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Cryptic molecular-geographical divergence among Iranian *Silene aucheriana* populations inferred from ISSR and climatic data

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Abstract

Silene aucheriana Boiss. belongs to the Sect. *Auriculatae* (Boiss.) Schischk. in the *Caryophyllaceae* family, widely distributed in Iran. In this study, 75 random samples from 15 populations of different geographical and climate conditions in Iran were collected. Inter Simple Sequence Repeat (ISSR) technique has been used to analyse the genetic diversity in Iranian *Silene aucheriana* populations. Analysis of molecular variance (AMOVA) demonstrated 87% of the genetic variation exists among populations and 13% within the populations, which is in agreement with the high genetic differentiation among population diversity ($Gst = 0.8$), and a low level of gene flow ($Nm = 0.07$). Two distinct gene pools in the main distribution areas have been identified by the STRUCTURE and the K-Means analyses. Our results suggest that genetic divergence is present amongst populations that is most likely caused by their climatic conditions.

Introduction

Silene L. is the largest genus in the *Caryophyllaceae* family with more than 800 species found around the world (Bittrich 1993; Greuter 1995). *Silene* subgeneric classification has been organized in different ways according to its great diversity and number of species (Greuter 1995; Oxelman and Lidén 1995; Oxelman et al. 2001). Initially Chowdhuri (1957) establishes 44 sections, then Melzheimer (1988) recognized 21 sections but more recently, based on molecular evidence the genus has been classified into three subgenera and 34 sections (Desfeux and Lejeune 1996; Oxelman et al. 1997, 2001; Eggens et al. 2007; Popp and Oxelman 2007; Petri and Oxelman 2011; Rautenberg et al. 2012;

Naciri et al. 2017). This genus is perennial, stems erect up to 40 cm, the inflorescence dichasial panicles, stem leaves linear-lanceolate, calyx ovate to cylindrical, teeth triangular, and capsule ovate (Melzheimer 1988). In Iran, section *Auriculatae* (Boiss.) Schischk. is one of the largest sections with 57 species, of which 27 are endemic (Jafari et al. 2019). Recent molecular analyses of the *Auriculatae* section show that *Silene aucheriana* Boiss. belongs to this section (Jafari et al. 2019; Safaeishakib et al. 2020). This species is found in Anatolia, Iraq, Persia, and Turcomania (Melzheimer 1988) and distributed in the west, northwest, the Alborz slope, northeast, east, and central part of Iran (Chowdhuri 1957; Rohrbach 1868; Melzheimer 1988; Greuter 1995; Gholipour and Amini Rad 2017).

Inter-Simple Sequence Repeats (ISSR) has been reported as a useful tool for investigating genetic variation and population structure (Zietkiewicz et al. 1994; Wang et al. 2008; Baruah et al. 2013; Coutinho et al. 2014; Ferreira et al. 2015; Hilooğlu et al. 2016; Safaei et al. 2016). Moreover, it has been used effectively to estimate the genetic differentiation and phylogenetic relationships among four subspecies of *S. scabriflora* from the Iberian Peninsula using ISSR and cpSSR markers (Ferreira et al. 2015). This molecular method has also been used for the mating system to develop *S. vulgaris* cultivars of high quality with 14 accessions based on

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morphological and biochemical traits (Egea-Gilabert et al. 2013). Genetic diversity may appear spatially structured at different scales, such as among populations and neighboring individuals (Escudero et al. 2003). Analysis of spatial genetic structure is a valuable method for inferring evolutionary forces like random drift and selective pressures (De Kort et al. 2013). Populations of *S. aucheriana* are widely distributed in different regions of Iran with different ecological conditions and therefore, it is expected to show genetic differences. Regarding the ability of ISSR markers to detect minor genetic differences (Godwin et al. 1997) we used it to illustrate genetic structure and variation among 15 populations from different regions of Iran. The main goals of this paper were to (a) estimate the genetic diversity, (b) to investigate the inter- and intra-population genetic structure of Iranian

S. aucheriana, and (c) assess the geographical distribution and similarity in climate conditions for studied populations.

Materials and methods

Plant material and sampling

Plant descriptions from *Flora Iranica* (Melzheimer 1988) and *Flora of Turkey* (Coode and Cullen 1967) were used to identify the collected populations. The climatic conditions were used to select the population of interest for this study. For the analysis, 15 populations were divided into two groups according to their elevation (Table 1, Fig. 1). Populations with an elevation cut-off limit higher than

Table 1 Localities and voucher numbers of *S. aucheriana* populations used for this study. In different regions, GPS was used to determine the location (longitude, latitude, and elevation). Average rainfall and temperature were obtained from (www.en.climate-data.org). Highlighted are areas with an elevation cut-off limit higher than 2800 m

| Populations	Locality	Longitude/Latitude	Elevation cut-off limits (meter)	Elevation (meter)	Average temp (°C)	Average rainfall (mm)	Voucher No
Pop 1	Haraz-Emamzadeh Hashem mountain (Em-ha)	52° 02′ 08"E 35° 50′ 04"N	**> 2800**	3050	6.8	374	IAUH000015357
Pop 2	Semnan-Firoozkooh to Sorkheh, Gorsfid	53° 31′ 59"E 35° 15′ 19"N	< 1800	1251	17.40	103	IAUH000015358
Pop 3	Hamedan-Heydareh	48° 27′ 45"E 34° 46′ 41"N	< 1800	1115	13.32	443	IAUH000015359
Pop 4	Esfahan-Khansar, Golestan Kooh	50° 27′ 12"E 33° 07′ 35"N	< 1800	1723	13.52	222	IAUH000015360
Pop 5	W-Azerbaijan, Qoshchi (to Salmas)	45° 03′ 32"E 37° 59′ 38"N	< 1800	1780	15.80	321	IAUH000015361
Pop 6	Chalous- Zangolehpass	51° 07′ 06"E 36° 28′ 15"N	**> 2800**	2840	6.5	1660	IAUH000015362
Pop 7	W-Azerbaijan-Silvana	44° 51′ 02"E 37° 23′ 57"N	< 1800	1720	19.49	982	IAUH000015363
Pop 8	Firoozkooh- Gaduk pass	52° 55′ 05"E 35° 49′ 29"N	**> 2800**	2840	8.5	530	IAUH000015364
Pop 9	Rudbar-Klishom village	49° 25′ 27"E 36° 46′ 32"N	< 1800	1780	12.9	907	IAUH000015365
Pop 10	Larijan-Rineh	52° 10′ 07"E 35° 52′ 52"N	**> 2800**	2889	11.82	1044	IAUH000015366
Pop 11	Chalous road- Dona village	51° 22′ 09"E 36° 11′ 46"N	< 1800	1790	16.73	1440	IAUH000015367
Pop 12	Tehran-Tochal	51° 24′ 20"E 35° 49′ 12"N	**> 2800**	3010	9.38	429	IAUH000015368
Pop 13	Zanjan-Tarom	4818 49′ ‴E 36° 39′ 29"N	< 1800	1010	15.45	386	IAUH000015369
Pop 14	Tehran-Dizin	51° 25′ 02"E 36° 02′ 57"N	**> 2800**	2823	7.33	591	IAUH000015370
Pop 15	Khorasan Razavi—HezarMasjed mountain	60° 38′ 21"E 36° 04′ 23"N	**> 2800**	2950	10.3	370	IAUH000015371
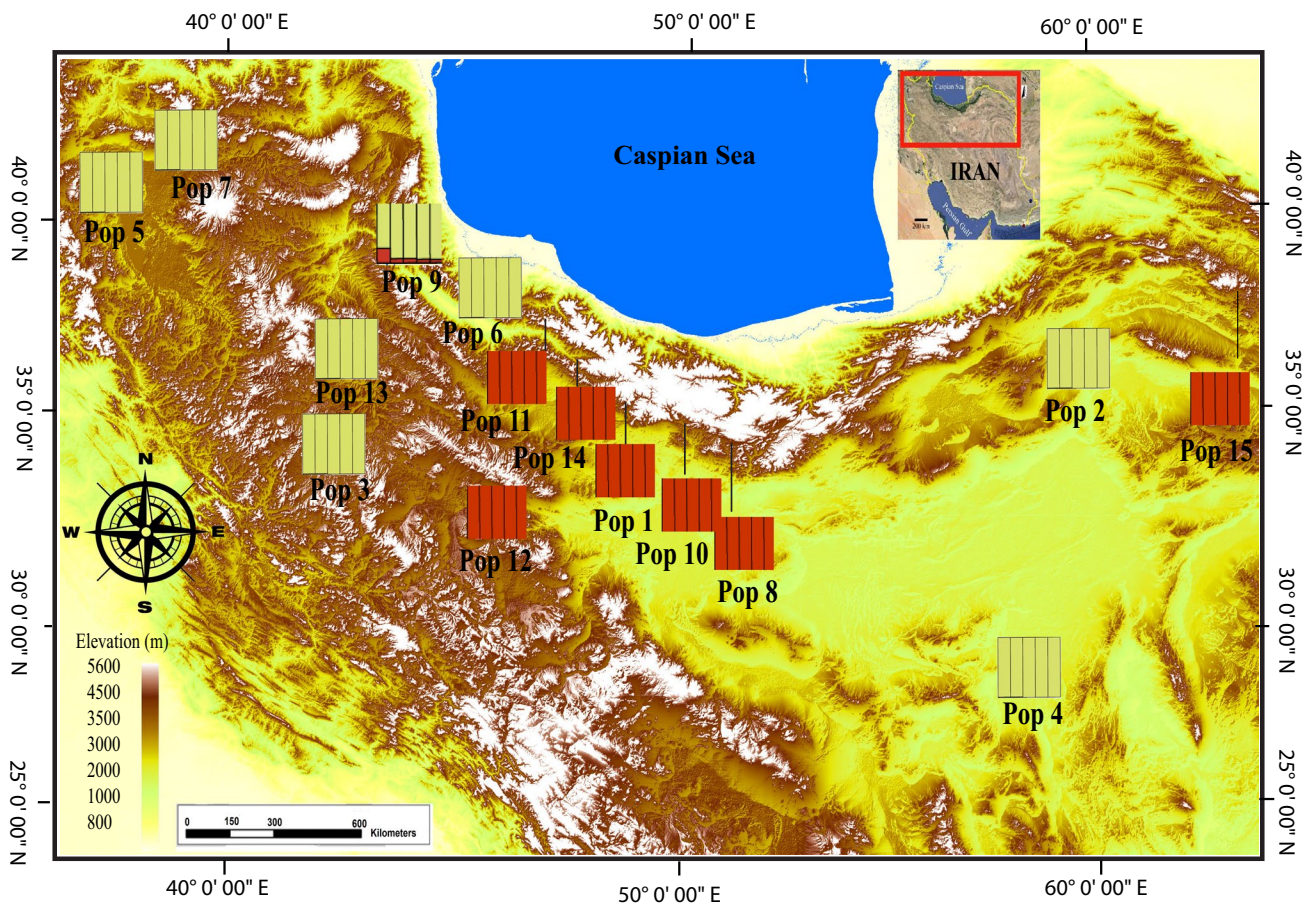
Fig. 1 Topographic map of the populations distribution of *S. aucheriana* in Iran showing the STRUCTURE plot based on k = 2. (Map designed using Arc GIS 10.2). Brown-colored segments reflect elevation cut-off limit higher than 2800 m, lower average temperature, and less rainfall (8.66 °C, 566.34 mm), whereas yellow-colored segments show different climate conditions with an elevation cut-off limit lower than 1800 m, higher average temperature, more average rainfall, (15.57 °C, 600 mm). Local names are presented in Table 1

2800 m (mountainous area and dry cold) in one group and those with an elevation cut-off limit lower than 1800 m (semi-arid, and sub-humid) placed in another group. These cut-off limits were based on the mean elevations of localities. The assessment of these climate differences and classification was based on the Köppen-Geiger (KG) climate classification scheme (Peel et al. 2007), www.WorldClim.org, (Fick and Hijmans 2017), www.weatherunderground.com, and (Hijmans et al. 2005) definitions. These populations of *S. aucheriana* were selected from different climate conditions and different geographic locations. Such information would be useful not only for breeding but also for effective genetic diversity and conservation. Therefore, further characterization and identification of genetic diversities of the existing populations under different climate conditions would be beneficial for genetic diversity among and within the populations (El-Kassaby and Ritland 1996). Five specimens from each population were collected randomly at about 200 m intervals of each other, for a total of 75 specimens. Fresh leaves were collected randomly from plant specimens and dried in silica gel. Voucher specimens were deposited at the herbarium of Islamic Azad University, Tehran, Iran (IAUH). Meteorological data on localities including average temperature and rainfall were obtained from www.climate-data.org (Table 1).

DNA isolation and ISSR-PCR amplifications

Total genomic DNA was extracted from silica-dried leaves (approximately 0.5 g material per sample) using CTAB activated charcoal protocol (Doyle and Dickson 1987; Doyle and Doyle 1987). The quality and integrity of extracted DNA was determined by running on 1% agarose gel electrophoresis. High-quality DNA samples were stored at -20 °C until required. A set of 16 ISSR primers were selected according to Agarwal et al. (2008) and Sharma et al. (2008) studies. These primers include UBC-801 $(AT)_8$ T, UBC-802 $(AT)_8$G, UBC-803$(AT)_8$C, UBC-804 $(TA)_8$A, UBC-805 $(TA)_8$C, UBC-806 $(TA)_8$G, UBC-807 $(AG)_8$ T, UBC-808 $(AG)_8$C, UBC-809 $(AG)_8$G, UBC-810 $(CA)_8$ T, UBC-812 $(GA)_8$A, UBC-818 $(CA)_8$G, UBC-834 $(AG)_8$YT, UBC-848 $(CA)_8$RG, UBC-855 $(AC)_8$YT, UBC-861 $(ACC)_6$

commercialized by UBC (the University of British Columbia). All of these primers were preliminarily assayed on a limited number of individuals. This screening allowed us to identify eight primers producing more variable bands: UBC-807 $(AG)_8$ T, UBC-808 $(AG)_8C$, UBC-810 $(CA)_8$ T, UBC-818 $(CA)_8G$, UBC-834 $(AG)_8YT$, UBC-848 $(CA)_8RG$, UBC-855 $(AC)_8YT$, UBC-861 $(ACC)_6$.

Polymerase Chain Reaction (PCR) were carried out in a 25 µl volume containing 10 mM Tris–HCl buffer at pH 8.0 mM KCl; 1.5 mM MgCl2; 0.2 mM of each dNTP (Bioron, Germany); 0.2 µM of each primer; 20 ng genomic DNA and three U of Taq DNA polymerase (Bioron, Germany). The PCR amplification was performed with Techne thermocycler (Sensoquest, Göttingen, Germany) using the following program profile: Pre-heating at 94 °C for 5 min, 30 cycles of denaturation for 40 s at 94 °C, annealing for 1 min at 48 °C, the extension for 1 min at 72 °C, and 7 min final extension at 72 °C (Doyle 1991). As a standard for determining the size of the amplified fragments, the 100 bp DNA marker size was used. The quality of the amplification products was initially checked on 1% agarose gel. The PCR products of NED, PET, 6-FAM, and VIC labeled products were pooled (2 pools for 8 primers). 2 µl of each compound product was added to 7.75 µl of HiDi formamide (Applied Biosystems, Foster City, CA, USA) and 0.25 ll ROX-500 internal size standard (Applied Biosystems) were then injected to a 3730xl capillary sequencer (Applied Biosystems-Hitachi). Raw ISSR data were visualized with GENE MAPPER version 4.0 (Applied Biosystems, Foster City, CA, USA). Output ISSR files were aligned with the internal size standard using GeneMarker version 1.95 (GeneMarker, SoftGenetics, State College, Pennsylvania). Each peak with a signal intensity of more than 500 RFU (Relative Fluorescence Units) was scored as the present. The presence (1)/ absence (0) binary matrixes of two primer sets were combined and prepared for analysis (Hulce et al. 2011).

Data analysis

The achieved ISSR bands were coded as binary datamatrix and used for genetic diversity analysis. Genetic diversity parameters including the average number of alleles per locus (*Na*), the number of effective alleles (*Ne*) (Kimura and Crow 1964), Shannon's information index (*I*) (Lewontin 1972), average observed heterozygosity (*Ho*), average expected heterozygosity (*He*), number of bands (*NB*), the percentage of polymorphic loci (*P%*), unbiased heterozygosity (*uHe*) (Weising et al. 2005; Freeland et al. 2011) were calculated using GenAlEx 6.4 (Peakall and Smouse 2006). Additionally, the Nei's genetic distance matrix (Nei 1973) were analysed using the POPGEN version 1.32 (Yeh et al. 1997). Moreover, population genetic differentiation of the studied genotypes was performed in GenoDive version 3 (Meirmans

and Tienderen 2004). The correlation between the populations' genetic and geographic distance was estimated by the Mantel test (Mantel 1967) as implemented in PAST version 3.18 (Podani 2000; Hammer 2012) performing 5,000 permutations. Analysis of molecular variance (AMOVA) test (1000 permutations) (Excoffier et al. 1992) were performed using of GenAlex version 6.4 (Peakall and Smouse 2006). Furthermore, the genetic differentiation was determined using PhiPT, a measure that permits intra-individual variation to be suppressed and is therefore ideal for comparing codominant and binary data with 999 permutations (Assoumane et al. 2012). The Bayesian clustering approach implemented in STRUCTURE version 2.3.4 (Falush et al. 2007) was used to infer groups or subpopulations in the dataset. The analysis was performed with an admixture linkage model with correlated allele frequencies. To evaluate the discontinuous group (K) numbers, the K number was set between 1 and 15 with 10 replicates, and the length of the burn-in period was set to 100,000 times (Evanno et al. 2005; Zhong et al. 2019). The online STRUCTURE HARVESTER tool (Earl and von-Holdt 2012) was used to apply the Evanno method (Evanno et al. 2005) to detect the value of the optimal K that best fit the data. In K-Means clustering, two summary statistics, pseudo-F, and Bayesian Information Criterion (BIC), provide the best fit for k (Meirmans 2012). A Neighbor -joining (NJ) phylogenetic tree was constructed based on the Nei's (1973) genetic distances for determining the genetic relationship among populations and principal coordinate analysis (PCoA) determined the relative genetic distance between individuals by using PAST version 3.18 (Hamer et al. 2001).

Results

Genetic diversity among the populations

The size of the amplified fragments ranged from 100 to 1700 bp. The UBC-807 primer amplified the largest number of loci (19) and the UBC-848 primer the smallest (11). The total number of loci amplified by the eight ISSR primers was 138, with an average of 15 loci per primer. For evaluating the genetic diversity of *S. aucheriana* populations a high number of reproducible and reliable bands from primers were achieved. The Tarom (Pop 13) population exhibited the highest number of private and common bands. While in the populations from Emamzadeh Hashem (Pop 1), Zangoleh (Pop 6), and Dizin (Pop 14) the value of the private bands became zero. The highest values of *He* (0.060), *uHe* (0.066), and *Ho* (0.041) were generated in the Klishom population (Pop 9). Whereas the lowest values of *He* (0.011), *uHe* (0.012), *Ho* (0.009), *I* (0.015), and *Na* (0.020) were achieved in Gorsfid (Pop 2) population. Furthermore, Silvana (Pop 7), and Klishom (Pop 9) had the

highest values of ($I = 0.088$, $Na = 1.112$) respectively. The other genetic diversity parameters ($NB = 57$, $Ne = 0.500$) showed the highest value in the Tarom (Pop 13) population and the lowest values of ($NB = 23$, $Ne = 0.196$) were gained in the Dona population (Pop 11). Additionally, analysis of the present data displayed that the percentage of polymorphic loci ($P\%$) ranged from (2.17%) in the Gorsfid population (Pop 2) to (16.67%) in the Silvana population (Pop 7) with a mean of (8.74%) (Table 2). The Genetic diversity within populations (Hs) and total expected heterozygosity (Ht) in the populations were (0.03, 0.27) respectively. A high level of genetic differentiation between populations ($Gst = 0.87$) with low and restricted gene flow ($Nm = 0.07$) confirmed divergence and differentiation have occurred among populations (Table 3).

Populational genetic structure

The Mantel test results reflected a highly significant correlation between genetic and geographic distances ($r = 0.2356$, $p = 0.0002$) after 5,000 replications. According to the pairwise population matrix of Nei's genetic identity and the genetic distance determined among the studied populations show that the highest genetic distance (0.583) occurred between Gorsfid (Pop 2), and Tarom (Pop 13) populations while the shortest genetic distance and close affinity (0.165) happened between Tochal (Pop12), and Zangoleh (Pop 6) populations (Fig. 2). These analyses were consistent with the result of AMOVA which estimate that 87% of the genetic variation exists among populations and 13% within the populations. PhiPT values

Table 3 Genetic differentiation parameters of *S. aucheriana* populations using ISSR marker

Ht	Hs	Gst	Nm
0.2724	0.0349	0.8720	0.0734

Total expected heterozygosity (*Ht*), genetic diversity within populations (*Hs*) degree of genetic.differentiation (*Gst*), gene flow (*Nm*)

were statistically significant and were assessed by AMOVA demonstrating more contribution by interpopulation variation (PhiPT value = 0.868, $p = 0.001$) (Table 4). STRUCTURE utilizes a model-based clustering analysis that groups individuals into genetic clusters (K) therefore, populations were divided into two groups. Method of Evanno et al. (2005) was resulted in finding K = 2 (Fig. 3A). The STRUCTURE plot revealed the Tarom (Pop 13), Klishom (Pop 9), Heydareh (Pop 3), Dona (Pop 11), Silvana (Pop 7), Qoshchi (Pop 5), Gorsfid (Pop 2), Golestan Kooh (Pop 4) populations were grouped together while the other studied populations such as Zangoleh (Pop 6), Emamzadeh Hashem (Pop 1), Rineh (Pop 10), Gaduk (Pop 8), Hezar Masjed (Pop 15), Dizen (Pop 14), and Tochal (Pop12) were placed in the same group. However, it can be acknowledged that there is a little allelic combination in the Klishom population (Pop 9) (Fig. 3B). By considering the environmental patterns it can be noticed that Tochal (Pop12), Zangoleh (Pop 6), Emamzadeh Hashem (Pop 1), Rineh (Pop 10), Hezar Masjed (Pop 15), Gaduk (Pop 8), Dizin (Pop 14) populations are placed together and distributed in elevation cut-off limit higher than 2800 m with less average rainfall,

Table 2 Genetic parameters evaluated in the populations of *S. aucheriana* using ISSR marker

Pop	NB	P%	Ne	Na	I	Ho	He	uHe
P01	46	10.14	0.435	1.065	0.056	0.028	0.038	0.042
P02	34	2.17	0.268	1.020	0.015	0.009	0.011	0.012
P03	29	5.80	0.267	1.047	0.036	0.022	0.025	0.028
P04	34	7.25	0.319	1.058	0.045	0.028	0.031	0.035
P05	32	11.59	0.348	1.083	0.067	0.032	0.046	0.051
P06	44	15.22	0.471	1.092	0.081	0.040	0.054	0.060
P07	42	16.67	0.470	1.101	0.088	0.039	0.058	0.065
P08	29	7.25	0.283	1.048	0.040	0.019	0.027	0.030
P09	39	13.77	0.420	1.112	0.085	0.041	0.060	0.066
P10	41	10.14	0.399	1.071	0.057	0.027	0.039	0.043
P11	23	2.90	0.196	1.021	0.017	0.010	0.012	0.013
P12	35	5.07	0.304	1.049	0.035	0.021	0.025	0.028
P13	57	8.70	0.500	1.075	0.056	0.026	0.039	0.044
P14	42	9.42	0.399	1.067	0.055	0.031	0.038	0.042
P15	39	5.07	0.333	1.039	0.030	0.011	0.021	0.023
Mean	37.7	8.74	0.361	1.063	0.051	0.028	0.035	0.039

Number of bands per population (*NB*), the percentage of polymorphic loci (*P%*), effective number of alleles (*Ne*), number of alleles per locus (*Na*), Shannon's information index (*I*), the observed hetrozygosity (*Ho*), the expected heterozygosity (*He*), unbiased heterozygosity (*uHe*)

Fig. 2 Pairwise population matrix of Nei's genetic distances among fifteen populations of *S. aucheriana*. The highest and the lowest genetic distance are highlighted
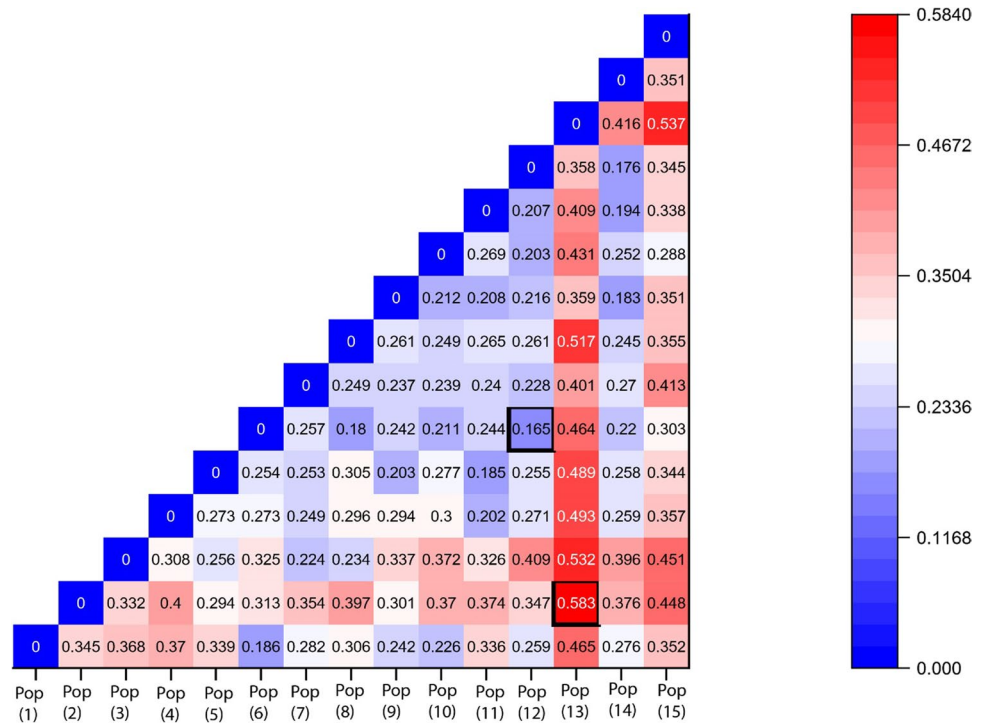
and lower average temperature (566.34 mm, and 8.66 °C) respectively. In contrast, Tarom (Pop 13), Klishom (Pop 9), Heydareh (Pop 3), Dona (Pop 11), Silvana (Pop 7), Qoshchi (Pop 5), Gorsfid (Pop 2), Golestan Kooh (Pop 4) populations separated based on the similarity in climates with more average rainfall, higher average temperature (600 mm, 15.57 °C), and elevation cut-off limit lower than 1800 m (Fig. 1).

Populations grouping based on genetic data

Further analysis with PCoA to ensure the correct grouping of populations was done along the first two axes. These first two axes (69.45% and 19.76%) explained 89.21% of the total variance (Fig. 4A). According to NJ phylogenetic tree and PCoA plot based on ISSR molecular data Hezar Masjed (Pop 15), Rineh (Pop 10), Emamzadeh Hashem (Pop 1), Tochal (Pop12), Zangoleh (Pop 6), and Dizin (Pop 14) populations

Table 4 Analysis of molecular variance (AMOVA) among and within populations of S. aucheriana based on ISSR data

Source	df	SS	MS	Est. Var	%
Among Pops	14	1325.333	94.667	18.37387%	
Within Pops	60	168.000	2.800	2.800	13%
Total	74	1493.333	21.173	100%	
PhiPT	0.868 P (rand > = data) = 0.001				

Df degree of freedom, *SS* sum of squares, *MS* mean squares, *Est. var* estimated variability, % proportion of genetic variability, *P* P-value, *PhiPT* values below diagonal. Probability based on 999 permutations is shown above diagonal

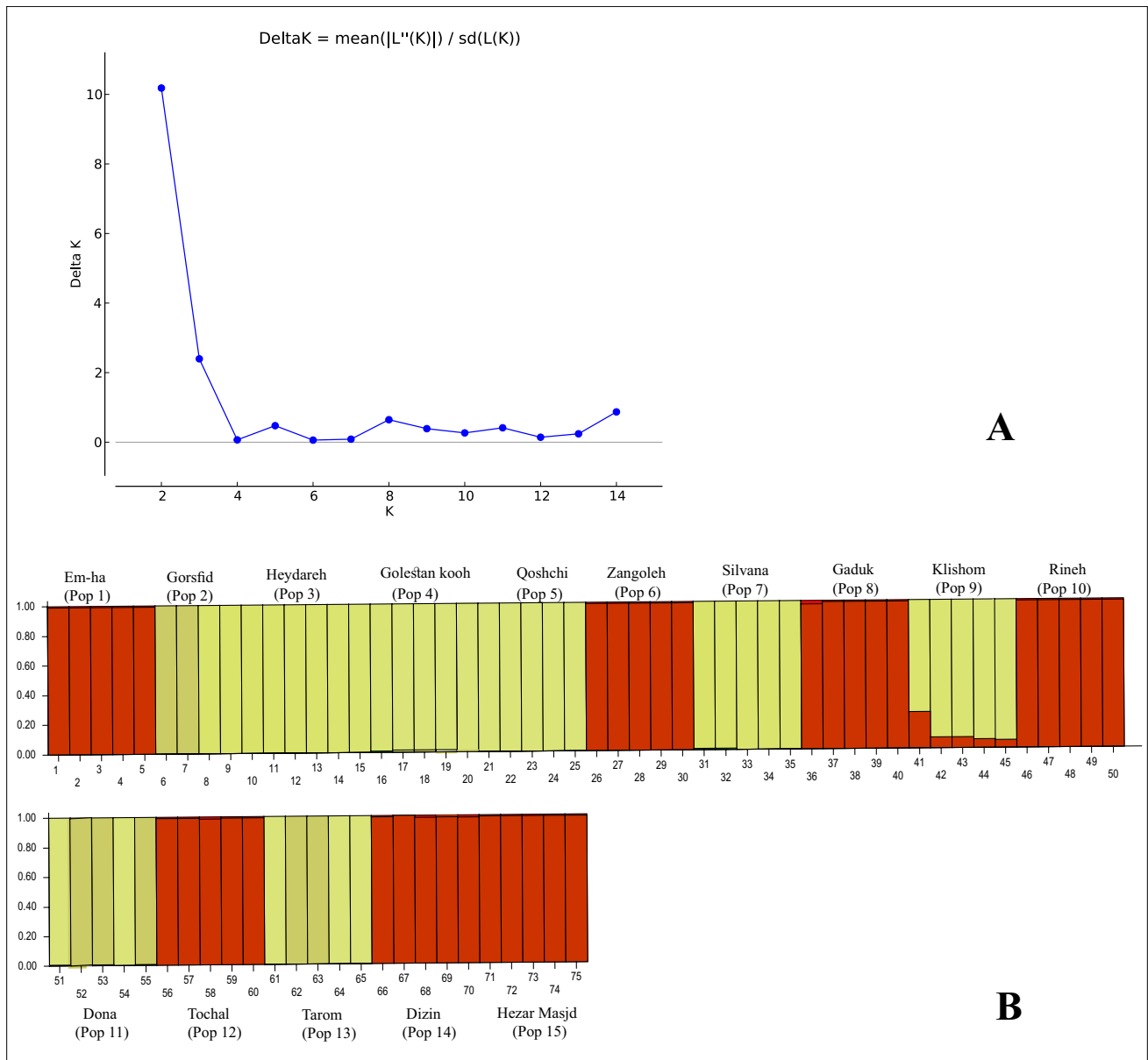were placed together in a closer distance. Also, Gorsfid (Pop 2), Golestan Kooh (Pop 4), Qoshchi (Pop 5), Dona (Pop 11), Tarom (Pop 13), Silvana (Pop 7), Heydareh (Pop 3) populations were placed in the different clusters (Fig. 4A, B). Klishom population (Pop 9) samples were placed near other populations which appears to show a slight gene flow in both populations which support by the PCoA and NJ approaches. The genetic cluster comprises all of the populations from different regions without geographical proximity.

Discussion

Populations of *S. aucheriana* have been reported from various regions of Iran in the present study. Thus, the investigation of genetic diversity should provide basic information about their current status in this country. According to the results, the value of gene flow was extremely low ($Nm = 0.07$). In the absence of a high gene flow dispersion of genetic drift mounting, decreasing the share of within-population gene diversity favors among-population genetic diversity intensifying the differentiation (Setsuko et al. 2007). Among the investigated populations Tarom (Pop 13) population (belonging to lower elevation) showed the highest genetic diversity in terms of the private allele while Emamzadeh Hashem (Pop 1), Zangoleh (Pop 6), and Dizin (Pop 14) populations (belonging to higher elevation) exhibited the least diversity, with no private alleles indicating a founder effect followed by genetic drift (Nei

Fig. 3 Population genetic structure of *S. aucheriana.* (**A**) Population genetic structure of the estimated delta K value with respect to K, according to the calculation method by Earl and vonHoldt (2012), graph showing the best value of K=2. (**B**) Population structure estimated by STRUCTURE (k=2)

et al. 1975; Slatkin and Takahata 1985). The present study confirms molecular markers are the major technique to detect the variability and differences within and among populations at the DNA level (Freeland 2020). The Mantel test offered clear geographic inclination in the distribution of genetic variation. Therefore, displayed the isolation of populations by geographic distances in shaping the present genetic structure of *S. aucheriana* populations. This geographical isolation played a major role in the high genetic differentiation among populations (Slatkin 1987). One hypothesis is that there is a geographic trend of higher

elevation populations (brown) that are closer to the Caspian Sea (NE) against the yellow populations that have a more southerly (SW) trend, except for the Gorsfid (Pop 2) population that breaks this trend and could indicate a recent introduction or a relict population of the yellow group. As Ferreira et al. (2015) concluded two subspecies of *Silene scabriflora* Brot. were closer to the sea; the Mediterranean and Atlantic coasts, and influenced by very specific and characteristic environments which can partially explain their divergence compared to the other subspecies. Similarly, As Brothers et al. (2016) have reported

Fig. 4 A) Principal coordinates analysis (PCoA) using the Nei's genetic distance computed from ISSR data. For an explanation of numbers, see Table 1. (**B**) Neighbor -joining (NJ) dendrogram based on Nei's genetic distance showing relationships among the *S. aucheriana* populations Climate conditions were labeled by colors that reflect groupings derived from STRUCTURE analysis
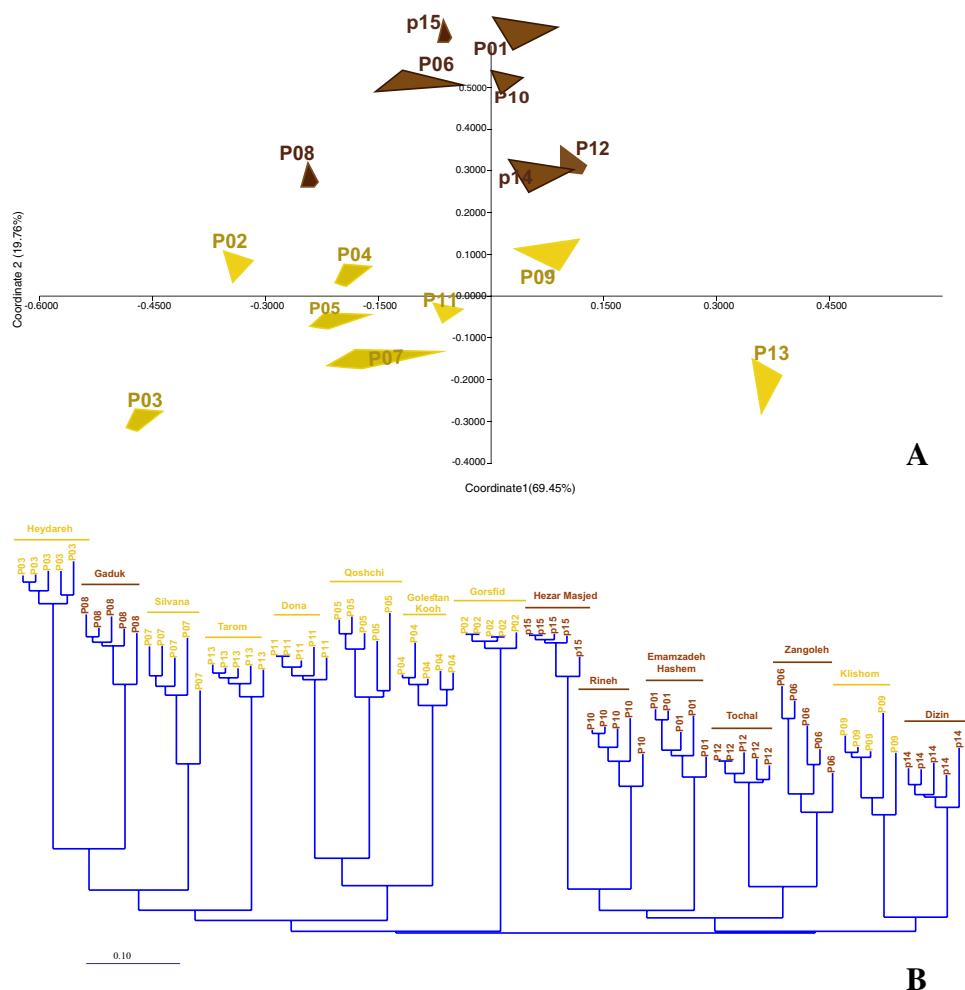
the population divergence in *Silene latifolia* Poir. was observed in two types of climate regions, which indicated differences in their morphology and phenology. Among-population differentiation in phenotypic traits and allelic variation is expected to occur as a result of isolation, drift, founder effects, and local selection (Jolivet and Bernasconi 2007). Consequently, based on the results conducted on European populations of *S. latifolia*, investigating molecular and genetic divergence is a pre-requisite for researches of local adaptation in response to selection under variable environmental conditions (Richards et al. 2003; Jolivet and Bernasconi 2007). Furthermore, new genotypes should be handled with care because of local adaptation to environmental and ecogeographic conditions (Vander Mijnsbrugge et al. 2010). According to the study by Karrenberg et al. (2018) based on environmental data containing 19 bioclimatic variables conducted on populations of *Silene dioica* (L.) Clairv. and *S. latifolia*, they found that ecological divergence has been the driver of speciation. Principally, climate conditions and some related parameters in a short time frame show the genetic composition

of plants (Allard 1988; Perez de la Vega et al. 1994). It can also be expressed that breeding systems represent an important effect on the amount and distribution of genetic variation among and within populations (Charlesworth 2003). The importance assigned to marker characterization (particularly, to the influence of the environment on the type of character) has caused a diminution in the importance of morphological descriptors for genetic diversity (Egea-Gilabert et al. 2013). In addition, the levels of the breeding system (inbreeding or outbreeding) and pollination modes can seriously affect the genetic structure of the populations (Hamrick and Godt 1996). Subsequently, ecogeographic variables associated with genetic variation would help the study of future breeding programs (Granberg et al. 2008; Hansen 2003; Taylor et al. 1999). The importance of this issue is undeniable that genetic variation and population structure are strongly correlated with population size, systems of mating, natural selection, life form, ecological tolerance, dispersal of seeds, and also the pattern of gene flow (Colautti et al. 2010). Environmental factors have also been shown to influence

flower size, and other reproductive traits (Worley et al. 2000; Delph et al. 2004). By providing more evidence for this research, it can be concluded that the divergence of populations has been revealed under the influence of two different types of climate. In our study, the clustering of each population was consistent with their climatic conditions, indicating genetic divergence along with their geographic distribution.

Conclusions

Present data provide information on the genetic diversity and population structure of *S. aucheriana* from different regions showing significant levels of genetic variation among populations. These results indicate that all individuals of populations shared two genetic pools as revealed by STRUCTURE. The geographic and genetic distance showed a significant correlation. Our results indicated two types of biogeographical groups have been formed (in terms of elevation) and what has been seen in relation to the climate data. In this regard, seven populations with an elevation cut-off limit higher than 2800 m were placed in one group, and the other eight populations with an elevation cut-off limit lower than 1800 m were placed together.

Data availability The data are available from the corresponding author upon reasonable contact.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Consent for publication Consent for publication submission is approved by all authors.

Ethical approval The article does not contain any studies with human participants or animals performed by any of the authors.

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