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Genetic relationships among *Achillea tenuifolia* accessions using molecular and morphological markers

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Abstract

ISSR and morphological markers were used to detect genetic diversity in several genotypes of *Achillea tenuifolia* from different geographical regions of Iran. Fifteen primers revealed 247 polymorphic bands, out of which 214 (86.78%) were polymorphic. The dendrogram was constructed using SM coefficient and UPGMA method. The generated dendrogram revealed three groups. The accessions originated from central regions of the country separated from others in group 3. The principle coordinate analysis (PCoA) confirmed the results of clustering (>90%). For morphological traits, North-western (NW) accessions had the highest values of leaf length, leaf width, leaf area, essential oil yield and the latest flowering time, while the Northern one (AtN76) had the highest flower diameter and number of florets in main inflorescence. Results showed the relatively broad genetic base of in most of the accessions evaluated in this study. The lowest and the highest gene diversity were obtained in North-western (AtNW) group (0.18) and Northern (AtN) accession (0.28) respectively. High genetic variation of *A. tenuifolia* might be attributed to its reproductive propagation and seed dispersal. So, conservation strategies should be provided to maintain such diversity aiming to improve future breeding programs.

Keywords: *Achillea tenuifolia*, genetic diversity, ISSR, morphology.
Abbreviations: ISSR: Inter Simple Sequence Repeat. AFLP: Amplified Fragment Length Polymorphism. CTAB: Cetyl Trimethyl Ammonium Bromide. EDTA: Ethylene Diamine Tetra Acetic acid. PCR: Polymerase Chain Reaction. UPGMA: Unweighted Pair Group Method. Average PCoA: Principal Coordinate Analysis. PIC: Polymorphic Information Content. SM: Simple Matching. AMOVA: Analysis of Molecular Variance.

Introduction

The genus of Achillea comprises more than 100 species worldwide. This genus is classified in Asteraceae family and Anthemideae sub-family (Rechinger, 1963). Some Achillea species have been applied for ornamental purposes because of their relatively high drought tolerance (Artimage, 1992; Evenor and Reuveni, 2004; Khalil et al., 2011). Furthermore, they have been used in folk remedies as an appetizer, wound healer, diuretic, carminative or menstrual regulator, anti fever, anti-inflammatory, asthma, bronchitis, cough reliever and heart cellular energy metabolism. (Asgarirad et al., 2010; Trumbeckaite et al., 2011). Nowadays, its spasmolytic, choleretic, treatment of wounds and anti-inflammatory activities has been applied for medicinal purposes (Rahimmalek et al., 2009a; Gharibi et al., 2011). Nineteen species of Achillea have been recognized in Iran (Rechinger, 1963). Achillea tenuifolia Lam. has been known for its antioxidant properties (Asgarirad et al., 2010), essential oil compounds (Aghjani et al., 2000; Shafaghat, 2009) and its seed oil quality (Goli et al., 2008). Natural evolution is one of the main causes of plants genetic diversity which has allowed plants to adapt to different climates. Negative ecological impacts of over-harvesting for traditional medicinal uses, rough grazing, plowing of rangelands for agricultural purposes have all resulted in restriction of the number of members of many plant species (Rahimmalek et al., 2009b). These phenomena could increase genetic erosion, that is, the reduction in the species gene pool (Han et al., 2007). One of the major constraints for medicinal plant researchers is lack of genetic variability, absence of suitable genotypes for different planting systems and selection of elite genotypes with high bioactive compounds. Therefore, research improvements for medicinal plants depend on the utilization of the available genetic diversity. Furthermore, drought is considered as a major problem for Iranian germplsams. This problem is more dramatic for non-domesticated medicinal plants which are grown extensively in nature. So, the assessment of genetic variability could increase the information on endangered condition of some species for developing new conservation strategies.In recent years, the use of molecular markers has become an important tool to study genetic diversity of plants. Different kinds of markers have been used for study of genetic variability of wide range of plant species (Rahimmalek et al., 2009c; Keivani et al., 2010; Golam et al., 2011). Among molecular markers, ISSRs are widely used in genetic diversity studies because they need no prior DNA sequence information, their development costs are low, and the laboratory procedures can easily be applied to any plant species (Aga et al., 2005; Zietkiewicz et al., 1994). So far, most studies on A. tenuifolia have focused on its bioactive compounds (Aghjani et al., 2000; Dokhani et al., 2005; Jaimand and Rezaee, 2001). There is only one report on genetic diversity of five Achillea species including A. tenuifolia using AFLP markers (Rahimmalek et al., 2009b). The study of genetic relationships of A. tenuifolia using ISSR

and morphological markers has not been reported.The main purposes of this study were to assess the genetic diversity of A. tenuifolia accessions from different geographical locations of Iran using ISSR and morphological markers, to determine whether the genetic distances among the accessions are correlated with their geographic distribution patterns and to identify regions with higher diversity in gene pool of this species and to make a comparison between the results by morphological and ISSR markers.

Results

Primer selection and amplification

Fifteen primers were used for amplification of A. tenuifolia accessions based on the number of amplification products, the quality of the profiles, the level of polymorphism, and the reproducibility of bands. The selected primers generated a total of 247 amplified fragments with an average of 16.4 fragments per primer (Table 3). The size of the scorable products ranged from 200 to 2000 bp and the number of products per primer varied from 14 in P9 [(AG)8 T)] to 24 in P11 [(CA)8 RT)]. The average percentage of polymorphic bands was 86.8% which is in agreement with Gharibi et al. (2011) results on A. millefolium. The number of polymorphic bands per primer varied from 8 to 23, with an average of 14.3 (Table 3). The average PIC value for the amplification products was 0.306 (Table 3). P11 [(CA)8 RT)] and P1 [(CA)8 G)] showed the highest and the least PIC values, respectively (Table 3). The primers anchored at 3` end gave clearer banding pattern as compared to those anchored at 5` end (Pradeep Reddy et al., 2002; Rahimmalek et al., 2009c; Gharibi et al., 2011). Previous reports showed that the particular motifs can produce clearer banding patterns in special species, but in most species the AC or AG motifs were in priority. In the Achillea genus more clarity of bands was observed in AG and AC motifs as it has been reported by Gharibi et al. (2011) for A. millefolium species. So, for A. tenuifolia the use of similar primers appeared to be more efficient in comparison with other motifs.

Genetic diversity analysis

Genetic diversity was indicated by the range of SM similarity coefficients. The least genetic similarity was observed between AtN18 and AtC79 with the similarity coefficient of 0.558, while the AtC65 and AtC68 were the most genetically similar accessions with the coefficient of 0.903 (Table 4). It is indicated from the SM similarity matrix that the values of similarity coefficients among most of the studied accessions tend to be rather high. It is caused likely by the fact that the genotypes were sampled from only one species. It is clear from the above results that most of the A. tenuifolia accessions used in the present study were randomly selected from less restricted germplasm pool and they were collected from diverse geographical regions in Zagros Mountains. This suggests the relatively broad genetic base for most of the genotypes evaluated in present study. This result is in accordance with Rahimmalek et al., (2009b) data for this species using AFLP markers.

Cluster and PCoA analyses

The cluster analysis was performed using SM similarity matrix and UPGMA method (Fig. 2). The highest co-phenetic correlation coefficient (r= 0.857) was obtained between the SM similarity data matrix and the cophenetic matrix, indicating a good fit between the dendrogram clusters and similarity matrices. Three groups were revealed according to the resulted dendrogram (Fig. 2). The group 1 mostly consisted of the genotypes collected from North-western and Northern locations and group 3 included Central ones (Fig. 2). In most cases, the collected genotypes indicated a considerable variation based on their geographical regions (Fig. 2). PCoA was performed to specify the association between accessions in more detail. The results showed that the first three principal coordinates explain 70.32% of the total variation. The results of PCoA analysis largely corresponded to those obtained through cluster analysis. Based on the results of PCoA analysis, the similarity between the groups 1 and 2 was more than that of the third group (Fig. 3). The high similarity of accessions from N to NW and W was also observed in PCoA analysis.

Relationships among accessions

In this study, the genotypes were grouped in four major groups (AtW, AtNW, AtN and AtC) according to their geographical distribution. The lowest and the highest gene diversity over loci were observed in AtNW (0.18) and AtN (0.28), respectively (Table 5). AMOVA analysis showed that all genetic distances in the matrix were significantly different ($P<0.001$). About 68.66% of total genetic variation was explained by differences among the assumed groups, while 31.34% of the total variation was observed within groups (Table 6). The fst value was 0.31 for accessions included in this study.

Morphological analysis

The mean, maximum, minimum and coefficient of phenotypic genetic variation for each trait are summarized in table 7. Among the accessions, the AtN76 had the highest flower diameter and number of florets in main inflorescence which can be used as a good candidate to facilitate the extraction of essential oil from flowers. It may also be considered as an appropriate ornamental flower. North-western (NW) accessions tended to have the highest leaf length, leaf width, leaf area and essential oil content per leaf. So, they can be introduced as high essential oil yielding accessions. Furthermore, NW accessions had the latest flowering time, while the Northern ones tended to be early flowering (Table 7). A. tenuifolia genotypes were grouped using Ward's method according to their morphological characteristics. In general, the morphological analysis confirmed the results of molecular analyses. As a result, the samples were classified into four groups (Fig. 4). Group 1 consisted of North-western accessions. Group 2 included Northern (N) and central (C) ones. Most of C accessions were classified in group 3 and western (W) ones were clustered in group 4 (Fig. 4).

Comparison of molecular and morphological analysis

A high degree of similarity was observed between morphological and molecular clusters, however the Northern genotypes tended to group with genotypes from central regions as revealed by morphological analysis (Fig. 4). It might be explained by higher similarity between

Table1. List of *A. tenuifolia* accessions included in this study.

No.	Accession code	Species	location	Essential oil yield (%)
1	AtW2	*A. tenuifolia*	Khoram abad,Lorestan ,Iran	0.59
2	AtW3	*A. tenuifolia*	Khansar, Isfahan, Iran	0.20
3	AtN18	*A. tenuifolia*	Taleghan, Tehran, Iran	0.17
4	AtNW27	*A. tenuifolia*	Salmas, Azarbaiegan gharbi, Iran	0.83
5	AtNW28	*A. tenuifolia*	Orumieh, Azarbaiegan gharbi, Iran	0.49
6	AtNW30	*A. tenuifolia*	Naqade, Azarbaiegan gharbi, Iran	0.84
7	AtNW31	*A. tenuifolia*	Mahabad, Azarbaiegan gharbi, Iran	0.76
8	AtNW33	*A. tenuifolia*	Divandare, Kordestan, Iran	0.55
9	AtW34	*A. tenuifolia*	Sannandaj, Kordestan, Iran	0.74
10	AtW39	*A. tenuifolia*	Borugerd, Lorestan, Iran	0.25
11	AtN47	*A. tenuifolia*	Qom-Tehran highway, Qom, Iran	0.18
12	AtC57	*A. tenuifolia*	Tiran, Isfahan, Iran	0.06
13	AtC65	*A. tenuifolia*	Semirom, Isfahan, Iran	0.25
14	AtC68	*A. tenuifolia*	Fereydan, Isfahan, Iran	0.07
15	AtC70	*A. tenuifolia*	Malayer, Hamedan, Iran	0.14
16	AtN76	*A. tenuifolia*	Karaj, Alborz, Iran	0.30
17	AtC79	*A. tenuifolia*	Salafchegan, Markazi, Iran	0.28

Fig 1. Geographical distribution of *A. tenuifolia* accessions used in this study.

traits evaluated in this study. In spite of molecular analysis, the North-Western accessions were grouped in separate group using morphological data (Fig.4). However, the molecular results revealed more distinct classification compared to morphological analysis.

Discussion

The analysis for assessment of genetic variability has been reported in several species of medicinal plants, for instance, Artemisia annua (Sangwan et al., 1999), Tanacetum vulgare (Keskitalo et al. 2001) and A. millefolium (Gharibi et al., 2011). The molecular studies on the Achillea genus have mainly been focused on A. millefolium (Guo et al., 2006; Guo et al., 2008; Gharibi et al., 2011). However one molecular study on the diversity of A. tenuifolia using AFLP markers has been reported (Rahimmalek et al., 2009b). In the present study, the genetic relationships of A. tenuifolia species were evaluated using ISSR markers and morphological traits. The results of present research were in accordance with those of previously reported study using AFLP markers (Rahimmalek et al., 2009b). Rahimmalek et al. (2009b) mentioned that A. tenuifolia has the highest gene diversity in comparison with A. filipendulina, A. millefolium, A. santolina and A. biebresteinii. They attributed the higher genetic diversity in A. tenuifolia to more extensively distribution of this species through the country. This variation was also observed with some morphological traits such as essential oil content (Table 1). The high variation of essential oil compounds in A. tenuifolia species was already reported by several researchers (Aghjani, et al., 2000; Jaimand and Rezaee, 2001; Dokhani et al., 2005; Rahimmalek et al., 2009a). The morphological data revealed species with potentially high essential oil content among the studied species (Table 7). The NW and N accessions were known as the latest flowering and earliest flowering species, respectively. The obtained results might be helpful in developing new genotypes with ornamental purposes as well as in setting time criteria for harvesting of flowers with optimum essential oil content to prohibit oil extraction parameters. The amount of genetic diversity plays an important role in improvement of breeding programs where

Table 2. Sequence and appropriate annealing temperature of ISSR primers used in this experiment

Primer name	Motif	Sequence	Annealing temperature (^{0}C)
P1	5′-$(CT)_8$ G-3′	5′-CTCTCTCTCTCTCTCT G-3′	53
P2	5′-$(CA)_8$ G-3′	5′- CACACACACACACACA G-3′	52
P5	5′-$(AC)_8$ G-3′	5′ -ACACACACACACACAC G-3′	50
P7	5′-$(AG)_8$ YT-3′*	5′ -AGAGAGAGAGAGAGAG YT-3′	49
P8	5′-$(AC)_8$ YG-3′*	5′ -ACACACACACACACAC YG-3′	53
P9	5′-$(AG)_8$ T-3′	5′ -AGAGAGAGAGAGAGAG T-3′	53
P11	5′-$(CA)_8$ RT-3′*	5′- CACACACACACACACA RT-3′	50
P12	5′-$(GA)_8$ T-3′	5′- GAGAGAGAGAGAGAGA T-3′	48
P14	5′-DBD $(AC)_7$-3′ *	5′-DBD ACACACACACACAC-3′	49
P15	5′-BDB $(TCC)_7$-3′ *	5′-BDB TCCTCCTCCTCCTCCTCCTCC-3′	60
P16	5′-HVH $(TCC)_7$-3′ *	5′- HVH TCCTCCTCCTCCTCCTCCTCC-3′	60
P17	5′-$(AG)_8$ C-3′	5′ -AGAGAGAGAGAGAGAG C-3′	49
P19	5′-$(AG)_8$ RC-3′ *	5′ -AGAGAGAGAGAGAGAG RC-3′	52
P22	5′-$(TCC)_5$ YR-3′*	5′- TCCTCCTCCTCCTCC YR-3′	52
P26	5′-CCA $(CT)_8$ -3′	5′-CCACTCTCTCTCTCTCTCT-3′	52

*type of degenerate nucleotide: R = A/T, Y = G/C, B = T/G/C; D = A/T/G, H = A/T/C, V = 3A/G/C.

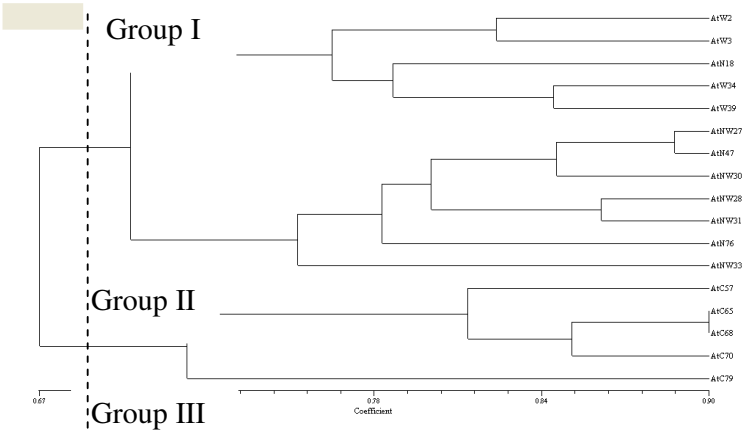

Fig 2. UPGMA dendrogram based on Jaccard similarity coefficient among *A. tenuifolia* accessions collected from different geographical regions of Iran.

successful variety development and achievement of breeding objectives depends largely on high available diversity. The level of genetic variability within a given species may be influenced by different factors such as the pollination system, pollinators, number of individuals within species, self-incompatibility and method of propagation (Fracaro et al., 2005; Rahimmalek et al., 2009c). While no observation on A. tenuifolia has been reported, self-incapability has mainly influenced the level of genetic variation in A. millefolium species (Lofgren, 2002). The method of propagation is considered as another determining factor in level of genetic diversity. Seeds of A. tenuifolia can be dispersed by wind to very distant locations. Since no rhizome has been found in A. tenuifolia (in contrast to A. millefolium which is mainly propagated by the rhizomes) the reproductive propagation has been known as the only way of its reproduction in nature In A. millefolium however, vegetative propagation (via rhizomes) is relatively more common in most habitats in comparison with reproductive propagation (Lofgren, 2002; Gharibi et al., 2011). Thus, higher genetic diversity and broader genetic base in A. tenuifolia as compared to rhizome containing species such as A. millefolium may be explainable by taking into account their way of propagation. On the other hands, self- incompatibility and some pollination barriers may cause increased seed abortion in this species. For example, unfavorable climatic condition could adversely affect the pollinator efficiency in some habitats and the resulted increase in seed abortion may in turn lead to decreased seed dispersal during a period of time. Furthermore, reduced fertilization and germination rates caused by simultaneous drought events may increase extinction risk of some populations (Fracaro et al., 2005; Rahimmalek et al., 2009c). In this study, the fst value was 0.31 indicating the relatively high genetic differentiation of assumed groups. There are similar reports for high fst value in Asteraceae family (Yang et al., 2007; Caujape´-castells et al., 2008). Natural barriers such as Zagros Mountains in Iran might decrease the pollination efficiency, seed dispersal and gene diversity during the years (Gharibi et al., 2011). The results of molecular analysis showed that the accessions from central regions were more divergent in comparison to W and NW accessions. It might be caused by situation of mountains in Iran. The central accessions of A. tenuifolia in Iran were less affected by Mountains. The higher altitudes were mostly in N and NW. So, the more divergent nature of central genotypes seems to be logical. On the other hands, the lowest gene diversity was found for AtNW genotypes, while the highest was obtained for AtN ones. The use of conservation strategies therefore will be beneficial to improve the low gene diversity groups. For example, besides the need for small

Table 3. Summary of ISSR primers characteristics in different *A. tenuifolia* accessions

Primer name	Number of scorable bands	No. polymorphic bands	Polymorphisms (%)	PIC/primer
P1	15	13	86.6	0.21
P2	14	11	78.5	0.24
P5	20	19	95	0.25
P7	13	12	92.3	0.31
P8	20	17	85	0.35
P9	14	12	85.7	0.33
P11	25	23	92	0.36
P12	15	13	86.6	0.29
P14	14	13	92.8	0.32
P15	18	14	77.7	0.34
P16	14	12	85.7	0.28
P17	16	15	93.7	0.31
P19	22	18	81.8	0.34
P22	10	8	80	0.35
P26	17	15	88.2	0.31
Total	247	215	-	
Average	16.4	14.3	86.78	0.306

Table 4. Similarity matrix of *A. tenuifolia* accessions using ISSR markers.

AtC79	AtN76	AtC70	AtC68	AtC65	AtC57	AtN47	AtW39	AtW34	AtNW33	AtNW31	AtNW30	AtNW28	AtNW27	AtN18	AtW3	AtW2	
																1	AtW2
															1	0.828	AtW3
														1	0.781	0.793	AtN18
													1	0.706	0.685	0.730	AtNW27
												1	0.820	0.719	0.689	0.706	AtNW28
											1	0.836	0.857	0.661	0.711	0.716	AtNW30
										1	0.846	0.865	0.782	0.689	0.678	0.696	AtNW31
									1	0.752	0.770	0.748	0.759	0.655	0.684	0.733	AtNW33
								1	0.737	0.658	0.693	0.685	0.702	0.795	0.718	0.773	AtW34
							1	0.838	0.707	0.683	0.698	0.680	0.690	0.787	0.743	0.806	AtW39
						1	0.775	0.734	0.752	0.760	0.841	0.783	0.891	0.714	0.700	0.778	AtN47
					1	0.655	0.718	0.651	0.621	0.615	0.642	0.605	0.613	0.695	0.702	0.676	AtC57
				1	0.841	0.669	0.696	0.692	0.686	0.682	0.691	0.702	0.655	0.707	0.700	0.711	AtC65
			1	0.903	0.847	0.634	0.668	0.654	0.625	0.672	0.644	0.628	0.618	0.670	0.687	0.670	AtC68
		1	0.839	0.869	0.765	0.653	0.647	0.649	0.634	0.703	0.705	0.671	0.658	0.635	0.644	0.611	AtC70
	1	0.782	0.743	0.777	0.668	0.806	0.687	0.641	0.762	0.674	0.820	0.770	0.776	0.644	0.661	0.709	AtN76
1	0.756	0.775	0.706	0.737	0.654	0.658	0.580	0.630	0.692	0.672	0.696	0.670	0.689	0.558	0.574	0.605	AtC79

Table 5. Basic gene diversity information of four *A. tenuifolia* groups according to their geographical regions

Sd	Mean	AtC	AtN	AtNw	AtW	Statistics
0.829	4.25	5	3	5	4	No. of gene copies
0.00	215	215	215	215	215	No. of loci
19.68	160	127	174	163	176	No. of usable loci
7.49	64.25	54	74	61	68	No. of polymorphic. loci
-	0.22	0.19	0.26	0.20	0.21	Expected heterozygosity
-	21.75± 0.14	0.20±0.12	0.28±0.21	0.181±0.11	0.21±0.13	Average gene diversity over loci

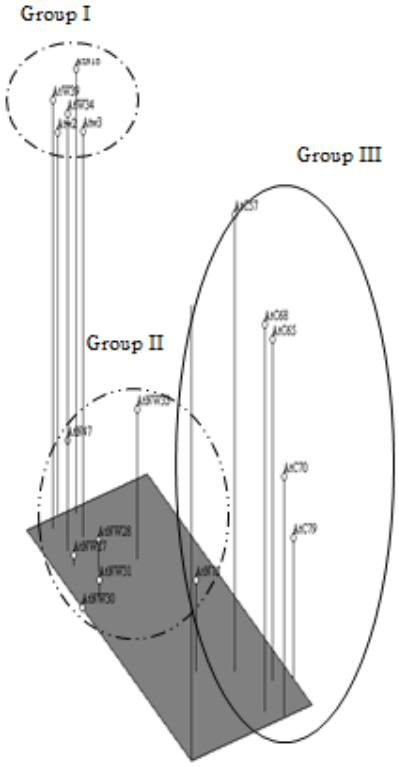

Fig 3. Patterns of relationships among *A. tenuifolia* accessions revealed by PCoA based on ISSR data.

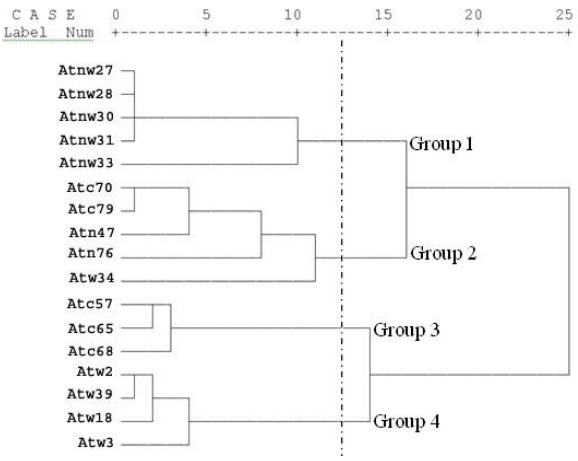

Fig 4. Morphological dendrogram based on Ward's method among *A. tenuifolia* accessions collected from different geographical regions of Iran.

conservation areas, it is necessary to constantly monitor the genetic variability and to transfer the plant material or genes from one location to another. In conclusion, assessment of wild medicinal plants not only provides information for in situ conservation, but also increases new insights for future breeding programs. In this study, molecular and morphological markers raveled useful information on the genetic diversity available in Iranian A. tenuifolia accessions. Furthermore, elite accessions were identified according to their morphological traits. The results of this study also discussed about the restricting factors which affects the genetic diversity within studied species. Consequently, prediction of new conservation strategies will allow improvement of breeding programs on the medicinal plants.

Materials and methods

Plant materials

Seventeen accessions of A. tenuifolia were collected from different geographical regions of Iran (Rechinger, 1963) (Table 1). Samples were identified as A. tenuifolia by V. Mozafarian. The accessions were chosen to represent a wide geographic range according to the distribution map of A. tenuifolia in Iran (Fig. 1). Young leaves were collected and transported to the laboratory and stored in a -800C freezer until use.

Morphological data analysis

Major morphological traits including plant height, leaf length, leaf width, leaf area, shoot dry weight, inflorescence length, flower diameter, day to flowering, day to 50% flowering, day to 100% flowering, number of florets in main inflorescence, number of florets in lateral inflorescence and essential oil yield of all genotypes were measured in three replications and the mean values were used for analysis. The dendrogram for morphological characters was constructed using SPSS ver. 9.

Extraction of genomic DNA

DNA from young leaves was extracted using the modified CTAB procedure as described by Murry and Thompson (1980). The quality and quantity of DNA was estimated spectrophotometrically and electrophoretically. The DNA was diluted to a working concentration of 10 ng/µl.

ISSR Analysis

A total of 18 ISSR primers were screened and 15 primers producing higher clarity and reproducibility were selected for the ISSR analysis of A. tenuifolia genotypes (Table 2). PCR reactions were carried out in a volume of 15 µl containing 10 ng total DNA, 10× PCR buffer, 2% formamide, 0.25 mM each dNTP, 10 pM each primer, 4 mM MgCl2, 1 U Taq DNA polymerase. The optimum annealing temperature was determined for each primer (Table 2). PCR cycling conditions for all accessions was as 2 min initial denaturation (940C); followed by 40 cycles of 1 min at 940C, 1 min at the specific annealing temperature, and 2 min at 720C; ending with a final extension step of 10 min at 720C. Amplified DNA fragments were separated in a 2% agarose gel at 100 W for 3 h in 1× TBE buffer (100 mM Tris–Borate, pH 8.0, 2 mM EDTA) and stained by ethidium bromide.

Molecular data analysis

The polymorphic bands were scored as present (1) or absent (0). The cluster PCoA analyses were conducted by the software NTSYSpc Version 2.02 (Rohlf, 1998). PIC values were calculated by applying the simplified formula (Anderson et al., 1993): PICi = 2fi (1 - fi), where fi is the percentage of the ith amplified present band. Genetic similarities among all accessions were calculated according to SM similarity index, using the similarity of qualitative data

Table 6. Analysis of molecular variance (AMOVA) among and within four *A. tenuifolia* groups according to their geographical regions.

Source of variation	d.f	Sum of squares	Mean of Squares	Variance component	Percentage of variation	p-value
Among groups	3	58.15	19.38	3.03	31.34	<0.001
Within groups	13	86.43	6.64	6.64	68.66	<0.001
Total	16	144.58	26.02	9.68		

Table 7. The mean, Maximum, Minimum and coefficient of phenotypic genetic variation of studied traits.

Phenotypic genetic variation (%)	Min/ Genotype	Max/ Genotype	Mean	Trait
41.91	11.2 (AtN76)	52.7 (AtNW33)	38.09	Plant height (cm)
1.26	11.445 (AtNW27)	1.98 (AtW2)	4.5	shoot dry weight (g)
4.79	1.42 (AtC68)	4.48(AtNW30)	2.97	Leaf length (cm)
29.44	0.12 (AtC57)	0.65 (AtNW31)	0.31	Leaf width (cm)
0.27	79.2(AtNW33)	50.6(AtW3)	65.8	Inflorescence length (cm)
3.81	5.3(AtC68)	2.15(AtN76)	3.26	Flower diameter (cm)
0.43	18(AtN76)	39(AtNW30)	28	Day to flowering (day no.)
0.49	49(AtN76)	74(AtNW33)	59	Day to 50% flowering (day no.)
0.88	70(AtN47)	92(AtNW33)	75	Day to 100% flowering (day no.)
3.81	5.3 (AtC68)	2.15 (AtN76)	52.29	No. of florets in main inflorescence
1.1	13(AtN76)	5 (AtNW31)	8.75	No. of florets in lateral inflorescence
10.44	0.14 (AtC70)	0.84 (AtNW30)	0.38	Essential oil yield of leaves%
8.75	0.17 (AtN47)	0.934 (AtNW27)	0.48	Leaf area (cm^2)

(Simqual) routine. The dendrogram was constructed using UPGMA clustering procedure. A Mantel test (Mantel, 1967) was used to detect the correlation between two dendrograms. The cophenetic correlation coefficient was generated by means of the COPH routine in order to check the goodness of fit between the clusters in the dendrogram and the similarity coefficient matrix. Gene diversity and AMOVA analysis were calculated among Iranian populations using Arlequin version 3 software.

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