Biosystematic studies of *Cirsium arvense* populations in Iran

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Abstract. Morphological studies were carried out in 13 populations of *Cirsium arvense* in the wild in Iran and compared with their molecular features. The aim of this study was to identify the genetic and morphological diversity of this species and to seek any new infraspecific taxonomic entities owing to geographical adaptations. The analyses showed significant correlation between some of the morphological characters, such as capitulum length and fruit length. PCA analysis of morphological characters revealed that characters like length of the leaf middle lobe, width of base in the leaf middle lobe and ratio of length/width of involucres, and the ratio of pappus length/fruit length are the most variable characters among the populations. Mantel test showed no correlation between geographical distance and morphological differences between populations, while significant correlation between geographical distance and both RAPD and ISSR molecular markers was observed, showing that with increase in geographical distance between the studied populations, increase in molecular difference occurs, but such molecular differences do not result in morphological differences. A consensus tree made on the basis of the morphological and molecular trees separated two populations from the others, which in terms of morphological characters are considered to be a new variety of *C. arvense*.

Key words: *Cirsium arvense*, Iran, ISSR, morphometry, RAPD

Introduction

The genus *Cirsium* Mill. (*Asteraceae*), with its about 250 perennial, biennial or annual species, grows in the various regions of the Northern Hemisphere, Europe, North Africa, Siberia, Central Asia, West & East Africa, and Central America (Zomlefer 1994, Bureš & al. 2004).

Extensive occurrence of interspecific hybridization is a common feature of the genus *Cirsium* which, in turn, makes it taxonomically a complex group and also leads to new forms in adaptation to various environmental conditions (Bureš & al. 2004). The hybrid plants persist primarily through vegetative growth, forming clusters of flowering shoots often connected by rhizomes. Because hybrids are usually fertile, they can often produce introgressive hybrids with the parental species or triple hybrids with other taxa (Bureš & al. 2004).

About 28 *Cirsium species* have been reported in *Flora Iranica* (Rechinger 1979) and classified in five sections. Studies into this genus in Iran are mainly confined to cytological reports (Ghaffari 1999, Nouroozi & al. 2010, 2011).

Canada Thistle is native to Southeast Europe and the Eastern Mediterranean, and was probably introduced to North America in the 1600s as a contaminant of crop seed and/or ship's ballast (Zouhar 2001). In addition to North America, Canada Thistle is invasive also in North and South Africa, the Middle East, Japan, India, New Zealand, Australia, and South America. It infests at least 27 crops in 37 countries and thrives in the temperate regions of the Northern Hemisphere (Zouhar 2001).

Cirsium arvense (L.) Scop., a rhizomatous perennial, reaches 30 cm – 1.5 m in height (Fig. 1). Its roots grow deep into the ground. Stems do not have conspicuous spines (Fig. 2). Leaves are dark-green and lanceolate to oblong-lanceolate (Fig. 3). They are glabrous above, but their undersides have short, white hairs. They may be pinnatifid and very prickly (Fig. 4). *C. arvense* has male and female plants. The female flowers are flask-shaped, 1–1.5 cm in diameter, and 1–2 cm high. They are fragrant, while the male flowers are not. The male flowers are smaller and more globose than the female flowers. Flowers range in colour from purple to pink or white. The plant blooms from June to August (Fig. 5). Fruits are tiny (Fig. 6), 2–3 mm long, about 1 mm in diameter, with a white to light-brown pappus (Blamey & al. 1989).

Fig. 1. *Cirsium arvense.*

Fig. 2. *C.arvense* – stem.

Fig. 5. *C. arvense* – seed and pappus.

Fig. 4. *C. arvense* – flower.

Fig. 3. *C. arvense* – leaf.

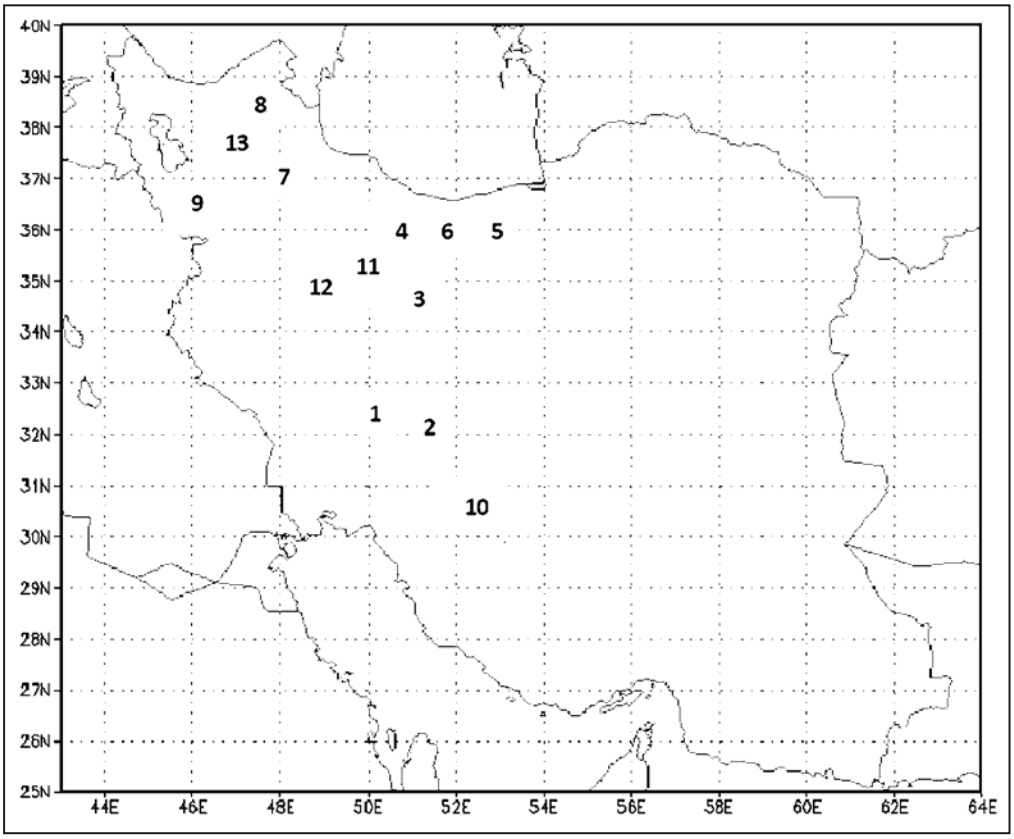

Fig. 6. Map of Iran with localities of the studied *C. arvense* populations. Population code (localities): **1**. Tafresh, **2**. Mahalat, **3**. Malard, **4**. Porkan, **5**. Shahrestanak, **6**. Asara, **7**. Khalkahl, **8**. Miandoab, **9**. Tabriz, **10**. Kashan, **11**. Talaghan, **12**. Kordan and **13**. Ghasemloo.

Cirsium arvense is considered an invasive weed in different regions of the world, and has been studied extensively with regard to inter-populations genetic diversity and structure in different regions of the world, but not in Iran (e.g. Bodo Slotta & Horvath 2005; Bodo Slotta & al. 2006, 2010).

Bodo Slotta & Horvath (2005) developed microsatellite (simple sequence repeat) and inter-simple sequence repeat (ISSR) markers for North American populations of *C. arvense*, obtaining an average of nine polymorphic alleles per microsatellite locus and 11 per ISSR locus to examine the genetic structure of *C. arvense* in the Northern Great Plains and their transferability to endemic *Cirsium* spp.

Bodo Slotta & al. (2006) studied the level of genetic diversity within and gene flow between Canada Thistle (*C. arvense*) and its relatives in the Northern Great Plains by using SSR and ISSR molecular markers. As with the ISSR markers, Bull Thistle had the greatest homology among microsatellite alleles. Alleles homologous within *Cirsium* were sequenced and surveyed for utility as phylogenetic tools in assessing relationships between the closely related species. Analyses have shown that introduced invasive thistles and native thistles do not hybridize, even when they co-occur.

Bodo Slotta & al. (2010) studied the genetic diversity of the same species in 85 localities of North America by using seven microsatellite markers. The populations exhibited greater intra-population diversity (60 %) than expected for a reported clonally reproducing species. Total diversity of sampled locations in North America (0.183) was less than the one reported earlier for European locations (0.715), but the greater mean difference between North American populations suggests strong founder effects or restriction of gene flow influencing the individual populations.

Since *Cirsium arvense* has a wide distribution in Iran and grows under various environmental conditions, we have previously studied 13 populations of this species from the standpoint of cytological and molecular (RAPD and ISSR markers) diversity. Here, we report morphometry of these populations and comparison of morphological and molecular results, trying also to identify the populations which differ most from the others on the basis of the obtained combined data. Moreover, we have tried to correlate the geographical distance of the populations with their morphological and molecular differences. New taxonomic forms of *C. arvense* in Iran are also suggested on the basis of the present investigation.

Material and methods

Plant material

Thirteen populations of *Cirsium arvense* (L.) Scop. were collected for morphological and molecular studies (Fig. 6; Table 1). Geographical distances of these populations are given in Table 2.

Table 1. Voucher number and locality details.

Voucher number	Code	Place of collection
HSBU 2010503	1	Markazi-Tafresh-altitude 1969-Norouzi 1387/2/25
HSBU 2010511	2	Markazi-Nahalat-altitude 1711-Norouzi 1387/2/25
HSBU 2010500	3	Karaj-Malard-altitude 1163-Seif 1389/2/25
HSBU 2010502	4	Karaj-Chalous road-Porkan-altitude 1542-seif 1389/2/28
HSBU 2010507	5	Karaj-Chalous road-Shahrestanak-altitude 2190-Seif 1389/2/28
HSBU 2010506	6	Karaj-Chalous road-Asara-altitude 1897-seif 1389/2/28
HSBU 2010508	7	Ardebil-Khalkhal-altitude 1848-Norouzi 1387/2/22
HSBU 2010510	8	West Azarbaiejan-Miandoab-altitude 1299-Norouzi 1387/3/22
HSBU 2010501	9	East Azarbaiejan-Tabriz-altitude 1796-Norouzi 1387/3/22
HSBU 2010509	10	Isfahan-Kashan-altitude 927-Norouzi i 1388/2/25
HSBU 2010505	11	Ghazvin-Talaghan-altitude 1804-seif 1389/2/25
HSBU 2010512	12	Tehran-Kordan-altitude 1418-Seif 1389/2/25
HSBU 2010504	13	West azarbaiehan-orumieh-Ghasemlo-altitude 2800-Norouzi 1387/3/25

Table 2. Geographical distance between studied populations.

Population	1	2	3	4	5	6	7	8	9	10	11	12	13
1	–	–	–	–	–	–	–	–	–	–	–	–	–
2	241	–	–	–	–	–	–	–	–	–	–	–	–
3	217	268	–	–	–	–	–	–	–	–	–	–	–
4	233	284	21	–	–	–	–	–	–	–	–	–	–
5	280	290	70	56	–	–	–	–	–	–	–	–	–
6	272	322	60	49	30	–	–	–	–	–	–	–	–
7	649	699	427	418	310	467	–	–	–	–	–	–	–
8	696	746	588	569	650	619	461	–	–	–	–	–	–
9	701	751	593	574	680	623	327	116	–	–	–	–	–
10	320	114	252	282	370	320	784	853	858	–	–	–	–
11	337	387	119	91	175	155	395	464	469	386	–	–	–
12	331	381	110	87	200	149	450	510	510	378	40	–	–
13	744	795	632	612	490	662	195	579	445	892	511	511	–

Abbreviations. Localities: 1. Tafresh, 2. Mahalat, 3. Malard, 4. Porkan, 5. Shahrestanak, 6. Asara, 7. Khalkahl, 8. Miandoab, 9. Tabriz, 10. Kashan, 11. Talaghan, 12. Kordan, and 13. Ghasemloo.

Morphological study

A total of 31 morphological characters, including 26 quantitative and five qualitative, were studied in 13 populations (Table 3). For the purposes of morphometry, at least five randomly selected plants were analyzed. These plant specimens were collected from the visited natural populations. The mean values of the quantitative characters were used (Table 3), while the qualitative characters were coded as binary (Table 4). The average, standard deviation and range of the quantitative characters are shown in Table 5. Data were standardized (mean = 0, variance = 1) and used for multivariate analyses.

Table 4. Morphological qualitative characters.

Characters \ Populations	1	2	3	4	5	6	7	8	9	10	11	12	13
A. Stem branching	0	0	1	0	0	0	1	0	0	1	1	1	0
B. Stem indumentum	0	0	1	0	0	0	1	1	1	1	0	0	0
C. Stem spine	1	0	0	1	0	0	0	0	0	0	1	1	0
D. Achene color	0	0	0	1	1	1	0	0	0	1	1	1	0
E. State of indumentum of bract phyllari	0	0	1	0	1	0	0	1	0	1	0	0	1

Legend. A. 0 – Branched from above, **1** – Branched from downwards; **B. 0** – Whitout hair, **1** – hairy; **C. 0** – Whitout spines, **1** – spiny; **D. 0** – Brown, **1** – Light-brown; **E. 0** – Whitout hair, **1** – hairy.

Table 5. Quantitative characters analysis.

Characters \ Analysis	Average	Standard deviation	Ranges
Capitulum length	18.738	2.068	15_21.6
Terminal spine length	2.747	3.725	1_5
Length of involucre leaflet	11.1	2.265	6_14
Width of inolucre leaflet	1.502	0.342	1_2.8
Ratio of length/width in involucre leaflet	7.7538	2.761	3.6_14
No of rows in involucre leaflet	6.392	1.008	4_7.6
Bracteiole No	81.361	21.464	37.3_116.3
Involucre length	13.553	2.977	3.6_16.6
Involucre width	9.392	2.037	6_13
Ratio of length/width of involucre	1.584	0.452	1.08_2.5
Corolla length	15.992	2.164	12.3_19
Stigma Length	4.084	1.435	2_6.6
Pistil length	11.353	2.769	5.3_15
Ratio of stigma/pistil	0.398	0.282	0.19_1.25
No. of corolla	75.250	22.054	43.3_120
Fruit length	3.130	0.604	2_4
Fruit width	1.347	0.201	1_1.7
Pappus length	22.553	4.135	17.5_30.3
Ratio of pappus/fruit length	7.478	1.140	5.4_9.3
Length of leaf lamella	67.833	33.626	30_130
Width of leaf lamella	11.93	2.737	6.6_16.6
Ratio of length/width in leaf	6.341	2.162	3.2_10.5
Length of leaf middle lobe	9.3584	3.088	4.3_15
Width of leaf middle lobe	8.792	3.421	3.5_15.6
Leaf lobe index	1.476	1.070	0.7_4.9
No. of leaf lobes	4.476	0.930	3_6

Table 3. Morphological quantitative characters.

Characters (mm) \ Populations	1	2	3	4	5	6	7	8	9	10	11	12	13
Capitulum length	20.6	19.3	19	19	15.3	19	21	19	21.6	15	17.3	18	17
Terminal spine length	1.25	1.83	1.75	1.58	1.6	3.3	1.3	2.1	1	2.1	1.5	1.41	1.5
Length of involucre leaflet	12	14	11.3	11.6	14	13.6	10.6	8	10.6	6	11.3	10.8	11.3
Width of inolucre leaflet	1.8	1.3	1.5	1.16	1	2	1.3	1.41	1.08	1.6	1.8	2.08	1.5
Ratio of length/width in involucre leflet	6.5	10.5	7.5	10	12	6.8	8	5.6	9.8	3.6	6.2	5	7.5
No of rows in involucre leaflet	7.6	7.3	6	7.3	7.3	7	6	6	4	5.3	6.3	6	7
Bracteiole N	99.3	90	97.3	116.3	90.6	81.3	64.6	62	74	50	96	92	62
Involucre length	14.3	15.6	13.3	15	13.3	14.6	15	13	15	12	16.6	15.5	14
Involucre width	10	13	10	6	8	7	11	11.5	10.3	10	7.3	10	7
Ratio of length/width of involucre	1.43	1.2	1.3	2.5	1.8	2.09	1.3	1.08	1.4	1.2	2.2	1.9	2
Corolla length	18.3	17	17.3	15.1	12.3	17	15	14.6	19	12.5	17	18.3	14.5
Stigma Length	3.3	4.1	5.3	3	2	4	6.6	3	6.6	2.6	3.6	4	5
Pistil length	15	12.6	12	12.1	10.3	13	5.3	9.6	13	7.6	13.3	14.3	9.5
Ratio of stigma/pistil	0.2	0.32	0.4	0.24	0.19	0.3	1	0.51	0.51	0.2	0.27	0.27	0.52
No of corrola	97	100	88.66	55.6	57.6	43.3	78.6	120	47	75	72	73.5	70
Fruit length	3.1	3.3	3.6	3.8	3.5	3.5	2.9	2.5	2	4	3.5	2.5	2.5
Fruit width	1.5	1.25	1.25	1.25	1.16	1.16	1.4	1.7	1.5	1.6	1.25	1	1.5
Pappus length	22.3	24	30.3	23.6	19	22.6	20	20	18	25	29.3	19.6	17.5
Ratio of pappus/fruit length	7.05	7.8	8.2	6.1	5.4	6.4	6.8	8	9	9.3	8.3	7.87	7
Length of leaf lamella	50	38	88.33	130	30	70	40.3	54.6	53.3	64.3	100	105	85
Width of leaf lamella	11.6	11.6	16.6	15	12.3	6.6	8.3	12	10.3	12	14.6	13.6	10
Ratio of length/width in leaf	4.3	3.2	5.3	8.6	8.1	10.5	4.84	4.3	5.1	5.3	6.8	7.6	8.5
Length of leaf middle lobe	10	9	11.66	11	7.3	11	7	6	6	10.3	15	13	6.1
Width of leaf middle lobe	6.3	4.5	13.3	12	10	8.3	7.1	5.3	5.6	9.3	15.6	13	8
Leaf lobe index	1.6	1.7	0.9	0.9	1.52	4.9	0.99	1.27	0.7	1.06	1.2	1.4	1.06
No. of leaf lobes	4.6	4.5	3.6	5.6	4.6	5.8	5	3	4	3.6	6	4.5	3.6

Morphological data analysis

For clustering, Manhatan distance and taxonomic distance were used. UPGMA (Unweighted Paired Group using Average) and NJ (Neighbor Joining) methods were applied. Cophenetic correlation was determined to check the fit of dendrograms. A consensus tree was used to combine the obtained morphological and molecular trees (Poodani 2000). Principal components analysis (PCA) was performed to identify the most variable characters among the studied populations (Poodani 2000). Coefficient of correlation was determined among the morphological characters to show their relationship, while Mantel test was performed to show the relationship between morphological distance, geographical distance of the populations and their molecular distance (Poodani 2000, Weising & al. 2005). SPSS ver. 9 (1998), NTSYS Ver. 2.02 (1998) and DARwin ver. 5 (2008) were applied for statistical analyses.

RAPD analysis

Thirty decamer RAPD primers of Operon technology (Alameda, Canada) belonging to OPA, OPH sets, were used in this study. DNA extraction was carried out by using the CTAB method (Murry & Tompson 1980) as modified by De La Rosa & al. (2002). The PCR reaction mixture consisted of 1 ng template DNA, 1 × PCR buffer (10 mM Tris-HCL pH 8.8, 250 mM KCL), 200 µM dNTPs, 0.80 µM 10-base random primers, and 1 unit of Taq polymerase in a total volume of 25 µl. DNA amplification was performed on a palm cycler GP-001 (Corbet, Australia). Template DNA was initially denatured at 92 °C for 3 min, followed by 35 cycles of PCR amplification under the following parameters: denaturation for 1 min at 92 °C, primer annealing for 1 min at 36 °C and primer extension for 2 min at 72 °C. A final incubation for 10 min at 72 °C was performed to ensure that the primer extension reaction has proceeded to completion.

The PCR amplified products were separated by electrophoresis on a 2 % agarose gels using 0.5 × TBE buffer (44.5 Mm Tris/Borate, 0.5 Mm EDTA, pH 8.0) or 6 % polyacrylamide gels. The gels were stained with ethidium bromide and visualized under UV light (Sambrook & al. 2001). A 100 bp DNA ladder (GeneRuler, Fermentas) was used as molecular standard in order to confirm the appropriate RAPD markers. These markers were named by primer origin, followed by the primer number and the size of amplified products in base pairs.

ISSR analysis

The total genomic DNA was extracted from fresh leaves using the CTAB method by Murry & Tompson (1980) as modified by De la Rosa & al. (2002). The six ISSR primers were $(GA)_9T$, UBC810, UBC811, UBC834, UBC849, and CA7GT, commercialized by UBC (the University of British Columbia). PCR reactions were performed in a 25 µL volume containing 10 mM Tris–HCl buffer at pH 8; 50 mM KCl; 1.5 mM $MgCl_2$; 0.2 mM of each dNTP; 0.2 µM of a single primer; 20 ng genomic DNA and 3 unit of Taq DNA polymerase (Bioron, Germany). Amplification reactions were performed in a Techne thermocycler (Germany) with the following program: 5 min initial denaturation step 94 °C, 30 s at 94 °C; 1 min at 50 °C, and 1 min at 72 °C. The reaction was completed by final extension step of 7 min at 72°. The amplification products were visualized by running on 2 % agarose gel, after ethidium bromide staining. Fragment size was estimated by using a 100 base pairs (bp) molecular size ladder (Fermentas, Germany) (Fig. 15).

Molecular data analysis

In order to determine the significant difference in meiotic characteristics, a paired sample χ^2 test was performed. Principal components analysis (PCA) was applied to show the meiotic differences among the studied populations.

The obtained RAPD and ISSR bands were treated as binary characters and coded accordingly (presence = 1, absence = 0). Jaccard similarity and Nei's genetic distance (Nei 1973) were determined among the studied populations and used for grouping of the genotypes by UPGMA (Unweighted Paired Group with Arithmetic Average) and NJ (Neighbor Joining) clustering methods and ordination based on principal coordinate analysis (PCO) and PCA (Podani 2000, Weising & al. 2005). NTSYS Ver. 2.02 (1998) and DARwin ver. 5 (2008) were used for clustering and PCO analyses. In order to determine the molecular difference between populations, AMOVA test was applied. For testing the agreement between the obtained RAPD and ISSR trees, Mantel test was run (Podani 2000) by GENALEX 6 (Peakall & Smouse 2006).

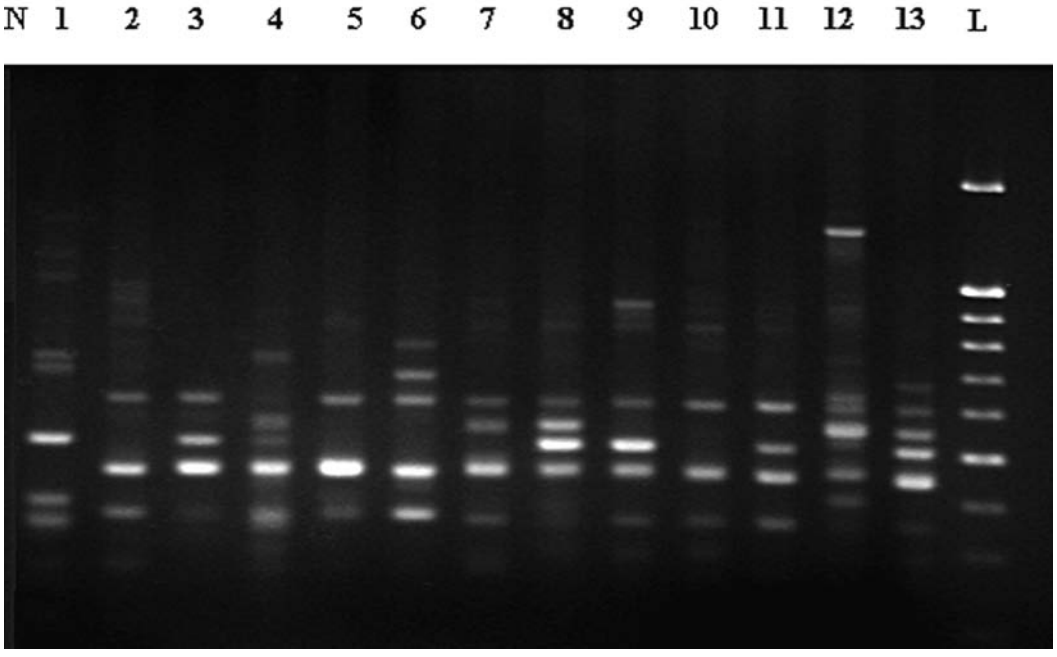

Fig. 15. CA7GT ISSR profile of the *Cirsium arvense* populations. Abbreviations: N. No DNA. **1**. Meyandoab, **2**. Khalkhal, **3**. Kashan, **4**. Tabriz, **5**. Mahallat, **6**. Ghasemloo, **7**. Tafresh, **8**. Shahrestanak, **9**. Malard, **10**. Taleghan, **11**. Kordan, **12**. Porkan, **13**. Asara. L. Ladder.

Results and discussion

The determined correlation coefficient showed a significant positive correlation ($p<0.05$) between the length of capitulum and corolla and between the length of involucre leaflet with ratio of leaflet length/width, between the number of leaflet rows and the number of bracteoles in the involucre. Similarly, there was correlation between the length of bracteoles in involucres with the number of leaf lobes and length of corolla, and between the ratio of length/width of involucres with the number of corolla, as well as between the length of fruit with the width of fruit and length of pappus.

There was a significant negative correlation ($p<0.05$) between the capitulum length and fruit length, between the width of involucre leaflet with ratio of leaflet length/width and the length of leaf central lobe. There was also correlation between the ratio of pappus length/fruit length and the ratio of leaflet length/width, as well as between the involucre length with index of leaf lobes, width of involucre with length of leaf lamella, ratio of length/width of the leaf lamella and base of the leaf middle lamella, as well as the number of corolla and ratio of length/width of involucre. The same holds true for the ratio of length/width of involucre with the length of leaf lamella.

A PCA analysis of the morphological characters has revealed that the first four factors comprise about 70 % of the total variation. In the first factor with about 25 % of the total variance, characters like length of the leaf middle lobe, width of base in the leaf middle lobe and ratio of length/width of involucre had the highest positive correlation (>0.70), while the width of involucre had the highest negative correlation (–0.72).

In the second factor with about 18 % of the total variance, the ratio of pappus length/fruit length had the highest positive correlation (>0.90). Corolla length and capitulum length had the highest positive correlation (>0.90) in the third factor, while the ratio of length/width of lamella in involucre leaflet had the highest positive correlation (>0.70) in the fourth factor. Therefore, these were the most variable morphological characters among the studied populations.

Canada Thistle is adaptable to a wide range of habitats. It occurs in nearly every upland herbaceous community within its range, particularly in prairie communities and riparian habitats (Zouhar 2001). It is most commonly found in disturbed areas as part of the initial post-disturbance community along roadsides, railroads, stream banks, ditches, lakeshores, seashores, sand dunes and other open sandy areas, in clearings and forest openings, and in wet and wet-mesic grasslands and prairie potholes (Zouhar 2001). It shows great morphological variations in the areas in which it grows, therefore morphological variations observed in the present study may partly show geographical adaptation of this species in Iran.

The UPGMA and NJ trees of morphological characters have grouped the species in a similar manner and since the UPGMA tree showed a higher cophenetic correlation ($r = 0.98$), it is discussed here.

In general, two major clusters are formed. In the first cluster, two populations of Kordan and Taleghan have shown the highest morphological similarities and are joined together, the Porkan population also joins them at some distance, while two other populations of Asara and Shahrestank are greatly distanced from these species.

The second major cluster contains two subclusters. In the first subcluster, two populations of Miandoab and Kashan have shown high morphological similarity and are joined together, while the Ghasemlo population is at a greater distance. Five other populations form the second subcluster, where the Khalkhal and Tabriz populations and the Tafresh and Mahalat populations have shown greater similarity and are placed close to each other. Mention deserves the fact that the populations of Asara, Porkan, Kordan, Taleghan, and Shahrestanak occur in three neighbouring provinces of Tehran, Ghazvin and Alborze, which form the first group/cluster in the morphological analysis, while the populations of Tafresh and Mahallat (Markazi Province), Ghasemloo and Meyandoab (West Azarbayejan), Tabriz (East Azarbayejan, Khalkhal (Ardebil) and Kashan (Isfahan), which comprise the second group/cluster, are located at much farther distance from the populations of the first cluster/group (Fig. 7).

An interesting agreement has been observed while comparing the morphological results of the present study with the recently reported molecular tree for the same populations (Sheidai & al. 2010). For example, the two populations of Taleghan and Kordan which showed the highest morphological similarities were very close to each other in the UPGMA tree obtained by RAPD molecular data and relatively close in the ISSR ordination plot (Sheidai & al. 2012). The same holds true for almost all other populations (Fig. 8 and 9). Therefore, a consensus tree was constructed on the basis of a combined data set of morphological and molecular data presented in Fig. 10. It shows a clear separation of the

Taleghan and Kordan populations which indicates their genetic and morphological distinctness.

A detailed molecular/morphological investigation of these populations was undertaken to determine the taxonomic place of these two populations. In *Flora Iranica*, two varieties of *incanum* and *arvense* have been cited for *C. arvense* of Iran. These two varieties differ from each other on the basis of their leaf characteristics. The first variety, i.e. *C. arvense* var. *incanum* Ledeb., has elongated leaves with indumentum and shallow lobes while, the second variety, i.e. *C. arvense* var. *arvense,* has broad leaves with deep lobes, without any indumentum.

Cirsium arvense is an extremely variable species with regard to leaf division and indumentum. Several authors recognize different varieties based primarily on differences in leaf morphology. But according to many botanists, it is doubtful that variety designation is meaningful and so they do not consider the described variants of species to be taxonomically significant. Among the 13 studied populations, 11 have shown morphological characters described for the variety *incanum* (Fig. 11), while the Taleghan and Kordan populations have broad leaves with deep lobes (similar to var. *arvense*) and have shown the presence of indumentum (similar to var. *incanum*) (Fig. 12). Furthermore, these two populations have had spines on the stem which were absent in the other populations (Fig. 13) (they have had indumentum covering their stems) (Fig. 14). Therefore, we suggest for these populations to be regarded as a new variety of *C. arvense*.

The geographical distance between the studied populations is presented in Table 2. According to the Mantel test, the geographical distance and morphological differences among the populations showed no significant correlation ($t = 1.43$, $p = 0.90$), while there was a significant correlation between the geographical distance and both RAPD and ISSR molecular markers ($t = 2.80$, $p = 0.002$ and $t = 2.66$, $p = 0.003$, respectively). These data indicate that with the increase in geographical distance between the studied populations, molecular difference increases, but such molecular differences do not result in morphological differences.

A Pearson correlation of the standardized morphological and of the molecular data (data not given) was carried out in order to investigate which ISSR loci are correlated with the morphological characters. During the first attempt, the most variable morphological characters (length of leaf middle lobe, the ratio of pappus length/ fruit length, ratio of length/ width of involucres and corolla length) were determined among the populations.

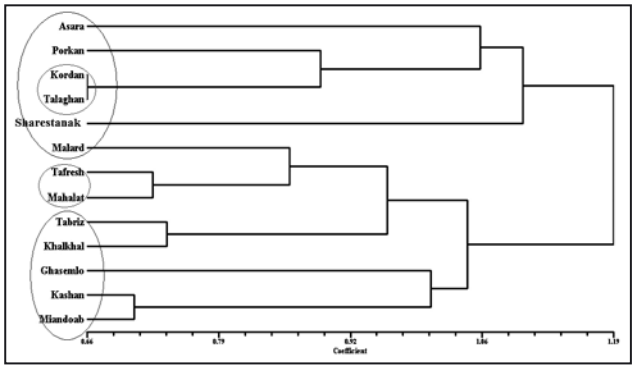

Fig. 7. UPGMA tree of morphological characters.

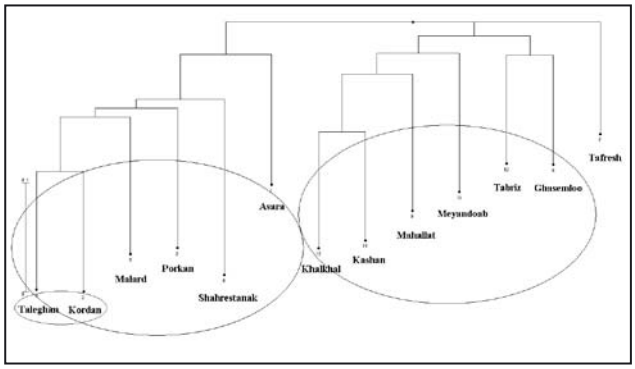

Fig. 8. UPGMA tree of RPDA data.

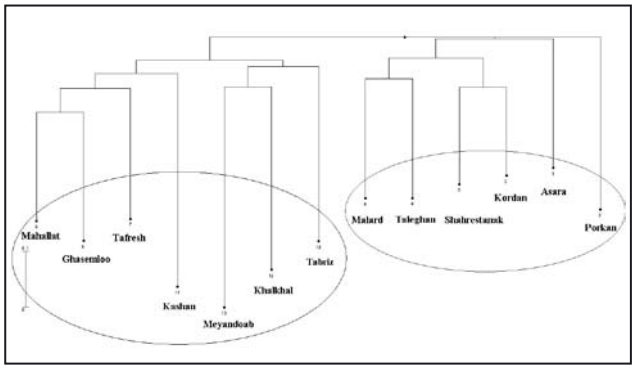

Fig. 9. UPGMA tree of ISSR data.

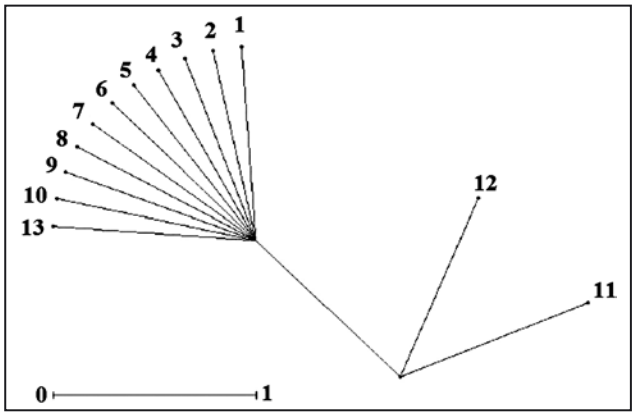

Fig. 10. Consensus tree of the morphological and molecular trees. Population codes as in Table 1.

Fig. 11. *C. arvense* var. *incanum* leaf.

Fig. 13. Spines in stems.

Fig. 12. Talaghan and Kordan population – leaf.

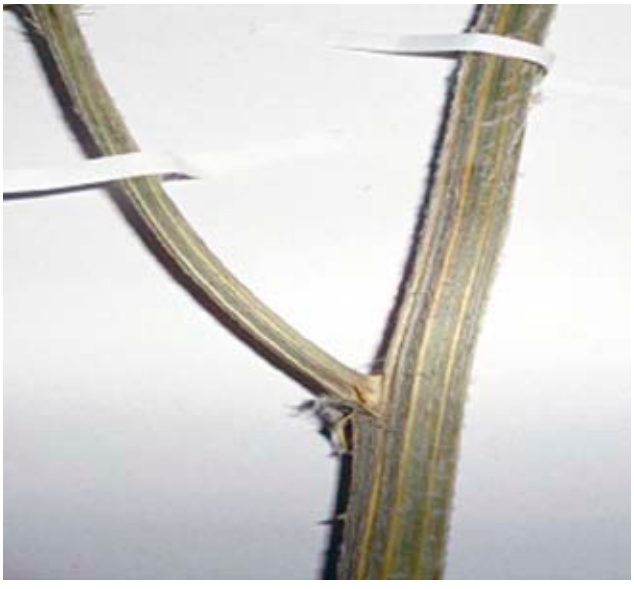

Fig. 14. *C. arvense* – stem.

The length of leaf middle lobe was negatively correlated with ISSR band 11 of the primer CA7GT, with 680 bp molecular weight ($r = -0.6$, $p<0.05$), the ratio of pappus length/ fruit length was positively correlated with ISSR band 10 (2800 bp) of ISSR primer 849 ($r = -0.6$, $p<0.05$), but negatively correlated with ISSR locus 13 (600 bp) of the same primer.

The ratio of involucre length/ width was positively correlated to ISSR band No 12 of ISSR primer 849, and band No 7 (2000 bp) and 9 (2100 bp) of the primer CA7GT. Similarly, the corolla length was positively correlated to ISSR loci 5 (1100 bp) of ISSR primer 849 and ISSR band No 4 (2200 bp) of the primer CA7GT ($r = 0.6$ $p = <0.05$), and negatively correlated to ISSR loci 6 (690 bp) of ISSR primer 849 and band No 3 (29000 bp) of the primer CA7GT ($r = -0.61$, $p = <0.05$). Therefore, we suggest that changes in these molecular loci may be responsible for change in the morphological variations occurring among the studied populations.

We have found correlation between some other morphological characters in this species and ISSR loci; for example, length of the spine in the terminal leaf of involucres has shown positive correlation with ISSR bands No 7 and 8 (600 and 4000 bp, respectively) of the ISSR primer 849. The involucre leaflet length has shown negative correlation with band No 10 (2800 bp) of ISSR primer 849, and was positively correlated to band No 13 (600 bp) of the same primer. Similarly, the ratio of length/width of

involucre leaf was positively correlated to bands No 2 (2100 bp) and 11 (1500 bp) of the ISSR primer 849.

Some of the ISSR loci also showed significant correlation to each other, for example, in ISSR primer 849, band 2 shows correlation with band 11, while, bands No 3 and No 4 were positively correlated. However, bands 5 and 6 were negatively correlated (r = –0.67). Similarly, band No 12 (1100 bp) of ISSR primer 849 was positively correlated to bands No 6 (2000 bp) and No 8 (1100 bp) of the primer CA7GT. These molecular loci may be related and inherited jointly.

In a similar study, Bodo Slotta & al. (2006) have studied the level of genetic diversity within and gene flow between Canada Thistle (*C. arvense*) and its relatives in the Northern Great Plains by using SSR and ISSR molecular markers. They have found that among the sampled *Cirsium* species, Bull Thistle had the greatest homology to Canada Thistle in ISSR loci and some microsatellite sequence regions were conserved between Eurasian and North American thistles.

In another attempt Bodo Slotta & al. (2010) have studied the genetic diversity of the same species in 85 localities of North America by using seven microsatellite markers. The populations exhibited a greater intrapopulation diversity than expected for a reported clonally reproducing species. The total diversity of the sampled locations in North America (0.183) was lower than the earlier reported one for European locations (0.715), but the greater mean difference between the North American populations suggested strong founder effects or restriction of gene flow influencing the individual populations.

In general, it seems that *Cirsium arvense,* which usually has a wide geographical distribution, shows extensive genetic variation due to local adaptations which, in turn, may lead to morphological variations. Such variations may be so great as to be able to form new taxonomic entities. However, as the present investigation indicates, the magnitude of morphological variations is not directly related to the geographical distance of the populations.

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