Investigation of phenotypic and molecular diversity of *Descurainia sophia* (L.) Webb ex Prantl and *Sisymbrium irio* (L.)

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Summary

Descurainia sophia and *Sisymbrium irio*, both from Brassicaceae family have potential medicinal effects. Seeds are consumed widely across Iran in sweet drinks. To determine the morphological and molecular diversity, 55 genotypes from these two genera were studied. All 11 ISSR primers detected polymorphism and generated 274 polymorphic loci. The average values of Polymorphism Information Content (PIC), Marker Index (MI) and Resolving Power (Rp) for ISSR primers were 0.278, 6.899 and 11.57, respectively. The AMOVA analysis revealed a high genetic variation (58%) within each genus. The UPGMA clustering based on molecular analysis separated all 55 genotypes into two main groups, correlating with division of plants into two genera. Moreover, seven morphological traits were evaluated to distinguish all 55 genotypes. Among them, rosette figure showed the most significant differentiation between two genera. The highest correlation was observed between two traits of seed color and seed weight at 0.758. The dendrogram obtained from the morphological traits corresponded with the UPGMA clustering. The chemical composition of essential oils of *D. sophia* and *S. irio* were identified via gas chromatography mass spectrum (GC-MS). Although GC-MS analysis detected β-pinene as the dominant component in both plants but different compounds were also detected. The present investigation clearly indicates that these morphological traits alone or combined with molecular analysis using 11 ISSR markers is considered as the true reflection of two genera partitioning, and hence the eligibility of both molecular and morphological criterion are proved. These findings could be used for future breeding programs in fields of seed production and medicinal extracts.

Key words

Descurainia sophia, Sisymbrium irio, morphological and molecular diversity, ISSR, GC/MS

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Introduction

Brassicaceae is a very large plant family with over 3000 species worldwide. *Descurainia sophia* L. (synonym: *Sisymbrium sophia*) and Sisymbrium irio L. in this family are known as "Khakshir" in Iran and are used as important medicinal plants. These two herbs, specially their seeds, are widely consumed for food, medicinal and industrial applications (Mitich, 1996; Peng et al., 1997; Bekker et al., 2005; Sun et al., 2005; Mohamed and Mahrous 2009; Li et al., 2010; Mosaddegh et al., 2012; Mokhtassi-Bidgoli et al., 2012). The seed oil is particularly rich in fatty acids, linoleic and linolenic acid, making it highly moisturizing and nourishing for skin. Although the both genera are distributed around the world, *S. irio* is originated from southern Europe, Africa and temperate and tropical regions of Asia (National Plant Germplasm System, 2012), whereas *D. sophia* is from Northern Africa and South of Europe (Zhang, 2003). These annual dicotyledonous plants grow immensely in fodder plains, pasturelands, farms, waysides and disarranged terrains (Mitich 1996; Li et al. 2011). *Descurainia* genus is designated in memory of F Decourain, a French botanist who lived from 1678 through the mid-18th century. Species within the *Descurainia* genus have complex relationships and properties. The species in *Descurainia* is *D. sophia*. *Sophia*, which is Greek for wisdom, it is an allegory for the plant's popular medicinal characteristics (Gledhill, 1989). Originally in 1753, Linneanus labelled *D. sophia* as *Sisymbrium sophia* in Species Plantarum context. Prior to this, descurainias were placed in the same genus by a botanist from United States. Yet, Michel Adanson in 1763 regrouped the genus as *Sophia* in the Familles des Plantes. By 1892 due to different viewpoints of systematists, a third main classification was developed. In the nineteenth century Philip Barker Webb appointed this class as *Descurainia sophia*. Finally, it was published in Adolph Engler and Karl Prand's Die Natürlichen Pflanzenfamilien by Karl Prand. In numerous ranges of different species of the Brassicaceae family, the genetic diversity within and between its species has been successfully determined by ISSR markers. The objective of this investigation was to assess phenotypic and genetic variation between and within species of *D. sophia* and *S. Irio*.

Materials and Methods

Plant materials and morphological evaluation

Seeds of all 55 genotypes were grown in a greenhouse at Bu-Ali Sina University, Hamedan in three replications. Plants including 32 *D. sophia* genotypes collected from Hamedan, Lorestan, Central, Fars, Yazd and Kerman provinces and 23 genotypes of *S. irio* from Hamedan, Fars, Gilan, Qom, Yazd and Ardebil (Fig. 1). Seven morphological characters including seed color, seed size, seed weight, rosette figure, number of seeds per silique, silique length and inflorescence length were studied (Table 1). Seed color of the fully mature seeds was visually scored and ranked into the five different classes ranging from bright yellow (1) to black (5). Seeds were divided into the two groups of smaller and bigger than one millimeter (mm). Specification of the rosette figures were implemented into the three groups of bipinnate (1), lacerate (2) and undulate leaves (3). The number of seeds per silique and silique length were averaged from three ripe siliques. Detecting correlations between the seven traits and morphological data analysis were performed through SPSS software version 22 (IBM Corp. 2013).

Table 1. The descriptive statistics of seven morphological traits in each genus with the number of genotypes (N) and standard deviation (SD)

		D. Sophia		*D. Sophia*	
Trait	Unit/Code	N	SD	N	SD
Seed color	1-5	32	0.504	23	0.815
Seed size	1-2	32	0.336	23	0.344
Seed weight	g	32	0.029	23	0.028
Rosette figure	1-3	32	0.000	23	0.487
Number of seeds per silique	-	32	4.255	23	8.387
Silique length	mm	32	0.936	23	1.506
Inflorescence length	mm	32	2.206	23	2.430

DNA extraction and ISSR analysis

Genomic DNA was isolated from young leaves according to the modified CTAB method (Doyle and Doyle, 1987). The amplification of DNA fragments was carried out by eleven ISSR primers. PCR products were separated on 1.2% agarose gels, stained with ethidium bromide and documented using a 3UV transilluminator system (Liu et al., 2007a,b).

Molecular analysis

The amplified ISSR fragments were scored for presence (1) or absence (0) of bands. Only clear and reproducible bands were scored. Jaccard's similarity coefficient was used to calculate the pairwise genetic similarities among individuals (Jaccard, 1908). To determine the quality of clustering, the cophenetic correlation coefficient (r) was measured based on UPGMA cluster analysis (Rohlf and Sokal, 1981). These calculations were carried out by NTSYS 2.02e software package (Rohlf, 1992). The principle coordinate analysis (PCoA) (Mohammadi and Prasanna, 2003) was also calculated to assess genetic diversity. The partitioning of genetic variance into two levels (among and within populations) was carried out by analysis of molecular variance (AMOVA) (Excoffier et al., 1992) in GenAlex version 6.4 (Peakall and Smouse, 2006). PIC value for each polymorphic locus was calculated according to Roldan-Ruiz et al. (2000): $PIC_i = 2f_i\ (1 - fi)$, where f_i is frequency of fragments present in that locus. The marker index (MI) was calculated based on $MI = EMR \times PIC$ formula (Powell et al., 1996). Resolving power (Rp) for each primer, which indicates discriminatory potential of applied primers, was calculated based on Prevost and Wilkinsin (1999) formula: $Rp = \sum Ib$, where Ib is the band informativeness. Ib is computed via $Ib = 1 - [2 \times (0.5 - p)]$, where p is the proportion of all 55 genotypes containing the band. To examine different genetic diversity data, between two species, PopGene 32 (Yeh and Boyle, 1997) software was used. Number of observed alleles (Na), number of effective alleles (Ne), Nei's gene diversity (H) and Shannon's information index (I) were calculated. Other genetic diversity parameters including number

Figure 1. Schematic map of Iran with locations where *D. sophia* and *S. irio* were collected. Caspian sea and Persian gulf in the north and south of country are depicted.

of polymorphic loci (PL), percentage of polymorphic loci (PPL) and gene flow (Nm) based on $Nm = 0.5 \times (1 - Gst) \div Gst$ were also calculated (McDermott and McDonald, 1993).

Gas Chromatography/Mass Spectroscopy

Three plants from each species (both from Hamedan, Fig 1) were grown and used for the experiment. Plant materials were dried. Leaves and seeds were grinded separately. Essential oils of two genuses were isolated by Ultrasonic Assisted Headspace Solid Phase Micro Extraction (UA-HS-SPME) method (Eloff, 1998), and identified by analytical gas chromatography mass spectrum (GC/MS). GC-MS analysis was performed using Agilent 6890N mass spectrometer interfaced with an Agilent 5973 chemstation. The chromatographic conditions were as follow: column oven program: 50°C (10 min, isothermal) to 250°C (10 min, isothermal) at 2°C min^{-1}; the injector and detector temperature was 250°C. Helium was carrier gas (flow rate 0.90 mL min^{-1}). A HP-5 MS capillary column (30×0.25 mm i.d., 0.25 µm stationary phase thicknesses) was utilized. Actual temperature in MS source reached approximately 250°C. The ionization voltage was 70 eV. The volatile compounds were identified by comparing their MS patterns and retention times with those of known compounds published in the literature.

Results

Morphological assessments

Seven morphological characters were evaluated for all 55 genotypes. Standard deviation (SD) and variance of seven morphological traits in each genus of (*D. sophia* and *S. irio*) were separately calculated (Table 1). Strong correlations were observed in the correlation matrix (Table 2). Seed color and rosette figure showed the highest negative correlation, while seed color and seed weight had the highest positive one. Based on the all seven morphological traits a dendrogram using single linkage was constructed (Fig. 2). The dendrogram at the rescaled distance cluster of 25 was branched into two groups, which correlate exactly with the division of the two genera. This partitioning based on the morphological characters was consistent with UPGMA clustering based on molecular analysis.

Molecular analysis

A total of 274 loci were amplified from 55 genotypes (Table 3). PCR products were in size range of 100-3100 bp with 29.90 loci per ISSR primer. All primers produced polymorphic loci. Primer analysis indicated a high diversity among the 55 genotypes (Table 3). Genotyping data obtained with 11 ISSR primers were used to assess marker performance through evaluation of three parameters: PIC, MI and Rp. The range of PIC was 0.188 (UBC 807) to 0.333 (M2), averaging 0.278. To determine general usefulness of the system of markers applied, MI for each primer was calculated. With the average of 6.899, the highest MI was observed for UBC 834 (8.840) and the lowest for primer UBC 807 (4.512). The mean of Rp for all primers was 11.57. The minimum Rp value was observed at 6.49 for UBC 807 and the maximum at 15.61 for UBC 841 (Table 3). Thirty-two genotypes of *D. sophia* produced 12 monomorphic loci via eight ISSR primers, but 23 genotypes of

Table 2. Correlation matrix of morphological traits

Trait	Seed color	Seed size	Seed weight	Rosette figure	Number of seeds per silique	Silique length
Seed size	0.658*	1.000				
Seed weight	0.758*	0.489	1.000			
Rosette figure	-0.884*	-0.674*	-0.632*	1.000		
Number of seeds per silique	-0.358	-0.381	-0.287	0.390	1.000	
Silique length	-0.481	-0.367	-0.322	0.487	0.645*	1.000
Inflorescence length	-0.672*	-0.600*	-0.514	0.694*	0.444	0.378

Table 3. Primer features include primer names, primer sequences, annealing temperature (Ta), size range (SR), number of total loci (NL), polymorphic information content (PIC_{avg}), resolving power (Rp) and marker index (MI) of the 11 ISSR primers. (Y*=C/T, R*=G/A)

Primer name	Primer sequence	Ta (C°)	size range	NL	PIC_{avg}	MI	Rp
UBC 807	(AG)8T	54	250-2500	24	0.188	4.512	6.49
UBC 834	(AG)8Y*T	53	100-2500	34	0.260	8.840	12.83
UBC 855	(AC)8YT	54	300-2525	22	0.275	6.050	10.63
UBC 818	(CA)8G	53	300-3000	19	0.318	6.042	9.47
UBC 844	(CT)8R*C	54	350-2700	20	0.287	5.756	9.91
UBC 848	(CT)8RG	42.5	240-3100	28	0.267	7.476	12.55
UBC 811	(GA)8C	51	200-2500	26	0.297	7.722	13.10
UBC 810	(GA)8T	52	200-2500	24	0.261	6.264	11.83
UBC 841	(GA)8YC	53	150-2500	25	0.321	8.025	15.61
UBC 823	(TC)8C	54	300-2500	27	0.255	6.885	9.32
M2	GGGC(GA)8	56	200-2500	25	0.333	8.325	15.53
Mean	-	-	-	29.90	0.278	6.899	11.57
Total	-	-	-	274	-	-	-

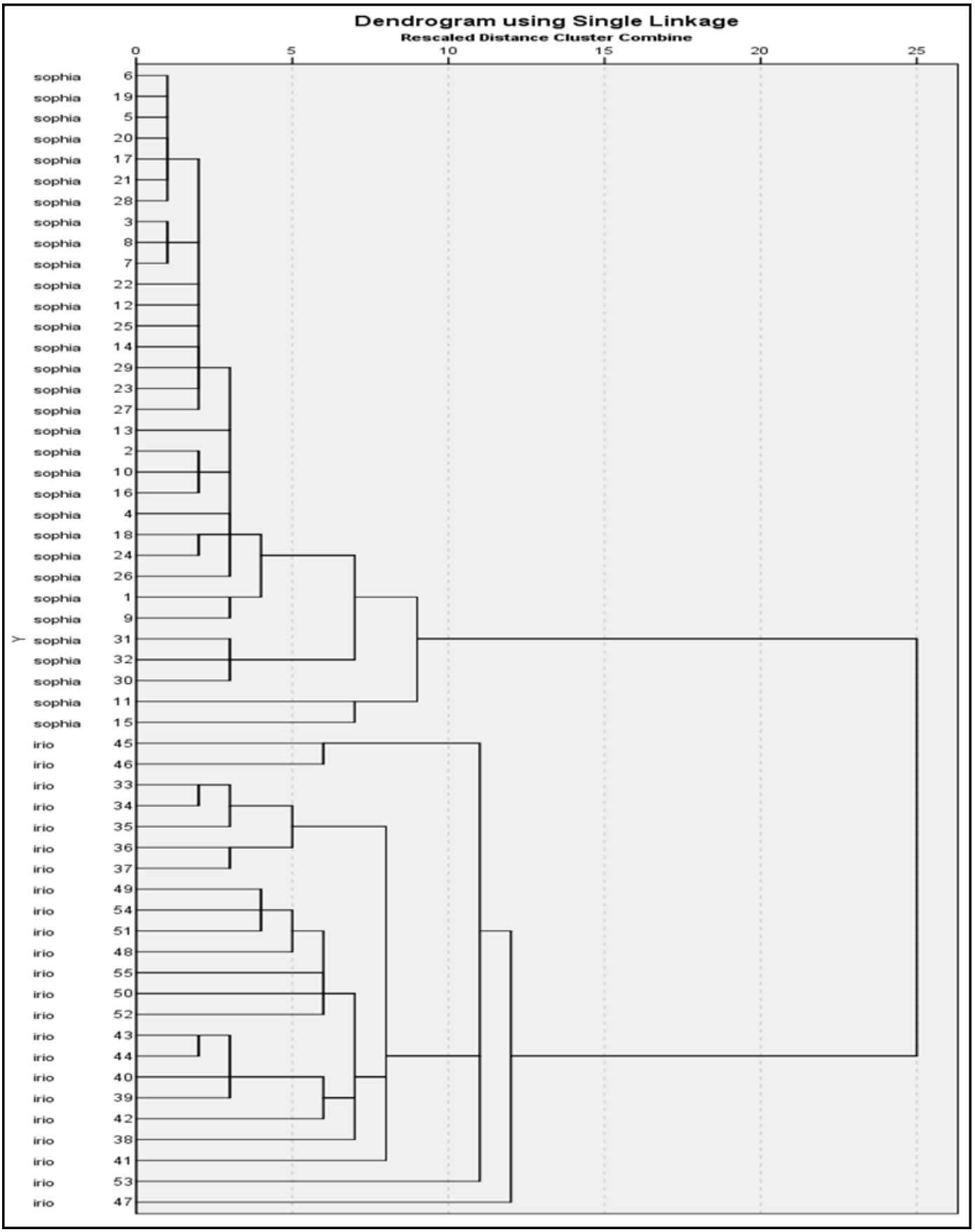

Figure 2. Dendrogram generated based on the seven morphological traits for the 55 genotypes

S. irio showed no monomorphic loci. Jaccard's pair-wise similarity coefficient for the 55 genotypes was in range of 0.043 to 0.813. The highest similarity was between *D. Sophia 13* and *D. Sophia 14* (both from Lorestan), while the minimum was between *D. sophia 13* (Lorestan) and *S. Irio 21* (Central) (Data not shown). The UPGMA clustering algorithm from ISSR analysis grouped the 55 genotypes into two clusters at a similarity index value of 0.11 (Fig. 3). This partitioning of genotypes showed a precise correlation with the two genera classification of genotypes. Top cluster consisted of 32 genotypes of *D. sophia* and 23 genotypes of *S. Irio* grouped in the second cluster. At similarity index of 0.81, *D. sophia 13* and *D. sophia 14* were placed in one cluster. A cophenetic value of 0.9798 indicated that cluster analysis strongly represented the similarity matrix (Wang et al., 2011), and there was a good correlation between the similarity matrix and UPGMA clustering. In principle component analysis the eigen values for first three PCAs were 16.33, 5.37 and 2.37, respectively, that represented 43.78% of total variation. Therefore, there was a proper distribution of ISSR markers through entire genome. The cumulative arrangement of 55 individuals, using genetic similarity based on ISSR markers, was shown in Fig. 3. The graph of PCoA analysis showed two main associations, which confirmed the partitioning results of UPGMA clustering into two genera. AMOVA analysis via GenAlex software was calculated with 1000 permutations. Among two genera there was 42% of genetic variation, and a significant 58% of the total genetic variation was within each genus. Also AMOVA analysis was separately calculated for each genus. Genetic variation within six groups of *D. sophia* and six groups of *S. irio* were as high as 86% and 78%, respectively (Table 4). Genetic diversity of each genus (*D. sophia* and *S. irio*) and at the genera level was calculated through PopGene (Table 5). Number of observed alleles (Na) and effective alleles (Ne) were 1.624-1.627 (*D. sophia - S. irio*) and 1.278-1.214 (*D. sophia - S. irio*), respectively. The mean of Nei's gene diversity (H) for two genuses was 0.1578 whereas H at the genera level was 0.207. Shannon's information index (I) at genera level was 0.339 and averaging at 0.252 for both genuses. Diversity amongst genera (Gst) and gene flow (Nm) were found to be 0.2323 and 1.6526, respectively.

Table 4. Analysis of Molecular Variance (AMOVA) within and among genera

Source	df	SS	MS	Est. Var.	%
Among genera	1	585.753	585.753	20.819	42%
Within genera	53	1514.065	28.567	28.567	58%
Total	54	2099.818	-	49.386	100%
Among *D. sophia* groups	5	223.717	44.743	3.946	14%
Within *D. sophia* groups	26	656.783	25.261	25.261	86%
Total	31	880.500	-	29.207	100%
Among *S. irio* groups	5	226.565	45.313	6.827	22%
Within *S. irio* groups	17	407.000	23.941	23.941	78%
Total	22	633.565	-	30.768	100%

Table 5. Genetic diversity data calculated at levels of each genus and at the level of the genera.

Population	SS	Na	Ne	H	I	PL	PPL	Gst	Nm
D. Sophia	32	1.6241	1.2782	0.1725	0.2693	171	62.41%		
S. irio	23	1.6277	1.2142	0.1432	0.2341	172	62.77%		
Mean	-	1.6259	1.2462	0.1578	0.2517	-	-		
Genera	55	2.000	1.3163	0.2068	0.3390	274	100%	0.2323	1.6526

SS: Sample Size, Na: Number of observed alleles, Ne: Number of effective alleles, H: Nei's gene diversity, I: Shannon's information index, PL: Number of polymorphic loci, PPL: Percentage of polymorphic loci, Gst: Diversity among populations, Nm: Gene flow

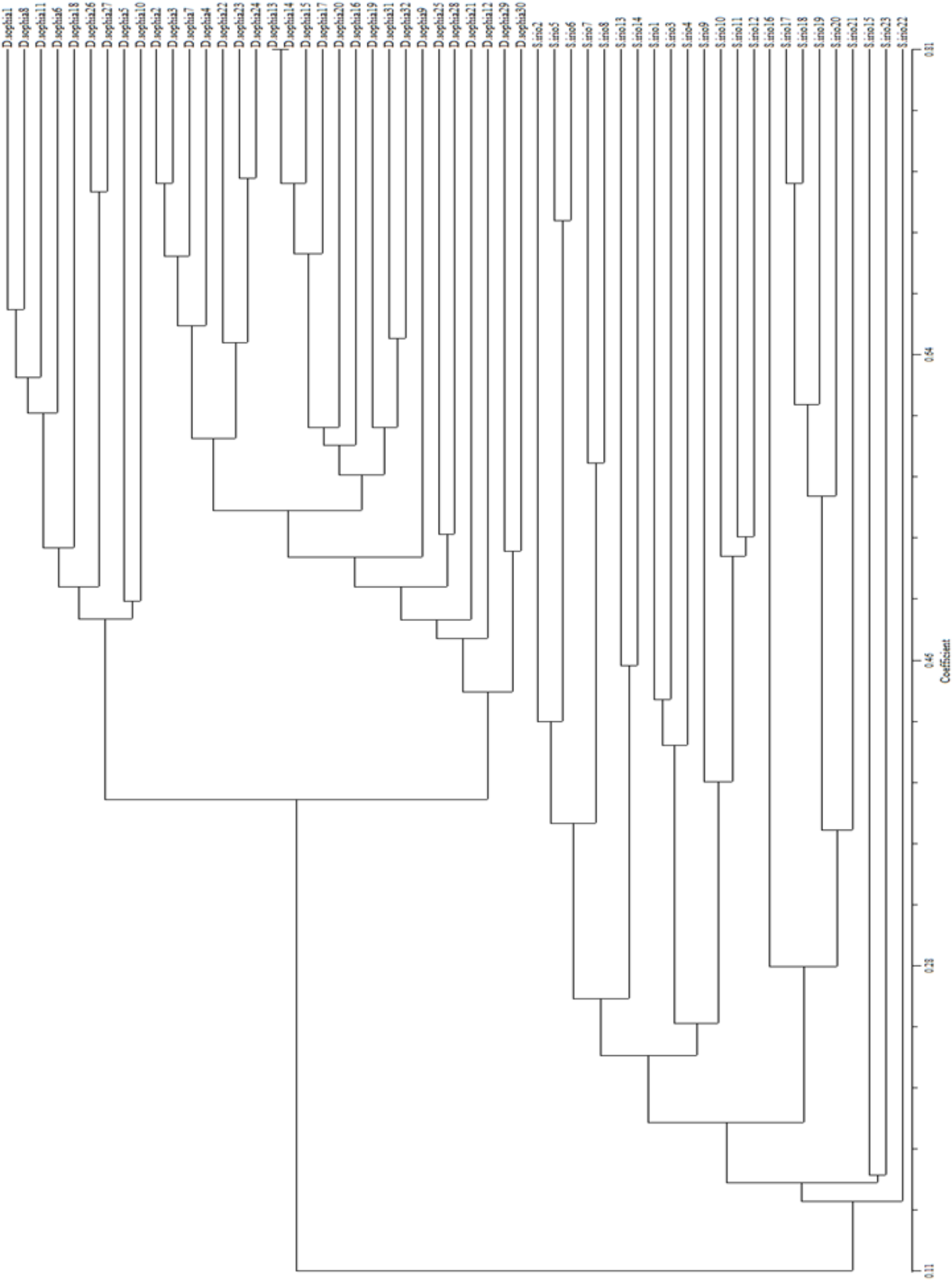

Figure 3. UPGMA clustering of 55 genotypes based on Jaccard similarity coefficient

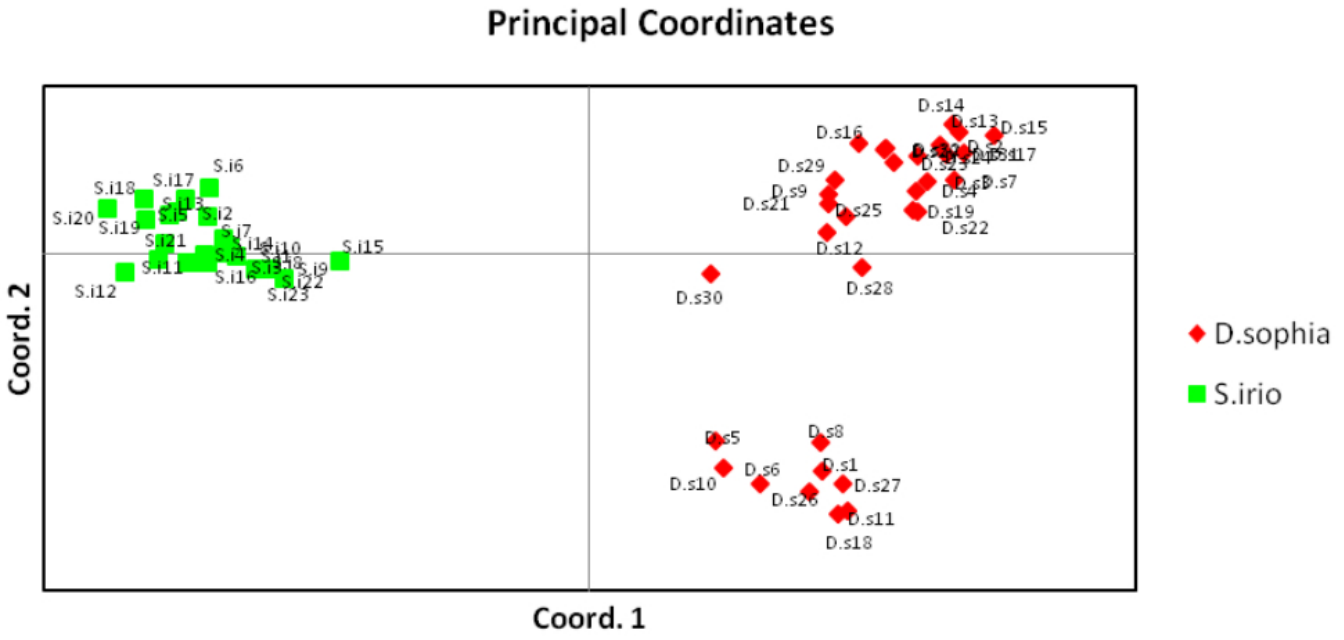

Figure 4. PCoA analysis of the total genotypes defined based on the ISSR data

GC/MS analysis

With GC/MS analysis, sixteen combinations and phytochemicals were detected in *D. sophia* seed essential oil. β-pinene was the major compound (31.17%) followed by menthol (16.92%), bicycloelemene (8.90%), α-bisabolene (6.15%) and, cis-β-ocimene (6.14%) (Fig. 5). GC/MS analysis of leaf essential oil of *D. sophia* identified eighteen phytochemicals. Again β-pinene was the major compound (35.36%) followed by menthol (19.92%), bicycloelemene (17.44%), α-bisabolene (7.03%) and, α-thujene (4.9%) (Fig. 5). In essential oil of *S. irio* seed β-pinene was the major component (23.75%), and the other chemical materials were bicycloelemene (13.52%), menthol (10.89%), isopropyl isothiocyanate (10.06%) and pulegone (5.71%) (Fig. 6). In leaf essential oil of *S. irio* 23 compounds were identified; β-pinene was the main component (28.74%), followed by menthol (10.59%), sabinene (6.92%) and isobutyl isothiocyanate (6.75%) (Fig. 6). A number of these compounds, such as β-pinene, sabinene, cis-β-ocimene, isoborneol, menthol, thymol, α-bisabolene in *D. sophia* and isopropyl isothiocyanate, n-(n-propyl) acetamide, indole, n-butyl isothiocyanate, 4-(2,5–dihydro-3-methoxy phenyl) butylamine in *S. irio*, are in agreement with the findings of the previous studies (Baydar et al., 2004; Mohamed and Mahrous, 2009; Al-Qudeh and Abu Zarga, 2010). The compounds α-thujene, piperitone, bicycloelemene, caryophyllene oxide, pulegone, cymene and copaene of *D. sophia* and α-thujene, β-pinene, menthol, bicycloelemene, piperitone, copaene, α-pinene, pulegone, sabinene, caryophyllene oxide, α-bisabolene of *S. irio* are reported here for the first time. The cis-β-ocimene (17.12%) was reported as the main component in essential oil of *D. sophia* (Mohamed and Mahrous, 2009). In our study β-pinene was the main component (31.17%), and cis-β-ocimene was the fifth compound (6.14%). Al-Qudah and Abu Zarga (2010) have reported that dioctyladipate (25.44%) and n-(n-proyl) acetamide (14.77%) were the significant components in essential oils of *S. irio*. In this study β-pinene was the main component (23.75%) in seeds and in leaves (28.74%). Similar in both plants were α-thujene, β-pinene, cymene, trans sabinene hydrate, 3-carene, menthol, bicycloelemene, β-bourbonene, trans caryophyllene, α-bisabolene, caryophyllene oxide. Only in *D. Sophia* were detected cis-β-ocimene, isoborneol, piperitone, thymol and phytol, while isopropyl isothiocyanate, n-(n-propyl) acetamide, pulegone, n-butyl isothiocyanate, copaene, tetradecane, 4-(2,5–dihydro-3-methoxy phenyl) butylamine, 3',5'-dimethoxy acetophenone were detected only in *S. irio*. Isopropyl isothiocyanate is strongly odorous and pungent compound, formed by the action of enzyme myrosinase on the glucosinolate when plant tissue is disrupted. Many crucifers including *S. irio* contain glucosinolates, which are precursors of many volatile compounds in particular isothiocyanates (Al-Qudah and Abu Zarga, 2010). This may cause the bitter taste of *S. irio*.

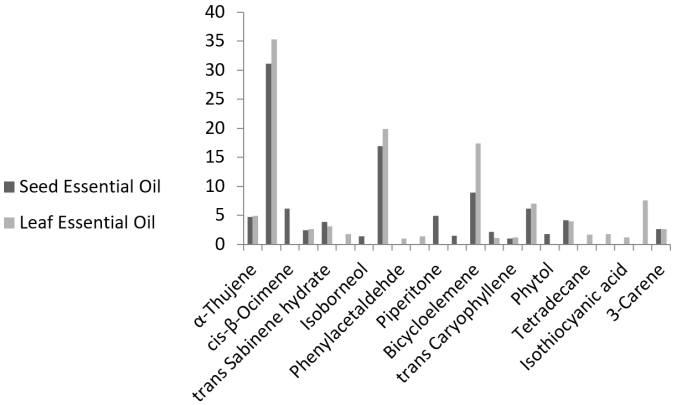

Figure 5. GC/MS analysis of seed and leaf essential oils from *Descurainia sophia*

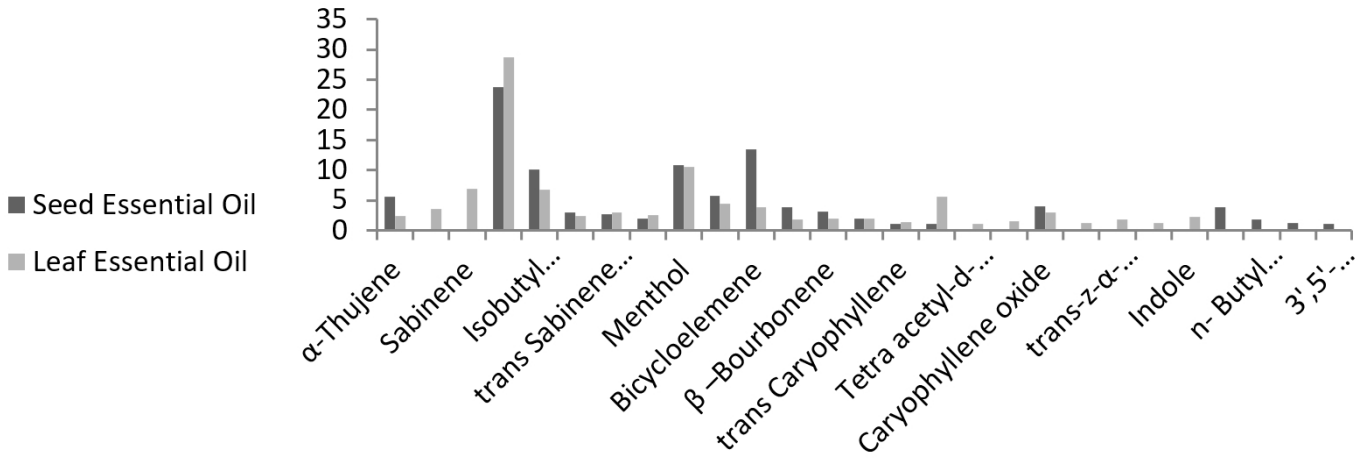

Figure 6. GC/MS analysis of seed and leaf essential oils from *Sisymbrium irio*

Discussion

Herbal drug technology together with accurate combination of customary science and modern techniques is applied for converting botanical materials into medicines. Verification of medicinal importance in plant genera and species was estimated extensively with DNA based methods alongside evaluation of morphological traits (Joshi et al., 2004). In addition to Iran's long history in traditional medicine and growing interest toward these plants, a distinct division and identification based on both molecular and morphological characters is required for later breeding programs and preservation of such unique germplasms. Seeds from the two herbs of *D. sophia* and *S. irio* are widely consumed by public in Iran as Khakshir. In this investigation 11 ISSR primers and seven morphological characters were used to distinguish a total of 55 genotypes into two genera of *D. sophia* and *S. irio*. ISSR markers were useful to study genetic diversity in *D. sophia* (Saki et al., 2015). Both molecular and morphological traits have successfully separated two genera in cluster analysis based on differences in banding patterns and dispersion of each trait from their average. Few studies on genetic and morphological diversity of these two herbs have been conducted. Our data in molecular analysis showed that from total genetic variation, 58% was observed within genera, indicating the high diversity among genotypes of each group. Of course with more molecular marker data, more differences in other fraction of genome may be detected. There is a small group within the *D. sophia* cluster that shows genetic variation within species, which can be due to gerplasm exchange between the regions. Since *D. sophia* and *S. irio* produce a great number of seeds, wind could help in its dispersal. The chemical composition of the essential oil derived from the plant can be influenced by many factors, such as climatic, seasonal, geographic and soil conditions, harvesting period and distillation technique (Baydar et al., 2004; Prabuseenivasan et al., 2006).

There were differences in chemical composition based on essential oil of seed and leaf of one individual per species, but a full chemical profile of all samples is needed to compare with morphological and molecular diversity data. Seven morphological traits classified the 55 genotypes into two clusters, of which the top cluster included *S. irio* genotypes and the smaller cluster, genotypes of *D. sophia*. At morphological assessments, rosette figure, seed color and seed weight had the most impact in genotype differentiation of *D. sophia* and *S. irio* from each other. Thus these traits could be used as morphological markers to distinguish two genera. As a result, diversity among the seven morphological traits of *S. irio* was higher than in *D. sophia* genotypes. By the molecular analysis eight primers (UBC807, UBC855, UBC844, UBC848, UBC811, UBC810, UBC841 and M2) for *D. sophia* genotypes showed 12 monomorphic loci, which could be applied as molecular markers for their distinction from *S. irio*.

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