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Original scientific paper

GENETIC AND MORPHOLOGICAL DIVERSITY IN *Cousinia calocephala* JAUB. & SPACH (ASTERACEAE; SECTION *CYNAROIDEAE* BUNGE) POPULATIONS

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The genus *Cousinia* Cass. of the tribe Cardueae with about 700 species is one of the most diverse genera in Central and SW Asia following *Senecio* and *Vernonia*. The section *Cynaroideae* with 89 species is the largest section of the genus. *Cousinia calocephala* is the only endemic species of the section distributed in 14 provinces of Iran from Alborz to Zagros mountains. In present study 65 plant specimens of 13 geographical populations of *C. calocephala* were investigated based on the morphological and genetic (ISSR) data. ANOVA test revealed a significant morphological difference among the studied populations. Similarly, AMOVA test yielded a significant genetic difference between the studied populations, suggesting that the studied populations are morphologically and genetically differentiated. AMOVA test revealed that 94% of the total genetic difference was due to inter-populations genetic differences, while 6% was due to within-species genetic variability. The discriminating power of ISSR loci as determined by Gst against Nm analysis, revealed that almost all ISSR loci have an excellent discriminating power. Thus, ISSR markers are efficient in differentiating of the studied *C. calocephala* populations. The mantel test, revealed a significant positive correlation between genetic and morphological distance and geographical distance of the studied populations. Genetic analysis results revealed that along with genetic drift, low level of gene flow and migration, adaptive loci also helped populations diverge and adapt these populations to their local condition. Thus, we have three different groups which can be considered as three ecotypes for *C. calocephala* based on the morphological and genetic data.

Keywords: *Cousinia calocephala, Cynaroideae,* ISSR, Morphometry.

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INTRODUCTION

The genus *Cousinia* Cass. of the tribe Cardueae (Family Asteraceae) with about 700 species is one of the most diverse genera in Central and SW Asia following *Astragalus* L. (*c*. 3000 species), *Senecio* L. (*c*. 1500 species) and *Vernonia* Schreb. (*c*. 1000 species) (FRODIN, 2004; ATTAR and GHAHREMAN, 2006; SUSANNA and GARCIA-JACAS, 2006; LOPEZ-VINYALLONGA *et al*., 2009; ATTAR and DJAVADI, 2010; GHAHREMANINEJAD, 2015; MEHREGAN and ASSADI, 2016; RASTEGAR *et al*., 2017, 2018). The genus *Cousinia* contains more than 400 species in South West Asia, with the highest number of species in the Flora Iranica area, out of which 379 are endemic. These species are distributed in mountainous regions of Iran, Afghanistan and Turkmenistan (RECHINGER, 1986; KNAPP, 1987; LOPEZ-VINYALLONGA *et al*., 2011). Though the exact number of species in this genus in Iran is in dispute (ATTAR, 2000; MEHREGAN, 2008; MEHREGAN, 2011; MEHREGAN and KADEREIT, 2008; ASSADI, 2009; ATTAR and MIRTADZADINI, 2009; MEHREGAN and ASSADI, 2009; ATTAR and DJAVADI, 2010), up to now, about 270 *Cousinia* species are reported in Iran, out of which nearly 200 species are endemic (TSCHERNEVA *et al*., 2005; DJAVADI *et al*., 2007; ZARE *et al*., 2013). The *Cousinia* species are classified into 70 sections (RECHINGER, 1986). The section *Cynaroideae* Bunge with 89 species is the largest section of the genus and contains Irano-Turkestanian elements (RECHINGER, 1979; 1986). Iran with 77 taxa, of which 66 are endemic, seems to be the center of the diversity of section *Cynaroideae* (ATTAR and GHAHREMAN, 2006). Most of the Iranian species are found in mountains ranges of Alborz, Zagros, and remote mountains of the central parts of Iran (ATTAR and DJAVADI, 2010). Two main morphological traits of the section are decurrent leaves and appendiculate bracts (ATTAR and DJAVADI, 2010). *Cousinia calocephala* Jaub. & Spach is the only endemic species of the section and the genus distributed in 14 provinces of Iran from Alborz and Zagros ranges (ZARE *et al*., 2013). It is a perennial herb, with completely decurrent stem leaves, 50-100 and 90-125 flowers and bracts respectively, triangular-rhombic appendage of median bracts, squarrose and regularly spinose at margin, corolla purple, 18-24 mm length, and limb as long as tube or slightly longer (ATTAR and DJAVADI, 2010).

Detailed population genetic investigation is an important step for genetic evaluation of endemic plant species as it provides insight on the spatial genetic structure, genetic diversity and gene flow versus genetic fragmentation of these plant species. It also produces data on the number of probable gene pools for conservation and breeding strategies for the studied taxa. An examination of the genetic diversity among populations within a species is essential for a better understanding of evolutionary processes and the nature of the species (SHEIDAI *et al*., 2013; 2014; 2016).

Different molecular markers have been used in identification of population genetic structure and probable gene pools; evaluation of genetic diversity and speciation process; but some of them such as inter-simple sequence repeats (ISSRs) seems to be very efficient in creation of high levels of resolution in genetic investigation due to their high polymorphism. These molecular markers are reproducible, easy detection by PCR, cheap; as well as provide useful data for evolutionary and population genetic investigation (See for example, SHEIDAI *et al*., 2012; 2013; 2014; MINAEIFAR *et al*., 2015; 2016).

C. calocephala is an endemic species which grows in divergent geographical regions of Iran from Alborz to Zagros mountains and organizes various local populations (ATTAR, 2000;

MEHREGAN and KADEREIT, 2008; ATTAR and DJAVADI, 2010; LOPEZ-VINYALLONGA *et al*., 2009). Accordingly, the main goals of the present study are: 1) to identify the population genetic structure and gene flow in thirteen local populations of this species using ISSR molecular marker; 2) To investigate the morphological diversity of these populations in Iran; and 3) compare the genetic variability revealed by ISSR and morphological data. This information can be used in conservation, breeding and sustainable management of this endemic plant species.

MATERIALS AND METHODS

Plant material

Genetic and morphological data investigated and discussed in present study are based on 65 samples from 13 populations in *C. calocephala*. The plant samples were collected from 13 geographical populations of Iran. The voucher specimens have been deposited in The Herbarium of Tehran University (TUH) (Table 1 and Fig.1).

Table 1. Investigated Cousinia calocephala populations and their voucher informatio.

Pop.	Province	Locality	Altitude (m)	Longitude	Latitude	Voucher no.
1	East Azarbaijan	Mianeh	1257	47° 35' 55"	36° 58' 49"	46276 (TUH)
2	East Azarbaijan	Maraqe	1645	46° 47' 32"	37° 19' 33"	38636 (TUH)
3	West Azarbaijan	Takab	1400	46° 45' 27"	36° 32' 37"	69880 (TUH)
4	West Azarbaijan	Takhte Soleiman	2479	47° 24' 36"	36° 36' 05	46280 (TUH)
5	Hamadan	Hamadan	1900	48° 30' 54"	34° 47' 54"	20547 (TUH)
6	Hamadan	Kabudar Ahang	1690	48° 44' 23"	35° 13' 04"	20556 (TUH)
7	Chahar Mahal& Bakhtiari	Farsan	1880	50° 35' 27"	32° 14' 16"	20549 (TUH)
8	Chahar Mahal& Bakhtiari	Bazoft	2050	50° 16' 23"	32° 10' 01"	17789 (TUH)
9	Alborz	Kandovan	2600	51° 18' 26"	36° 08' 36"	9784 (TUH)
10	Alborz	Karaj	2650	51° 20' 39"	35° 57' 50"	23290 (TUH)
11	Mazandaran	Yoush	1600	51° 48' 52"	36° 12' 15"	21221(TUH)
12	Kurdestan	Zarrineh	2100	46° 55' 31"	36° 03' 44"	40507(TUH)
13	Lorestan	Shoulabad	1700	49° 11' 37"	33° 11' 12"	21875(TUH)

Fig. 1. Distribution map of the studied *Cousinia calocephala* populations in Iran. Populations are marked with numbers from 1-13 according to the Tab. 1.

DNA extraction and ISSR assay

Total genomic DNA was extracted from leaf tissue using protocol of the CTAB-activated charcoal and Polyvenyl Pyrrolidone (PVP) method (SHEIDAI *et al*., 2013). Quality of extracted DNA was examined by running on 0.8% Agarose gels. Ten ISSR primers, UBC 807, UBC 810, UBC 811, UBC 834, CAG(GA)7, (CA)7AC, (CA)7AT, (CA)7GT (GA)9A, and (GA)9T, commercialized by the University of British Columbia, were used.

Each PCR amplification was performed in a 25µL volume containing 20 ng of genomic DNA, 10 mM Tris-HCl buffer at pH 8, 50 mM KCl, 1.5 mM MgCl2, 0.2 µM of a single primer, 0.2 mM of each dNTP and 3 U of Taq DNA polymerase (Bioron, Ludwigschafen, Germany).

The PCR amplification program was performed in a Techne thermocycler (Germany) with the following program: 5 min at 94°C, followed by 45 cycles of 30 s at 94°C, 30 s at 54.6°C, and 2 min at 72°C, with a final extension step of 10 min at 72°C. The amplification products were apeared by running on 2% agarose gel, followed by ethidium bromide staining. The fragments size was evaluated by using a 100-bp molecular size ladder (Fermentas, Germany). The experiment was reproduced 3 times and constant ISSR bands were utilized for further analyses.

Data analyses

Morphological analysis

In total, 14 morphological characteristics (quantitative and qualitative), including head diameter, flowers number, bracts number, appendages length of median

bracts, appendages width of median bracts, corolla length, stem leaves, uppermost leaves, position of median bracts, appendages shape of median bracts, appendages margin of median bracts, corolla color, ratio limb to anther tube and anther tube color were studied (Table 2). The morphological characteristics were coded accordingly. The analysis of variance (ANOVA) test was performed to reveal significant morphological difference among the studied populations. Data were standardized (Mean = 0, variance = 1) and used for multivariate analyses. UPGMA (Unweighted paired group using average), and Ward (Minimum spherical variance) clustering based on Euclidean distance and Gower distances as well as principal coordinate analysis (PCoA) and multidimensional scaling (MDS) methods were used for grouping of the populations. Principal components analysis (PCA) was used to identify the most variable morphological characters. (PODANI, 2000; SAFAEI *et al*., 2016). The Mantel test was performed to estimate correlation between geographical distance and morphological distance of the studied populations (PODANI, 2000). Data analyses were performed by PAST ver. 2.17 (HAMMER *et al*., 2012).

Table 2. Morphological characters and their codes.

Character	Codes			
Head diameter (cm)	x<1.5	1.5 ≤x≤ 2.5	x>2.5	
Flowers number	x<90	90 ≤x≤ 130	x>130	
Bracts number	x<90	90 ≤x≤ 130	x>130	
Appendages length of median bracts (mm)	x<1.5	1.5 ≤x≤ 2.5	x>2.5	
Appendages width of median bracts (mm)	x< 1.5	1.5 ≤x≤ 2.5	x>2.5	
Crolla length (mm)	x< 18	18 ≤x≤ 20	x>20	
Stem leaves	Interruptedly decurrent	Countinuously decurrent		
Uppermost leaves	Distant from the head	Close to the head	Surrounding the head	
Position of median bracts	Spreading	Recurved	Spreading-Recurved	
Appendages shape of median bracts	Triangular	Rhombic		
Appendages margin of median bracts	1-2 spines	Spinose		
Corolla color	Dark-Purple	Pink	Purple	White
Ratio limb to anther tube	Longer	As long as		
Anther tube color	Pink	Purple		

Molecular analysis

The obtained ISSR bands were treated as binary characters (presence = 1, absence = 0). The genetic diversity parameters like Nei's gene diversity (H), Shannon information index (I), number of effective alleles, and percentage of polymorphism were determined for each population (FREELAND *et al*., 2011). Nei's genetic distance was utilized for clustering (WEISING *et al*., 2005). Neighbor Joining (NJ), WARD and UPGMA (Unweighted paired group using average) clustering as well as principal coordinate analysis (PCoA) and multidimensional scaling (MDS) methods were utilized for the grouping of the populations after 100 times permutation (FREELAND *et al*., 2011).

The consensus tree was constructed from the obtained morphological and ISSR trees. Similarly, tree distance was estimated accordingly.

The Mantel test was performed to estimate correlation between geographical distance and genetic distance of the studied populations (PODANI, 2000). PAST ver. 2.17 (HAMMER *et al*., 2012) and DARwin ver. 5 (PERRIER and JACQUEMOUD-COLLET, 2006) programs were used for these analyses.

AMOVA (analysis of molecular variance) (with 1000 permutations) as implemented in GenAlex 6.4 (PEAKALL and SMOUSE, 2006) was used to determine species genetic differentiation. Gene flow was determined by: (1) calculating Nm an estimate of gene flow from Gst by PopGene ver. 1.32 (1997) as: Nm 1⁄4 0.5(1 e Gst)/Gst, (2) reticulation analysis that is based on the least square method as performed in T-REX (BOC *et al*., 2012).

In order to investigate geographical separation of the studied populations, ISSR data were also analyzed by TCS Networking as implemented in PopART (Population Analysis with Reticulate Trees) program (http://popart.otago.ac.nz) (LEIGH and BRYANT, 2015).

RESULTS

Morphometry

ANOVA test revealed a significant difference for all quantitative morphological characteristics among the studied populations ($P < 0.01$). The WARD (Figure 2), UPGMA tree (Figure not given) and PCA plot (Figure not given) of morphological characteristics clearly separated the studied populations.

PCA analysis of morphological data revealed that the first two PCA components accounted for about 90% of total variations (Table 3). Morphological characteristics such as, shape and length of the appendages of median bracts, diameter of the heads, color and length of the corolla, as well as the number of flowers and bracts had the highest value of correlation with these components and were the most variable morphological features across the studied taxa. Indeed, these morphological features are of taxonomic value in divergence across the studied populations.

WARD tree (Figure 2) separated the studied populations based on all morphological characteristics including both quantitative and qualitative characteristics. In general, two major clusters were formed in WARD tree (Figure 2), with populations 1, 12, 2, 3, and 4 showing morphological similarity and placed in the first major cluster. On the other hand, populations 5, 6, 7, 13, 8, 11, 9 and 10 formed the second major cluster. UPGMA tree and PCA plot (Figures not given) supported the grouping made by the WARD tree.

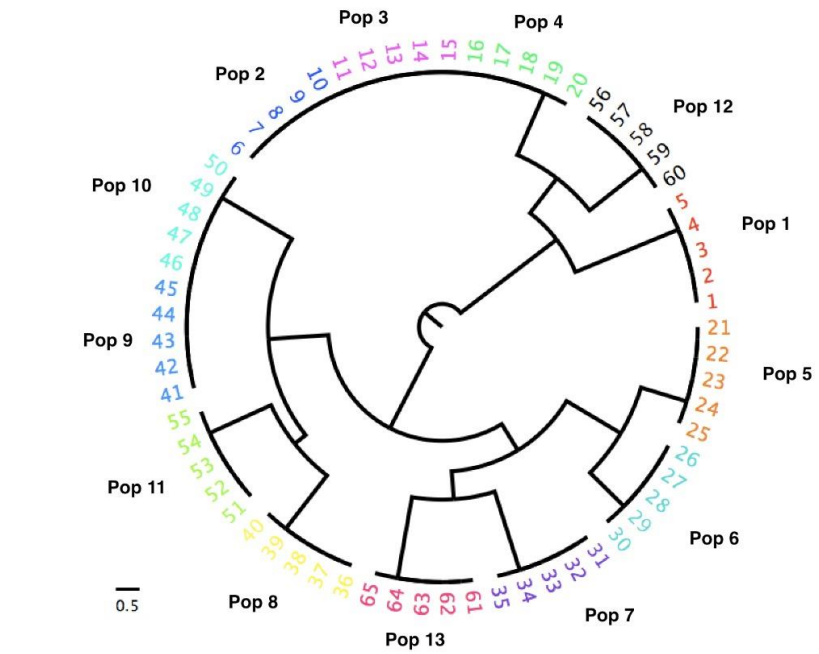

Fig. 2. WARD dendrogram of the studied populations based on morphological data. (populations 1-13 are according to Table 1).

Table 3. PCA analysis of morphological data.

Principal Components	Eigenvalue	% Variance
1	6.12557	83.276
2	0.566931	7.7073
3	0.256792	3.491
4	0.155087	2.1084
5	0.134554	1.8292
6	0.0939183	1.2768
7	0.0166137	0.22586
8	0.00630884	0.085767

The mantel test, after 10000 times of permutation, revealed a significant correlation (r = 0.098, P = 0.0028) between geographical distance and morphological distance in the populations. Thus, as the populations deviate from each other, they become more divergent in morphological characteristics.

ISSR assay

ISSR primers produced 27 reproducible bands/loci. The discriminating power of ISSR loci as determined by Gst against Nm (migration) analysis (Table 4), revealed that almost all ISSR loci have an excellent discriminating power. Thus, ISSR markers are efficient in differentiating the studied *C. calocephala* populations.

Table 4. Discrimination power of ISSR loci in studied populations.

Locus	Sample Size	Ht	Hs	Gst	Nm*
Locus1	65	0.0549	0.0358	0.3491	0.9321
Locus2	65	0.0814	0.0380	0.5329	0.4382
Locus3	65	0.1316	0.0738	0.4391	0.6387
Locus4	65	0.2979	0.0358	0.8800	0.0682
Locus5	65	0.4462	0.0358	0.9198	0.0436
Locus6	65	0.4734	0.0000	1.0000	0.0000
Locus7	65	0.4462	0.0358	0.9198	0.0436
Locus8	65	0.4970	0.0000	1.0000	0.0000
Locus9	65	0.3550	0.0000	1.0000	0.0000
Locus10	65	0.2604	0.0000	1.0000	0.0000
Locus11	65	0.2604	0.0000	1.0000	0.0000
Locus12	65	0.4734	0.0000	1.0000	0.0000
Locus13	65	0.3550	0.0000	1.0000	0.0000
Locus14	65	0.1420	0.0000	1.0000	0.0000
Locus15	65	0.3779	0.1096	0.7101	0.2041
Locus16	65	0.2604	0.0000	1.0000	0.0000
Locus17	65	0.3550	0.0000	1.0000	0.0000
Locus18	65	0.4260	0.0000	1.0000	0.0000
Locus19	65	0.3550	0.0000	1.0000	0.0000
Locus20	65	0.2604	0.0000	1.0000	0.0000
Locus21	65	0.0814	0.0380	0.5329	0.4382
Locus22	65	0.3972	0.0380	0.9042	0.0529
Locus23	65	0.2604	0.0000	1.0000	0.0000
Locus24	65	0.1420	0.0000	1.0000	0.0000
Locus25	65	0.2604	0.0000	1.0000	0.0000
Locus26	65	0.0814	0.0380	0.5329	0.4382
Locus27	65	0.4462	0.0358	0.9198	0.0436
Mean	65	0.2955	0.0190	0.9355	0.0345
St. Dev		0.0191	0.0008		

* Nm = estimate of gene flow from Gst or Gcs. E.g., Nm = 0.5(1 - Gst)/Gst

AMOVA test yielded a significant genetic difference between the studied populations (P = 0.001), suggesting that the studied populations are genetically differentiated. AMOVA revealed that 94% of the total genetic difference was due to inter-populations genetic differences, while 6% was due to within-species genetic variability. These results indicated a of high level of genetic variability among *C. calocephala* populations.

The populations' relationship illustrated by WARD dendrograms based on morphological features (Figure 2) and molecular data (Figure 3) were congruent in most cases. This was also illustrated in the consensus tree (Figure 4) of these dendrograms. This tree revealed that in most cases (except for the population 11), the studied populations showed the same relationship in both morphological and molecular trees. For instance, populations 1, 12, 2, 3, and 4 were placed close to each other. The same holds true for populations 5, 6, 7, 13, and 8 well as for populations 9, 10. These results are supported by TCS network (Figure 5). TCS network (Figure 5) showed different numbers of loci among the studied populations; based on this network, the highest difference in loci occurred in population 9.

The population grouping based on ISSR data by NJ, UPGMA tree and PCO, MDS plot (Figures not given) showed similar results. Further, CCA plot of genetic data and environmental features (Figure 6) revealed that altitude, longitude, and latitude are effective factors in separation of studied *C. calocephala* populations.

The mantel test after 10000 times of ermutation performed between geographical distance and genetic distance in the studied populations yielded a significant correlation (P < 0.01). These results indicate that as the studied populations moved apart from each other, they diverged in genetic characteristics.

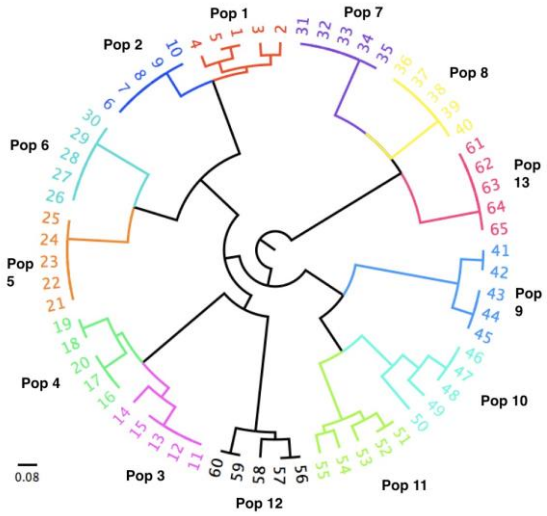

Fig. 3. WARD dendrogram of the studied populations based on ISSR data. (populations 1-13 are according to Table 1).

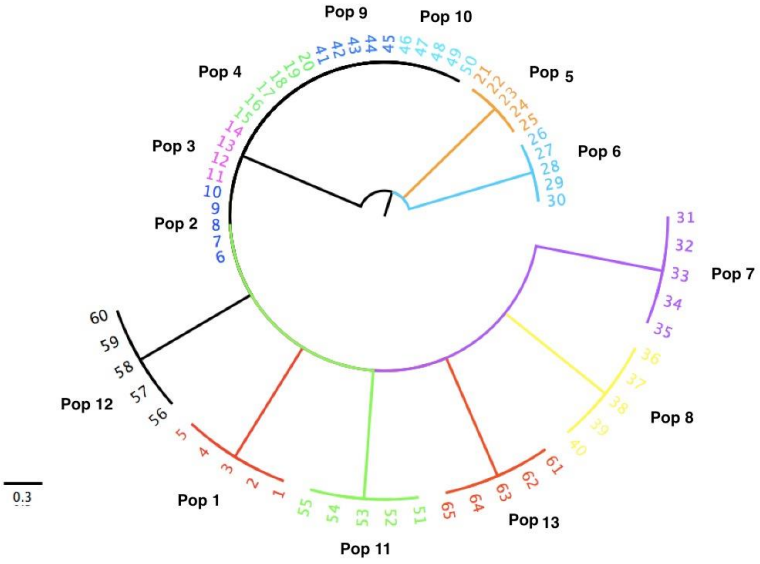

Fig. 4. Consensus tree based on morphological and ISSR dendrograms in studied populations. (populations 1-13 are according to Table 1).

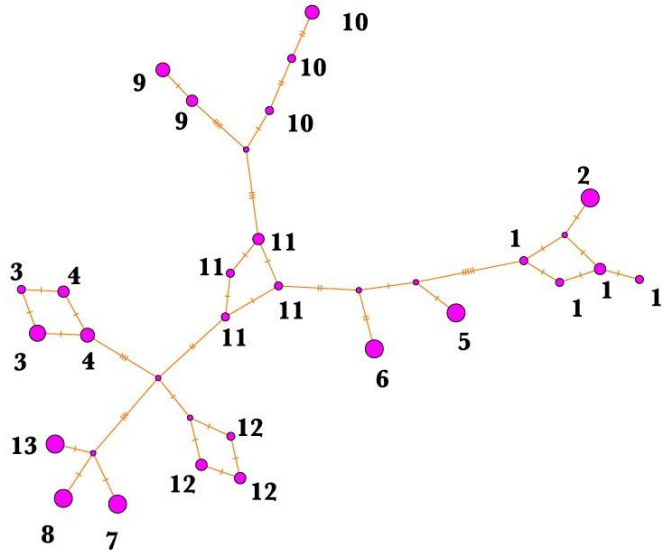

Fig. 5. TCS Network of studied populations based on ISSR data (populations are according to Table 1). (Numbers on branches show number of diffrent loci among studied populations).

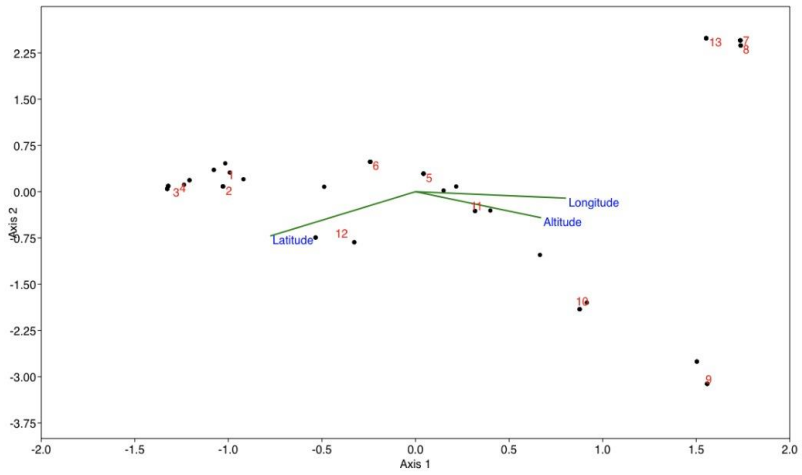

Fig. 6. CCA plot of ISSR data and environmental features in studied populations. (populations 1-13 are according to Table 1).

DISCUSSION

The genetic diversity is of fundamental importance in the continuity of plant species as is used to trigger the necessary adaptation to the cope with environmental changes. The general populations with high levels of genetic diversity have a better chance of survival compared to populations with a lower degree of genetic variability (SHEIDAI *et al*., 2012; 2013; 2014). This is particularly expected in *C. calocephala* with extensive geographical distribution in Iran. In the present study, we studied the geographical characteristics of the studied populations in order to predict the genetic diversity.

A high level of inter-population genetic variability (94%) was observed in *C. calocephala*, which is highly outcross, where the studied populations are morphologically and genetically differentiated. This usually occurs when the populations are isolated from each other or have tendency to interbreed. Assessment of the genetic diversity among populations within a species is crucial for a better understanding of evolutionary processes and the nature of the species. AMOVA test showed a significant genetic difference among the studied populations (P = 0.001), suggesting that the studied populations are genetically differentiated. Further, the low value of Nm (0.03) obtained for the studied populations showed a low value of gene flow. Due to low degree of gene flow among the studied populations, genetic differences increase. In cases with complete absence or very low value of gene flow, genetic drift is a strong evolutionary force and brings about high level of within-population genetic homogeneity.

This may lead to necessary adaptation to local habitats (HOU and LOU, 2011). Indeed, many plant species grow within a range of distinct habitats and have developed adaptive strategies suited to their particular habitat (SCHNELLER and LIEBT, 2007).

ISSR data showed genetic affinity between populations 1, 2, 3, 4, and 12. Indeed, these populations also differed in their morphological characteristics. The populations are different from other populations in some characteristics such as appendages of median bracts shape (triangular), appendages of median bracts length (>2.5 mm), head diameter (>2.5 cm), color (white, pink) and length of corolla (>20 mm), position of bracts (spreading-recurved), as well as flowers' and bracts' number (>130). It is interesting to mention that these populations are located in close proximity of each other geographic areas (Figure 1).

ISSR data revealed close affinity between populations 5, 6, 7, 8 and 13; it is in agreement with morphological data such as appendages of median bracts shape (rhombic), appendages of median bracts length (1.5-2.5 mm) head diameter (1.5-2.5 cm), color (dark-purple, pink) and length of corolla (18-20 mm), position of bracts (recurved), as well as flowers' and bracts' number (90-130). These populations have the same distribution area (Figure 1). The same holds true for populations 9, 10 and 11 due to the morphological characteristics such as appendages of median bracts shape (rhombic), appendages of median bracts length (1.5> mm) head diameter (1.5> cm), color (purple) and length of corolla (18-20 mm), position of bracts (recurved), flowers and bracts number (90>). These populations are located geographically near each other (Figure 1).

It can be concluded that environmental features (latitude, longitude, and altitude) simultaneously affect the gene flow, and the genetic structure of studied populations. Thus, we suggest that these diverged populations may represent different taxonomic groups bellow the species level within *C. calocephala.*

In many studies, different ecotypes have been reported because of inter-populations genetic differences followed by population morphological divergence of the population (SHEIDAI *et al*., 2012; 2013; 2014; MINAEIFAR *et al*., 2015, 2016).

Assessments of levels of inter-population genetic variations was have been used to prioritize populations for conservation efforts (TORO and CABALLERO, 2005); all else being equal, more weight was given to those exhibiting higher levels of inter-population variation, as well as to those that are more genetically divergent from others.

Genetic analysis results revealed that along with genetic drift, low level of gene flow and migration, adaptive loci also helped populations diverge and adapt these populations to their local condition. Thus, in the current study, we suggested three different groups which can be considered as three ecotypes for *C. calocephala* based on the morphological and genetic data.

CONCLUSION

The current study proved that the morphological characters and ISSR molecular data are useful in differentiating of studied *C. calocephala* populations. Both quantitative and qualitative morphological characteristics are important and suitable for differentiating of studied *C. calocephala* populations. The present study revealed a high level of inter-population genetic variability in *C. calocephala*. WARD cluster analyses based on the morphological features and molecular data revealed the significant information among the studied populations' relationships.

As a general conclusion, based on the morphological studies of the observed specimens and the effect of the environmental features (latitude, longitude, and altitude) on the genetic structure of studied populations, three different ecotypes were suggested for *C. calocephala*.

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REFERENCES

ASSADI, M. (2009): Four new species of the genus *Cousinia* Cass. (Asteraceae*)* from Iran. Iranian Journal of Botany, *15* (1): 36-44.

ATTAR, F. (2000): The systematic studies of **Cousinia** (Asteraceae) sect. **Cynaroideae** in Iran (Ph.D. thesis). Tehran University.

ATTAR, F., M., MIRTADZADINI (2009): Two new species of *Cousinia* Cass. sect. *Pugioniferae* Bunge (Asteraceae, Cardueae), from east and southeast of Iran. Iranian Journal of Botany, *15* (2): 146-152.

ATTAR, F., A., GHAHREMAN (2006): A synopsis of sect. *Cynaroides* (*Cousinia*, Compositae), distribution patterns and diversity centers. *Rostaniha* 7 (Supplement 2): 315-342.

ATTAR, F., S.B., DJAVADI (2010): A taxonomic revision of *Cousinia*, sect. *Cynaroides* (Asteraceae, Cardueae) in the flora of Iran. Iranian Journal of Botany, *16* (1): 130-184.

BOC, A., A.B., DIALLO, V., MAKARENKOV (2012): T-REX: a web server for inferring, validating and visualizing phylogenetic trees and networks. Nucleic Acid Res., *40*: W573-W579.

DJAVADI, S.B., F., ATTAR, M., ESKANDARI (2007): *Cousinia papilosa*, A new species from eastern Iran, Including chromosome count and palynological studies. Rostaniha, Vol. *8* (2): 63-73.

FREELAND, JR., H., KIRK, S.D., PETERSON (2011): Molecular Ecology, second ed. Wiley-Blackwell, UK, pp. 449.

FRODIN, D.G. (2004): History and concepts of big plant genera. Taxon., *53* (3): 753-776.

GHAHREMANINEJAD, F. (2015): Notes about *Astragalus* (Leguminosae) in Iran. *Annalen des Naturhistorischen Museums in Wien, B 117*: 279-281.

HAMMER, Q., D.A.T., HARPER, P.D., RYAN (2012): PAST: Paleontological Statistics software package for education and data analysis. Palaeontologia Electronica, *4*: 9.

HOU, Y., A., LOU (2011): Population genetic diversity and structure of a naturally isolated plant species, *Rhodiola dumulosa* (Crassulaceae). *PLOS ONE, 6*: 24-497.

KNAPP, H.D. (1987): On the distribution of the genus *Cousinia* (Compositae). Pl. Syst. Evol., *155*: 15-25.

LEIGH, J.W., D., BRYANT (2015): PopART: Full-feature software for haplotype network construction. Methods in Ecology and Evolution, *6*(9):1110–1116.

LOPEZ-VINYALLONGA, S., I., MEHREGAN, N., GARSIA-JACAS, O.V., TSCHERNEVA, A., SUSANNA, J.W., KADEREIT (2009): Phylogeny and evolution of the *Arctium-cousinia* complex (compositae, cardueae-caruinae). Taxon., *58*: 153-171.

LOPEZ-VINYALLONGA, S., K., ROMASCHENKO, A., SUSANNA, N., GARSIA-JACAS (2011): Systematics of the Arctioid group: Disentangling *Arctium* and *Cousinia* (Cardueae, Carduinae). Taxon., *60* (2): 539-554.

MEHREGAN, I. (2008): Systematics, phylogeny and biogeography of *Cousinia*-Asteraceae Dissertation. Fachbreich Biologie der Johannes Gutenberg, Universitat Mainz.

MEHREGAN, I. (2011): Notes on the taxonomy of *Cousinia* sec. *Haussknechtianae* (Asteraceae; Cardueae). Iranian Journal of Botany, *17*: 137–149.

MEHREGAN, I., M., ASSADI (2009): *Cousinia* sect. *Argenteae* (Asteraceae, Cardueae), a new section including a new species from NE Iran. Willdenowia, *39*: 265-271.

MEHREGAN, I., M., ASSADI (2016): A synopsis of *Cousinia* sect. *Pseudactinia* (Cardueae, Asteraceae) including a new species from NE Iran. Phytotaxa, *257*(3): 271.

MEHREGAN, I., J.W., KADEREIT (2008): Taxonomic revision of *Cousinia* sect. *Cynaroideae* (Asteraceae, Cardueae). Wildenowia, *38*: 293-362.

MINAEIFAR, A., M., SHEIDAI, F., ATTAR (2015): Genetic and morphological diversity in *Cousinia cylindracea* (Asteraceae) populations: Identification of gene pools. Biodiversitas, *16*: 288-294.

MINAEIFAR, A., M., SHEIDAI, F., ATTAR, Z., NOORMOHAMMADI, S., GHASEMZADEH-BARAKI (2016): Biosystematic study in the genus *Cousinia* Cass. (Asteraceae), section *Cousinia*. Biochemical Systematics and Ecology, *69*: 252-260.

PEAKALL, R., P.E., SMOUSE (2006): GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes, *6*: 288-295.

PERRIER, X., J.P., JACQUEMOUD-COLLET (2006): DARwin Software. http://darwin.cirad.fr/darwin.

PODANI, J. (2000): Introduction to the Exploration of Multivariate Data. Backhuyes: Leiden; pp. 407.

RASTEGAR, A., S.A., AHMAD, F., ATTAR (2017): *Cousinia azmarensis* (Asteraceae, Cardueae), a New Species from Kurdistan, Iraq. Harvard Papers in Botany, *22*(1): 71-73.

RASTEGAR, A., F., ATTAR, M., MIRTADZADINI (2018): *Novelties in Iranian* Cousinia *sect.* Cynaroideae *(Asteraceae, Cardueae): new taxa and taxonomic notes*. Phytotaxa, *343* (3): 227.

RECHINGER, K.H. (1979): Compositae III-Cynareae. *Cousinia* in KH Rechinger (ed.). Flora Iranica, no. 139A: 108-153 Graz, Austria.RECHINGER, K.H. (1986): *Cousinia*: morphology, taxonomy, distribution and phytogeographical implication. Proceedings of the Royal Society of Edinburgh, *89* B: 45-58.

SAFAEI, M., M., SHEIDAI, B., ALIJANPOOR, Z., NOORMOHAMMADI (2016): Species delimitation and genetic diversity analysis in Salvia with the use of ISSR molecular markers. Acta Bot. Croat., *75*: 45-52.

SCHNELLER, J., B., LIEBST (2007): Patterns of variation of a common fern (*Athyrium filix-femina*; Woodsiaceae). population structure along and between altitudinal gradients. Amer J Bot., *94* (6): 965-971.

SHEIDAI, M., E., SEIF, M., NOUROOZI, Z., NOORMOHAMMADI (2012): Cytogenetic and molecular diversity of *Cirsium arvense* (Asteraceae) populations in Iran. J. Jap. Bot., *87*: 193-205.

SHEIDAI, M., S., ZANGANEH, R., HAJI-RAMEZANALI, M., NOUROOZI, Z., NOORMOHAMMADI, S., GHASEMZADEH-BARAKI (2013): Genetic diversity and population structure in four *Cirsium* (Asteraceae) species. Biologia, *68*: 384−397.

SHEIDAI, M., S., ZIAEE, F., FARAHANI, S.M., TALEBI, Z., NOORMOHAMMADI, HASHEMINEJAD, Y., AHANGARI FARAHANI (2014): Infra-specific genetic and morphological diversityin *Linum album* (Linaceae). Biologia, *69*: 32-39.

SHEIDAI, M., F., TABAN, S.M., TALEBI, Z., NOORMOHAMMADI (2016): Genetic and morphological diversity in *Stachys lavandulifolia* (Lamiaceae) populations. Biologia, *62*(1): 9-24.

SUSANNA, A., N., GARCIA-JACAS (2006) [2007]: Tribe Cardueae Cass. (1819). Pp. 123-147. In: Kadereit JW and Jeffrey C (volume ed.), the families and genera of vascular plants 8. Berlin, etc.

TORO, M.A., A., CABALLERO (2005): Characterization and conservation of genetic diversity in subdivided populations. Philosophical Transactions of the Royal Society B, *360*:1367-1378.

TSCHERNEVA, O.V., M., JOHARCHI, F., GHAHREMANINEJAD (2005): A new species of the genus *Cousinia* (Asteraceae) from Iran. Botaniceskii Zhurnal, *90* (3): 411-414.

WEISING, K., H., NYBOM, K., WOLFF, G., KAHL (2005): DNA Fingerprinting in Plants. Principles, Methods, and Applications, second ed.472. CRC Press, Boca Rayton, Fl, USA, pp. 1-17.

ZARE, M., A.R., KHOSRAVI, M.R., JOHARCHI (2013): Distribution patterns of the genus *Cousinia* (Asteraceae) in Iran. Iranian Journal of Botany, *19* (1): 127-141.

GENETIČKI I MORFOLOŠKI DIVERZITET KOD *Cousinia calocephala* JAUB. & SPACH (ASTERACEAE; ODELJAK *CYNAROIDEAE*) POPULACIJA

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Izvod

Rod Cousinia Cass. plemena Cardueae sa oko 700 vrsta jedan je od najraznolikijih rodova u srednjoj i jugozapadnoj Aziji posle *Senecio* i *Vernonia*. Odeljak Cinaroideae sa 89 vrsta najveći je deo roda. *Cousinia kalocephala* je jedina endemska vrsta sekcije koja se distribuira u 14 provincija Irana od Alborza do planina Zagros. U ovom istraživanju istraženo je 65 uzoraka biljaka iz 13 geografskih populacija *C. calocephala* na osnovu morfoloških i genetskih (ISSR) podataka. ANOVA test otkrio je značajnu morfološku razliku između proučavanih populacija. Slično tome, AMOVA test je doneo značajnu genetsku razliku između proučavanih populacija, sugerišući da su proučavane populacije morfološki i genetski diferencirane. AMOVA test je otkrio da je 94% ukupne genetske razlike nastalo zbog međupopulacijske razlike, dok je 6% nastalo zbog genetske varijabilnosti unutar vrsta. Diskriminirajuća moć ISSR lokusa utvrđena Gst-om u odnosu na Nm analizu. Stoga je yaklučeno da su su ISSR markeri efikasni u razlikovanju proučavanih populacija *C. kalocefale*. Mantel test je pokazao značajnu pozitivnu povezanost između genetske i morfološke distance i geografske udaljenosti proučavanih populacija. Rezultati genetske analize otkrili su da su, pored genetičkog drifta, niskog nivoa protoka gena i migracije, adaptivni lokusi takođe doprineli divergentnosti populacijai njihovoj adaptiranosti na lokalne uslove. Dakle, imamo tri različite grupe koje se na osnovu morfoloških i genetskih podataka mogu smatrati kao tri ekotipa *C. kalocephala*.

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