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The Morphological and Genetic Variation of a Species of Lettuce (*Lactuca undulata* Ledeb.): Geographically Widespread but Locally Endangered

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ABSTRACT

Lactuca undulata Ledeb. (Asteraceae) is an annual plant that has valuable compounds such as caffeic acid and its derivatives. This study aims to understand if there is detectable genetic diversity in the Iranian populations of *L. undulata*. For this purpose, the diversity of five *L. undulata* populations collected from different regions of Iran was evaluated based on the morphological characteristics and ISSR markers. A common garden experiment was conducted to assess the morphological variation of five *L. undulata* populations. The results of the study of morphological features showed that most of the studied traits have a high diversity among populations. In the PCoA performed, five populations of *L. undulata* were identified based on their morphological characteristics. In genetic analyses, using ISSR markers, a total of 60 bands were produced using four primers. The percentage of total polymorphism was 100% and no monomorphic band was obtained. The Nei gene diversity index (H) and Shannon diversity index (I) indicated high genetic diversity (0.24) in the populations. Analysis of molecular variance (AMOVA) showed that 73% of the genetic diversity was within the populations and another 27% was among the populations. The highest and the lowest genetic diversity were observed in Cheshmeh Ali and Qom populations, respectively. PCoA nearly separated genetically different populations into distinct groups. Five *L. undulata* populations were found to differ significantly in their morphological and genetic polymorphism. The studied populations were found in highly degraded habitats with a limited number of individuals. Degradation and loss of habitats are the major concerns in determining the conservation status of the species in general and at the population level in particular.

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Introduction

Biodiversity research and conservation of plants serve as the foundation for a better understanding of evolution, domestication, and plant systematics. Genetic variation within and among natural populations is essential for the health and long-term survival of a species. An accurate estimate of the genetic variation among or within the populations of a rare or endangered species could be helpful to understand the mechanisms of its rarity (Ding *et al.*, 2013), and provide fundamental information for designing conservation programs (Frankham *et al.*, 2002). Determining the contents of genetic diversity in plant material is the first step in identifying and maintaining hereditary reserves and the primary basis for genetic research and breeding programs (Govindaraj *et al.*, 2015). The evolutionary potential of species and their ability to withstand adverse environmental conditions depend on the extent of genetic diversity (O'Hanlon *et al.*, 2000). Many aspects of natural resources conservation, such as the loss of genetic diversity and the revival of endangered plant populations, can be seen through careful study of

genetics and the structure of plant populations. The genus *Lactuca* L. is one of the most valuable plants in the Asteraceae family represented by more than 100 species of annual, biennial or perennial herbs (Bhellum and Singh, 2015). Most of the species are distributed throughout the temperate and warm regions of the world while the greatest diversity is confined to Southwest Asia and the Mediterranean basin (Lebeda *et al.,* 2013). There are 16 species in Europe, 12 in North America, 43 in Africa and 51 in Asia (Doležalová *et al.,* 2002).

Several species of this genus have been cultivated since ancient dates, the most famous of which is edible lettuce (*L. sativa*) due to the importance of its leaves in human nutrition. Another valuable species of this genus is *Lactuca undulata* Ledeb., an annual plant with a height of 10-50 cm. This species is mostly distributed along the central and west Asia (Safavi *et al.,* 2013). Akhani (1998) mentioned *L. undulata* as a vulnerable species in Golestan National Park Flora. The presence of secondary metabolites such as caffeic acids and its derivatives in *L. undulata* shows a great potential for domestication and production of valuable metabolites such as cichoric, chlorogenic and caffeic acids in industrial scale (Ramezannezhad *et al.,* 2019).

Morphological characteristics and molecular markers are commonly used in the determination of the genetic relationships of different masses, populations, cultivars and plant varieties. The molecular markers are of high importance since, unlike morphological data and isozyme markers, they are not affected by environmental conditions and show the existing polymorphisms well (Brito *et al.*, 2018).

ISSR markers (Inter-Simple Sequence Repeats) have the ability to detect polymorphism and genetic diversity within and between populations and genotypes. They have been used on a large scale for structural analysis of various populations and identification of different variants (Souframanien and Gopalakrishna, 2004). It does not require previous genomic sequence information, which makes ISSR technically simpler than many other marker systems (Bornet and Branchard, 2001).

Morphological characteristics of populations have been studied in different species of lettuce, including *L. sativa* L. (Doležalová *et al.,* 2003) *L. serriola* L. (Doležalová *et al.,* 2005; Novotná *et al.,* 2011). Also, different markers have been used frequently to assess the genetic variation in *L. sativa* varieties (Sharma *et al.,* 2018) and *L. Serriola* populations (Jemelková *et al.,* 2018). Genetic diversity of other species, including *L. saligna*, *L. virosa*, *L. dregeana*, *L. altaica*, *L. aculeate*, *L. tenerrima*, *L. perennis*, *L. tatarica*, *L. sibirica*, *L. quercina*, *L. viminea* and *L. indica* have been studied using different molecular and protein markers (Koopman *et al.,* 2001; Lebeda *et al.,* 2012).

So far, there has been no study on the morphological variability and genetic diversity of *L. undulata*. The aim of the present study is to investigate the variation of *L. undulata* populations in Iran based on morphological characters and ISSR markers.

Materials and Methods

Plant materials

During May and June 2018, a total of 100 samples were randomly collected from five naturally occurring populations of *L. undulata* in different regions of Iran, including Pardisan Town of Qom, Firoozkooh, Cheshmeh Ali Damghan, Dezian Village located in Biarjmand and Mirzabaylu Plain located at the east of Golestan National Park (Table 1, Fig. 1). Fresh leaves were placed in paper bags and then transferred to sealed bags containing silica gel. Herbarium voucher specimens from each population were deposited with Golestan University Herbarium (Table 1).

Table 1. List of monitoring sites with wild *L. undulata* populations in Iran in 2018. Herbarium Vouchers were lodged at Golestan University Herbarium.

Population	Longitude	Latitude	Altitude (m)	Herbarium Voucher
Firoozkooh	52° 49' 17.2"	35° 47' 12.1"	1950	6288
Mirzabaylu	56° 15' 21.0"	37° 21' 49.9"	1350	6293
Biarjmand	55° 59' 34.19"	36° 0' 5.07"	1226	6290
Qom	50° 70' 93.55"	34° 50' 41.34"	930	6289
Cheshmeh Ali	54° 5' 11.63"	36° 16' 43.40"	1516	6292

Morphological characteristics

A common garden experiment was designed and six seeds from each population were planted in 15 cm wide and 20 cm deep pots containing gardening soil. The pots were placed in light/dark 16/8 h and 25 ± 0.5 C temperature.

Fig. 1. Geographic distribution of five sampled populations of *L. undulata*

Various factors, including leaf length and width, hair density on abaxial and adaxial leaf surfaces, number of leaf lobes and leaf area in vegetative phase and also in reproductive phase, stigma length, style length, stamen length, the number of flowers per head, the length and width of the seeds, and the length of the seed beak were photographed using a microscope (OptixPenPix Z5 Digital Microscope) and all the measurements were conducted using ImageJ Software (Fig. 2).

Then, the data were sorted in MS-Excel and analyzed in R and SPSS ver. 22. Principal Coordinates Analysis (PCoA) was used to investigate the level of variation within and among the populations.

DNA extraction

For each sample 0.2 g of ground dry leaf was transferred to a 2-ml sterile microcentrifuge tube and 500 mL of extraction buffer (10% w/v SDS; 1 M, Tris-Cl pH 8.0; 0.5 M EDTA, pH 8.0) preheated at 65°C was added. The reaction tube was incubated at 65°C for 30 min. Then, the tube was stored at 4°C for 15 minutes. After that, 250 µL of 6 M ammonium acetate was added and stirred vigorously and placed at 4°C for another 15 min. After centrifuging the tube for 15 min at 13000 ×g, 600 µL of supernatant was transferred to a new tube and 360 µL isopropanol was added and centrifuged for 15 min at 13000 ×g.

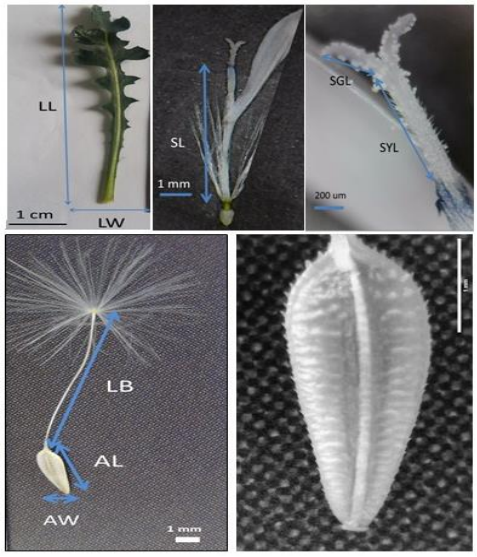

Fig. 2. Morphological characteristics of *L. undulata*:LL= leaf length; LW= leaf width; SL= stamen length; SGL= Stigma length; SYL= style length; LB= length of beak; AL= achene length; AW= achene width; Achene of a sample from Biarjmand population.

Then, 500 µL of 70 % ethanol was added and centrifuged for 15 min at 13000×g and the supernatant discarded. The tubes were stored at room temperature overnight with the lids open. The pellet was dissolved in 200 µL LTE (Light - Tris-EDTA) and after 24 h, it was centrifuged for 20 min at 13000 ×g. Finally, 150 µL of supernatant was transferred to a new tube. The quantity and quality of extracted DNA were estimated on 1% agarose gels.

ISSR amplification

The ISSR analysis was performed using the procedure as described in (Gross *et al.*, 2012). Ten ISSR primers (obtained from MacroGen, Seoul, Korea) were tested for screening polymorphisms; four primers (ISSR-06, ISSR-07, ISSR-08 and ISSR-10) that produced reproducible ISSR bands were selected to amplify DNA samples of five *L. undulata* populations (Table 2).

Table 2. Primers characteristics for ISSR analysis.

Primer	Sequence (5′-3′)	Annealing temperatures (°C)	GC%	Mw
ISSR-06	ACACACACACACARG	48.2	50	48052
ISSR-07	AGAGAGAGAGAGAGYC	48.2	50	502707
ISSR-08	GTGGTGGTGGTGRC	46	64.3	43988
ISSR-10	CACCACCACCACRC	46	64.3	41148

ISSR reactions were performed in a 10 µL reaction mixture containing 1 µL of template DNA solution, 1 µL of primers, 3 µL of deionized water and 5 µL of master mix containing: 200 µM of mix dNTP, 1.5 mM Mg^{2+}, 1×polymerase buffer and 1U *Taq* DNA polymerase (Ampliqon, Denmark). Amplification reactions were conducted with a PCR Thermal Cycler (Hettich, Germany). The cycling parameters were 95°C, 10 min and 30 cycles of 30 s at 95°C, 30 s at 45°C and 1 min at 72°C and a final extension of 10 min at 72°C. PCR products were separated on 1 % agarose gels in a 1×TBE buffer system. DNA stain (Smobio, Taiwan) was used for visualizing DNA bands and DNA ladder of 100 bp to 3000 bp (Smobio, Taiwan) as a size marker. The electrophoresis was run at 200 V and 90 mA for about one hour. Bands of DNA in the gel were revealed and photographed using Gel Documentation System (Bio Rad Co., USA).

Data analysis

For each ISSR reaction, only distinct, well-resolved, reproducible bands were scored as present (1) or absent (0) and the resulting matrix was organized in MS-Excel. Statistical analysis of data was performed using GenAlEx 6.51b2 Software and various parameters of genetic diversity: the percentage of polymorphic bands (*P* %), Nei's gene diversity (*H*), Shannon's information index (*I*), the observed number of alleles (*Na*) and the effective number of alleles (*Ne*) were estimated. The Analysis of Molecular Variance (AMOVA) was used to describe the molecular variance components and their significance levels for variation among individuals within and among the populations (Peakall and Smouse, 2012). A Principal Coordinate Analysis (PCoA) was performed to further examine the genetic relationships among the populations on the basis of the same ISSR data. A Mantel test was performed to explore the correlation between the geographic and genetic distances (Mantel, 1967).

Results

Morphological traits

The analysis of variance of morphological data indicated a significant difference between the means of morphological features in five *L. undulata* populations grown in a common garden experiment (Supplement 1). The results of the study of morphological features showed that most of the studied traits have a high diversity among populations (Supplement 2). The highest leaf length (12.18 cm), hair density on adaxial surface of leaf (3.66), number of flowers in inflorescence (23) and length of beak (11.24 mm) were observed in Mirzabaylu population while the lowest hair density on abaxial surface of leaf (0.83) and stigma length (0.37 mm) were observed in Biarjmand population. Also, Biarjmand population had the highest leaf surface area (7.06 cm), leaf width (2.33 cm) and the achene width (1.17 mm). The Qom population showed the highest achene length (3.19 mm) and the lowest leaf length (6.91 mm) and the number of flowers in inflorescence (16.33). Firoozkooh population had the highest hair density on abaxial surface of leaf (7.33) and style length (1.07 mm) and the lowest hair density on adaxial surface of leaf (1.16), number of leaf lobes (8.66) and leaf width (1.37). The highest length of the stamen was observed in Cheshmeh Ali (6.32 mm) and Firoozkooh (6.09 mm) populations and the highest stigma was observed in Mirzabaylu (0.55 mm) and Firoozkooh populations (0.51 mm).

Significant differences in stem elongation (bolting) and flowering time were observed among the five populations. Specimens from Qom and Biarjmand populations began flowering in less than two months while flowers in Mirzabailu, Cheshmeh Ali and Firoozkooh populations appeared more than a month later

(Fig. 3). In the PCoA performed, five populations of *L. undulata* were identified based on their morphological characters (Fig. 4); the first component explains about 31% of the variation, while the second explains about 19%. The ordination pattern shows a first group in the sector on the right, composed of individuals of Biarjmand population classified by the seed length, seed width, leaf width and leaf area.

Fig. 3. Plants obtained from sowing seeds of five *L. undulata* populations: M) Mirzabayloo; F) Firoozkooh; B) Biarjmand; CH) Cheshmeh Ali; Q) Qom.

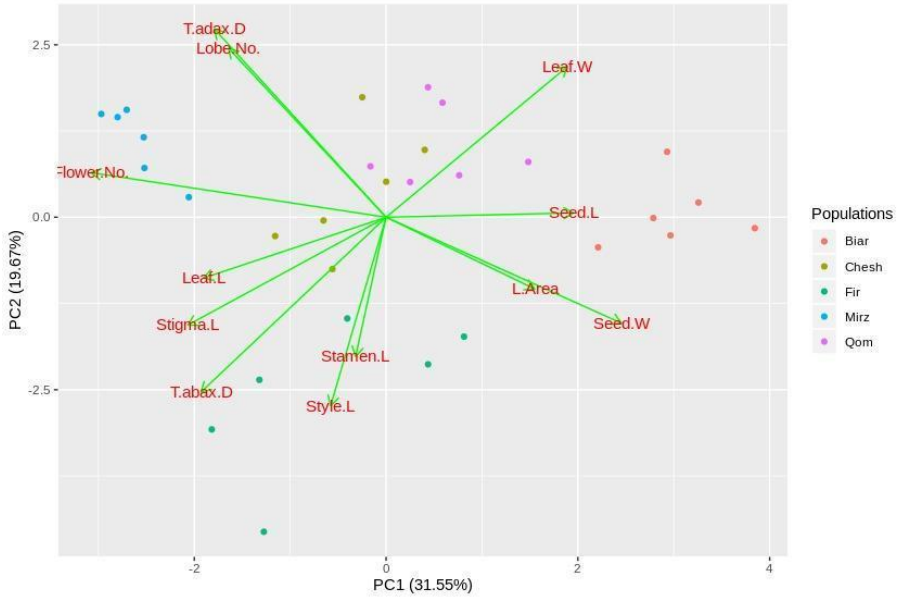

Fig. 4. PCoA analysis of morphological characteristics of five *L. undulata* populations in Iran.

The second group is composed of individuals of Mirzabaylu population which is classified by the number of flowers, hair density on adaxial leaf surface and number of leaf lobes. Firoozkooh population is in the lower left part of the plot. Members of this population have the highest hair density on abaxial leaf surface and the longest style. Individuals of this population are classified by the leaf length, stigma length, stamen length, style length and hair density on abaxial leaf surface. Qom and Cheshmeh Ali populations were not classified under distinct groups. However, they were clustered at the center of the plot.

Genetic diversity

Four selected ISSR primers were used to perform PCR and amplify DNA fragments of all 100 samples from five natural populations of *L. undulata* (Table 1, Fig. 5), which yielded 60 discernible and bright bands in the size-range of 300-2500 bp.

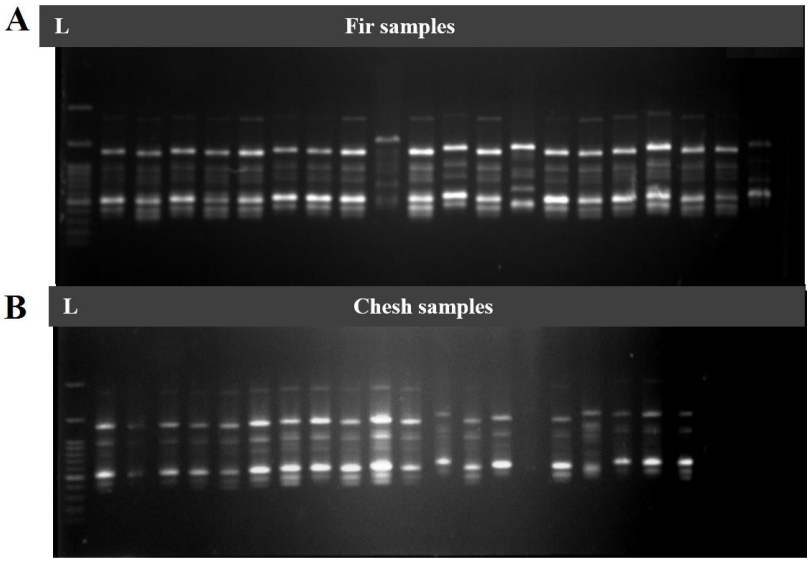

Fig. 5. A representative ISSR bands pattern of *L. undulata* populations obtained by primer ISSR-07: A) Firoozkooh populations; B) Cheshmeh Ali populations; L= DNA Ladder (100 bp to 3000 bp).

The results of the analysis of genetic diversity showed that the percentage of polymorphic bands (*P*) ranged from 60% (Qom) to 78.3% (Chesh) (Table 3) with an average of 67% at the population level. The mean observed number of alleles (*Na*) ranged from 1.26 (Qom) to 1.56 (Cheshmeh Ali) while the mean effective number of alleles (*Ne*) varied from 1.38 (Qom) to 1.50 (Cheshmeh Ali). Shannon's information indices (*I*) varied from 0.32 (Qom) to 0.42 (Cheshmeh Ali), with an average of 0.36 and Nei's genetic diversity (*H*) ranged from 0.22 (Qom) to 0.28 (Cheshmeh Ali), with an average of 0.24. The highest and lowest genetic diversity was observed in Cheshmeh Ali *I*= 0.42, *H*= 0.28) and Qom populations (*I*= 0.32, *H*= 0.22), respectively. Comparing Nei's genetic distances between pairs of populations showed that the lowest genetic distance was between Qom and Biarjmand populations (0.047) and the highest genetic distance was between Qom and Firoozkooh populations (0.176) (Table 4). The hierarchical AMOVA revealed that the major variance component (73%) was observed within the populations while only 27% variance was reflected among the populations. Both variance components proved highly significant values ($p < 0.001$) (Table 5).

Table 3. Genetic diversity estimates of *L. undulata* populations based on ISSR loci.

Pop	*N*	*Na*	*Ne*	*I*	*H*	*%P*
Firoozkooh	20	1.33	1.39	0.34	0.22	61.67
Mirzabaylu	20	1.43	1.40	0.35	0.23	68.33
Biarjmand	20	1.40	1.42	0.36	0.24	66.67
Qom	20	1.26	1.38	0.32	0.22	60.00
Cheshmeh Ali	20	1.56	1.50	0.42	0.28	78.33
Mean	20	1.40	1.42	0.36	0.24	67.00

Na: No. of Different Alleles; *Ne*: No. of Effective Alleles; *I*: Shannon's Information Index; *H*: Nei's gene diversity; and %*P*: Percentage of polymorphic loci.

Table 4. Nei's genetic distances and identity among populations of *L. undulata*.

POP	Firoozkooh	Mirzabaylu	Biarjmand	Qom	Cheshmeh Ali
Firoozkooh	****	0.118	0.173	0.176	0.131
Mirzabaylu	0.889	****	0.084	0.098	0.076
Biarjmand	0.841	0.919	****	0.047	0.059
Qom	0.838	0.907	0.954	****	0.056
Cheshmeh Ali	0.877	0.927	0.943	0.946	****

Above diagonal: Nei's genetic distances; below diagonal: Nei's genetic identity.

Table 5. Analysis of molecular variance (AMOVA) for *L. undulata* by ISSR.

Source of variation	df	Sum of squares	MS	Variance components	% Variation
Among Pops	4	262.720	65.680	2.900	27%
Within Pops	95	728.850	7.672	7.672	73%
Total	99	991.570		10.573	100%

*Based on the Mantel test, the correlation between genetic and geographical distances was weak (r= 0.148, P <0.01) (Supplement 3).

Principal coordinate analysis (PCoA) was used to provide spatial representation of the relative genetic distances among the individuals. The first two principal coordinates explained 15.96% and 11.52% of total variation, respectively. A total of 34.73% was explained by the first three components (Fig. 6).

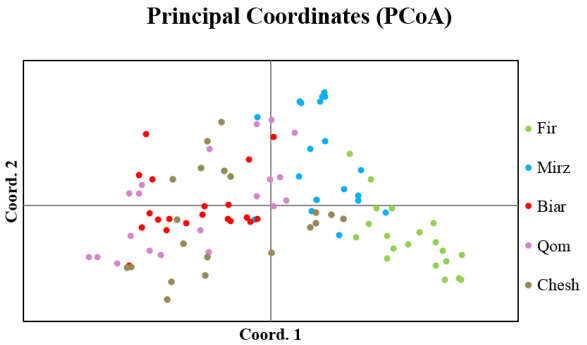

Fig. 6. A two-dimensional plot of the Principal Coordinate Analysis (PCoA) of ISSR data showing the clustering of populations of *L. undulata*.

The three populations of Qom, Cheshmeh Ali, and Biarjmand were clustered in one area, while the two populations of Mirzabaylu and Firoozkooh were separated. However, some members of Qom and Cheshmeh Ali were clustered within the population of Mirzabaylu (Fig. 4).

Discussion

High morphological and genetic polymorphism was found in *L. undulata* populations. The total number of bands scored was 60, 100% of which were polymorphic, indicating a relatively high level of genetic diversity in the populations under study. Similar results were reported on *L. sativa* varieties and genotypes in which band polymorphism of 67 to 100% was found (Al-Redhaimam *et al.*, 2005; Hwang *et al.*, 2002; Sharma *et al.*, 2018; Tardin *et al.*, 2003). Also, our findings are comparable with Jemelková *et al.'s* (2018) study in which they reported 44.8 to 84.4% polymorphism in *L. serriola* populations in Sweden and Slovenia using SSR and AFLP markers. Liang *et al*. (2014) recorded 87.9% polymorphism in chicory accessions. Mean gene diversity has been reported by Liang *et al.* (2014) to be *I*= 0.572 and *H*= 0.384 among chicory accessions. This is while in this study, we found mean gene diversity of *I*= 0.326 and *H*= 0.220 in Qom population to *I*= 0.420 and *H*= 0.285 in Cheshmeh Ali population.

The results of the common garden experiment showed that morphological traits in the five populations of *L. undulata* vary. The characteristics of lettuce achenes, including the length and width and the length of the beak are important morphological traits used to classify different subspecies of lettuce (Křístková *et al*., 2014). In the current study, achene length ranged from 2.52 mm in Mirzabaylu population to 2.76 mm in Firoozkooh population and 3.5 mm in Qom population. The increase in achene length is therefore in an east-west transect. This finding is consistent with the results of Novotná *et al*. (2011) who studied the morphology of achenes of *L. serriola* populations in Europe. They concluded that achene length and width increased along an east–west transect. In the present study, this result was not observed for achene width. Achenes in Mirzabaylu, Biarjmand and Firoozkooh populations had significantly longer beaks (11.24, 10.55 and 9.08 mm respectively) compared to achenes of Qom population (7.87 mm) (Supplement 2). Mirzabaylu, Biarjmand and Firoozkooh populations were collected from undulating plains and Qom population was collected from plain fields. This finding is consistent with the results of Novotná *et al*. (2011) which reported that the length of beaks could be partly influenced by landform. Cody and Overton (1996) reported that dispersal ability reached higher values in the longer-beaked populations, and diaspores with beaked achenes have significantly lower settling velocities than diaspores with unbeaked achenes. The mean value of the L/W index ratio decreased along a

west-east transect from 5.05 in Qom to 3.11 in Firoozkooh and 2.81 and 2.55 in Cheshmeh Ali and Biarjmand, respectively, with the exception of the Mirzabaylu population that had a ratio of 3.93 (Supplement 2). This finding is consistent with the results of Novotná *et al.* (2011) who reported that the mean value of the L/W index ratio in achenes of *L. serriola* populations decreased along a west-east transect in Europe. On the other hand, by increasing the latitude, the length of beak increased while the length of the achene decreased. This is in accordance to the findings of Křístková *et al*. (2014) who reported that the latitude had a significant influence on the morphological characters of *L. serriola* achene, so that as the latitude increases, the length of beak increases, while the length of the achene decreases. Křístková *et al.* (2014) reported that although climatic conditions, altitude, latitude and longitude, and soil type affect these differences, the observed differences are likely to be the result of genetic variation between individuals and populations. A high level of variation in plant phenotypes has been reported in greenhouse experiments among samples of two widely distributed species, *L. saligna* and *L. serriola* (Doležalová *et al.,* 2005; Lebeda *et al.,* 2007; Novotná *et al.,* 2011). Lebeda *et al.* (2013) hypothesized that these variations resulted from the evolutionary adaptation of plants under different ecological and climatic conditions in their original habitats. The phenotypic variation observed in *L. undulata* seems to be due to the widespread geographical distribution of this species compared to *L. aculeata*, which has made it to adapt to different climatic conditions.

Within the genus *Lactuca*, a high level of variations has been reported in phenological characteristics among accessions grown in greenhouse experiments. Samples of *L. serriola* from various countries have shown substantial differences in the time of flowering (Doležalová *et al.,* 2005). A similar pattern of flowering has been recorded for *L. saligna* samples (Křístková *et al.,* 2011). Persistence of differences in developmental rates of plants grown in a uniform environment demonstrates their genetic basis (Lebeda *et al.,* 2013). Differing flowering time in plants plays a major role in their reproductive success and is also considered an important trait in agriculture. Several mechanisms have been adopted by plants to synchronize flowering, so that they can maximize seed yields by undertaking fertilization and seed development at the optimal time. Several distinct environmental cues, such as photoperiod, vernalization, and higher ambient temperatures, as well as endogenous cues, such as ageing, the phytohormones (gibberellin), and accumulation of the carbohydrate are responsible for promoting flowering processes. These signaling cues are mostly perceived in the leaves and shoot apical meristem to initiate flower formation (Kinoshita and Richter, 2020). Extensive genetic studies over the last decades have identified key regulators responsible for flowering. These regulators function in some discrete flowering pathways (Kinoshita and Richter, 2020). Bolting is a key process in the growth and development of lettuce (*Lactuca sativa* L.) and the bolted lettuce undergoes various physical and chemical changes (Sung *et al.,* 2016). Unlike most other flowering plants, transition from vegetative to reproductive phase in lettuce is induced by high temperatures and bolting via auxin synthesis and accumulation of carbohydrates (Hao *et al.,* 2018). Han *et al.* (2016) examined the phenology of two bolting-sensitive and bolting-resistant lines of edible lettuce and reported that the concentrations of auxin and jasmonic acid were significantly higher in bolting-sensitive lines. Proper timing of flowering is a major developmental decision in the life history of plants. In the present study, Cheshmeh Ali, Mirzabaylu and Firoozkooh Areas have higher altitudes and lower average temperatures than Qom and Biarjmand. Therefore, the minimum temperature that the seeds can withstand during the winter is lower in these populations. On the other hand, the onset of the drought period in Qom and Biarjmand Regions occurs earlier and this causes the plant to have a very short period of time to produce seeds and complete its life cycle. These factors may have caused differences in flowering time in these populations through evolution by altering the metabolism of growth regulators, especially auxin and gibberellin. Subsequent research could show the role of genetics, environmental factors, and growth regulators in these areas.

Results of AMOVA analysis revealed that most of the variations in *L. undulata* are within the populations (73%) and lesser amounts of the variations are among the populations (27%; Table 5). This result is consistent with the finding of Jemelková *et al*. (2018) that observed a major variance component (66%) within *L. serriola* populations and 34% of the total variation among the populations. This finding is, however, in contrast with the report of Sheidai *et al*. (2016) who observed 54% of the total genetic variability among *Cirsium aduncum* populations and 46% of it within the population. Also, the high genetic diversity within the *L. undulata* populations is inconsistent with the results of Rauscher and Simko (2013) who used SSR markers in *L. sativa* and reported that genetic diversity among different lettuce accessions (97%) is more than within the accessions (3%). Hossein-Pour *et al.* (2019) stated that higher variations within populations and genotypes could be the result of adaptation, selection, gene flow, variation in ecotypes, genetic drift, and the pollination syndromes. Higher variations could also be due to human activities and environmental changes over time (Solouki *et al.*, 2008).

The results of PCoA analysis of morphological traits showed that the two populations of Qom and Cheshmeh Ali are adjacent to each other in the plot, indicating the similarity between the two groups of samples. The three populations of Cheshmeh Ali, Mirzabaylu and Firoozkooh are classified into separate groups. These results are somewhat consistent with the results of the PCoA of genetic data. The PCoA plot of genetic data revealed some degree of genetic admixture between five populations. Some populations were placed in overlapped (intermixed) regions in the molecular plot. For example, Cheshmeh Ali and Biarjmand populations were placed close to Qom population and similarly, Mirzabaylu population was placed close to Firoozkooh population. According to the PCoA analysis, despite the long geographical distance between the populations of Firoozkooh and Mirzabaylu (compared to the populations of Mirzabaylu and Biarjmand), these two populations are in the same group. This result was also observed in the populations of Qom and Biarjmand, which was consistent with the low correlation between the geographical and genetic distances obtained from the Mantel test. The fact that these two populations with a greater geographical distance are genetically similar may indicate that the pressure of natural selection on these populations has not yet been greatly affected, or possibly due to a gene flow (seed migration) which is sometimes faster than expected (Hamrick *et al.*,1992).

Various species of lettuce, including *L. serriola*, *L. saligna*, and *L. aculeata*, have been used to produce edible lettuce hybrids and improve their quality (Lebeda *et al.*, 2012). However, so far, no research has been done on the possibility of hybridization and domestication of *L. undulata* and *L. sativa*.

Because of the presence of valuable phenolic compounds such as caffeic acid and its derivatives, it seems that the increase in these compounds in *L. sativa* and also the production of pest-resistant cultivars through the production of hybrids between *L. undulata* and *L. sativa* is worthwhile. High genetic diversity within the populations is itself a crucial factor in preventing them from extinction. Maintaining genetic diversity is one of the main goals of endangered species conservation (Hamrick and Godt, 1997). Due to the high diversity of morphology and genetics among the *L. undulata* populations, planning to protect this rich gene source is of great importance.

The high rate of genetic diversity in the population of Cheshmeh Ali (*I*= 0.420, *H*= 0.285, *P*= 78 %,) and its low rate in the population of Qom (*I*= 0.326, *H*= 0.220, *P*= 60 %) indicates the ability of the population of Cheshmeh Ali and the greater sensitivity of the population of Qom in the face of changing environmental conditions. Due to the relatively high genetic diversity within the five populations of *L. undulata* and the authors' field observations of a small number of individuals in all five habitats and plant extinction in areas mentioned in previous studies as habitats, the declining population of this species in nature seems to have occurred in recent years. Because to date, there is no study on the protection and cultivation of this species, seed collection, domestication and cultivation are recommended.

Conclusion

Several genetic diversity indices revealed the presence of higher diversity in the studied samples, which can be used for future conservation programs. For the first time, genetic and morphological diversity of *L. undulata* populations were studied. It was found that the ISSR markers have a high capability in identifying polymorphic regions and studying the diversity and conservation of *L. undulata* populations.

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Conflict of interests

The authors declare that they have no conflict of interest.

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