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Evaluation of genetic diversity among some wild populations of *Achillea bieberstenii* Afan. from Iran using morphological and agronomical traits

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Abstract: In this study, genetic diversity among 25 populations (each population 6 plants) of *Achillea bieberstenii* Afan. collected from 12 provinces of Iran using a total of 35 morphological and agronomical characteristics including 31 quantitative and 4 qualitative traits was evaluated as an important step for possible use in the breeding programs of this medicinal plant. According to the results of analysis of variance, there were significant differences among the studied populations for some important characters. Also, results of simple correlation analysis were shown the significant positive and negative correlations among some important characters. Factor analysis was also used for defining of the determinant factors and the characters constituted in each factor. In Principal component analysis (PCA), 10 main and independent factors with over Eigen values than two explained 85.36% of the total variation related to main effective characters. Based on the constructed dendrogram, 25 populations of *A. biebersteinii* were clearly divided into 11 main clusters. Groups mainly have differences in yield and yield components. In this investigation not only was observed high variation for studied morphological characteristics but also some native populations were clearly shown their valuable breeding potential in some agronomical traits for breeding programs and medicinal purposes.

Keywords: *Achillea bieberstenii* Afan.*;* Breeding; Genetic diversity; Medicinal plant; Morphological traits; Natural populations

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Introduction

Morphological variability is a characteristic of all organisms and one of the basic characteristics in the world. Measurement, description and analysis of variations are fundamental steps to answer questions of biological adaptability (Ge and Hong, 1995). Morphological characters are the outside exhibition of organism, and in the natural habitats, they are not only affected by the genetic background of the species, but also by the environmental conditions as well. Plants have the potential to response to the changed environments by changing their morphology and there for, the intra-specific variation in plant characteristics is usually regarded as the adaptive mechanism to different environments (Mal and Doust, 2005). Studies on the morphological variations of plant species according to the habitat differences suggest their relationships with environmental factors clearly, and help us understand the manner, mechanism and influencing factors of plant adaptation and evolution (Yang, 1991).

The genus *Achillea* L. (commonly known as yarrow) belongs to the Aster family (Asteraceae) and comprises more than 100 species worldwide (Rahimmalek et al., 2009). These often medicinal and rhizomatous perennial plants are native to Europe, Western Asia and North Africa although they are also found in Australia, New Zealand and North America (Huber Morath, 1986; Chevallier, 1996). In traditional systems of medicine, *Achillea* species have a long history of use as medicinal plants mainly due to their anti-inflammatory, anti-spasmodic, diaphoretic, diuretic, carminative, tunic, vermifugal and emmenagogic properties and are used as a cure for hemorrhage, pneumonia, rheumatic and abdominal pains, stomach-ache and wounds (Zargari, 1996; Baris et al., 2006; Esmaeili et al., 2006). Nowadays, different medicinal properties of these plants such as spasmolytic, choleretic, anti-inflammatory and wound healing are documented (Benedek, 2007). In recent years, the anticancer activity of essential oils isolated from some *Achillea* species has been reported and shown that they can modulate macrophages activities (Paulo, 2005). Due to hair growth promotion property, yarrow are used in cosmetic industries for production of hair shampoos as well creams (Karlova, 2001; Lewis, 2006).

Iran represents a significant source of germplasm of different medicinal plant species, particularly for the genus *Achillea* (Zargari, 1996). In the Flora of Iran, the genus *Achillea* is represented by nineteen species, of which seven are endemic (Huber Morath, 1986). One of these species is *A. bieberstenii* Afan. which occurs naturally in many parts of the country in the central, North, Northwest, West and Northeast with the local name of “Bumadarane Zard” (Huber Morath, 1986; Mozaffarian, 1996). This plant is a perennial villose herb with 10-100 cm height and radiate heads which are borne in large dense compound corymbs on the erect stems (Huber Morath, 1986).

A. biebersteinii is used in folk medicin of Jordan because of its carminative properties, while in Turkey the plant is also used for abdominal pain, stomach-ache and for wound healing (Bader et al., 2003). To date, many investigations considered the volatile oil and extract of *A. biebersteinii* from the chemical constituents to biological activities points of view (Rustaiyan et al., 1998; Bader et al., 2003; Sokmen et al., 2004; Baris et al., 2006; Esmaeili et al., 2006; Kordali et al., 2009; Rahimmalek et al., 2009). Based on the results of these studies, the plant has considerable different biological activities including antibacterial, antifungal, antioxidant, insecticidal, herbicidal and wound healing.

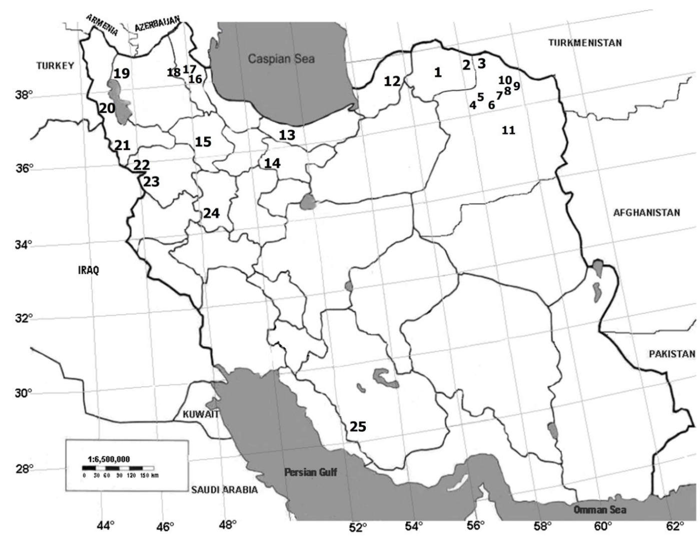

Figure 1. Collection sites of studied *Achillea biebersteinii* populations. For codes see Table 1.

In Iran, most of the studies on the genus *Achillea* were done using one species originated from a limited geographical area and there is especially no comprehensive research considered variation in morphological characteristics of wild populations of *A. biebersteinii*. Since Iran is one of the important origins of genus *Achillea* and there is substantial diversity in *A. biebersteinii* plants in the country, therefore, the main aim of the present study was to expand the knowledge on morphological and agronomical characteristics of 25 populations of *A. biebersteinii* growing wild in the different parts of Iran in order to find promising populations of this species which have the potential to be use as initial materials for breeding programs.

Material and methods

Plant materials

A. biebersteinii plants were collected at the full flowering stage from their natural habitats in different parts of Iran between April and May 2009 (Figure 1). Sites of collections were determined based on previous collections cited in Flora Iranica (Huber Morath, 1986) and local information as well. Geographical and climatic conditions of each habitat were obtained from the nearest meteorology station (Table 1). Voucher specimens of each population were deposited at

Table 1. Origin, geographical, climatic of natural habitats of 2 Iranian wild populations of *A.biebersteinii*

No.	Site name (origion)	Abb.	Longitude	Latitude	Altitude (m)	T_{min}	T_{max}	H_{min}	H_{max}	H_{total}	P_{annual}	Sun
1	**Havar**, North Khorasan	H	E 57°11'	N 37°28'	2980	6.8	19.7	0.4	0.8	0.4	272.4	2714
2	**Golool**, North Khorasan	GOL	E 58°11'	N 37°37'	2100	6.8	17.5	0.4	0.7	0.53	252.7	2714
3	**Chelmir**, Khorasan-e-Razavi	CH	E 58°34'	N 37°31'	1584	6.8	19.7	0.4	0.8	0.58	272.4	2714
4	**Adag**, Khorasan-e-Razavi	AD	E 58°53'	N 36°11'	1260	6.7	21.8	0.3	0.7	0.49	239.8	3072.2
5	**Akhlamad**, Khorasan-e-Razavi	AK	E 58°59'	N 36°11'	1155	6.7	21.8	0.3	0.7	0.49	239.8	3072.2
6	**Buzhan**, Khorasan-e-Razavi	BU	E 59°03'	N 36°61'	1600	6.7	21.8	0.3	0.7	0.49	239.8	3072.2
7	**Golmakan**, Khorasan-e-Razavi	GLM	E 59°13'	N 36°29'	1315	6.6	20.2	0.35	0.69	0.48	212.6	2898.2
8	**Azghad**, Khorasan-e-Razavi	AZ	E 59°24'	N 36°19'	1800	7.1	21.1	0.37	0.74	0.55	255.2	2892.4
9	**Goojgi**, Khorasan-e-Razavi	GO	E 59°56'	N 36°31'	2100	7.1	21.1	0.37	0.74	0.55	255.2	2892.4
10	**Ortokand**, Khorasan-e-Razavi	O	E 59°51'	N 36°48'	1480	7.1	21.1	0.45	0.75	0.55	255.2	2892.4
11	**Aman Abad**, Khorasan-e-Razavi	AM	E 59°32'	N 35°58'	1210	7.1	21.1	0.37	0.74	0.55	255.2	2894.4
12	**Tangehgol**, Golestan	T	E 55°49'	N 37°23'	220	11.7	23.9	0.55	0.82	0.68	564.1	2439.1
13	**Siahbisheh**, Mazandaran	SI	E 51°33'	N 36°23'	1990	6.3	14.8	0.47	0.8	0.63	503.4	1959.4
14	**Mohammad Shahr**, Tehran	MO	E 50°32'	N 35°48'	1140	7.8	21.2	0.32	0.69	0.47	243.8	2959.7
15	**Zanjan**, Zanjan	ZN	E 48°45'	N 36°30'	1640	4	18	0.37	0.75	0.54	313.1	2843.2
16	**Sardabeh**, Ardabil	SAR	E 48°15'	N 38°37'	1840	2.8	15.3	0.53	0.89	0.71	303.9	2454.3
17	**Meshkin Shahr**, Ardebil	ME	E 47°38'	N 38°24'	1394	5.9	15.4	0.45	0.75	0.6	383.9	2503.2
18	**Sati**, Ardebil	SA	E 47°24'	N 38°15'	1920	5.9	15.4	0.45	0.75	0.6	383.9	2503.2
19	**Mishoodagh**, East Azarbaijan	MI	E 45°38'	N 38°19'	2450	6.9	18	0.37	0.71	0.54	288.9	2794.3
20	**Ghasemloo**, West Azarbaijan	GH	E 44°43'	N 37°29'	1340	5.4	17.6	0.42	0.78	0.6	341	2829.3
21	**Piranshahr**, West Azarbaijan	PI	E 45°04'	N 36°41'	1842	6.2	17.9	0.37	0.71	0.51	672.7	2766.4
22	**Nenor**, Kordestan	N	E 46°00'	N 35°52'	1830	8.7	18.6	0.34	0.58	0.44	689.3	2884.6
23	**Zaribar**, Kordestan	ZR	E 46°08'	N 35°32'	1285	5	20.6	0.34	0.77	0.53	991.2	2967.9
24	**Eberoo**, Hamedan	E	E 48°28'	N 34°41'	2250	3.3	19.1	0.36	0.77	0.54	316.6	2929.1
25	**Firooz Abad**, Fars	F	E 52°37'	N 28°48'	1600	10.1	26.7	0.36	0.65	0.49	416.6	3358.6

T_{min} :Average of minimum temperature in year (C°); T_{max}: Average of minimum temperature in year (C°); H_{min} : Average of minimum relative humidity in year (%); H_{max}: Average of maximum relative humidity in year (%); H_{total}: Total relative humidity in year (%); P_{annual}: Total of precipitation in year (mm); Sun: total of sunshine hours

the Herbarium of Horticulture Department, Faculty of Agriculture and Natural Resources, University of Tehran, Karaj, Iran.

Table 2. traits, range of variability, mean and coefficient of variations for qualitative and quantitative traits from 25 populations of *A. biebersteinii*

No.	Trait	Abbreviation	Unit	Min	Max	Mean	CV (%)
1	Shoot diameter (between 1st and 2nd nodes)	SD(1&2)	mm	2.18	4.36	3.21	18.9
2	Shoot diameter (near inflorescence)	SD(NI)	mm	1	2.88	2.10	22.1
3	Number of leaves	NL	-	16.08	37.17	28.26	18.9
4	Leaf length	LL	mm	27.15	63.9	50.26	21.5
5	Leaf width	LW	mm	3.81	10.94	7.21	30.5
6	Leaf length/width ratio	LL/LW	Ratio	4.76	12.8	7.32	24.9
7	Internodes distance	ID	mm	14.53	30.71	22.22	21.0
8	Plant height	PH	cm	22.67	62.54	44.53	25.1
9	Number of inflorescence man rays	NIMR	-	5.25	14	7.60	26.7
10	Number of inflorescence heads (Capitula)	NC	-	77.43	265	177.07	26.4
11	Peduncle length	PL	mm	5.81	33.24	17.36	42.6
12	Length of head peduncle	LP	mm	2.05	10.16	3.78	49.2
13	Inflorescence length	IL	mm	26.79	97.09	61.90	37.6
14	Inner bract length	IBL	mm	2.67	3.57	3.21	7.3
15	Outer bract length	OBL	mm	1.41	2.13	1.75	12.1
16	Inner bract width	IBW	mm	0.58	2.67	1.02	44.3
17	Outer bract width	OBW	mm	0.9	1.77	1.31	14.1
18	Inflorescence width	IW	mm	32.85	69.56	51.49	20.5
19	Capitulum width	CW	mm	2.07	3.72	2.52	17.0
20	Capitulum length	CL	mm	3.283	57.585	6.07	176.9
21	Number of ray florets per capitulum	NRPC	-	4.38	5.46	4.92	5.4
22	Length of bract (outside at the base of inflorescence)	LB	mm	8.9	28.83	17.55	29.9
23	Peduncle diameter	PeduD	mm	1.24	4.28	2.07	32.5
24	Pedicel diameter	PediD	mm	0.2	1.29	0.45	50.6
25	Distance of inflorescence from the upest leaf	DIUL	mm	10.74	48.54	26.01	38.1
26	Number of outer bracts	NIB	-	4.31	6.65	5.34	9.6
27	Number of inner bracts	NOB	-	6.67	9.02	7.65	8.3
28	Ray floret length	DFL	mm	2.37	3.74	3.00	10.9
29	Disc floret length	RFL	mm	1.8	2.69	2.26	10.2
30	Number of main stem	NMS	-	1	4.5	2.29	50.1
31	Essential oil content	EOC	(W/V)	0.41	1.62	0.79	44.7
32	Leaf pubescence	LP	Code	1	3	-	-
33	Stem color	SC	Code	1	4	-	-
34	Flower color	FC	Code	1	3	-	-
35	Leaf color	LC	Code	1	5	-	-

Evaluation of morphological and agronomical characters

In this study a total of Four qualitative and 32 quantitative morphological traits were assessed (Table 2, 3). Of each population, six plants were selected and their characters were measured using a appropriate instruments. To measure the characters, five random samples were evaluated from each plant.

Table 3. The list of qualitative traits and their character states

Traits	Characters				
Codes	1	2	3	4	5
Leaf pubescence	sparse	intermediate	dense	-	-
Stem color	light green	green	dark green	green-purple	-
Flower color	light yellow	yellow	dark yellow	-	-
Leaf color	light olive	olive	dark olive	Light green	dark green

Data analysis

In order to evaluate morphological diversity and to establish relationships among studied populations, several statistical procedures were conducted. Quantitative data were computed using the SAS software ver. 9.1 (SAS, 2003) to perform analysis of variance, comparison of mean and to calculated coefficient of variation (CV). Simple correlations, factor and cluster analysis and scatter plots were carried out using SPSS® ver. 11.0. Factor analysis was done by Varimax factor rotation technique. Cluster analysis was also done using SPSS® ver. 11.0 based on the matrix resulted from the Euclidian distances and the Wards method.

Results

Analysis of variance

Comparison of means for quantitative parameters showed that all parameters except for number of leaves, peduncle length, length of head peduncle, inner bract width, inflorescence width, length of bract (outside at the base of inflorescence), peduncle diameter and pedicel diameter were significant ($P \leq 0.01$) (Table 4).

Table 4. Means comparison of 32 quantitative traits in 25 populations of *A. biebersteinii*

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
No.	population	SD(1& 2)	SD(NI)	NL	LL	LW	LL/LW	ID	PH	NIMR	NC	PL	LP	IL	IBL	OBL	IBW
1	H	3.00	1.82	22.68	59.00	4.61	13.71	23.43	59.32	7.42	132.47	8.63	8.66	54.95	3.34	2.07	1.00
2	GOL	2.70	1.75	34.50	39.74	4.34	9.27	20.26	31.80	5.67	143.32	5.81	2.05	39.50	3.07	1.58	1.00
3	CH	3.04	2.19	31.00	59.05	6.88	8.65	29.73	46.79	7.33	181.50	20.82	2.68	90.69	3.22	1.50	0.58
4	AD	2.83	2.45	20.77	48.98	9.36	5.74	20.91	39.85	6.83	139.67	12.20	3.15	38.98	3.21	1.76	0.81
5	AK	3.45	2.11	29.08	63.90	8.56	7.75	22.47	45.85	6.33	186.67	16.80	3.55	53.18	3.28	1.90	0.70
6	BU	3.32	2.35	28.83	47.48	9.98	5.82	17.45	39.93	10.00	182.67	16.15	2.61	96.93	3.27	1.56	0.71
7	GLM	3.82	2.88	27.67	42.81	6.24	7.09	26.91	59.95	8.67	192.25	28.70	10.16	78.03	3.40	1.41	1.14
8	AZ	4.36	2.50	35.83	54.79	7.78	7.68	26.73	62.50	7.92	178.77	21.48	3.83	46.97	3.57	1.84	1.17
9	GO	3.54	2.41	37.17	57.08	9.95	5.82	30.25	62.54	7.17	152.88	27.09	4.81	57.52	3.31	2.02	1.03
10	O	4.18	2.83	32.33	61.03	10.94	5.76	30.71	49.90	9.17	236.17	24.30	3.45	97.03	3.51	1.92	2.67
11	AM	3.32	2.38	25.08	61.17	6.23	9.99	23.47	39.59	8.83	170.65	23.67	3.78	76.24	3.09	1.98	1.01
12	T	3.79	2.11	23.67	27.15	4.60	6.33	14.53	27.42	8.17	77.43	10.90	2.91	27.69	3.39	1.69	1.07
13	SI	2.73	2.02	19.40	44.23	6.74	8.73	22.96	44.10	6.00	225.92	15.80	3.64	75.97	3.16	1.83	0.68
14	MO	2.26	1.47	31.36	55.63	9.65	5.83	23.93	54.00	5.25	99.08	8.61	2.16	36.57	2.79	1.47	0.59
15	ZN	3.78	2.24	26.43	49.19	9.15	5.45	19.93	48.85	6.00	222.25	22.67	2.91	85.85	3.57	2.13	2.06
16	SAR	2.50	1.45	30.50	29.84	4.13	7.37	15.47	25.77	7.21	179.55	9.69	2.19	42.06	3.31	1.85	1.20
17	ME	3.38	2.11	30.33	44.75	6.28	7.27	24.46	41.37	14.00	156.67	18.46	3.78	41.10	3.13	1.65	0.84
18	SA	2.64	1.97	16.08	46.63	6.83	7.50	22.04	43.25	5.92	167.85	25.76	4.87	82.08	3.45	1.76	0.74
19	MI	2.18	1.40	24.58	29.88	3.82	7.94	16.74	32.28	6.17	93.50	5.92	2.65	26.79	3.15	1.70	0.76
20	GH	2.76	1.71	24.79	43.70	4.30	10.58	15.96	36.60	8.80	187.39	13.25	2.82	59.40	2.81	1.58	0.91
21	PI	3.32	2.09	34.50	62.14	10.75	6.25	23.27	52.67	6.47	224.37	13.16	3.81	60.70	3.27	2.12	1.21
22	N	3.28	2.10	29.33	48.90	5.83	9.08	26.53	49.52	5.83	248.00	18.66	4.07	74.21	3.06	1.73	0.81
23	ZR	4.05	2.40	33.94	62.77	8.25	8.07	22.12	53.17	7.00	265.00	20.34	3.01	79.23	3.31	1.62	1.02
24	E	3.71	2.80	25.37	54.46	7.23	8.89	19.54	43.51	11.67	184.82	33.24	4.00	97.10	2.92	1.63	0.86
25	F	2.40	1.00	31.17	62.27	7.83	8.00	15.68	22.67	6.25	197.83	12.00	2.96	28.75	2.67	1.44	0.95
	MSD	2.05	1.48	29.05	33.22	7.30	5.71	13.86	24.26	7.97	175.51	34.25	11.46	87.09	0.71	0.76	2.24

MSD: Minimum Significant Difference, If difference between two means to be under MSD, are not significant different at 1% level of probability using Tukey's Studentized Range (HSD) Test

Table 4 (cont.) Means comparison of 32 quantitative traits in 25 populations of *A. biebersteinii*

		17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
No.	population	OBW	IW	CW	CL	NRPC	LB	PeduD	PediD	DIUL	NOB	NIB	RFL	DFL	NMS	EOC
1	H	1.35	46.32	2.64	4.22	4.78	12.57	1.77	0.31	13.47	6.22	8.60	3.34	2.41	2.50	0.9
2	GOL	1.49	44.25	2.11	3.28	4.58	13.65	1.24	0.31	17.36	5.27	7.38	2.51	1.98	3.50	1.6
3	CH	1.23	36.24	2.09	3.97	4.89	21.46	1.75	0.21	37.37	5.08	7.58	2.71	1.99	1.33	1.6
4	AD	1.26	47.80	2.48	4.04	4.93	15.84	1.63	0.39	30.88	5.00	6.95	3.20	2.62	2.83	0.5
5	AK	1.41	53.13	2.63	4.11	4.52	19.39	1.78	0.44	27.64	5.00	7.36	3.74	2.64	2.00	1.1
6	BU	1.16	43.65	2.22	3.86	5.33	23.27	3.38	0.32	34.37	5.19	7.50	3.23	2.34	1.17	0.7
7	GLM	1.35	50.57	2.47	4.04	5.31	18.53	2.28	0.38	26.87	4.92	8.15	3.23	2.36	1.00	0.6
8	AZ	1.55	61.06	2.80	4.44	4.94	22.83	2.14	0.43	22.34	5.91	8.39	3.11	2.61	1.17	1.2
9	GO	1.34	56.30	2.70	3.99	4.89	19.00	2.08	0.40	48.54	5.80	9.02	3.34	2.20	3.67	0.6
10	O	1.53	59.99	3.72	3.95	5.46	27.97	2.50	0.41	29.57	5.66	8.59	3.17	2.28	1.00	0.6
11	AM	1.50	49.29	2.64	3.60	4.99	19.68	4.28	0.37	32.59	5.22	7.63	3.27	2.31	2.50	1.1
12	T	1.42	39.85	2.15	3.64	5.06	17.19	2.40	0.32	10.74	4.67	7.76	2.87	2.07	2.00	0.8
13	SI	1.05	48.53	2.28	3.65	4.38	15.30	1.82	0.31	30.87	5.59	7.29	2.48	2.04	1.17	0.5
14	MO	0.90	39.56	2.33	3.63	4.87	14.78	1.28	0.20	19.67	6.65	8.25	2.37	1.81	1.33	0.4
15	ZN	1.77	68.00	3.54	5.33	5.22	18.31	2.55	0.54	21.74	5.71	8.21	3.14	2.49	4.50	0.7
16	SAR	1.21	57.82	2.58	4.04	5.00	12.66	1.54	0.40	14.68	5.44	8.10	3.09	2.26	1.67	0.7
17	ME	1.22	48.68	2.21	4.06	5.33	12.00	1.90	1.29	33.24	4.31	6.67	2.84	2.11	2.67	0.6
18	SA	1.19	42.56	2.08	4.15	4.78	16.70	1.91	0.51	31.19	4.89	7.39	2.90	2.31	4.50	0.7
19	MI	1.27	32.85	2.19	3.83	4.64	8.90	1.32	0.45	13.50	5.00	6.67	2.75	2.30	1.00	1.0
20	GH	1.12	49.50	2.30	3.34	4.80	12.17	1.68	0.87	18.35	5.78	7.00	2.68	2.09	3.00	0.7
21	PI	1.52	69.56	3.11	3.64	5.00	24.93	2.03	0.47	18.56	5.77	7.98	3.26	2.35	4.17	0.7
22	N	1.25	43.27	2.07	3.50	4.98	9.68	2.19	0.33	46.05	5.09	7.13	2.76	1.96	2.00	0.4
23	ZR	1.37	66.98	2.49	3.97	4.76	18.00	2.73	0.49	24.64	5.30	7.36	2.69	2.16	3.33	0.6
24	E	1.19	62.24	2.77	3.95	4.71	28.84	2.20	0.81	30.14	4.99	7.33	3.06	2.69	2.33	0.6
25	F	1.18	69.17	2.44	3.30	5.00	15.20	1.50	0.43	15.83	5.00	6.92	3.24	2.22	1.00	1.5
	MSD	0.56	63.53	1.48	1.32	1.12	25.25	3.15	1.29	34.27	2.02	2.42	1.11	0.81	3.51	0.56

MSD: Minimum Significant Difference, If difference between two means to be under MSD, are not significant different at 1% level of probability using Tukey's Studentized Range (HSD) Test

Table 5. Correlation matrix among 36 morphological characteristics

		1	2	3	4	5	6	7	8	9	10	11	12
		SD(1&2)	SD(NI)	NL	LL	LW	LL/LW	ID	PH	NIMR	NC	PL	LP
1	SD(1&2)	1											
2	SD(NI)	0.82**	1										
3	NL	0.33	0.06	1									
4	LL	0.30	0.26	0.34	1								
5	LW	0.36	0.40	0.34	0.64**	1							
6	LL/LW	-0.23	-0.30	-0.14	0.11	-0.66**	1						
7	ID	0.42*	0.54**	0.36	0.53**	0.42*	-0.05	1					
8	PH	0.52**	0.56**	0.27	0.52**	0.45*	-0.05	0.78**	1				
9	NIMR	0.40*	0.44*	0.03	-0.03	-0.03	0.00	0.04	-0.02	1			
10	NC	0.40*	0.33	0.23	0.46*	0.36	-0.07	0.29	0.26	-0.01	1		
11	PL	0.64**	0.76**	0.02	0.36	0.35	-0.21	0.51**	0.47*	0.40*	0.45*	1	
12	LP	0.26	0.36	-0.20	0.13	-0.11	0.29	0.37	0.55**	0.14	0.04	0.36	1
13	IL	0.45*	0.65**	-0.09	0.37	0.35	-0.10	0.40*	0.38	0.23	0.64**	0.72	0.20
14	IBL	0.60**	0.53**	0.00	-0.07	0.19	-0.29	0.32	0.42*	-0.01	0.14	0.30	0.29
15	OBL	0.27	0.18	-0.03	0.22	0.23	0.00	0.20	0.31	-0.14	0.16	0.08	0.10
16	IBW	0.51**	0.35	0.23	0.11	0.31	-0.23	0.21	0.15	0.08	0.35	0.25	0.04
17	OBW	0.62**	0.39	0.27	0.16	0.15	-0.03	0.18	0.20	-0.04	0.22	0.20	0.10
18	IW	0.45*	0.20	0.40*	0.47*	0.46*	-0.19	0.01	0.17	0.07	0.62**	0.33	0.01
19	CW	0.52**	0.40*	0.26	0.42*	0.56**	-0.26	0.27	0.35	0.06	0.39	0.33	0.07
20	CL	0.43*	0.37	-0.08	0.08	0.26	-0.25	0.19	0.42*	0.06	0.12	0.38	0.21
21	NRPC	0.43*	0.35	0.24	0.01	0.33	-0.37	0.18	0.09	0.48*	0.14	0.27	0.15
22	LB	0.63**	0.68**	0.25	0.50*	0.65**	-0.39	0.33	0.33	0.31	0.32	0.61**	0.02
23	PeduD	0.56**	0.56**	-0.02	0.26	0.25	-0.09	0.12	0.15	0.36	0.31	0.50**	0.10
24	PediD	0.14	0.08	-0.05	-0.08	-0.12	0.06	-0.14	-0.11	0.71	0.09	0.24	-0.02
25	DIUL	0.26	0.52**	0.10	0.33	0.38	-0.22	0.64**	0.40*	0.20	0.36	0.63**	0.10
26	NOB	-0.07	-0.14	0.25	0.32	0.28	0.09	0.22	0.49*	-0.40*	0.03	-0.19	0.02
27	NIB	0.41*	0.32	0.36	0.25	0.35	-0.13	0.48*	0.61**	-0.13	0.01	0.24	0.35
28	RFL	0.36	0.30	0.08	0.38	0.34	-0.06	0.09	0.18	0.16	0.12	0.29	0.37
29	DFL	0.38	0.42*	-0.17	0.22	0.24	-0.11	-0.07	0.17	0.20	0.10	0.35	0.26
30	NMS	0.07	0.07	-0.07	0.11	0.11	0.01	-0.04	0.13	-0.13	0.12	0.14	-0.02
31	EOC	-0.16	-0.29	0.52	0.57	-0.30	0.42*	-0.05	-0.30	-0.14	-0.18	-0.20	-0.15
32	LP	-0.14	-0.22	0.31	-0.08	-0.08	0.02	-0.05	-0.20	-0.34	-0.18	-0.23	-0.05
33	SC	0.10	0.07	0.01	-0.03	-0.17	0.27	0.10	0.07	0.14	0.28	0.10	-0.11
34	FC	-0.02	-0.20	-0.06	0.16	-0.11	0.26	-0.20	-0.09	-0.06	0.21	-0.19	-0.32
35	LC	0.27	0.24	0.24	0.23	0.27	-0.13	0.33	0.24	0.01	0.65**	0.56**	0.44*

**Correlation is significant at the 0.01 level (2-tailed) * Correlation is significant at the 0.05 level (1-tailed)

Table 5. (cont.) Correlation matrix among 36 morphological characteristics

		13	14	15	16	17	18	19	20	21	22	23	24
		IL	IBL	OBL	IBW	OBW	IW	CW	CL	NRPC	LB	PeduD	PediD
13	IL	1											
14	IBL	0.29	1										
15	OBL	0.10	0.53**	1									
16	IBW	0.27	0.50*	0.45*	1								
17	OBW	0.13	0.64**	0.59**	0.66**	1							
18	IW	0.18	0.13	0.35	0.52**	0.43*	1						
19	CW	0.31	0.42*	0.63**	0.85**	0.63**	0.70**	1					
20	CL	0.28	0.71**	0.46*	0.40*	0.48*	0.27	0.52**	1				
21	NRPC	0.22	0.30	-0.02	0.55**	0.27	0.20	0.38	0.25	1			
22	LB	0.60**	0.31	0.17	0.38	0.33	0.45*	0.58**	0.22	0.28	1		
23	PeduD	0.56**	0.29	0.24	0.25	0.37	0.18	0.26	0.15	0.43	0.45*	1	
24	PediD	-0.02	-0.16	-0.04	0.03	-0.05	0.24	0.04	0.11	0.17	-0.06	-0.03	1
25	DIUL	0.54**	0.06	0.04	-0.13	-0.11	-0.09	-0.07	0.06	0.13	0.22	0.33	0.05
26	NOB	0.01	0.00	0.34	0.21	-0.01	0.17	0.39	0.10	-0.14	0.07	-0.13	-0.37
27	NIB	0.19	0.52**	0.47*	0.50**	0.36	0.26	0.57	0.38	0.27	0.39	0.16	-0.40*
28	RFL	0.10	0.35	0.48*	0.26	0.45*	0.42*	0.49*	0.36	0.28	0.40*	0.33	0.00
29	DFL	0.16	0.39	0.40*	0.21	0.42*	0.43*	0.49*	0.55**	0.04	0.45*	0.20	0.17
30	NMS	0.10	0.19	0.45*	0.11	0.37	0.27	0.17	0.26	-0.11	-0.01	0.06	0.28
31	EOC	-0.20	-0.13	-0.21	-0.09	0.24	0.51	-0.18	-0.20	-0.13	0.01	-0.10	-0.24
32	LP	-0.43*	0.00	-0.15	0.07	0.23	-0.18	-0.36	-0.27	0.12	-0.09	-0.15	-0.08
33	SC	0.33	0.10	-0.17	0.12	-0.03	0.12	-0.01	0.04	0.01	-0.10	0.08	-0.19
34	FC	0.24	-0.03	0.12	0.10	0.11	0.09	0.18	-0.26	-0.24	0.15	0.02	-0.17
35	LC	0.45*	0.11	0.18	0.37	0.11	0.54**	0.23	-0.02	0.31	0.16	0.46	0.32

**Correlation is significant at the 0.01 level (2-tailed) * Correlation is significant at the 0.05 level (1-tailed)

Table 5. (cont.) Correlation matrix among 36 morphological characteristics

		25	26	27	28	29	30	32	33	34	35	36
		DIUL	NIB	NOB	DFL	RFL	NMS	EOC	LP	SC	FC	LC
25	DIUL	1										
26	NIB	-0.19	1									
27	NOB	0.03	0.65**	1								
28	DFL	0.10	-0.09	0.31	1							
29	RFL	-0.05	-0.11	0.09	0.75**	1						
30	NMS	0.07	0.04	0.06	0.05	0.10	1					
31	EOC	-0.24	-0.12	-0.08	-0.03	-0.09	-0.12	1				
32	LP	-0.13	-0.28	-0.12	-0.03	-0.20	-0.10	0.39	1			
33	SC	0.02	0.23	0.14	-0.15	-0.24	0.07	0.07	-0.55**	1		
34	FC	-0.39	0.28	0.21	-0.20	-0.15	0.11	0.08	-0.37	0.29	1	
35	LC	0.40*	0.04	0.04	0.14	-0.05	0.22	-0.25	-0.02	0.26	-0.16	1

**Correlation is significant at the 0.01 level (2-tailed) * Correlation is significant at the 0.05 level (1-tailed)

The highest value for leaf length , leaf width and leaf length/width ratio were observed in the Akhlamad (63.9mm), Ortokand (10.9mm) and Havar (13.7mm) populations, respectively and the minimum observed in populations of Tangehgol (27.2mm), Mishoodagh (3.8mm) and Zanjan (5.4mm), respectively . The longest values for the plant height were determined in the population of Goojgi and the shortest in the population of Firooz Abad with 62.5 and 22.7 cm, respectively. The mean of highest values for the Number of inflorescence main rays and number of inflorescence heads (capitula) was observed in the populations of Meshkinshahr (14 main rays) and Zaribar (number of 265 capitula) and lowest values for them were observed in the Mohammadshahr (6 main rays) and Tangehgol (77 capitula), respectively. The highest value for the inflorescence length was observed in the population of Eberoo and the lowest in Mishoodagh being 79.1 and 26.8 mm, respectively. The mean of lowest dry weight for each plant were in Zanjan population at about 16.8 g, and the highest in population of Mishoodagh with 1.6 g. The highest essential oil content was detected in population of Golool at about 1.62% (v/w) and lowest percentage in Mohammad shahr population with 0.41% (v/w). Mean values of the studied morphological and agronomical parameters showed large variations among the populations for almost of measured parameters. Mean values and range of the variability for the different characters of each population are shown in Table 2.

Simple correlations

The bivariate correlations between studied characters are shown in Table 5. The most significant positive correlation was found between the capitulum width and inner bract width (r=+0.85), shoot diameter (between 1st and 2nd nodes) and shoot diameter (near inflorescence) (r= +0.82), plant height and internodes distance (r=+0.78), disc floret length and ray floret length (r=+0.75), inflorescence width and capitulum width (r=+0.70). Also some vegetative characters were significantly correlated with reproductive ones that are observable in correlation Table.

Factor analysis

The aim of factor analysis is determining the number of main factors for reducing the number of effective parameters to discriminate populations. For each factor, a factor loading of more than 0.63 was considered as being significant (Table 6). According to factor analysis, 18 of the morphological characters accounted for 44.4% of the variance as the four first main factors, and the other parameters scattered within six factors determined 85.4% of the total variance. The largest portion of the variance at the first factor belongs to variables, shoot diameter (between 1st and 2nd nodes), shoot diameter (near inflorescence), peduncle length, inflorescence length, length of bract (outside at the base of inflorescence) , peduncle diameter and distance of inflorescence from the upest leaf that negative effects indicated them 14.6% of the total variance.

Table 6. Eigen values of rotated factors and cumulative variance (%) of 10 factors contributing to 100% of total variance

Factor	1	2	3	4	5	6	7	8	9	10
Cumulative variance (%)	14.59	25.80	35.96	44.43	52.88	59.91	66.70	73.27	79.56	85.36
Eigen value	5.25	4.04	3.66	3.05	3.04	2.53	2.45	2.37	2.26	2.09
Parameters	Factor loading									
SD(1&2)	**0.63****	0.50	0.21	0.07	0.19	0.12	-0.06	-0.05	0.25	0.17
SD(NI)	**0.84****	0.28	0.19	-0.03	0.16	0.08	-0.05	-0.10	0.17	-0.09
NL	-0.05	0.17	0.48	0.25	-0.14	-0.05	-0.27	-0.10	0.18	**0.63****
LL	0.30	-0.27	0.54	0.44	0.38	0.05	-0.07	0.12	-0.04	0.24
LW	0.30	0.08	0.44	0.34	0.24	0.00	**-0.65****	-0.05	-0.06	-0.08
LL/LW	-0.17	-0.30	0.02	-0.04	0.05	0.06	**0.81****	0.22	0.03	0.26
ID	0.48	0.07	**0.67****	0.13	-0.10	-0.01	0.09	-0.35	-0.11	0.08
PH	0.38	0.10	**0.80****	0.03	0.09	0.19	0.11	-0.22	-0.05	-0.14
NIMR	0.37	0.08	-0.13	-0.06	0.08	-0.19	0.07	-0.06	**0.84****	-0.03
NC	0.37	0.07	0.03	**0.81****	0.02	0.10	0.00	0.11	-0.07	-0.03
PL	**0.79****	0.07	0.13	0.25	0.16	0.13	0.02	-0.15	0.21	-0.11
LP	0.20	0.14	0.28	0.01	0.31	-0.11	**0.65****	-0.31	-0.01	-0.24
IL	**0.82****	0.06	0.08	0.31	-0.03	0.04	0.06	0.25	-0.06	-0.22
IBL	0.34	**0.72****	0.09	-0.19	0.17	0.26	0.04	-0.18	-0.23	-0.09
OBL	-0.02	0.39	0.22	0.08	0.42	0.51	0.00	0.08	-0.23	-0.14
IBW	0.08	**0.81****	0.13	0.32	0.10	0.03	-0.10	0.22	0.05	0.03
OBW	0.18	**0.67****	-0.01	0.07	0.31	0.41	0.05	0.04	-0.10	0.41
IW	-0.02	0.26	0.16	**0.66****	0.41	0.24	-0.20	0.18	0.23	0.13
CW	0.10	0.58	0.35	0.33	0.43	0.14	-0.22	0.30	0.08	-0.04
CL	0.20	0.52	0.18	-0.11	0.30	0.38	0.02	-0.05	0.06	-0.30
NRPC	0.21	**0.63****	-0.01	0.23	-0.02	-0.34	-0.14	-0.24	0.32	-0.01
LB	**0.64****	0.16	0.24	0.08	0.36	-0.07	-0.40	0.27	0.10	0.18
PeduD	**0.67****	0.25	-0.15	0.17	0.14	-0.01	0.01	0.11	0.00	0.05
PediD	-0.03	-0.07	-0.22	0.15	0.04	0.28	0.03	-0.13	**0.85****	-0.13
DIUL	**0.65****	-0.25	0.18	0.24	-0.13	0.06	-0.08	-0.44	-0.03	-0.14
NOB	-0.27	0.08	**0.77****	0.06	-0.05	0.07	-0.04	0.38	-0.21	-0.12
NIB	0.11	0.53	**0.71****	-0.04	0.10	0.01	0.04	0.09	-0.18	-0.01
RFL	0.15	0.23	0.06	0.12	**0.86****	-0.03	0.03	-0.14	0.00	0.05
DFL	0.20	0.15	-0.06	-0.08	**0.88****	0.19	-0.05	-0.01	0.13	-0.10
NMS	-0.01	0.04	-0.03	0.15	-0.01	**0.90****	-0.02	-0.01	0.02	-0.03
EOC	-0.09	-0.06	-0.12	-0.09	0.01	-0.08	0.27	0.09	-0.14	**0.81****
LP	-0.19	0.05	-0.25	-0.07	-0.06	-0.08	-0.29	-0.52	-0.33	0.56
SC	0.16	0.15	0.11	0.30	-0.35	-0.01	0.60	0.31	0.11	-0.02
FC	0.02	0.02	-0.04	0.04	-0.12	0.00	0.11	**0.87****	-0.22	0.03
LC	0.27	0.12	0.05	**0.86****	-0.09	0.18	0.06	-0.18	0.09	-0.05

**Significant factor loadings (considered values above 0.63)

The second factor with 11.2% of total variance included significant positive parameters of the inner bract length, inner bract width, outer bract width and number of ray florets per capitulum. Characters such as internodes distance, plant height, number of inner bracts and number of outer bracts were existed in the third factor contributing to 10.2% of the overall variance. The fourth factor with 8.5% of the total variance included three parameters of number of inflorescence heads (capitula), inflorescence width and leaf color.

Cluster analysis

In order to reveal relationships among the populations of *A. biebersteinii*, a cluster analysis was performed based on 10 factors in this study. All populations at approximately a distance of 10 out of 25 were grouped into 10 main branches (Figure 2).

Group A: This branch was divided into two sub-clusters consisting of five populations, Adag, Akhlamad, Piranshahr, Azghad and Goojgi. These populations had similarity in some characters such as number of leaves and outer bract width. Members of the first group had the highest value for these parameters than other populations.

Group B: This cluster contained only one member namely Mohammadshahr. This population has highest values for leaf length/width ratio and number of inner bracts. Also, population of Mohammadshahr have lowest value for number of inflorescence man rays, pedicel diameter, disc floret length, ray floret length and essential oil content among others.

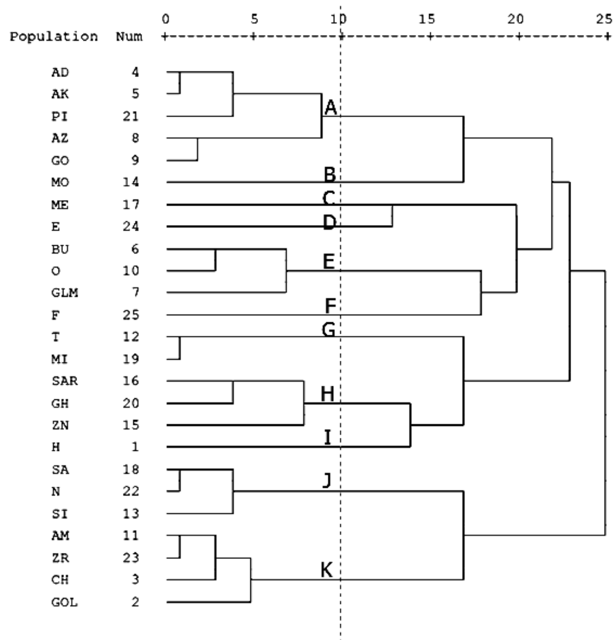

Figure 2. Dendrogram of the similarities among the 25 Iranian wild populations of *A.biebersteinii* , using Wards method based on 10 factors.

Group C: This branch only include one member namely Meshkinshahr population. This population has highest value for number of inflorescence main rays and pedicel diameter. length of head peduncle and number of inner bracts were lowest in this population.

Group D: This branch was consisted of population of Eberoo only. This population has utmost value for peduncle length, inflorescence length, length of bract (outside at the base of inflorescence) and ray floret length, which caused the population to be classified into separate cluster.

Group E: This branch was divided into two sub-clusters including three populations of Buzhan, Ortokand and Golmakan. These populations had a similar shoot diameter (near inflorescence), internodes distance, inner bract length, inner bract width, number of ray florets per capitulum and peduncle diameter in comparison to the other populations.

Group F: This cluster only consist of one member namely Firooz Abad. This population has the highest values for leaf length and essential oil content. The lowest value for shoot diameter (near inflorescence), plant height, inner bract length, outer bract length, capitulum length and number of main was also observed for this population in this study.

Group G: This cluster comprises two populations including Tangehgol and Mishoodagh. Members of this group contained leaves with high degree of pubescence. Also, this group has the lowest value of leaf length, number of inflorescence heads (capitula), peduncle length and inflorescence length.

Group H: This branch divided into two sub-clusters, in which the first sub-cluster consisted of two populations (Sardabe and Ghasemloo), while second sub-cluster was made of Zanjan population. These plants were similar in terms of capitulum width, capitulum length and number of inner bracts.

Group I: Only includes population from Havar. This population was among the populations whit the highest leaf length/width ratios, plant height, outer bract length, number of outer bracts and disc floret length.

Group J: This branch was divided into two sub-clusters including three populations of Sati, Nenor and Siahbishe. They were similar from the points of number of inflorescence heads and distance of inflorescence from the upest leaf.

Group K: This cluster divided into two sub-clusters, the first sub-cluster consisted of three populations of Aman abad, Zaribar and Chelmir, and the Second sub-cluster included population of Golool. The common features of these plants were some qualitative parameters such as degree of leaf pubescence, stem color and flower color.

Discussion

It has widely been accepted that there is a correlation between environmental conditions and morphological variation of a certain plant species, and that species that can occupy a wide range of habitats are more variable in morphology than species with a narrow range of habitats (Baker, 1974; Sultan, 2001; Richards and Pennings, 2005). Morphological variation also is representative of the adaptability of populations to different habitat, species that has a greater morphological variation would be more adaptive to environment than species with a small morphological variation (Pang and Jiang, 1995).

Achillea species have a wide range of distribution in Iran. They differ widely in morphology, phenology, flowering and fruiting patterns (Mozaffarian, 2008). Some of which have significant morphological differentiation within them. It has been shown that there is a close relationship between the leaf area and there essential oil content; this parameters was reported to vary in a great degree among different populations of some *Achillea* species growing wild in different port of Iran (Rahimmalek et al., 2009).

Results of present study showed that there is a high morphological variation within populations of *A. biebersteiini* collected from different parts of Iran. As shown in Fig. 2 populations of Adag and Akhlamad, Tangehgol and Mishoodagh, Sati and Nenor, Aman Abad and Zaribar were the most similar than other studied populations and, in country, populations of Adag and Golool have the least similarity. The population of Firooz Abad has the lowest similarity with others, which has different mean value for many characteristics than other populations such as the highest values for leaf length and essential oil content, Also it has lowest value for shoot diameter (near inflorescence), plant height, inner bract length, outer bract length, capitulum length and number of main stem.

In conclusion, morphological diversity is the observable physical variation present in populations and includes both genotypic and environmental components (Schlichting and Levin, 1984; Tulig and Clark, 2000; Yeater et al., 2004). Genotypic variability is the component of variation that is due to the genotypic differences among individuals within a population or among populations within a species (Humphreys, 1991; Loos, 1993). Although morphology cannot be directly related to genotype, it has a strong genotypic basis. Therefore, morphological characters can be used as a measure of genetic variations between populations (Schlichting, 2002). In total, we observed in present study that the studied populations of *A.bieberstenii* are diverse and variations between them are high. Therefore, selection of suitable traits for use in breeding programs of this plant is possible.

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