Genetic diversity and population structure in *Senecio leucanthemifolius* subsp. *vernalis* (*Asteraceae*) in Iran

Rosa Eftekharian[1], Masoud Sheidai[1], Farideh Attar[2] & Zahra Noormohammadi[3]

[1] Faculty of Biological Sciences, Shahid Beheshti University, Tehran, Iran,
e-mail: rozaeftekharian@yahoo.com (corresponding author), msheidai@yahoo.com

[2] Faculty of Biological Sciences, Tehran University, Tehran, Iran,
e-mail: fattar@khayam.ut.ac.ir

[3] Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran

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Abstract. *Senecio leucanthemifolius* subsp. *vernalis*, known under the vernacular name of Eastern Groundsel, is one of the annual Eurasian subspecies of the genus *Senecio*. This taxon contains important alkaloids and carotenoids. In animals, carotenoids serve as a source of vitamin A and other retinoids, and as photoprotective and cancer prevention agents. We studied the genetic diversity, population structure and morphological variability of 16 geographical populations of *Senecio leucanthemifolius* subsp. *vernalis* in Iran. AMOVA and Gst analyses revealed significant molecular differences among the studied populations. Mantel test did not show any significant correlation between the genetic distance and geographical distance of the studied populations. Reticulation analysis revealed that gene flow occurred among most of the studied populations. STRUCTURE analysis revealed the populations' genetic stratification. MANOVA test of quantitative morphological characters showed a significant difference among the studied populations. The obtained consensus tree of the morphological and genetic trees revealed that some of the studied populations differed from others in their genetic and morphological features.

Key words: gene flow, genetic stratification, Iranian flora, *Senecio leucanthemifolius* subsp. *vernalis*

Introduction

Senecio L. (*Asteraceae*, *Senecioneae*) is one of about 50 plant genera comprising over 500 species (Mabberley 1997). It has a worldwide distribution, particularly in South Africa, the Mediterranean floristic region and in the temperate areas of Asia and America. The species of this genus are of diverse biological types: annuals, perennials, aquatics, climbers, shrubs, and small trees (Matthews 1975).

The aerial parts of *Senecio* species are used for their medicinal properties: astringent, antidiarrhoeal, diuretic, diaphoretic, emmenagogue, galactagogue, expectorant. A homeopathic remedy is used in the treatment of internal haemorrhages and dysmenorrhoea. An emollient poultice is made from the leaves. A decoction of the root is used for internal bruises and wounds.

Senecio leucanthemifolius subsp. *vernalis* (Waldst. & Kit.) Greuter (≡ *S. vernalis* Waldst. & Kit.), known under the common name of Eurasian Groundsel, is one of the annual Eurasian subspecies of the genus (Greuter & Raab-Straube 2006). This taxon contains important pyrrolizidine alkaloids (PA) (Skaanild & al. 2001) that are responsible for killing more grazing livestock than all other poisonous plants together (Jeffrey 1978). Because of their cumulative effect, pyrrolizidine alkaloids have been reported by the World Health Organization to be toxic for animals and humans. This *Senecio* species poses insignificant risk for

humans, the aerial parts being used under the generic name of *Senecionis herba*. However, some sensitive individuals can develop an allergic reaction, because of the sesquiterpene lactones, which can cause dermatitis. *Senecio leucanthemifolius* subsp *vernalis* also contains carotenoids neoxanthin, violaxanthin, lutein-5,6-epoxide, lutein, β-cryptoxanthin, and α- and β-carotene (Mogoşanu & al. 2009). In animals, carotenoids serve as a source of vitamin A and other retinoids, and as photoprotective and cancer prevention agents (Mogoşanu & al. 2009). The worldwide distribution of *S. leucanthemifolius* subsp. *vernalis* covers West Asia, Caucasus, Central Asia, Central Europe, East Europe, and Southeast Europe. The texon is considered native in different regions of Iran and comprises many local geographical populations.

Population genetic analyses can provide data on a variety of important evolutionary parameters, including the levels of genetic variation, partitioning of variability within/between populations, inbreeding, selfing versus outcrossing rates, effective population size, and population bottleneck. These analyses may be of help in developing some effective management strategies for the endangered and/or invasive species (Chen 2000; Ellis & Burke 2007). *Senecio leucanthemifolius* subsp. *vernalis* forms several local populations in Iran. So far, no investigation has been reported about the population genetic structure of this valuable plant species in the country. Therefore, we carried out a population genetic analysis of 16 populations of the taxon for the first time in Iran.

Table 1. The studied populations, their locality and geographical features.

	Province	Locality	Altitude (m)	Longitude	Latitude	Voucher No.
Pop1	Zanjan	Avarezi	1631	36.40	48.24	2014330
Pop2	EastAzarbayejan	Soofian	1514	38.17	45.56	2014331
Pop3	EastAzarbayejan	Jolfa	1731	38.56	45.41	2014332
Pop4	EastAzarbayejan	Hashtrood	1847	37.16	47.12	2014333
Pop5	Mazandaran	Polesefid	571	36.07	53.03	2014334
Pop6	Tehran	Tochal	1910	35.53	51.25	2014335
Pop7	Ghazvin	Alamout	1723	36.29	50.23	2014336
Pop8	Mazandaran	Ghaemshahr	58	36.27	52.51	2014337
Pop9	Mazandaran	Behshahr	21	36.41	53.33	2014338
Pop10	Mazandaran	Babolsar	-20	36.42	52.39	2014339
Pop11	Mazandaran	Kiasar	1250	36.14	53.32	2014340
Pop12	Mazandaran	Alasht	1684	36.04	52.50	2014341
Pop13	Alborz	Hashtgerd	1273	35.57	50.40	2014342
Pop14	Gilan	Fooman	33	37.13	49.18	2014343
Pop15	Tehran	Tehran	1197	35.41	51.25	2014344
Pop16	Semnan	Chashm	2209	35.53	53.15	2014345

We have used ISSR molecular markers as these markers were shown to be informative for genetic diversity and population structure studies (e.g., Sheidai & al. 2012, 2013; Azizi & al. 2014).

Material and methods

Plant material. One hundred and forty-one plant accessions were collected from 16 geographical populations and used for the present study. Details of localities are provided in Table 1 and Fig. 1. The studied populations were in the northeastern, northern and northwestern parts of the country. They were absent in the southern part. Voucher specimens are deposited in the Herbarium of Shahid Beheshti University (HSBU). Fresh leaves were collected and used for DNA extraction and molecular study.

Morphological studies. Morphological characters, including qualitative and quantitative, are shown in Table 2. The analysis of variance test (MANOVA) was performed to show any significant morphological difference between the studied populations. For grouping of the plant specimens, UPGMA (unweighted paired group with arithmetic average) and CVA (Canonical Variates Analysis) were applied. Morphological data for these analyses were standardized (mean = 0, variance = 1) (Podani 2000).

Molecular studies. ***DNA extraction and ISSR assay***: Fresh leaves were collected randomly from 10 plants in each of the studied populations and dried in silica gel powder. Genomic DNA was extracted using CTAB activated charcoal protocol (Sheidai & al. 2013). The quality of extracted DNA was examined by running on 0.8 % agarose gel. Ten ISSR primers (AGC)5GT, (CA)7GT, (AGC)5GG, UBC810, (CA)7AT, (GA)9C, UBC807, UBC811, UBC834, and UBC823 commercialized by UBC (the University of British Columbia) were used. PCR reactions were performed in a 25 μl volume containing 10 mM Tris–HCl buffer at pH 8; 50 mM KCl; 1.5 mM $MgCl_2$; 0.2 mM of each dNTP (Bioron, Germany); 0.2 μM primer; 20 ng genomic DNA, and 3 U *Taq* DNA polymerase (Bioron, Germany). The amplification reactions were performed in a Techne thermocycler (Germany), with the following program: a 10 min initial denaturation step at 94 °C, 30 sec at 94 °C; 30 sec at 54.7 °C, and 2 min at 72 °C. The reaction was

Fig. 1. Distribution map of *Senecio leucanthemifolius* subsp. *vernalis* populations studied in Iran. For population numbering, see Table 1.

Table 2. Morphological characters.

Quantitative characters	Qualitative characters
plant height	type of stem
length of basal leaf	stem indumentum
width of basal leaf	blackness of calyculus bracts
thickness in base of stem	blackness of involucral bracts
length of stem leaf	
width of stem leaf	
number of capitula	
length of peduncle	
length of bract on a peduncle	
number of involucral bracts	
length of involucral bracts	
number of calyculus bracts	
number of ray flowers	
length of tube in ray flowers	
length of lamina in ray flowers	
width of lamina in ray flowers	
length of style in ray flowers	
length of corolla	
length of corolla tube	
length of corolla lamina	
length of anther in disk flower	
length of style in disk flower	
length of nut	
length of papus	

completed by a final extension step of 10 min at 72 °C. The amplification products were visualized by running on 2 % agarose gel, followed by the ethidium bromide staining. The fragment size was estimated by using a 100 bp molecular size ladder (Fermentas, Germany).

***Data analysis*:** The obtained ISSR bands were coded as binary characters (presence = 1, absence = 0). Genetic diversity parameters were determined for dominant molecular markers in each population. These parameters were Nei's gene diversity (H), unbiased gene diversity, Shanon information index (I), number of effective alleles, and percentage of polymorphism (Weising & al. 2005; Freeland & al. 2011). Nei's genetic distance was determined among the studied populations and used for clustering. For grouping of the plant specimens, Neighbor Joining (NJ) clustering method was applied after 100 times bootstrapping (Freeland & al. 2011; Huson & Bryant 2006). Mantel test was performed to check correlation between the geographical distance and genetic distance of the studied populations (Podani 2000; Weising & al. 2005). PAST ver. 2.17 (Hammer & al. 2012), DARwin ver. 5 (2012) and SplitsTree4 V4.13.1 (2013) programs were used for these analyses.

Significant genetic difference among the studied populations and provinces were determined by: 1 – AMOVA (Analysis of Molecular Variance) test (with 1000 permutations) for dominant molecular markers as implemented in GenAlex 6.4 (Peakall & Smouse 2006), and 2 – Nei's Gst analysis of dominant markers as implemented in GenoDive ver.2 (2013) (Meirmans & Van Tienderen 2004). Furthermore, the populations' genetic differentiation was studied by G'ST est = standardized measure of genetic differentiation and D_est = Jost measure of differentiation (Jost 2008). In order to overcome the potential problems caused by dominance of the ISSR markers, a Bayesian program, Hickory (ver. 1.0) (Holsinger & Lewis 2003) was used to estimate the parameters related to genetic structure (Theta B value). Three runs were conducted with default sampling parameters (burn-in = 50000, sample= 250000, thin = 50) to ensure consistency of results (Tero & al. 2003). Genetic structure of geographical populations and provinces was studied by structure analysis (Pritchard & al. 2000) for dominant markers (Falush & al. 2007). Model-based clustering, as performed by STRUCTURE software ver. 2.3 (Pritchard et al. 2000), was carried out to group the studied populations on the basis of genetic affinity. This program was also used to reveal the genetic admixture of studied populations. For this analysis, the admixture ancestry model under the correlated allele frequency model was used. Markov Chain Monte Carlo simulation was run 20 times for each value of K (2–16) for 20 iterations after a burn-in period of 10**5**. All other parameters were set at their default values. Data were scored as dominant markers and analysis followed the method suggested by Falush & al. (2007). STRUCTURE Harvester web site (Earl & von Holdt 2012) was used to visualize the STRUCTURE results and also to perform Evanno method for identifying the proper number of K (Evanno & al. 2005). The choice of the most likely number of clusters (K) was carried out comparing the log probabilities of data [Pr (X|K)] for each value of K (Pritchard & al. 2000), as well as by calculating an *ad hoc* statistic ΔK based on the rate of change in the log probability of data between successive K values, as described by Evanno & al. (2005). We have used two summary statistics to present K-means clustering: 1 – pseudo-F (Calinski & Harabasz 1974) and 2 – Bayesian Information Criterion (BIC) (Schwarz 1978). The clustering with the highest value for pseudo-F is regarded as providing the best fit, and the clustering with the lowest value for BIC is regarded as providing the best fit (Meirmans 2012). Similarly, non-metric multidimentional scaling (MDS) (Podani 2000) was performed for studying the genetic distinctness of the provinces. Indirect evaluation of the gene flow (Whitlock & Mccauley 1999) among populations was checked by reticulation analysis and population assignment test, using the maximum likelihood method as implemented in GenoDive ver.2 (2013) (Meirmans & Van Tienderen 2004). Recently, Frichot & al. (2013) introduced the statistical model called latent factor mixed models (LFMM) that tests correlations between environmental and genetic variation, while estimating the effects of hidden factors that represent the background residual levels of population structure. We have used this method to check if ISSR markers show correlation with the environmental features (longitude, latitude and altitude) of the studied populations. The analysis was done by a LFMM program Version 1.2 (2013).

In order to reveal agreement between the genetic tree and morphological trees, we have obtained a consensus tree based on ISSR tree and morphological trees. All 141 specimens were used in both analyses.

Results

Populations genetic diversity

We have obtained a high number of reproducible bands from almost all used ISSR primers, and finally a data matrix of 141 × 59 was formed for further analysis. Genetic diversity parameters determined in 16 geographical populations of *S. leucanthemifolius* are presented in Table 3. Both common genetic diversity indices and unbiased gene diversity parameter (which is free from the sampling size) have produced similar results.

The highest value of percentage polymorphism (55.88 %) was observed in Mazandaran, Ghaemshahr (Population 8). This population had also the highest values for unbiased gene diversity (0.195), Shanon information index (0.278), and genetic diversity due to populations (Hst) as estimated in AMOVA (0.267).

Soofian population (Population 2) had the lowest values for unbiased gene diversity (0.044), percentage

polymorphism (16.18 %), Shanon's information index (0.067), and Hs (0.065). Genetic diversity due to each population (Hs) ranged from 0.065 and 0.087 in Populations 2 and 1, to 0.261 and 0.216 in Populations 8 and 11.

AMOVA (Table 4) revealed a significant molecular difference among the studied populations (P = 0.01). It also revealed that 40 % of the total genetic variability occurred among the studied populations, while 60 % occurred within the populations.

Table 3. Genetic diversity parameters determined in the studied populations.

Pop	N	Na	Ne	I	He	UHe	%P
Pop1	10	0.456	1.103	0.095	0.062	0.065	19.12%
Pop2	10	0.456	1.064	0.067	0.042	0.044	16.18%
Pop3	10	0.721	1.161	0.149	0.097	0.102	32.35%
Pop4	10	0.647	1.116	0.118	0.075	0.079	26.47%
Pop5	6	0.926	1.225	0.205	0.134	0.147	41.18%
Pop6	11	0.912	1.214	0.198	0.129	0.135	44.12%
Pop7	9	0.897	1.208	0.193	0.125	0.133	41.18%
Pop8	7	1.191	1.293	0.278	0.181	0.195	55.88%
Pop9	6	0.691	1.156	0.145	0.095	0.104	27.94%
Pop10	10	1.059	1.245	0.230	0.149	0.157	50.00%
Pop11	9	1.103	1.264	0.243	0.159	0.168	51.47%
Pop12	9	0.956	1.236	0.223	0.145	0.154	45.59%
Pop13	9	0.971	1.199	0.196	0.124	0.132	45.59%
Pop14	6	0.706	1.162	0.150	0.098	0.107	30.88%
Pop15	10	1.074	1.236	0.221	0.143	0.150	50.00%
Pop16	9	0.662	1.128	0.124	0.079	0.084	27.94%
Average	8.813	0.839	1.188	0.177	0.115	0.122	37.87%

Abbreviations: **N** = Number of specimens, **Na** = Number of different alleles, **Ne** = No. effective alleles, **I** = Shanon Information Index, **He** = Gene diversity, **UHe** = Unbiased gene diversity, and **%P** = Percentage polymorphism.

These results indicate a high level of genetic diversity both within and among *Senecio leucanthemifolius* populations. Significant genetic difference among the populations was also obtained by Gst analysis and Hickory test. The Gst value obtained among populations after 999 permutations was 0.389 (P = 0.001), while the Bayesian approach based on Hickory test produced theta-II value of 0.50, which is high and significant.

Moreover, the populations differentiation parameters determined among the studied populations produced high values for G'st (0.466, P = 0.001) and Jost differentiation index (D-est = 0.126, P = 0.001). These results indicate that the geographical populations of *Senecio leucanthemifolius* are genetically differentiated from each other.

Grouping of the plant populations obtained by NJ tree and Neighbor-Net method produced similar results. Therefore, only the NJ tree based on Nei's genetic distance is presented in Fig. 2. Three major clusters were formed. Populations 1, 5 and 8 formed the first major cluster and showed higher degree of genetic affinity.

The second major cluster contained three subclusters. Plant specimens of Population 14 mainly formed the first subcluster. Plants of Populations 2 and 9 comprised the second subcluster, while members of Populations 12, 13, 15, and 16 formed the third subcluster.

Table 4. Pair-wise Fst value (above diagonal) and their respective P value (bellow diagonal) among the studied populations (for population numbering, see Table 1).

	Pop001	Pop002	Pop003	Pop004	Pop005	Pop006	Pop007	Pop008	Pop009	Pop010	Pop011	Pop012	Pop013	Pop014	Pop015	Pop016
Pop001	–	0.593	0.394	0.506	0.5	0.38	0.479	0.336	0.545	0.407	0.398	0.493	0.5	0.593	0.558	0.642
Pop002	0.001	–	0.569	0.654	0.593	0.555	0.53	0.458	0.483	0.499	0.525	0.452	0.377	0.612	0.536	0.587
Pop003	0.001	0.001	–	0.302	0.472	0.239	0.307	0.344	0.48	0.208	0.231	0.382	0.348	0.413	0.407	0.538
Pop004	0.001	0.001	0.001	–	0.482	0.264	0.341	0.357	0.538	0.318	0.397	0.477	0.452	0.481	0.455	0.62
Pop005	0.001	0.001	0.001	0.001	–	0.343	0.436	0.305	0.466	0.328	0.372	0.375	0.428	0.502	0.42	0.507
Pop006	0.001	0.001	0.001	0.001	0.001	–	0.146	0.256	0.438	0.164	0.209	0.335	0.34	0.353	0.389	0.523
Pop007	0.001	0.001	0.001	0.001	0.002	0.001	–	0.28	0.439	0.215	0.266	0.37	0.335	0.394	0.352	0.501
Pop008	0.001	0.001	0.001	0.001	0.001	0.001	0.001	–	0.224	0.285	0.305	0.324	0.351	0.317	0.362	0.511
Pop009	0.001	0.001	0.002	0.001	0.002	0.001	0.001	0.002	–	0.437	0.439	0.378	0.403	0.441	0.47	0.559
Pop010	0.001	0.001	0.001	0.001	0.002	0.001	0.001	0.001	0.001	–	0.088	0.273	0.28	0.37	0.356	0.48
Pop011	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.004	–	0.325	0.325	0.374	0.418	0.491
Pop012	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	–	0.168	0.315	0.253	0.384
Pop013	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	–	0.279	0.25	0.43
Pop014	0.001	0.001	0.001	0.001	0.003	0.001	0.001	0.001	0.002	0.002	0.002	0.001	0.001	–	0.29	0.579
Pop015	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.002	–	0.433
Pop016	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	–

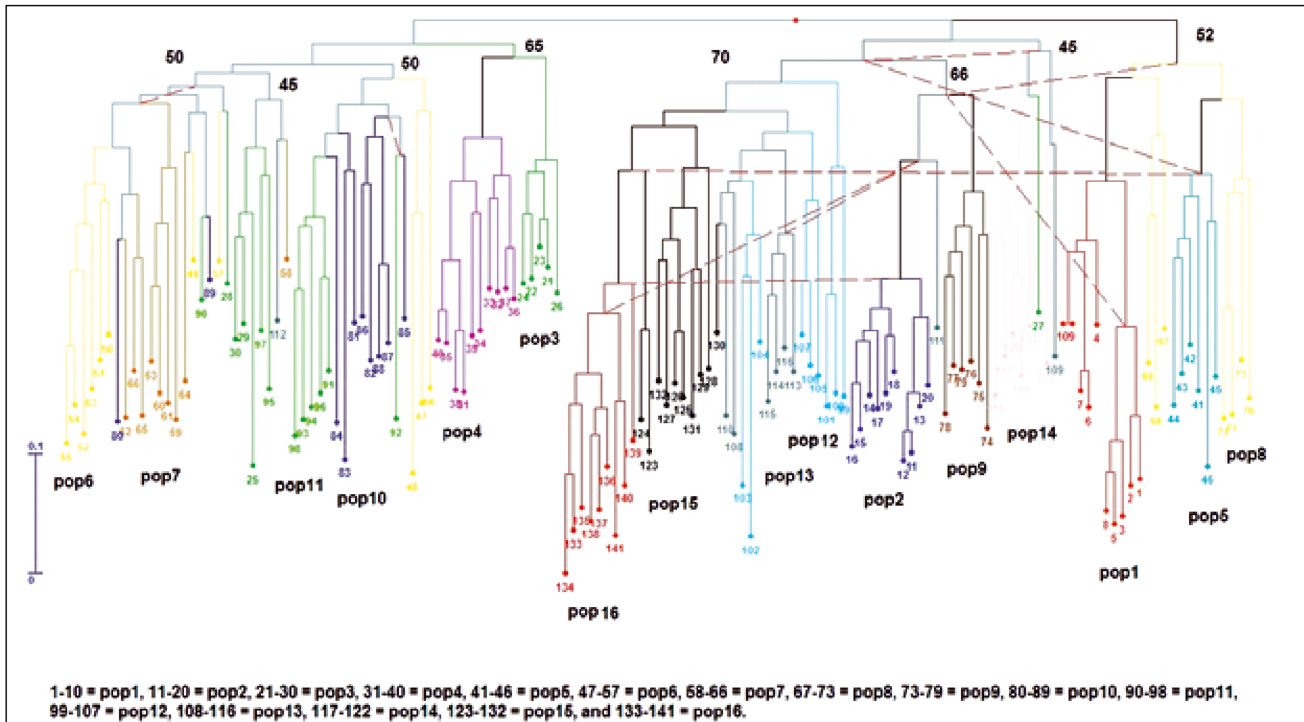

Fig. 2. Reticulation tree based on Nei's genetic distance of the studied populations (values above nodes are bootstrap values).

The other populations comprised the second major cluster and were distributed into three subclusters. Populations 3 and 4 formed the first subcluster, while Populations 10 and 11 formed the second subcluster. Plants of Populations 6 and 7, along with a few plants of Population 3 comprised the third subcluster.

Mantel test did not show a significant correlation between the genetic distance and geographical distance of the studied populations (Fig. 3). Therefore, no isolation by distance occurred among these populations.

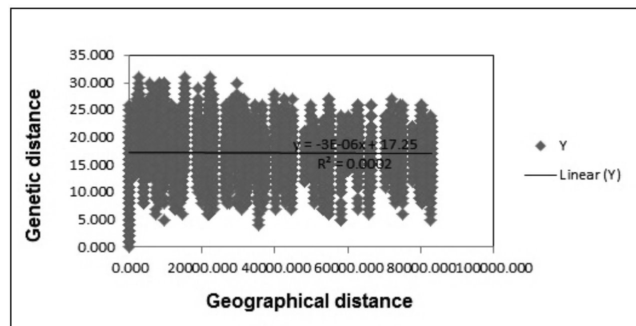

Fig. 3. Mantel test plot of genetic distance versus geographical distance in the studied populations.

Populations genetic structure

The Evanno test produced delta k = 4 as the best number of genetic groups (Fig. 4). A STRUCTURE plot based on k = 4 in presented in Fig. 5. In general, Populations 1, 3 and 4 are genetically more alike and form the first genetically similar group. The same holds true for Populations 2, 9 and 16 that compose the second group. Populations 5–8 and 10 and 11 form the third genetic group, while Populations 12–15 compose the forth group.

Gene flow among populations

The STRUCTURE plot based on k = 4 (Fig. 5) has revealed the allele combination difference among the studied populations and the occurrence of genetic admixture among them. The highest degree of genetic admixture was observed in Populations 5–8, 10, 11, and 13.

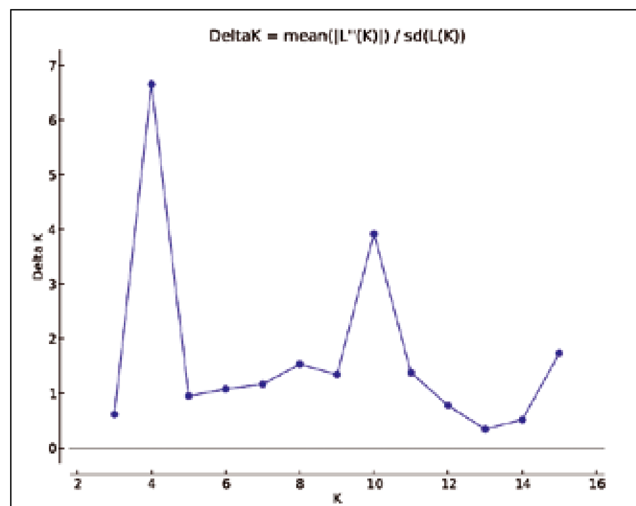

Fig. 4. Delta k plot of Evanno test based on STRUCTURE analysis.

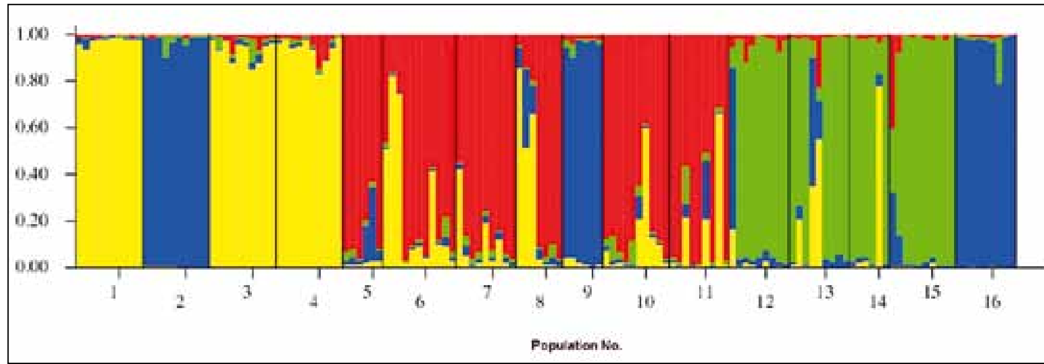

Fig. 5. STRUCTURE plot of 141 plant accessions based on k = 4 (for population numbering, see Table 1).

A reticulogram (Fig. 2) presented before also revealed a certain degree of gene flow among these populations. These reticulations may be due to shared ancestral alleles or ongoing gene flow that we do not know for sure.

Population assignment test has revealed that individual number 90 of the presumed Population 11 (Mazandaran, Kiasar population) was inferred as a member of Population 10 (Mazandaran, Babolsar). The two geographical populations are in the same province and may have a frequent gene flow. Similarly, it has revealed that plant number 57 of Population 6 (Tochal) was inferred to be a member of Population 7 (Alamout). The two geographical populations are in two neighboring provinces and may have a frequent gene flow.

Adaptation to local environments often occurs through natural selection acting on a large number of loci, each having a weak phenotypic effect. One way to detect these loci is to identify genetic polymorphisms that exhibit high correlation with environmental variables used as proxies for ecological pressures. We have used the computer program Latent Factor Mixed Model (LFMM) written by Frichot & al. (2013) for this purpose. The results (Table 5) showed that 21 ISSR loci had –log10 (p-value) equal or bigger than 1.00 and were significantly correlated to the studied environmental parameters (P = 0.05).

Morphological variability

MANOVA test performed for quantitative morphological characters has shown a significant difference (p = 0.01) among the studied populations. Moreover, a CVA plot based on all morphological characters (quantitative and qualitative) separated these populations from each other (Fig. 6). Therefore, these populations differed significantly in morphological features.

Table 5. LFMM result showing ISSR loci with significant correlation with geographical features.

Name	Zscore	–log10 (p-value)	p-value
ISSR4	3.93499	4.07988	8.31991E-05
ISSR6	2.82565	2.3262	0.00471841
ISSR7	2.70184	2.16142	0.00689576
ISSR8	1.95033	1.29126	0.0511372
ISSR9	2.10633	1.45375	0.0351761
ISSR10	4.25956	4.68861	2.04826E-05
ISSR11	4.52473	5.21843	6.04737E-06
ISSR12	3.58371	3.47013	0.000338746
ISSR17	7.98424	14.8496	1.41394E-15
ISSR18	3.85222	3.93163	0.00011705
ISSR19	2.12465	1.47345	0.033616
ISSR22	2.97673	2.5356	0.00291342
ISSR25	3.51588	3.35823	0.000438302
ISSR27	2.2703	1.63471	0.0231897
ISSR28	2.6042	2.03579	0.00920901
ISSR35	2.55261	1.97094	0.0106919
ISSR45	2.32683	1.69953	0.0199743
ISSR48	3.00296	2.5729	0.00267365
ISSR56	2.18926	1.54397	0.0285778
ISSR62	3.42667	3.21393	0.000611041
ISSR63	4.06324	4.31518	4.83967E-05

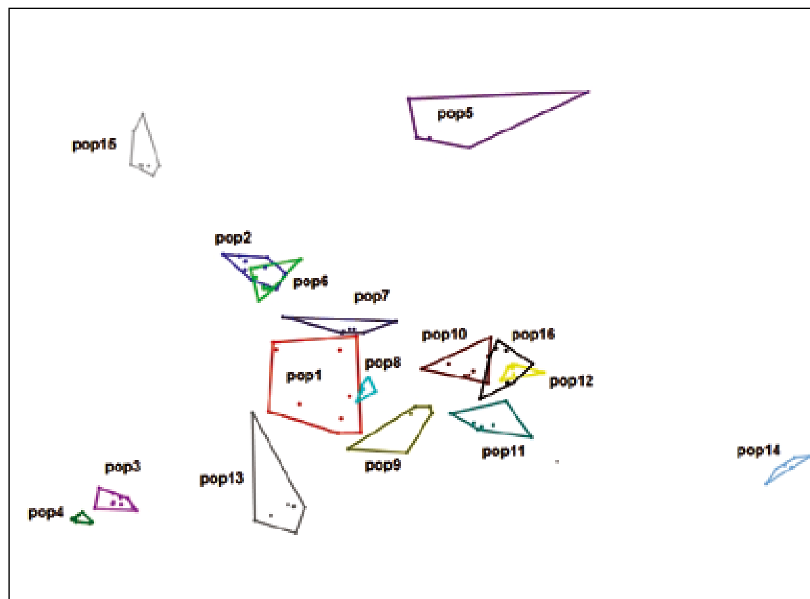

Fig. 6. CVA plot of morphological data (for population numbering, see Table 1).

A consensus tree of both morphological and ISSR data is presented in Fig. 7. Some of the populations like populations 1, 2, 4–6, 9, 13, and 16 differed in both morphological and genetic features from the other populations and formed a separate cluster.

Discussion

Senecio leucanthemifolius populations showed a high degree of genetic variability. Genetic diversity is important for geographical populations to face changes in their local environment (Erfmeier & al. 2013; Sheidai & al. 2014).

Significance among the populations' genetic differentiation was observed, and a STRUCTURE plot identified four different genetic groups in this species. The presence of different genetic groups in plant populations of a species may serve as different genetic sources for breeding and hybridization programs for crop plants and as a good source of genetic variability for adaptation of the plant taxa (Sheidai et al. 2014).

Genetic differentiation among the populations is due to a limited gene flow among populations and a possible genetic drift (Jolivet & Bernasconi 2007; Hou & Lou 2011). Our data showed a high degree of gene flow among the populations of each genetic group. The occurrence of high genetic diversity within populations is due to the outcrossing nature of species (Bodo-Slotta & al. 2010; Sheidai & al. 2013). The same may hold true for *Senecio leucanthemifolius* populations.

A Mantel test revealed the lack of isolation-by distance across the distribution range of the studied populations. Therefore, in spite of genetic differentiation

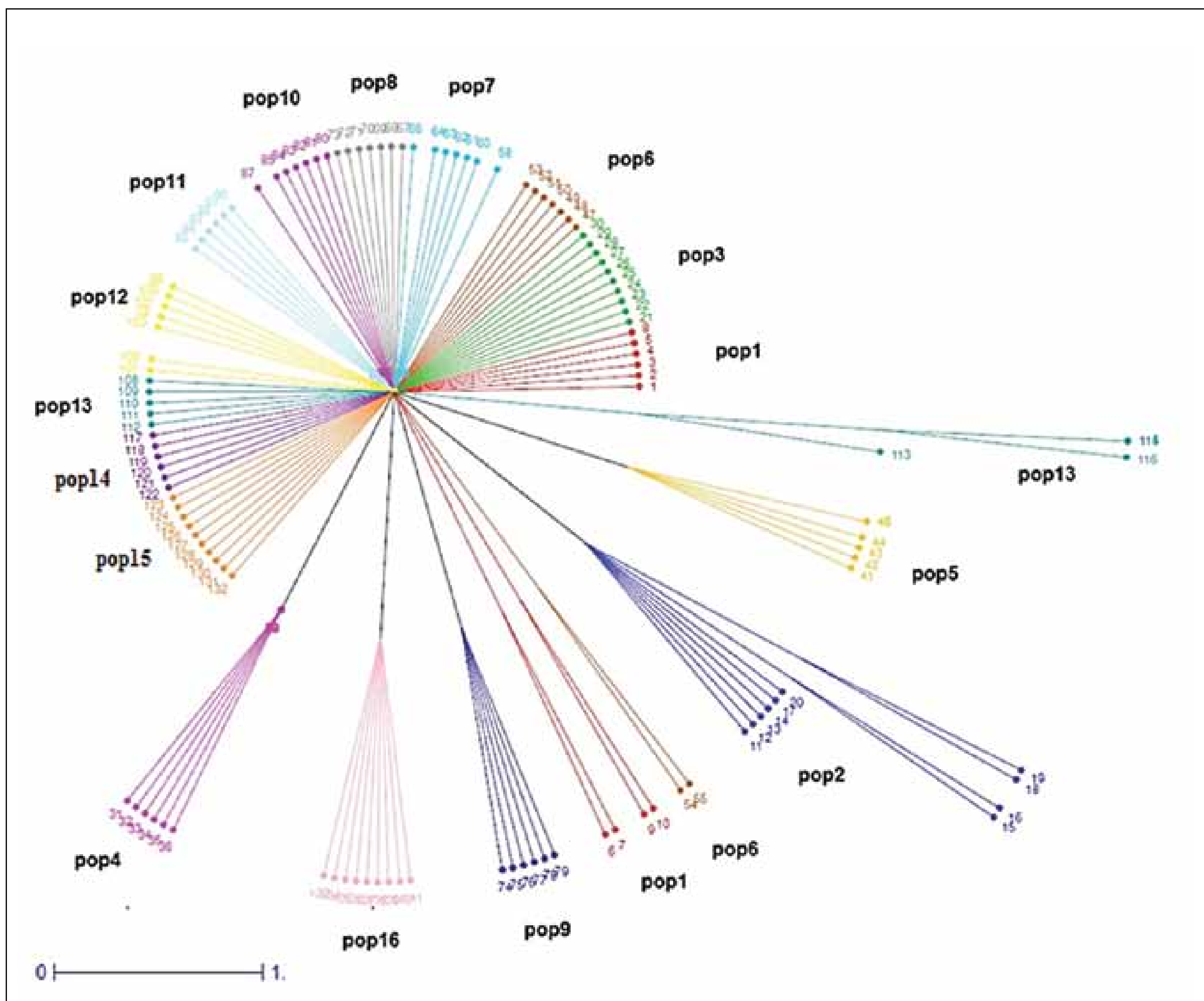

Fig. 7. Consensus tree of morphological and genetic data (for population numbering, see Table 1).

among the studied *Senecio leucanthemifolius* populations, they are not totally isolated and still some amount of gene flow has occurred between them. Our study has indicated that a gene flow occurred between the two most distant populations and between those that are in close vicinity.

Therefore, we suggest that a combination of genetic drift, limited gene flow and local adaptation may have played a part in the genetic divergence of *Senecio leucanthemifolius* populations in Iran.

The present study has revealed that some of the studied geographical populations differed from the rest in both genetic and morphological features, as evidenced in the consensus tree. Significant morphological differences among the geographical populations of a single species and separation of populations on the PCA plot have been considered adequate reasons for regarding these populations as separate ecotypes (Ockendon 1971). Therefore, in these populations morphological changes accompanied genetic differences and they may be considered as infra-taxonomic forms.

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