- elliptic	Subglobose			
Antophore indumentum	Present				
Testa cell projections	Mammillae				

the primer UBC 823, and similarly, Zardkooh population of this species was the only population having ISSR band (1050 bp) of the primer UBC823.

In *S. indeprensa*, Sandooghshekan population had one specific band (1470 bp) of the primer UBC 849, while Gholeghorhood population of this species had one specific band (1600 bp) of the primer UBC 849. Taltoor population of *S. crispans* had specific band (910 bp) of the ISSR primer $(GA)_9T$. The genetic diversity parameters determined in each species is given in Table 3. The highest values of Shannon index (I) and Nei's genetic diversity (He) were observed in the populations of *S. gynodioica* (0.27 and 0.17 respectively), but this result may have been affected by the different number of populations studied in each species. The AMOVA test showed significant molecular difference ($p<0.002$) with 24% of variance occurring among the species and 76% occurring within species (data not shown). The PCOA plot of ISSR data also almost separated the populations of 3 species studied based on their genetic differences (Figure 4), supporting AMOVA result.

A consensus tree was constructed (Figure 5) from morphological and molecular trees obtained, separating the populations of the 3 species studied in different

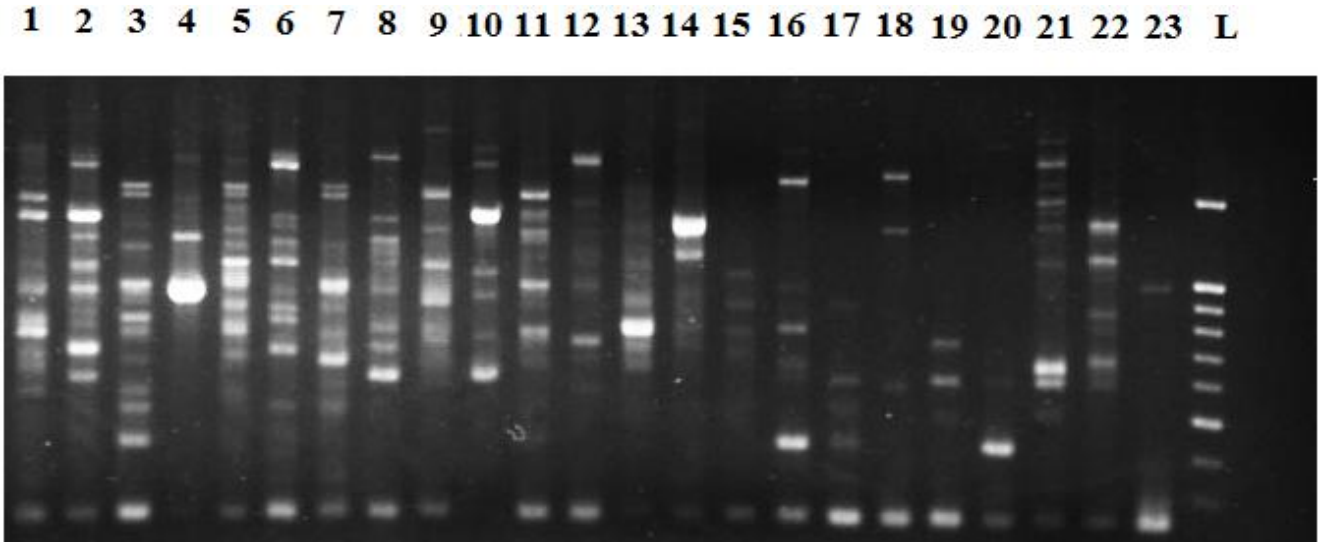

Figure 3. ISSR profile of primer UBC849 showing molecular polymorphism in the species and populations studied. Species and populations are from left to right as in the Table 1 (1-23), L = Molecular ladder.

Table 3. Genetic diversity parameters in *Silene* species studied.

Pop		N	Na	Ne	I	He
Pop1 (G)	Mean	16.000	1.490	1.254	0.277	0.170
	SE	0.000	0.071	0.022	0.019	0.013
Pop2(C)	Mean	2.000	0.301	1.074	0.063	0.043
	SE	0.000	0.054	0.018	0.016	0.011
Pop3(I)	Mean	5.000	0.825	1.158	0.167	0.104
	SE	0.000	0.080	0.021	0.019	0.013
Total	Mean	7.667	0.872	1.162	0.169	0.106
	SE	0.291	0.046	0.012	0.011	0.007

Ne: Number of effective alleles, Na ; number of Different Alleles, I: Shannon index and He: Nei's genetic diversity. Pop 1 (G) = *S. gynodioica*, pop2 (C) = *S. crispans* and pop3 (I) = *S. indeprensa*.

clusters. In the first major cluster formed by *S. gynodioica*, three subclusters were present. Two populations of Soltaneiye (gyn13) and Gheydar (gyn14) formed a single subcluster and stood far from the other populations studied, while Vardasht (gyn3) and Vardasht-Zardkooh (gyn11) populations joined each other and form another subcluster. Furthermore, in the major cluster formed by populations of *S. indeprensa* two subclusters were also formed. Two populations of Ghorkhood (indep1) and Hezarmasjed (indep2) formed a single subcluster which is placed far from the other 3 populations in this species. ANOVA and AMOVA tests showed significant difference for both quantitative morphological characters ($p<0.01$) and molecular characteristics ($p<0.001$) among the subclusters obtained in both *S. gynodioica* and *S. indeprensa* (data not shown).

Geographical distances of the populations studied in each species are given in Tables 4 and 5. Mantel test performed between morphological distance and geographical distance as well as between morphological distance and molecular distance did not show significant correlation in both *S. gynodioica* and *S. indeprensa*. A comparison of the morphological and molecular trees obtained arrived at the same conclusion. For example in the morphological tree, the first subcluster is formed by five *S. gynodioica* populations, viz. Sisakht, Padena, Dena mountain, Ghavamabad and Bel mountain (gyn8, gyn9, gyn1, gyn6 and gyn12 respectively). The first 3 populations are in close geographical vicinity in two provinces of Kuhgyloieh-va-Boyerahmad and Esfehan, while, two populations of Ghavamabad and Bel mountain

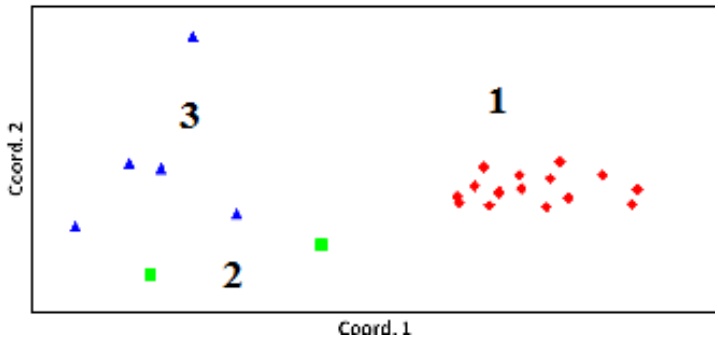

Figure 4. PCO plot of ISSR data. Pop 1-3 are: *S. gynodioica*, *S. indeprensa* and *S. crispans*, respectively.

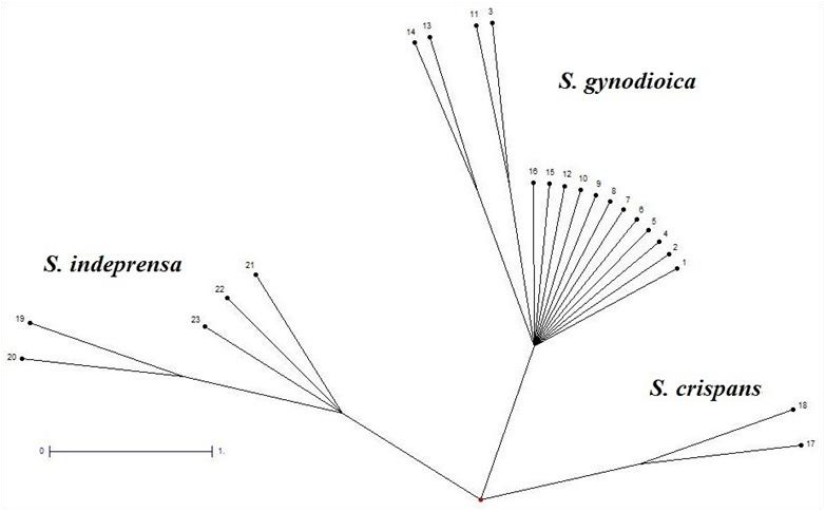

Figure 5. Consensus tree of morphological and ISSR trees.

Table 4. Geographical distance (Km) among *S. gynodioica* populations (populations 1-16 are as in Table 1).

Parameter	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1		197	124	10	304	236	787	5	4	311	163	138	655	639	302	51
2			125	208	194	430	602	204	208	200	72	313	293	450	127	246
3				125	180	301	691	131	129	192	101	168	564	553	217	152
4					299	232	787	9	6	317	171	129	670	645	307	38
5						489	512	312	314	9	138	332	390	381	109	339
6							997	230	223	498	389	136	868	856	520	193
7								795	800	501	624	843	129	164	489	833
8									4	322	173	134	667	647	304	43
9										317	171	130	670	646	308	40
10											165	339	381	375	112	348
11												254	498	474	134	205
12													720	705	387	90
13														41	360	697
14															340	682
15																350
16																

Table 5. Geographical distance (Km) among *S. indeprensa* populations (populations 1-5 are as in Table 1).

Parameter	1	2	3	4	5
1		244	35	284	225
2			220	67	89
3				256	192
4					76
5					

are from two distant regions in the Fars province. Therefore the populations forming this subcluster are from different provinces with different geographical distances. The same holds true for the second subcluster in which five *S. gynodioica* populations are placed close to each other. Two populations of Vardasht (gyn3) and Vardasht to Shahrekord (gyn11) with 101 km distance (both from Esfahan province) along with Kakan population (gyn16, from Kuhgyloieh-va-Boyerahmad are placed close to each other and two populations of Miane to Khalkhal (gyn7, from Ardebil province) and Vaneshan village (gyn15, from Esfahan province) with 520 km distance joined the others in the same subcluster. All these populations are from different geographical regions with different environmental conditions but show morphological similarities and are placed in a single subcluster.

DISCUSSION

Species comparison

Cluster tree and ordination plot of morphological characters confirm the species distinctness as they are separated in different groups containing all their populations together. ANOVA test indicated morphological difference of the species studied, while PCA analysis identified 8 morphological characters (petal claw length, seed width, ratio of seed length/width, ratio of basal leaf width/length, length of calyx tooth, calyx, seed length and cauline leaf) as the most variable morphological characters among the species studied. Therefore, combination of quantitative and qualitative morphological characters is well able to separate these species from each other. The AMOVA showed significant molecular differences among these species studied, indicating the genetic divergence.

All 8 ISSR primers used produced high level of molecular polymorphism ranging from 88.80% in the primer UBC807 to 100.00% in most of the other primers. The presence of ISSR polymorphism and population-specific bands of the species studied indicate the presence of inter populations genetic polymorphism, and the genetic divergence accompanied with the event of speciation.

Populations' diversity

Among-population differentiation in phenotypic traits and allelic variation is expected to occur as a consequence of isolation, drift, founder effects and local selection (Jolivet and Bernasconi, 2007). Therefore, investigating molecular and genetic divergence is a pre-requisite for studies of local adaptation in response to selection under variable environmental conditions (Jolivet and Bernasconi, 2007). In the present study, both morphological and molecular trees obtained showed intra-specific (inter-populations) diversity in the *Silene* species studied. Such inter-populations genetic diversity possibly occurred during the populations' divergence/ adaptation to different environmental conditions.

A comparison of morphological differences between two populations of Soltaneyeh and Gheydar (gyn13 and gyn14) in *S. gynodioica* shows that they differ in alar length, pistil pubescence and calyx length, which are important morphological characters at subspecies level. The plant specimens of these populations were collected from the locality reported for *S. gynodioica* subsp. *peduncularis*, according to the Flora Iranica (Reschinger, 1979). Therefore, based on morphological and molecular differences of these populations from the others and also their distribution localities, we considered them as *S. gynodioica* subsp. *peduncularis*. The plant specimens of the other populations were collected from the localities reported for *S. gynodioica* subsp. *glandolusa*.

Similar consideration for the two populations of Vardasht and Vardasht-Shahrekord (gyn3 and gyn11) in *S. gynodioica* subsp. *glandolusa* showed that these populations differ from the others in morphological characters like antophore length, calyx teeth length calyx length and lamella width. However, they showed morphological similarities to *S. gynodioica* subsp. g*landolusa* and in the consensus tree also showed affinity to this subsp. Therefore, due to their morphological and molecular differences from the other populations, we considered them as a new variety in *S. gynodioica* subsp. *glandolusa*.

In case of *S. indeprensa*, separation of two populations of Hezarmasjed and Chamanbid (indep 1 and indep2) from the other populations was supported by significant morphological and molecular differences. Detailed mor-

phological study showed that these two populations differ from the other populations in the capsule and calyx teeth length, which are important morphological characters in this species. Therefore, due to morphological and molecular differences we considered them as a new variety in *S. indeprensa*.

Richards et al. (2003) studied intra-specific genetic diversity in *Silene latifolia* by using different allozymes and reported significant positive correlation between the demographic and genetic variables. They suggested that some of the populations were released from the demographic consequences of inbreeding depression by gene flow. Tero et al. (2003) also studied the inter-populations genetic diversity in *S. tatarica* in northern Finland by using the amplified fragment length polymorphism (AFLP) markers and concluded that only one panmictic population exists, which comprised seven clusters with distinct subpopulations. They reported no correlation between geographical and genetic distances among the subpopulations, and no correlation between the subpopulation census size and amount of genetic variation.

In the present investigation, Mantel test showed no significant correlation between geographical, morphological and molecular distances among the populations studied in both *S. gynodioica* and *S. indeprensa*. Therefore, morphological and molecular differences observed among the populations may be a response against the local selection pressures imposed on them and at present we do not see any evidence of isolation-by-distance. In a similar study, Jolivet and Bernasconi (2007) studied genetic and morphological diversity in six populations of *S. latifolia* showing significant molecular and genetic differentiation in them. Geographic and phenotypic distances were significantly associated and age at first flowering increased significantly with latitude. By contrast, no evidence of isolation-by-distance and no significant association between molecular and phenotypic distances were found. In general, considering our previous cytological work (Sheidaei et al., 2010) and the present morphological and molecular findings, we can conclude that the populations divergence in the *Silene* species studied was accomplished by change in the chromosome number and structure, change in the frequency of chiasmata formation, change in the genome content (total chromosome length), change in DNA sequences (polymorphism in ISSR loci) and change in the morphological features.

The species relationship in the genus *Silene* is complicated and different authors have treated their affinities differently (for example, Rettig et al., 1992; Sheidai et al., 2010), partly due to the complex nature of the genus as frequent hybridization occurs among its species and also due to rapid species/subspecies radiation in the genus as also evidenced in the present study. Various geographical populations existing in each species may lead to the formation of new taxonomic and biological forms (as also reported here), which add to the complication of the genus taxonomic treatment.

REFERENCES

De la Rosa R, James C, Tobutt KR (2002). Isolation and characterization of polymorphic microsatellite in olive (*Olea europaea* L.) and their transferability to other genera in the Oleaceae. Primer Note. Mol. Ecol. Notes 2: 265-267.

Greuter W (1995). *Silene* (Caryophyllaceae) in Greece: A subgeneric and sectional classification. Taxon. 44: 543-581.

Jolivet C, Bernasconi G (2007). Molecular and quantitative genetic differentiation in European populations of *Silene latifolia* (Caryophyllaceae). Genetics 177 (2): 1239-1247.

Melzheimer V (1980). Caryophyllaceae. In: Rechinger KH (eds) Flora Iranica, No. 163. Akademische Druck-U, Verlagsanstalt, Graz, Austria. Pp. 353-508.

Minder AM, Rothenbuehler C, Widmer A (2007). Genetic structure of hybrid zones between *Silene latifolia* and *Silene dioica* (Caryophyllaceae): evidence for introgressive hybridization. Mol. Ecol. 16: 2504-2516.

Murray MG, Thompson WF (1980). Rapid isolation of high molecular weight plant DNA. Nucleic Acid Res. 8: 4321-4325.

Nei M (1973). Genetic distance between populations. Amer. Naturalist 106: 283-292.

Peakall R, Smouse PE (2006). GENALEX 6: Genetic analysis in Excel. Population genetic software for teaching and research. Mol. Ecol. Notes 6: 288-295.

Podani J (2000). Introduction to the Exploration of Multivariate Data. English translation. Backhuyes Publishers, Leide, pp. 407.

Rapson GL, Wilson JB (1992). Genecology of Agrostis capillaris L. (Poaceae) – an invader into New Zealand. Responses to light, soil fertility, and water availability. New Zealand Journal of Botany, 30, 13–24.

Rettig JH, Wilson HD, Manhart JR (1992). Phylogeny of the Caryophyllales: Gene sequence data. Taxon. 41: 201-209.

Richards CM, Emery SN, McCauley DE (2003). Genetic and demographic dynamics of small populations of *Silene latifolia***.** Heredity, 90: 181-186.

Sheidai M, Gholipour A, Noormohammadi Z (2010). Species relationship in the genus *Silene* L. Section *Auriculatae* (Caryophyllaceae) based on morphology and RAPD analyses. Acta Biol. Szeged. 54: 25-31.

Tero N, Aspi J, Siikamäki P, Jäkäläniemi A, Tuomi J (2003). Genetic structure and gene flow in a metapopulation of an endangered plant species, *Silene tatarica***.** Mol. Ecol. 12: 2073-2085.