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Original Article

Assessment of Genetic Diversity among Wild Populations of *Achillea bieberstenii* Afan. Using Agro-morphological and Germination Traits

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Abstract

There is substantial diversity in *Achillea biebersteinii* plants in Iran. It has considerable different biological activities including antibacterial, antifungal, antioxidant, insecticidal, herbicidal and wound healing. Research improvement for medicinal plants depends on the available genetic diversity. The assessment of genetic variability could increase the information for breeding programs. To evaluate possible diversity among different populations of *A. biebersteinii,* agro-morphological and germination traits of the populations were investigated. To evaluate agro-morphological traits, seeds of 22 populations were sown in the field. The experiment was laid out using Randomized Complete Block Design (RCBD) with three replications. A total of six agro-morphological traits including plant height, plant diameter, lateral shoot number, flower diameter, flower number and shoot yield, were collected. For evaluation of seed germination traits, seeds of 10 populations were sown in standard condition ((20±2 °C) and pre-cold treatment (7 days at 4 °C followed by standard condition). The seed germination traits were studied as hypocotyl length, radicle length, radicle to hypocotyl length ratio, seedling length, germination percentage, germination rate and vigor index. Based on the results the population 20187 (Golestan) had the highest value for all of agro-morphological traits. For germination traits the populations 10140 (Minoodasht) and 17310 (Shahrood) were the best performing populations. The result showed that pre cold treatment reduced radicle length in most cases but population 17310 (Shahrood) produced significantly longer radicle under pre-cold treatment. This population was recommended for dry cold regions.

Key word: *Achillea biebersteinii,* Agro-morphology, Germination, Cold treatment

Introduction

Achillea L. (Yarrow) is a perennial herb and well-known medicinal plant, widely used in folk medicine as appetizer, wound healer, diuretic, carminative or menstrual regulator, anti fever, anti-inflammatory, asthma, bronchitis, cough reliever and heart cellular energy metabolism [1,2].

These plants are native to Europe and western Asia but are also found in Australia, New Zealand, and North America. *Achillea* L. comprises more than 100 species worldwide. This genus is classified in Asteraceae family and Anthemideae sub-family**.** Nineteen species of *Achillea* have been recognized in Iran of which seven are endemic [3]. *A. biebersteinii* Afan. which belongs to Section Filipendulinae (D.C.) Boiss. is a perennial villous herb with 10-100 cm height, simple or branched from the base and radiate yellow heads which are borne in large dense compound corymbs (a flat-topped inflorescence: one in which the flower stalks arise at different levels on the main axis and reach about the same height on the erect stems) [3].

Iran is one of the important origins of the genus *Achillea* and there is substantial diversity in *A. biebersteinii* plants in the country. Many researchers reported the biological activities of volatile oil and extract of *A. biebersteinii* [4-8]. The result of these studies showed, *A. biebersteinii* had considerable different biological activities including antibacterial, antifungal, antioxidant, insecticidal, herbicidal and wound healing. Some *Achillea*

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species including *A. bieberstinii,* had been applied for ornamental purposes because of their large beautiful flowers and/or relatively high drought tolerance [9-13].

Morphological variability is a characteristic of all organisms and one of the basic characteristics in the world. The intra-specific variation in plants characteristics is usually regarded as the adaptive mechanism to different environments [14]. Measurement, description and analysis of variations are fundamental steps to answer questions of biological adaptability [15]. Negative ecological impacts of climate change, over-harvesting for traditional medicinal uses, rough grazing and using rangelands for agricultural purposes, have all resulted in restriction of the number of members of many plant species [16] and genetic erosion [17]. Research improvement for medicinal plants depends on the available genetic diversity. In the absence of knowledge about genetic diversity, selection of elite genotypes for different planting conditions or high bioactive compounds is not possible. Furthermore, drought is considered as a major problem for Iranian germplasms. This problem is more dramatic for non-domesticated medicinal plants which are grown extensively in nature i.e. *Achillea* spp. So, the assessment of genetic variability could increase the information for breeding programs and endangered condition of some species to develop new cultivars and conservation strategies.

There are many reports related to evaluating genetic diversity of *Achillea* based on molecular markers but researches based on agro-morphological traits are few. Rahimmalek *et al.* [18] had studied inter- and intra-genetic diversity of *Achillea* species using amplified fragment length polymorphism markers. Gharibi *et al.* [19] applied ISSR markers to study genetic variation in two sub-species of *A. millefolium*. Mirahmadi *et al.* [20] conducted a comparative study on chemical constituents of essential oils from different populations of *A. biebersteinii*. Mirahmadi *et al.* [21] evaluated genetic diversity among some wild populations of *A. bieberstenii* using morphological and agronomical traits. Shariati *et al.* [22] investigated the effect of dormancy breaking treatments on *A. millefolium* and Kaye *et al.* [23] reported that *A. millefolium* seed germination decreased under dark condition.

The aim of the present study was to expand the knowledge on morphological, agronomical and germination characteristics of *A. biebersteinii* populations, collected from different parts of Iran, in order to domesticate the species and achievement of promising populations with potential use as initial materials for breeding programs. This information will facilitate efficient breeding programs for better yielding adaptive verities to promote better environment with secondary product resource.

Materials and Methods

Seed collection and experiment layout

Seeds of *A. biebersteinii* populations from their natural habitats in different parts of north, northwest and central provinces of Iran (13 provinces) were collected (Table 1).

22 populations were tested for Agro-morphological traits**.** The experiment conducted in Alborz research complex with moderate semi-arid climate, mean annual precipitation 248 mm, mean annual maximum temperature 44 °C, mean annual relative humidity 40-50% and loamy soil with the PH level of 7.5-8.5. A total of 30 seedlings for each population were grown in jiffy pots forty days before transplanting in the field in March 2010. The field trial was arranged in a Randomized Complete Block (RCBD) design with three replications. Each plot included 36 plants (spaced 40 x 40 cm). The field was irrigated twice a week during summer.

In laboratory experiments, 10 populations were tested for germination characteristics in standard condition (20±2 °C) and pre-cold treatment (7 days at 4 °C followed by standard condition). The normal ISTA (1993) laboratory germination test procedure was used with three replications. 150 seeds of each population were sterilized with 70% ethyl alcohol for five minutes, and then washed with distilled water. Three replicates of 50 sterilized seed were placed in Petri dishes on double Whatman papers (TP). The samples were kept in germinator at 20±2 °C with 1000 lux light for 15 days. In the pre-cold treatment condition, the samples incubated at 4 °C for 7 days before transferring to standard condition (20±2 °C). A factorial experimental design of 10 populations and 2 treatments with 3 replications was used for germination laboratory trial.

Agro-morphological traits

During the two-year investigation (2010 and 2011), A total of six agro-morphological traits, including plant height , plant diameter , lateral shoot number (it should be mentioned that some of the plant produced lateral shoots but most of them didn't) , flower diameter , flower number and shoot yield , were recorded. The plant height was measured from the ground level to the top of the plants, the diameter of the largest corymb (special type of inflorescence in *Achillea* genous) was measured as flower diameter. The fresh yield was measured for 5 spaced plants from each experimental unit and expressed as gr. per plant. Similarly, the agro-morphological data were collected and averaged from 5 randomly selected plants from each plot. The data presented in this study are average values over two years.

Germination traits

The seed germination traits were hypocotyl length, radicle length, radicle to hypocotyl length ratio, seedling length, germination percentage, germination rate and vigor index. Number of germinated seed was recorded at 3, 6, 9, 12 and 15 days. Hypocotyl and radical length of 10 randomly-selected seedlings (15-day old) from each Petri dish were measured. Seed vigor index was estimated by multiplying germination percentage and seedling length [24].

Data analysis

Analysis of variance was computed on collected data for each agro-morphological and germination traits. Means comparisons were made using Duncan's Multiple Range Test (DMRT) and phenotypic correlation coefficients between traits were estimated using the SAS9.1 software. The Euclidean distances of populations were computed on phenotypic and germination traits and then they were used for the UPGMA cluster analysis method using Minitab 16.0 Software.

Results

Agro-morphological traits

Result of ANOVA suggested significant differences among 22 wild populations of *A. bieberstinii* for all the six phenotypic traits. Moderate to low CV values were obtained for all traits (Table 2). The result of Duncan's Multiple Range Test (DMRT)

Table 1 Collecting region and Geographic information of different *A. biebersteinii* populations.

Populations	Province	City	Latitude(N)	Longitude(E)	Altitude (m)
4280	Ghom	Jafarabad	-	-	-
8818	Lorestan	Khoramabad	33°28' 00"	48°36'00"	1720
9530	kordestan	Marivan	35°32'11"	46°08'36"	1348
9914	Esfahan	Fereydoon Shahr	32°57'00"	50°06'00"	2550
10075	Hamadan	Malayer	34°08'58"	49°01'02"	1990
10140	Golestan	Minoodasht	37°20'00"	56°02'00"	1120
10619	Golestan	Kordkoy	36°40'10"	54°07'33"	2200
12391	Ardabil	Ardabil	38°29'62"	47°57'09"	1327
13781	Golestan	Gorgan	36°43'07"	54°35'01"	865
13866	Golestan	Kordkoy	36°40'10"	54°07'33"	2200
14831	Markazy	Arak	-	-	-
15926	Yazd	Shahediyeh	-	-	12
16278	Golestan	Maravetapeh	37°46'44"	55°56'14"	618
17310	Semnan	Shahrood	37°13'00"	55°43'00"	1550
18406	Golestan	Kordkoy	36°40'9"	54°07'32"	1450
18412	Golestan	Kordkoy	36°40'10"	54°07'33"	2203
18478	Golestan	Minoodasht	37°20'00"	56°02'00"	837
19494	West-Az	Malakan	37°01'53"	46°16'45"	1372
19542	East-Az	-	-	-	-
20187	Golestan	-	36°40'10"	54°07'33"	10
21172	Gilan	Syahkal	36°52'09"	49°52'50"	1423
21173	Gilan	Syahkal	36°52'86"	50°00'36"	1453
21175	Gilan	Rostamabad	36°54'09"	49°27'47"	515
21604	Semnan	Semnan	36°04'02"	53°35'82"	2031
21693	Semnan	Shahrood	37°18'37"	55°49'39"	1362
22801	East-Az	Chaldoran	38°39'49"	44°41'91"	2163
22825	East-Az	Salmas	38°09'57"	44°30'41"	2323

(Table 3) showed that there were significant differences among the genotypes for all of the traits. Based on the results population 20187 (Golestan) had the highest value for all of the investigated agro-morphological traits, population 22825 (Salmas), also had higher mean values for flower number, plant diameter, and flower diameter. The lowest mean values for all of the traits were observed in 9530 (Marivan).

The 22 populations were clustered based on agro-morphological traits, dendrogram was created following Ward's method [25]. It produced three major clusters showing distance within the genotype by forming clusters with more homogenous group (Fig. 1). Cluster 1 contained populations 9530, 9914, 13781, 10075, 21173, 10140, 21604, 16278. Cluster 2 contained populations 15926, 21172, 20187 and cluster 3 contained populations 1016, 12391, 21693, 18406, 19494, 13866, 14831, 22801, 18412, 19542, 22825.

Means comparison for agro-morphological traits in three cluster of *A. bieberstenii* are shown in Table 4. Results showed that the cluster 2 had the highest values for all trait and the lowest values were observed in cluster 1.

Results of correlation analysis showed the significant correlations among some important characters (Table 5). Plant height was positively correlated with shoot yield and flower diameter ($P<0.05$). Flower number had significant positive correlation with lateral shoot number and plant diameter but non-significant positive correlation with shoot yield and flower diameter.

Germination traits

For identification of germination differences of 10 populations of *A. bieberstinii* 8 germination traits data were collected under pre-cold treatment (4 °C for 7 days) and control (20±2 °C) conditions.

Table 2 Results from the ANOVA on six Agro-morphological traits

Source of variation	Df	MS Plant Diameter	Plant Height	Flower Number	Flower Diameter	Lateral Shoot Number	Shoot Yield
Populations	21	1037.7**	921.93**	702.12*	6.09**	215.32*	110951**
rep	2	319.47*	123.43^{ns}	218.2*	0.33^{ns}	15.51^{ns}	1773.86^{ns}
Error	44	97.55	46.313	69.26	0.49	40.48	8420.5
C.V.		15.3	11.02	19.48	9.53	25.03	22.06

**Significant at 1% probability levels * Significant at 5% probability levels

Table 3 Means of the agro-morphological traits of 22 populations of *A. bieberstenii*

Populations Name	Plant Height (mm)	Plant diameter (mm)	Flower number	Flower diameter (mm)	Shoot Yield (gr/p)	Lateral Shoot number
9530	39.30 g	33.8 k	13.0 f	3.23 g	204.2 h	-
9914	51.40 efg	45.7 ijk	19.0 ef	6.75 ef	285.6 fgh	16.53 b
10075	64.70 dc	55.1 g-j	24.7 def	8.67 ab	212.5 gh	24.73 ab
10140	53.50 def	55.2 g-j	26.7 def	9.14 a	525.0 bc	32.92 a
10619	48.30 fg	60.3 f-i	47.6 abc	6.55 ef	213.9 gh	-
12391	49.20 gf	64. 7 d-h	54.3 ab	6.93 def	389.4 c-g	-
13781	74.50 c	42.9 ijk	17.8 f	5.73 f	241.9 fgh	17.80 b
13866	58.10 def	79.6 b-e	59.9 ab	7.47 b-e	523.6 bc	-
14831	57.70 def	84.3 abc	55.4 ab	6.80 ef	465.3 b-e	-
15926	100.9 ab	71.6 c-g	48.1 abc	9.53 a	351.4 c-h	-
16278	64.49 c-d	44.7 ijk	50. 7ab	8.60 ab	611.1b	-
18406	50.59 fg	60.9 e-i	44.4 bc	6.93 def	509.7 bcd	-
18412	52.50 def	79.3 b-e	61.6 a	6.20 ef	387.5 c-g	-
19494	58.35 def	67.3 c-g	51.6 ab	7.20 cde	520.8 bcd	-
19542	57.55 def	80.0 bcd	54.3 a	6.20 ef	343.1 e-h	-
20187	106.15 a	95.9 ab	54.00 a	8.73 ab	1116.7 a	-
21172	92.60 b	76.6 c-f	35.1dc	8.25 a-d	548.3 bc	35.08 a
21173	65.50 cd	36.7 jk	34.4 cde	8.33 abc	347.2 c-h	-
21604	45.80 fg	47.1 ijk	26.8 def	8.25 a-d	422.2 cde	-
21693	46.30 fg	58.9 f-h	51.4 ab	6.65 ef	322.8 e-h	-
22801	64.50 cde	77.7 b-f	57.5 ab	7.20 cde	419.4 c-f	-
22825	57.10 def	101.2 a	57.1ab	8.27 a-d	376.4 c-h	-

The means of column with the same letters were not significantly different based on DMRT $P<0.05$

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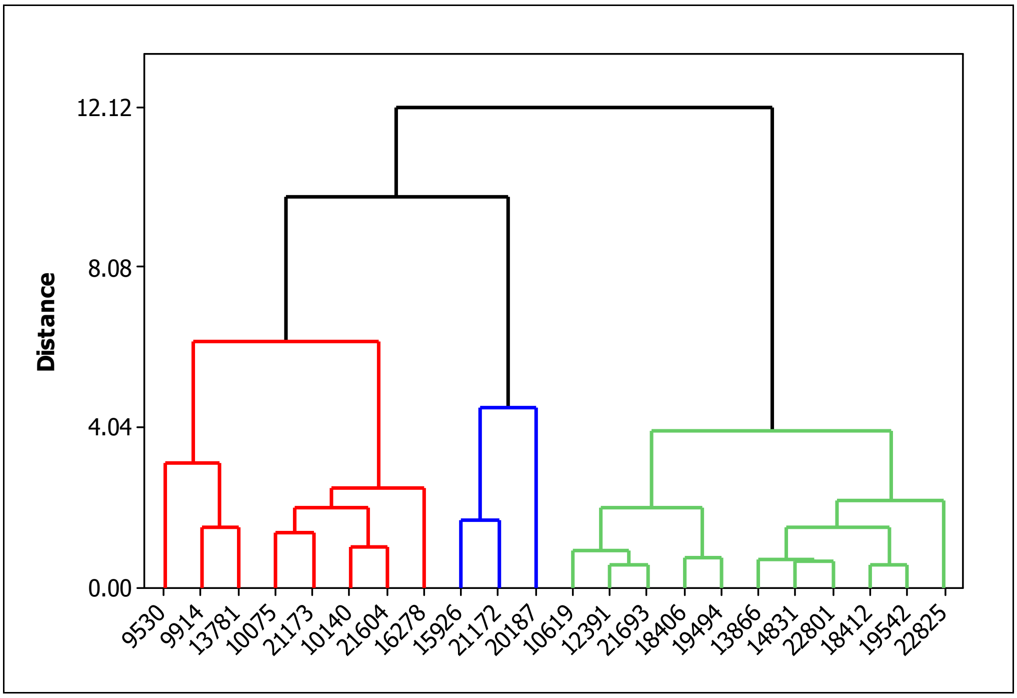

Fig. 1 Denderogram of the 22 wild populations of *A. bieberstenii* based on six agro-morphological traits.

Table 4 Means comparison for agro-morphological traits in three cluster of *A. bieberstenii*

Cluster No	Plant Height	Plant Diameter	Flower Number	Flower Diameter	Shoot Yield
Cluster 1(n=8)	57.4b	45.1b	26.6b	7.34ab	356b
Cluster 2(n=3)	99.9a	81.4a	45.7a	8.84a	672a
Cluster 3(n=11)	54.5b	74.0a	54.1a	6.95b	407ab

The means of cluster with the same letters were not significantly different based on DMRT P<0.05

Table 5 Pearson correlation analysis for the relationships between phenotypic parameters of 22 wild populations of *A. bieberstenii*

Traits	Plant Diameter	Plant Height	Flower number	Flower Diameter	Lateral Shoot number
Plant height	0.368		-	-	-
Flower number	0.758**	0.026	-	-	-
Flower diameter	0.246	0.536**	0.143	-	-
Lateral shoot number	0.860	0.401	0.932*	0.781**	-
Shoot Yield	0.383	0.481*	0.323	0.352	0.847**

**Significant at 1% probability levels * Significant at 5% probability levels different

ANOVA suggested significant differences among populations of *A. bieberstinii* for all of the seven germination traits. A low to moderate CV values were obtained for all traits (Table 6). Among the studied populations, variation for traits related to germination observed and populations exhibiting adequate performance for investigated traits were identified. The result of Duncan's Multiple Range Test (DMRT) (Table 6) showed that there were significant differences among the genotypes in all traits and it revealed that populations 10140 and 17310 collected from Minoodasht and Shahrood respectively were the best performing populations considering seed germination (Table 7). 17310 showed the maximum amount for all of the traits except hypocotyl length and 10140 had high values of germination rate, germination percentage, radicle length and vigor index. Results of overall means comparison for germination traits of 10 *A. bieberstenii* populations under pre-cold and control condition are shown in Table 8. In general, cold treatment induced a decrease in radicle length and radicle/hypocotyl length ratio.

Cold treatment did not show any significant effect on populations germination percentage, only germination percentage of 15926 (57.33) was significantly lower under control condition (Table 9). Cold treatment had negative effect on *A. bieberstinii* radicle length in many cases, but some populations responded differently i.e. radicle length of 17310, 21172, 21175 and 19542 was longer than control. Germination rate of most of the populations decreased under pre-cold treatment, except 10140 (16.45) and 21172 (12.18), germination rate of these two populations was very low under control condition; (germination rate of 21172 was 6.31 which was the lowest germination rate under control condition).

For grouping of 10 *A. bieberstinii* populations using data of seven germination traits, dendrogram was created (Fig. 2). It produced two major clusters. Means comparison of germination traits by cluster are shown in Table 10. Results showed that the cluster 2 had significant higher values for all traits except germination rate and percentage.

Discussion

Agro-morphological traits

Features of Achillea make it easy to adapt as a crop. Since they are perennial, the plants grow back after harvesting of above-ground parts. Some species of Achillea including *A. bieberstinii* has ornamental values, and some species are recommended as a groundcover to control soil erosion on slopes and hillsides. The capacity of *A. bieberstinii* and those species which spread by rhizomes make them valuable for this application.

Table 6 Results from the ANOVA of 10 Populations of *A. bieberstinii* for 7 germination traits under pre-cold (4 °C for 7 days followed by control condition) and control condition (20±2 °C).

Source of Variation	df	MS						
		Germination rate	Germination percentage	Radicle Length (mm)	Hypocotyl Length (mm)	Seedling Length (mm)	Vigour index	Radicle/ hypocotyle length ratio
Populations (P)	9	51.97**	407.26**	27.11**	39.36**	47.13**	40.82**	2.38**
Treatment (T)	1	2.18ns	290.4*	19.49**	2.053^{ns}	8.8935^{ns}	0.00216^{ns}	0.15*
P xT	9	43.62**	84.18^{ns}	3.67**	1.42^{ns}	3.75^{ns}	4.89^{ns}	0.26**
Error	40	1.48	60.8	1.08	1.64	4.14	4.15	0.032
C.V.		12.4	8.8	15.4	16.95	14.2	16.17	17.09

**Significant at 1% probability levels * Significant at 5% probability levels.

Table 7 Means comparison of the germination traits of 10 populations of *A. bieberstenii*.

Populations name	Germination Rate	Germination percentage	Radicle Length (mm)	Hypocotyl length (mm)	Seedling length (mm)	Vigor Index	Radicle / hypocotyl length ratio
10140	10.82 abc	88.7 ab	6.13 c	13.60 a	19.73 a	17.51 a	0.466 e
15926	1.90 d	68.0 c	9.73 a	6.75 bc	16.48 b	11.25 b	1.442 b
17310	12.37 a	94.7 a	9.87 a	6.65 bc	16.52 b	15.61 a	1.483 b
19494	10.70 bc	94.7 a	8.50 b	8.07 b	16.57 b	15.67 a	1.092 c
19542	11.00 abc	92.7 ab	7.97 b	3.30 d	11.27 c	10.47 b	2.501 a
21172	9.58 c	88.7 ab	4.88 cd	8.23 b	13.12 c	11.61 b	0.591 de
21173	9.57 c	83.3 b	5.72 c	7.58 bc	13.30 c	11.06 b	0.779 d
21175	11.89 ab	89.3 ab	4.27 d	7.58 bc	11.85 c	10.59 b	0.564 de
21604	9.57 c	86.7 ab	6.18 c	6.10 c	12.28 c	10.56 b	1.018 c
22801	10.85 abc	96.68 a	4.28 d	7.85 b	12.13 c	11.73 b	0.547 e

The means of populations (column) with the same letters were not significantly different based on DMRT P<0.05

Table 8 Overall Means of the germination traits of *A. bieberstenii* populations under pre-cold and control condition.

Treatment	Germination Rate	Germination percentage	Radicle Length (mm)	Hypocotyl length (mm)	Seedling length (mm)	Vigour Index	Radicle / hypocotyl length ratio
Cold	9.630 a	90.53a	6.18 b	7.76 a	13.94 a	12.61 a	0.996 b
Control	10.01a	86.13b	7.32 a	7.39 a	14.71 a	12.60 a	1.096 a

The means of treatments (column) with the same letters were not significantly different based on DMRT P<0.05
Control = 20±2 °C, Cold=4 °C for 7 days followed by control condition,

Table 9 Means comparison of the germination traits of 10 genotypes of *A. bieberstenii* under pre-cold treatment and control condition

Population	Germination Rate		Germination Percentage		Radicle Length (mm)		Hypocotyl Length (mm)		Seedling Length (mm)		Vigor Index		Radicle /Hypocotyl Length ratio	
	Control	Cold	Control	Cold	Control	Cold	Control	Cold	Control	Cold	Control	Cold	Control	Cold
10140	5.18 d	16.4 a	82.7 a	94.7a	7.53 bc	4.7 def	12.9 a	14.3a	20.4 a	19.1 a	16.96 a	18.06 a	0.60 d	0.33 e
15926	2.31 e	1.48l f	57.3 b	78.7b	9.87 ab	9.6 a	6.43 bc	7.07b	16.3 abc	16.7 bc	9.40 c	13.1 bcd	1.52 b	1.36 b
17310	13.81 a	10.93 bc	93.3 a	96 a	11.6 a	8.7 ab	7.07 b	6.23de	18.1 ab	14.9 b-f	16.87 a	14.4 bc	1.56 b	1.40b
19494	14.11 a	7.28 e	97.3 a	92 ab	9.33 abc	7.7 c	6.93 b	9.2 b	16.3 abc	16.9 b	15.78 ab	15.5 ab	1.35 bc	0.83 cd
19542	11.91 ab	10.1 cd	90.7 a	94.7a	7.80 cb	8.2 bc	3.80 c	2.8 f	11.5 c	10.97f	10.50 c	10.4 de	2.04 a	2.96 a
21172	6.31 d	12.8 de	89.3 a	88 ab	4.43 de	5.3 de	8.07 b	8.4 bc	12.5 c	13.7 de	11.10 c	12.1 cde	0.53 d	0.63 cde
21173	9.20 c	9.95 cd	81.3 a	85.3ab	7.67 cb	3.8 fg	7.27 b	7.9 cd	14.9 bc	11.7 f	12.80 cb	9.94 e	1.08 c	0.48 de
21175	13.2 ab	10.5 c	89.3 a	89.3ab	4.00 e	4.5 efg	7.40 b	7.77 cd	11.4 c	12.3 ef	10.20 c	10.9 cde	0.54 d	0.58 cde
21604	11.17 cb	7.96 de	84.0 a	89.3ab	6.77 cd	5.6 d	6.03 bc	6.17 e	12.8 c	11.7 f	10.60 c	10.5 de	1.12 c	0.91 c
22801	12.89 ab	8.81 de	96.0 a	97.3a	4.87 de	3.7 g	7.97 b	7.73 cd	12.8 c	11.4 f	12.30 cb	11.1 de	0.61 d	0.48 de

Means with the same letter within the same column are not significantly different. Control = 20±2 °C, cold=4 °C for 7 days followed by control condition,

Table 10 Means comparison for germination traits in two cluster of *A. bieberstenii*

Cluster No	Germination Rate	Germination Percentage	Radicle Length(mm)	Hypocotyl Length(mm)	Seedling Length(mm)	Vigor Index	Radicle /hypocotyl Length ratio
Cluster 1 (n=5)	9.4 a	87.7 a	8.4 a	7.7 a	16.1a	14.1 a	1.4 a
Cluster 2 (n=5)	10.3 a	88.9 a	5.1 b	6.0 a	12.6b	11.0 b	0.7 b

The means of cluster with the same letters were not significantly different based on DMRT P<0.05

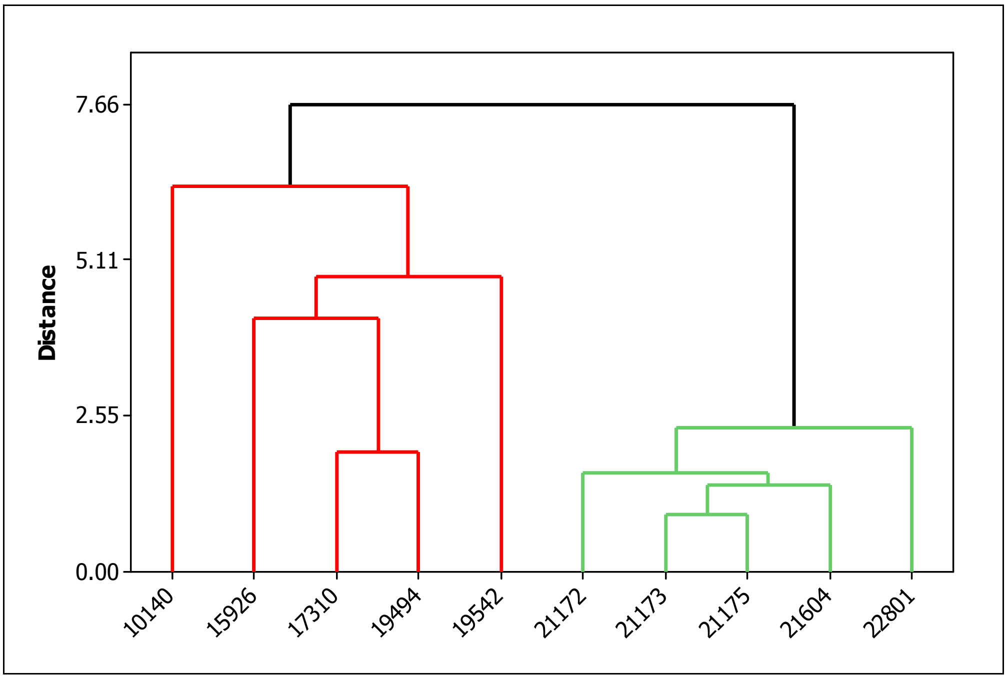

Fig. 2 Denderogram of the 10 wild populations of *A. bieberstenii* based on seven germination traits.

With its capability for regional adaptation and ability to grow in poor soils of various moisture regimes, *Achillea* is a relatively undemanding plant that can be grown throughout much of Iran. *Achillea* species had a wide range of distribution in Iran. They differ widely in morphology, phenology, flowering and fruiting patterns [26]. It has widely been accepted that species that has a greater morphological variation would be more adaptive to environment than species with a small morphological variation [27-30]. 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 [31]. This was supported by Gharibi *et al.* [19] report; they investigated genetic diversity of 40 accessions from eight populations of *A. millefolium* and concluded that morphological analysis in most cases corresponded to those obtained through molecular analyses.

The overall result of the agro-morphological traits shows that the studied populations of *A. bieberstenii* are diverse; therefore selection of suitable traits for breeding programs is possible. Our results had the similarity with the observation of Mirahmadi *et al.* [21] and Gharibi *et al.* [19]. Flowers are the main source of *Achillea* essential oil [18, 32] therefore 18412 and 13866 both collected from Kordkoy were promising populations for higher amount of essential oils. *A. bieberstenii* also had ornamental potential [33] therefore those populations with larger flowers and higher flower number i.e. 22825, 16278 and 20187 collected from Salmas, Maraveh-tapeh and Golestan respectively, are good selections for developing low maintenance ornamental varieties. Result showed that some important traits were positively correlated (Table 5) i.e. plant diameter and flower number also plant height and flower diameter; this means that populations that produce larger plants in diameter, produce more flowers and taller populations produce larger flowers.

Means comparison for agro-morphological traits in three clusters is shown in Table 4. Although cluster 2 showed to have higher values for all of the traits but cluster 3 has some useful characteristics such as shorter plants with many flowers. This makes it more suitable for landscaping and amount of essential oils of populations in this cluster might be more than cluster 2.

Germination traits

All of the populations showed high percentage of germinations under both control and pre-cold treatment this shows that this species does not need any specific treatment for germination. Our results is similar to the observation of Kay *et al.* [23] and Ghasemi Pirbalouti *et al.* [34] who reported that all of the treatments, including gibberellic acid and potassium nitrate, had no effect on the germination percentage of *Achillea* species. But not similar to

Shariati *et al.* [22] who reported that gibberellic acid and potassium nitrate increased *A. millefolum* germination percentage.
The result of analyses of variance (Table 6) showed the main effect of population was significant for all of the traits. The effect of pre-cold treatment was not significant on germination rate but the interaction between population x treatment was highly significant. On the other hand the effect of pre-cold treatment was significant on germination percentage but the interaction of population with pre-cold treatment was not significant. This indicates that population responded differently to pre-cold treatment considering germination rate but germination percentage of different populations were similar under pre-cold treatment, Table 7 shows germination percentage similarities and germination rate differences of the populations. Radicle length has important role in plant establishment, the correlation between radicle length and seedling establishment has been widely studied [35, 36]. Zu *et al.*[37] investigating effects of drought stresses on germination of *Pinus sylvestris* concluded that under drought stress condition, the root develops faster than the hypocotyls to acclimatize the drought stress. Therefore, the growth of radicle should reflect the adaptability of plant to drought stress; hence it can be concluded that in our study those populations with higher germination rates and longer radicel under pre-cold treatment can establish faster and are suitable populations for cold dry regions. The reduction of radicle growth under low- temperature condition observed in the present study (Table 8) was also observed by other authors [38,39]. Germination percentage was not affected by pre-cold treatment and all of the populations had high germination percentage under both pre-cold treatment and control condition (Table 9) this was also observed by other authors [22]. Hypocotyl length was not much affected by pre-cold treatment so populations with longer radicle had higher values of radicle/hypocotyl length ratio and seedling length and vice versa. Population 10140 and 19494 are the only populations with longer hypocotyls under pre-cold treatment (Table 9) and they might start the autotroph stage earlier than the others, in cold environment.
Means comparison of germination traits by cluster (Table 10) revealed that the cluster 1 had significant higher values for all traits. The main reason that cluster 1 had higher values for germination traits is higher radicel length, in fact Cluster 1 obtained higher values for seedling length, radicle/hypocotyl length ratio and vigor index due to higher radicle length. According to ISTA (International Seed Testing Association) and AOSA (Association of Official Seed Analysts) procedures for seed vigor testing [40,41], radicle length is an important trait that affects seed vigor, and some researchers proved it in their experiments [42]. As a result it can be concluded that populations in cluster 1 had more vigorous seeds.
As the germination test is conducted in an optimum condition specific to different species, it is not always possible to get an idea of the performance of a seed lot in the field on the basis of germination test in the laboratory. In our experiments some populations with good performing seeds showed high values for agro-morphological traits as well, but some did not. Referring to Table 3 and 7, populations 15926, 19494 and 19542 showed high values for both germination and agro-morphological traits. They were within cluster 1 of germination traits and cluster 3 of agro-morphological traits. But population 10140 which showed high performance in germination traits, had low values for agro-morphological traits. They were dropped within cluster 1 of germination and agro-morphological traits (table 10 and 4).

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