Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 6, No. 6, p. 125-135, 2015
http://www.innspub.net

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RESEARCH PAPER

OPEN ACCESS

Interspecific genetic diversity of Iranian *Achillea* species based on morphological traits and total protein profiling

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Article published on June 08, 2015

Key words: *Achillea* spp., protein, genetic diversity, morphology.

Abstract

Total protein banding patterns and morphological traits were used to assess genetic diversity among six *Achillea* species including *A. millefolium*, *A. filipendulina*, *A. biebersteinii, A. nobilis, A. tenuifolia* and *A. vermicularis*. Variance analysis of morphological traits showed that all evaluated traits were significantly different among species. High genetic variation was observed for both total protein profiles and phenotypic traits. Among the six *Achillea* species, the mean polymorphism% (*PPL*) and expected heterozigosity (*He*) values were 54.82% and 0.192, respectively. *A. tenuifolia* (*PPL* and *He* values: 89.47% and 0.315, respectively) had the highest level of variability, whereas *A. millefolium* had the lowest level of variability (*PPL* and *He* values: 34.21% and 0.132, respectively). The highest genetic distance and the lowest genetic similarity was detected between *A. millefolium* and A. *nobilis* (0.278 and 0.238, respectively), which allocated them in separated groups and made them a potential pair for hybridization to reach to high heterosis effects in their hybrids. Molecular variance (AMOVA) revealed that the differences among species accounted for 30% of the total variation, whereas differences within species were 70%. The principle coordinate analysis (PCoA) confirmed the results of clustering analysis. Morphological analysis in most cases corresponded to those obtained through protein analyses. These results showed that conservation strategies should be provided to maintain high diversity aiming to improve future breeding programs.

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Introduction

The genus *Achillea* is one of the most important genera of the Asteraceae family and is presented by about 85 species widespread throughout the world (Chevalier, 1996). This genus is represented by 19 species in the flora of Iran and seven of them are endemics (Huber-morath, 1986). *Achillea* spp. is an herbaceous perennial with flowers that range in color from white to yellow to red (Griffiths, 1994). Capitula of the genus *Achillea* L. are composed of ligulate florets which are female and tubulate florets which are bisexual (Dabrowska, 2002). *Achillea* byproducts have cosmetic and medicinal uses (Bartram, 1995), e.g. recent pharmacological studies have shown that essential oils of *Achillea* species have antimicrobial (Kharma and Hassawi, 2006; Aburjai and Hudaib, 2006; Al-Qura'n, 2008), anti-allergic and anti-inflammatory activities (Al-Qura'n, 2008).

Within the framework of a research project the genus *Achillea* has been studied with respect to morphological traits. For example, Gurevitch (1992) investigated sources of variation in leaf shape among two populations of *Achillea lanulosa*. Valant & Stner (2000) by studying details of leaf structure and floral characters stated that some characters may be useful for species delimitation. Also, Sulborska and Chmielewska (2005) focused on Morphology and ultrastructure of floral nectaries among some populations of *A. millefolium*. In addition, some researchers have used pollen characters e.g. shape, length of Polar axis (P), diameter of Equatorial axis (E), P/E ratio, spine length, number of spine rows to characterize *Achillea* species (Azani *et al.,* 2009; Meo and Khan., 2003). However, by little differences between species from length of polar axis, diameter of equatorial axis and spine length (Azani *et al.,* 2009), palynological markers could not classify species accurately. Other method to distinguish species is application of biochemical genetic techniques based on Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE); consist of enzymes and total protein markers (Salehi shanjani *et al.* 2012). These approaches display an important potential to provide an indirect, rapid, cheap and accurate test, to analyze proteins reflecting structural differences. Also, by using preserved proteins as electrophoretical markers, some disadvantages like morphological characters being affected by growth environment could be overcome (Lioi *et al.,* 1999). In *Achillea* spp., karami *et al.* (2012) and Nadiri *et al.* (2012) used soluble protein marker to show genetic diversity and differentiation between and among species. In other taxa, El-Shanshoury (2002) used seed proteins by SDS-PAGE to study the genetic variability between 30 *Lathyrus sativus* samples collected from different countries. Ertugrul *et al.* (2010) characterized *Consolida* taxa in Turkey by protein electrophoresis. Uysal *et al.* (2010) Determinate the relationship between 47 *Centaurea* species from Turkey using SDS-PAGE methods. Other discriminal methods in *Achillea* species include karyology (Vetter *et al.,* 1996) and DNA polymorphisms methods (Guo *et al.,* 2005, Abd-Eltwab and Zahran, 2010). However, Different approaches in genetic diversity analyses reveal different level of polymorphism (Porter and Smith, 1982). So, scientists prefer to utilize two or more of methods together to differentiate between the different populations and species, precisely (Morsy, 2007). For example, Salehi shanjani *et al.* (2012) used morphological characters and SDS-PAGE method to identify genetic variation and relationships between local and exotic germplasm of *Dactylis glomerata*. Also, Pirkhezri *et al.* (2010) studied distinguish in different populations of *Matricaria chamomilla* L. growing in southwest of Iran, based on morphological and RAPD markers. Genetic relationships among *A. tenuifolia* accessions using ISSRs and morphological markers was considered by Rahimmalek (2012). Morsy (2007) identified molecular variations of *A. fragrantissima* growing in five areas of south Sinai by protein electrophoresis, isozymes electrophoresis and RAPD systems.

Although application of morphological traits and SDS-PAGE Method together is a facile and accurate way to distinguish between populations and species (Salehi shanjani *et al.* 2012), no data has been presented relating to diversity of *Achillea* germplasm based on combination of morphological traits and protein electrophoresis. So, this work was done to: (1) analyzes

variation and determines the level of genetic diversity and differentiation among six species of *Achillea* including *A. millefolium*, *A. filipendulina*, *A. biebersteinii, A. nobilis, A. tenuifolia* and *A. vermicular* which are growing in Iran using morphological and protein markers, and (2) to compare the results of molecular and morphological classifications.

Materials and methods

Plant materials

The study was conducted during 2012–2013 in Alborz Research Center, Karaj, Iran. The seeds of six species of *Achillea* were obtained from National Natural Resources Gene Bank, Iran. The species were consisted of *A. millefolium*, *A. filipendulina*, *A. biebersteinii, A. nobilis, A. tenuifolia* and *A. vermicularis* which were originated from Golestan, Kurdestan, Markazi, Hamedan, Kurdestan and Kurdestan province, respectively.

Morphological data

The seeds were planted in pot and after growing in the glasshouse, in April the 15 plantlets of each species were transplanted to field with 50×30 cm spacing in a Randomized Complete Block Design (RCBD) with two replications. The cultural operations consisted of manual elimination of weeds, frequent irrigation in order to maintain the soil wet and fertilizer administration.

Major morphological traits including plant height (cm), stem diameter (mm), inflorescence number, leaf length (mm), leaf width (mm), inflorescence length (cm), inflorescence width (cm), capitulum number, involucre length (mm), involucre width (mm), ligulate florets number, ligulate florets number, ligulate florets length (mm), ligulate florets width (mm), tubular florets length (mm), tubular florets width (mm), floret number, seed length (mm), seed width (mm) and 1000 seeds weight (g) of all species were measured in three replications.

Leaf total protein extraction

A total of 60 entries were selected from six species. Freeze-dried leaves were ground to fine powder with mortar and pestle. Sample buffer was added to 0.04 g of grounded leaf as extraction liquid and mixed thoroughly in Eppendorf tube with vortex. The extraction buffer contained the following concentration: protein extraction 0.05 M Tris-HCL, pH=8, 0.2% SDS, 5 M urea, 1% B-mercaptoethanol. Standard SDS-PAGE was performed on a vertical slab gel based on Laemmli system (1970) by using 11% (w/v) separating gel and 5% (w/v) stacking gel. Following electrophoresis, the coomassie blue was added to the supernatant as tracking dye in order to observe the movement of protein in the gel. Molecular weights of protein bands were estimated by their relative mobility. "MW-SDS-70 Kit" was used as marker proteins.

Data analysis

Quantitative analysis of morphological traits was carried out using the SAS Ver. 8.02 (SAS Institute Inc). Analysis of Variance (ANOVA) was performed and then the means of results were compared by Duncan's multiple range tests. In order to determine the degree of associations among the characteristics, Pearson's coefficients were used. The SPSS software was used to produce a distance matrix and a dendrogram based on morphological data. Average Euclidian distance was calculated for each species-pair and the resulting distance matrix was used to construct a phenotypic dendrogram among different species using Average Linkage (between groups) cluster analysis (Mohammadi and Prasanna, 2003).

About protein profile data, the polymorphic bands were scored visually as present (1) or absent (0). Then, POPGENE, Ver.1.32 (Yeh *et al.*, 1999) software was used to calculate the indices of genetic diversity, such as the percentage of polymorphism (*PPL*) and expected heterozigosity (*He*) based on gene frequencies. At the same time, the genetic structure within and among species were detected using the software AMOVA-PREP 1.01 (Miller, 1997) and WINAMOVA (Excoffier, 1995) in order to partition the genetic variation among species and among individuals species. The significance of each variance component was tested with permutation tests (Excoffier *et al.*, 1992). Genetic distances were estimated according to Nei (1978) and WINAMOVA (Excoffier, 1995) in order to partition the genetic variation among

species and among individuals species. The NTSYS-pc ver. 2.02 (Rohlf, 1993) was used to estimate genetic similarities with the Jaccard's coefficient. The matrix of generated similarities was analyzed by the Unweighted Pair Group Method with Arithmetic Average (UPGMA), using the SAHN clustering module. The cophenetic module was applied to compute a cophenetic value matrix using the UPGMA matrix.

Results

Morphological Analysis

Twenty morphological traits were measured among species. ANOVA suggested significant differences among six *Achillea* species for almost all the 20 traits, except seed width (Table 1). Maximum coefficients of variability were belonging to Leaf width (45.4%), 1000 seed weight (35.9%) and capitulum number (33.5%). Table 2 represents correlation coefficients between morphological traits. Pearson correlation showed a positive relationship between tubular florets number and floret number (0.99), plant height and stem diameter (0.95), inflorescence length and capitulum number(0.91), leaf width and capitulum number (0.90), leaf length and leaf width (0.89), involucre width and tubular florets number(0.87), ligulate florets length and seed width (0.87) etc.

Table 1. Mean, coefficient of variability (CV %), Mean Square and Duncan test for comparisons of morphological traits among different species of *Achillea*.

	Plant height (cm)	Stem diameter (mm)	Inflore-scence no	Leaf length (mm)	Leaf width (mm)	Inflorescence length (cm)	Inflorescence width (cm)	Capitulum no.	Involucre length (mm)	Involucre width (mm)	Ligulate florets no.	Tubular florets no.	Ligulate florets length (mm)	Ligulate florets width (mm)	Tubular florets length (mm)	Tubular florets Width (mm)	Flore no.	Seed length (mm)	Seed width mm	1000 seed Weigh (g)
A. millefolium	37.7c	6.5c	5b	38.7b	3.7c	3.2c	1.9bc	49.3d	3.7c	2.2c	2.3a	8b	1.8d	0.9c	3.5ab	1.1b	10b	1.5ab	0.6a	0.1b
A. filipendulina	91.3a	10.8a	1d	93a	19.7a	6.3a	3.2ab	181.7a	3.8c	2.2c	2b	13.3b	2.2dc	1.1bc	2.3c	0.9b	15.3b	1.8a	0.6a	0.3a
A. biebersteinii	48.9bc	8bc	2cd	30bc	5bc	5.8ab	3.8a	113b	4.8a	2.7b	4.3a	24.7a	2.7b	1.3ab	3.1abc	0.9b	29a	1.6ab	0.8a	0.1b
A. nobilis	42c	7.7bc	2.7c	25.7c	9.8b	3.9bc	3ab	95bc	4bc	2.5bc	3ab	12.7b	2.4bc	1.2bc	2.5c	0.9b	15.7b	1.2b	0.6a	0.1b
A. tenuifolia	81.2a	10.3a	9.7a	25c	2.1c	4.5bc	2.2c	59bcd	4.6ab	3.7a	2.3a	25.7a	3.3a	1c	3.7a	1b	28a	1.7a	0.8a	0.2ab
A. vermicularis	54.2b	9.3ab	2.7c	21c	1.8c	2.7c	1.7c	11d	3.9c	3.8a	2.7b	29.3a	2.7b	1.4a	2.8bc	2.2a	32a	1.4ab	0.7a	0.2ab
Mean	59.2	8.8	3.8	38. 9	7.0	4.4	2.7	84.8	4.1	2.8	2.8	18.9	2.5	1.1	3	1.2	21. 7	1.5	0.68	0.17
Mean square	444 **	8.4**	29**	2216**	140**	7.3*	2.1*	1058 **	0.6*	1.6**	2.1*	225**	0.8**	0.1**	0.8*	0.8**	247**	0.2**	0.03n	0.01*
CV%	10.71	10.8	21.8	16.1	45.4	26.5	25.3	33.5	8.3	8.3	30.6	28.8	9.9	11.4	13.9	20.7	25.4	17.8	20.9	35.9

Means with same or two letters are non-significant, with different letters are significant at 0.05 level.

*, **: significant at 0.05 and 0.01 levels, respectively.

Table 2. Simple correlation between morphological traits among different species of *Achillea*.

	Plant height	Stem dia-meter	Inflor-escence no.	Leaf length	Leaf width	Inflore-scence length	Inflore-scence width	Capit-ulum no.	Involucre length	Involucre width	Ligulate florets no.	Tubular florets no.	Ligulate florets length	Ligulate florets width	Tubular florets length	Tubular florets width	Flore no.	Seed length	Seed width
Stem diameter	0.95**																		
Inflorescence no.	0.17	0.10																	
Leaf length	0.62	0.45	-0.28																
Leaf width	0.49	0.38	-0.58	0.89*															
Inflorescence length	0.58	0.44	-0.28	0.65	0.66														
Inflorescence width	0.10	0.03	-0.50	0.33	0.54	0.83*													
Capitulum no.	0.49	0.33	-0.47	0.81	0.90*	0.91*	0.80												
Involucre length	0.08	0.11	0.32	-0.42	-0.37	0.39	0.45	0.03											
Involucre width	0.18	0.40	0.51	-0.60	-0.65	-0.40	-0.51	-0.66	0.37										
Ligulate florets no.	-0.49	-0.40	-0.30	-0.45	-0.28	0.21	0.60	0.02	0.69	-0.01									
Tubular florets no.	0.21	0.44	0.20	-0.47	-0.51	-0.06	-0.11	-0.39	0.61	0.87*	0.35								
Ligulate florets length	0.37	0.52	0.50	-0.46	-0.42	0.10	0.01	-0.23	0.76	0.82*	0.25	0.85*							
Ligulate florets width	-0.14	0.15	-0.60	-0.21	-0.07	-0.13	0.09	-0.16	0.06	0.38	0.42	0.60	0.23						
Tubular florets length	-0.16	-0.25	0.85	-0.52	-0.76	-0.29	-0.42	-0.55	0.42	0.35	0.03	0.18	0.31	-0.53					
Tubular florets width	-0.13	0.13	-0.11	-0.34	-0.43	-0.64	-0.64	-0.67	-0.27	0.64	-0.11	0.56	0.16	0.70	-0.09				

	Plant height	Stem dia-meter	Inflor-escence no.	Leaf engtl	Leaf widtl	Inflore-scence length	Inflore-scence width	Capit-ulum no.	nvol-ucre engtl	nvol-ucre width	igulat florets no.	ubular florets no.	igulat florets length	igulat florets width	ubular florets length	ubular florets width	loret no.	Seed engtl	Seed width
loret no.	0.16	0.40	0.16	-0.49	-0.51	-0.03	-0.05	-0.36	0.65	0.84*	0.43	0.99**	0.85*	0.62	0.17	0.53			
eed length	0.79	0.63	0.25	0.56	0.25	0.67	0.18	0.45	0.30	0.01	-0.21	0.17	0.25	-0.34	0.24	-0.29	0.14		
eed width	0.38	0.44	0.54	-0.32	-0.49	0.22	0.01	-0.19	0.82*	0.69	0.285	0.81	0.87*	0.06	0.56	0.09	0.80	0.57	
1000 seed Weight	0.82*	0.77	-0.06	0.76	0.65	0.27	-0.16	0.41	-0.50	-0.36	-0.80	-0.41	-0.09	-0.11	-0.42	0.06	-0.21	0.47	-0.14

*, **: significant at 0.05 and 0.01 levels, respectively.

Dendrogram was drawn to display the phenetic relationships among different species of *Achillea* based on Euclidean distances from morphological data matrix. All species were represented into 3 main groups (Fig. 1). In group A, *A. biebersteinii* and*A. nobilis* were placed, also *A. millefolium*, *A. tenuifolia* and *A. vermicularis* were settled in B group. Finally, *A. filipendulina* is placed in group C.

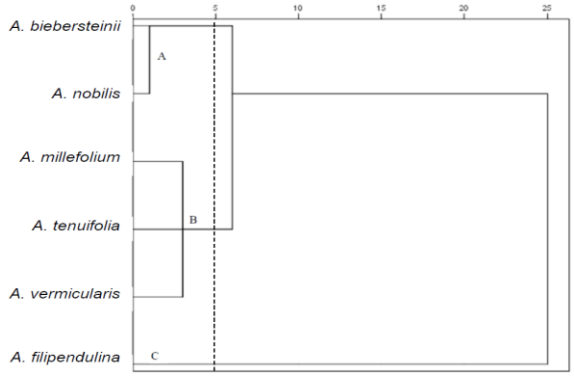

Fig. 1. Dendrogram showing the phenetic relationships among different species of *Achillea* based on Euclidean distances from morphological data matrix.

The principal components analysis (PCA) was performed to evaluate the contribution of each morphological parameter to the ordination of species. In PCA analysis (Table 3), first three components explained about 81.3% of total variation. First component, explaining about 37% of variation, was linked positively to properties related to involucre (length and width) and both kinds of florets (length, width and number), also inflorescence number and seed width. It was linked negatively to other characters. Second component that was responsible for 26.3% of variations was linked positively to length and width of tubular florets and ligulate florets number. It was linked negatively for the rest of them. Third component, explaining only 18.0% of variation, was linked positively to width of leaf, length and width of inflorescence, capitulum number, involucre length, properties related to ligulate florets (length, width and number), tubular florets number, floret number and seed width. It was linked negatively to other.

Table 3. Eigenvectors from the first three components of different species of *Achillea*.

	First Compo-nent	Second Compo-nent	Third Compo-nent
Plant height	-0.092	-0.384	-0.209
Stem diameter	-0.021	-0.381	-0.211
Inflorescence no	0.193	-0.061	-0.202
Leaf length	-0.319	-0.168	-0.101
Leaf width	-0.337	-0.130	0.009
Inflorescence length	-0.189	-0.314	0.235
Inflorescence width	-0.160	-0.154	0.432
Capitulum no	-0.298	-0.209	0.177
Involucre length	0.195	-0.222	0.326
Involucre width	0.319	-0.131	-0.167
ligulate florets no	0.122	0.013	0.489
Tubular florets no	0.290	-0.217	0.029
ligulate florets length	0.260	-0.276	0.038
ligulate florets width	0.112	-0.020	0.112
Tubular florets length	0.218	0.024	-0.053
Tubular florets width	0.194	0.072	-0.227
Floret no	0.289	-0.211	0.074
Seed length	-0.060	-0.350	-0.074
Seed width	0.248	-0.296	0.053
1000 seed Weight	-0.188	-0.207	-0.363
Eigen Value	7.40	5.26	3.61
Percentage of Variance	37.0	26.3	18.0
Cum percentage of variance	37.0	63.3	81.3

Proteins Assay

On the basis of the relative mobility of total proteins on the gel, 38 polypeptide bands of different sizes ranging from 10.35 to 80.25 kDa, from six *Achillea* species, were identified. The percentages of polymorphic bands over the total bands detected ranged from 34.21% to 89.47% with an average of 54.82%. The lowest and the highest *He* was observed with 0.132 and 0.315, respectively (Table 4). The probability that two randomly sampled polypeptides in a given species are different was 19.2% (*He* mean=0.192).

Table 4. Genetic diversity parameters of different species of *Achillea*.

Species	Bands Number	Polymo-rphism%	He
A. millefolium	22	34.21	0.132
A. filipendulina	16	42.11	0.141
A. biebersteinii	14	36.84	0.146
A. nobilis	23	57.89	0.156
A. tenuifolia	34	89.47	0.315
A. vermicularis	26	68.42	0.261
Mean	23.17	54.82	0.192

The Nei's genetic distances (Table 5) ranged from 0.081(between *A. filipendulina* and A. *biebersteinii*), to 0.278 (between *A. millefolium* and *A. nobilis*) with an average of 0.165. The Jaccard's similarity coefficients ranged from 0.238 (between *A. millefolium* and *A. nobilis*) to 0.898 (between *A. filipendulina* and *A. biebersteinii*), with an average of 0.635. The highest similarity coefficient (1.506) was observed between *A. tenuifolia* and *A. vermicularis*. The lowest similarity (0.238) was *A.millefolium* and *A.nobilis*. According to the UPGMA dendrogram (Fig. 2), at a similarity level of 0.07 the species were divided into three main groups. Group A involved *A. millefolium* and*A. vermicularis*, while *A. tenuifolia* was located in group B. Also, *A. nobilis* was placed insubgroup 1C and *A. filipendulina* and *A. biebersteini*i were placed in subgroup 2C. The principle coordinate analysis (PCoA) indicated that the first 3 principal components accounted for more than 83% of the total observed variation. First and second components accounted for 42.15% and 27.99% of the total variation (Fig. 3). AMOVA using total protein profiles revealed that variation among and within species accounted for 30% and 70% of the total variance, respectively (Table 6). This difference was statistically significant ($p< 0.001$) based on the permutation test.

Table 5. Pair-wise values for Nei's genetic distances (above diagonal) and Similarity coefficients (below diagonal) among different species of *Achillea* based on proteins data.

Species	***A. millefolium***	***A. filipenduinla***	***A. biebersteinii***	***A. nobilis***	***A. tenuifolia***	***A. vermicularis***
A. millefolium		0.187	0.184	0.278	0.178	0.123
A. filipendulina	0.329		0.081	0.113	0.219	0.136
A. biebersteinii	0.277	0.898		0.104	0.249	0.152
A. nobilis	0.238	0.798	0.720		0.189	0.165
A. tenuifolia	0.590	0.571	0.431	0.741		0.147
A. vermicularis	0.775	0.886	0.635	0.777	0.856	

Table 6. Analysis of Molecular Variance among and within different species of *Achillea*.

Source of variation	df	Sum of squares	Mean of Squares	Percentage of variation	*p*-value
Among species	5	111.651	22.330	30%	0.001
Within species	51	221.261	4.338	70%	0.001
Total	56	332.912	26.669		

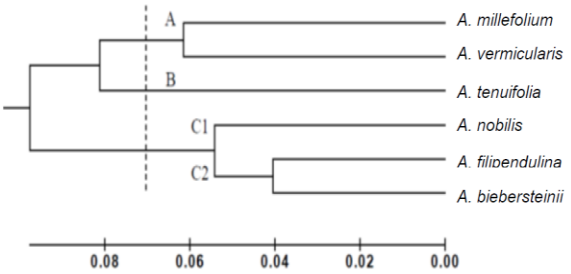

Fig. 2. UPGMA dendrogram based on Jaccard similarity coefficient different species of *Achillea*.

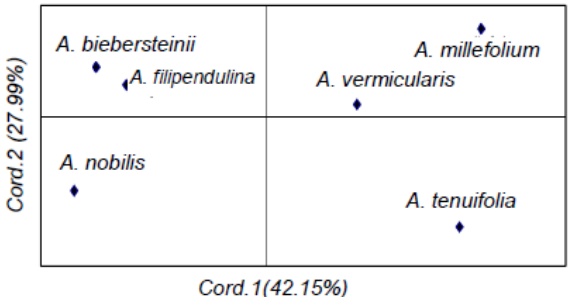

Fig. 3. Bi plot based on proteins data to classify different species of *Achillea*.

Discussion

Phenotypic variability among species from important traits such as inflorescence number and Inflorescence length, number of ligulate and Tubular florets and 1000 seed weight are made promising results for future breeding program. This wide domain of variability can be considered as an available gene pool to breeders for improvement through selection and hybridization breeding programs. High heterosis effects will be expected for hybrids of these species.

Flora Iranica (Huber-morath, 1986) categorizes *Achillea* species into three sections, including: Santolinoidea (consist of *A. tenuifolia* and *A. vermicularis*), Millefolium (consist of *A. millefolium* and *A. nobilis*) and Filipendulinae (consist of *A. filipendulina* and*A. biebersteinii*). Nonetheless, in recent study species from 3 sections were intermingled when they were classified based on morphological data. Inconsistency between classical category and category resulted of clustering *Achillea* species from morphological variables, has been reported by Azani *et al.* (2009). It is because of morphological traits are based on phenotypic expressions of the genotypes and are influenced by environmental and ontogenetic factors (Heywood, 2002). In this research, presence of significant differences between *A. Filipendulina* and other species from leaf characters (leaf length and leaf width) and capitulum number, allocated *A. Filipendulina* in a separated group (A group), solely. Further, based on significant differences between other species from leaf width, inflorescence length, involucre width and ligulate florets width, they were divided into 2 groups (B and C groups). By mean comparisons, *A. Filipendulina* shows the highest value ofleafparameters (length and width) that defines leaf area as a key factor on reduction of dehydration level (Khan *et al.,* 2011). So, it seems may be grown successfully on limited moisture areas. Among the species, the *A. tenuifolia* had the highest Inflorescence number and its Inflorescence length and number of florets is more than mean. Therefore, it 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 (Rahimmalek, 2012).

The amount of genetic diversity plays an important role in improvement of breeding programs where successful variety development and achievement of breeding objectives depends largely on high available diversity. In recent study, between different species of *Achillea* from polymorphism %, *A. tenuifolia* showed the highest variation among individuals of species. This result was in accordance with those of previously reported study using AFLP markers (Rahimmalek *et al.,* 2009) which mentioned that*A. tenuifolia* has the highest gene diversity in comparison with A. filipendulina, *A. millefolium* and *A. biebresteinii*, because of the wide distribution through Iran. So, conservation strategies should be provided to maintain such diversity aiming to improve future breeding programs. On the other hand, *A. millefolium* represents the lowest variation among individuals of specie in comparison with others. So, it faces with genetic drift that resulting with a poor gene pool. Lofgren (2002) reported self-incapability has mainly influenced the level of genetic variation in *A. millefolium* species.

By heterosis effect, species which have high genetic distances (and less genetic Similarity) could create more genetic variability than ones that have less genetic distances (and high genetic similarity). In the present investigation, the results of genetic distances indicated that genetic differences between Pair-wises of *A. nobilis* and *A. millefolium*, *A. biebresteinii* and *A. tenuifolia*, *A. filipendulina* and *A. tenuifolia*, are high. One the other hand, genetic Similarity among prior pair-wises is low. Classifying species based on their genetic similarity coefficients, put species with high genetic similarity in a same group. So, pairs like *A. nobilis* and *A. millefolium* which are located in different groups from clustering by poly peptide bands can be selected as fairly diverse with a high level of confidence and used as parents in a hybridization program. The dendrogram generated by poly peptide bands revealed 3 major groups corresponding to 6 species. The PCoA data confirmed the results of the clustering, as species which were placed in same group by dendrogram resulted by genetic similarity coefficients, were laid in same quarter.

The explained genetic variation by differences among the different species of is significantly less than observed variation within species. This is probably by out-crossing pollination system in this genus that facilities gene flow within species. Conversely, It has been reported that the levels of genetic diversity among accessions are more than within accessions in selfing species and taxa, e.g. *Phaseolus vulgaris* L. (Martins *et al.,* 2006), *Arbutus unedo* L. (Takrouni & Boussaid, 2010) and *Lathyrus sativus* (Nosrati*et al., 2012*).

Proteins analysis in most cases confirmed the results of morphological data and groups of species were relatively similar groups from morphological data. But, proteins analysis put *A. filipendulina* in a group with *A. nobilis* and *A. biebresteinii*, while it was located in a separated group from morphological data.

Conclusion

In conclusion, It is strongly recommended that both morphological and proteins assays could be used as complementary methods in describing the genetic diversity in the species of *Achillea*, because it provides the most accurate and effective results from identification and hybridization species with the highest genetic distance. As in recent research, although *A. filipendulina* and *A. biebresteinii* seems be favor for hybridization from morphological characters, they were unsuitable for it from poly peptides bands. So, we should hybrid them with suitable pairs, i.e. *A. millefolium*, *A. vermicularis* and *A. tenuifolia*, according to both of marker systems.

Because of some differences among polypeptides may be caused by pre-and post-transcriptional modifications and/or translation (Vogel *et al.,* 1994), applying molecular assays beside morphological and protein markers, is recommended and needed to express all the variability of *Achillea* gene pools.

Acknowledgement

This work was supported by the Research Institute of Forests and Rangelands (RIFR), Iran, Project no. 12-09-09-8901-89005.

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