INNSPUB

Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 7, No. 1, p. 403-412, 2015
http://www.innspub.net

RESEARCH PAPER OPEN ACCESS

Evaluation of genetic diversity of *Anthemis haussknechtii* and *Anthemis altissima* populations through agro-morphological characteristics

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Article published on July 26, 2015

Key words: *Anthemis haussknechtii*, *Anthemis altissima*, Genetic diversity, Population, Agro-morphological traits.

Abstract

Anthemis altissima L. and *Anthemis haussknechtii* are two valuable medicinal plants that antioxidant and antibacterial activity of their essential oil is reported. Most of the medicinal plants are harvested from wild resources. Introducing high yielding varieties could reduce pressure on natural resources and prevent genetic loss. Genetic diversity is the foundation of the improvement of crops. The goal of this investigation is to determine the extent of variability existing among 21 *A. haussknechtii* and 5 *A. altissima* populations through agro-morphological characteristics. A total of 8 agro-morphological traits namely, height, flower number, fresh weight, dry weight, percentage of dry matter, plant canopy, GDD(Growing Degree Day) to flowering and GDD to maturity were recorded. Although 5 population of *A. altissima* were collected from 5 different provinces result of ANOVA and Duncan's Multiple Range Test showed low diversity among these populations. *A. altissima* Populations had larger plants with more flowers. According to principal component analysis, the two first components explained 82% of total variability of the data in *A. haussknechtii*. Results of correlation analysis showed the significant correlations among most of the characters. The first component including plant canopy, height, fresh and dry weight, explained 67% of total variation. Populations of *A. haussknechtii* were classified into 4 clusters through hierarchical cluster analysis. Based on the result it was recommended that crosses could be made between cluster 1 and cluster 2 to get early matured variety with high flower yield. Wide range of variations among populations suggests good potential for selection in breeding programs.

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Introduction

As people search for alternative and herbal forms of medicine, and provided that there are no suitable synthetic substitutes for many of the compounds or difficulty in profiling and mimicking complex compound mixtures in the volatile oils, the original plant extracts will continue to be used long into the future. Meanwhile the demand for herbal plants as alternatives to synthetic drugs (due to the side effects that they may elicit) for treating numerous ailments, such as cancer, is an increasing global trend. Climate change, using rangelands for agricultural purposes, over-harvesting for traditional medicinal uses, heavy grazing and land degradation have all resulted in genetic loss (Han *et al.* 2007) and restriction of the number of members of medicinal plant species (Rahimmalek *et al.,* 2009). Producing high yielding adaptive verities will promote better environment with secondary product resources. For the effective improvement of medicinal plants, it is critical to understand the extent and distribution of genetic diversity within species. Evaluating agro-morphological characteristics is a reliable and easy way to assess genetic diversity.

The aim of present study was to find out agro-morphological variability among wild populations of two valuable medicinal plant; *Anthemis altissima* L. and *Anthemis haussknechtii* Boiss. & Reut., used as Chamomile in Iran. It is useful to know that different members of 4 genera from Asteraceae family; *Anthemis*, *Matricaria*, *Tripleurospermum* and *Tanacetum,* have been used as Chamomile (Babooneh) in Iran. Multitherapeutic, cosmetic, and nutritional values of Chamomile have been established through years of traditional and scientific use and research. *Anthemis* L. is one of the biggest genera of Asteracese family, this genus comprises of nearly 210 species (Bremer and Humphries, 1993; Bremer, 1994). There are 39 species of *Anthemis* in Iran among which 15 are endemic (Mozaffarian, 2008). Antibacterial and antioxidant activity of the essential oil obtained from the flowering parts of *A. altissima* and *A. haussknechtii* have been reported by different researchers (Samadi *et al.*, 2012; Konstantinopoulou *et al.*, 2003; Salehi *et al.*, 2008). Many Researchers interested in genetic diversity of *Anthemis* species in recent years, some of the research are based on molecular or biochemical markers (Tarighi and Salehi Shanjani, 2013; Oberprieder *et al.*, 2001) and some used the agro-morphological characteristics (Solouki *et al.*, 2008; Dandrea, 2002). Both *A. haussknechtii* and *A. altissima* had a wide range of distribution in Iran, it is anticipated that their populations show high diversity because, widespread species exhibit considerable phenotypic plasticity (Schlichting, 1986) and they must adapt to a broad range of environmental conditions (Hamrick and Godt, 1989). According to the literature this is the first time that wild populations of *A. altissima* and *A. haussknechtii* are being evaluated. The results of this study will expand the knowledge on, genetic variation of *A. altissima* and *A. haussknechtii* populations, for achievement of promising populations with potential use in breeding programs.

Materials and methods

Plant materials

A total of 5 *A. altissima* and 21 *A. haussknechtii* populations were included in the experiment for the evaluation of Agro-morphological traits during the spring and summer of 2012. The experimental material, collected from their natural habitats in different provinces of Iran, was obtained from natural resources gene bank at Research Institute of Forests and Rangelands , Tehran, Iran (Table 1 and 2).

Field planting and layout

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 early March. The field trial was arranged in a Randomized Complete Block design (RCBD) with three replications. Each plot included 36 plants (spaced 40 x 40 cm). The field was irrigated twice a week.

Characterization

A total of 8 agro-morphological traits namely, height, flower number, fresh weight, dry weight, percentage of dry matter, plant canopy, GDD to flowering (Growing Degree Day to first flower emergence) and GDD to maturity(half bloom) were recorded. 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 daily of GDD was calculated using the standard formula of degree day;

$$GDD = (T_{max} + T_{min}/2) - T_{base}$$

(T=temperature, T_{base}= 6 °C).

Statistical analysis

Analysis of variance was computed on collected data for each agro-morphological trait. Means comparisons were made using Duncan's Multiple Range Test (DMRT) and Pearson correlation coefficient was calculated for all agro-morphological variables using the SAS 9.1 software. Clustering was done following Ward's (1963) method using Minitab 16.0 Software. Principal Component Analysis (PCA) was carried out according to the usual way based on the covariance matrix.

Results

Anthemis haussknechtii

To identify the significant differences among traits of 21 *A. haussknechtii* populations 8 morphological data was collected and analysis of variance was performed (Table 3). The result of Duncan's Multiple Range Test (DMRT) showed that there were significant differences among the populations for all of the traits (Table 4). Based on the results population 13818 from Golestan had the highest value for all of the traits except GDD to flowering and GDD to maturity, population 10791 from Ilam showed the lowest mean values for plant canopy, height and flower number (Table 4). Population 17018 from kordestan, with the highest GDD to flowering and GDD to maturity, represents a late season population. Results of correlation analysis showed the significant correlations among most of the characters (Table 5). There was no correlation between flower number and dry matter percentage. GDD to flowering and GDD to maturity showed no correlation with the specified traits.

Table 1. The location of the sampled *Anthemis altissima* populations.

Population	Province	City	Altitude (m)	Longitude(E)	Latitude(N)
3119	Yazd	Shahediyeh	1220	54°16'00"	31°55'00"
8835	Lorestan	Khoramabad	1720	48°36'00"	33°28'00"
9885	kordestan	Sanandaj	1331	---	---
12790	Hamadan	Asadabad	---	46°58'51"	34/58/95
29610	Chaharmahal-Bakhtyary	Shahrekord	2095	50°55'56"	32°18'00"

Dendrogram was created following Ward (1963) and populations were represented into four main groups (Fig. 1). From total 21 *A. haussknechtii* populations, Cluster 1, 2, 3 and 4 contained 7, 1, 11 and 2 populations, respectively. The mean performance of all the characters in different clusters is presented in Table 6. Cluster 2 contained only one population which was a late season population (the highest GDD to flowering and GDD to maturity) with the highest amount for flower number. Populations in cluster 3 had the lowest amount for most of the specified traits. Populations 9021, 9483, 10058, 10791, 13472, 17021, 17886, 18050, 19545, 21296, 25962 and 25962 were in this cluster. Members of cluster 4 (populations 13818 from Golestan and 24761 from Ilam) showed the highest amount for all of the traits except GDD to maturity and flower number (Table 6).

Principal Component Analysis (PCA) was used as a data reduction technique to summarise the information of agro-morphological observations. Equations of every three first components have been shown in Table 7. The two first components explained 82% of total variability of the data.

Table 2. The location of the sampled *Anthemis haussknechtii* populations.

Population	Province	City	Altitude (m)	Longitude(E)	Latitude(N)
7517	Lorestan	Koohdasht	1130	47°27'00"	33°17'00"
9021	Lorestan	Koohdasht	1272	47°41'00"	33°33'00"
9483	Lorestan	Khoramabad	1350	47°51'00"	33°37'00"
9796	kordestan	Baneh	1610	46°06'53"	36°11'42"
10058	kordestan	Baneh	1900	46°05'03"	35°57'31"
10791	Ilam	Ivan	1120	---	---
13472	Ilam	Darehshahr	1000	---	---
13818	Golestan	Gorgan	2200	---	---
14923	Kermanshah	---	---	---	---
17018	kordestan	Sanandaj	2400	46°46'00"	35°29'00"
17021	kordestan	Sanandaj	2200	46°51'00"	35°28'00"
17886	Fars	---	---	---	---
17915	Fars	---	---	---	---
18050	West-Az*	Piranshahr	1949	45°30'77"	36°44'79"
19545	West-Az	---	---	---	---
21296	Ilam	---	1500	---	---
24761	Ilam	---	---	---	---
25962	kordestan	Saghez	1569	46°30'09"	36°04'56"
25976	kordestan	Qorveh	2195	47°49'55"	35°02'26"
26041	kordestan	Divandareh	1786	47°03'42"	35°49'43"
26044	kordestan	Divandareh	1841	47°15'10"	35°48'15"

West-Az =West Azarbyjan.

Table 3. Results from the ANOVA on 8 Agro-morphological traits of *Anthemis haussknechtii*.

Source of variation		ms							
	df	plant canopy	height	flower number	fresh weight	dry weight	dry matter percentage	GDD flowering	GDD maturity
population	20	2148780**	99.26**	1585.7**	86043**	15808**	25601**	4088.8**	87.63
rep	2	62203	11.75	1070.4*	34	166	528	4.9	3.343
Error	40	48707	4.83	266.6	223	1252	999	86.7	9.246

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

The first component explained 67% of total variation and includes plant canopy, height, fresh and dry weight. The second component explained 15% of total variation, GDD to flowering and GDD to maturity are main parts of this component. The third component describes only 0.09 % of the variation. On the graph being the result of PCA method, populations grouped the same as dendrogram, PCA divided the 21 *A. haussknechtii* populations into 4 groups (Fig. 2). Population 17018 from kordestan with the highest

GDD to flowering and maturity is well separated from the other pop and is located on one side of axis 2. Populations 13818 from Golestan and 24761 from Ilam with highest amount for most of the traits are separated from the other populations as well, and are located on right side of the axis 1(Fig. 2).

Table 4. Means of the agro-morphological traits of 22 populations of *Anthemis haussknechtii.*

Population	plant canopy (cm²)	height (cm)	flower number	fresh weight (gr)	dry weight (gr)	dry matter percentage	GDD Flowering (°C)	GDD Maturity (°C)
7517	728.2 bc	14.64 b-f	66.64 ab	90.73 b	22.2 b	25.01 c-f	560 f	1073 c
9021	340.8c	12.29 c-f	30.9 bc	27.07 b	7.73 c	28.74 b-f	973 bc	1073 c
9483	247.8 c	10.65 def	21.73 bc	23.87 b	6.53 c	27.41 b-f	973 bc	1073 c
9796	608.8 bc	17.2 bcd	53.98 abc	96.6 b	25.4 b	26.53 b-f	560 f	1015 c
10058	379.3 c	13.17 c-f	30 bc				973 bc	1073 c
10791	219.4 c	8.61 f	20.56 bc				1073	1220 b
13472	242.4 c	11.13 def	15.04 c	31.27 b	8.73 c	28 b-f	994 bc	1103 c
13818	2186 a	36.27 a	90.87 a	343.33 a	140 a	40.64 a	1015 b	1090 c
14923	646.8 bc	15.87 b-e	57.1 abc	83.53 b	24.87 b	29.57 b-e	663e	1025 c
17018	830.9 bc	21.25 b	93.28 a	77.3 b	18.3 b	23.9 def	1073 a	1362 a
17021	503 bc	14.96 b-f	37.74 ab	73.8 b	14.33 b	19.47 f	973 bc	1073 c
17886	173.2 c	9.35 ef	24.36 bc	78.13 b	12.6 b	19.58 f	973 bc	1073 c
17915	294.6 c	13.55 c-f	34.33 bc	30.07 b	9.47 c	31.15 bcd	560 f	1073 c
18050	321.9 c	14.94 b-f	30.73 bc	39.8 b	10.8 b	27.06 b-f	973 bc	1073 c
19545	452 c-g	15.05 b-f	54.52 abc	82.2 b	16.6 b	20.32 ef	973 bc	1073 c
21296	233.5 c	11.18 def	26.29 bc	31.33 b	7.8 c	28.36 b-f	962c	1073 c
24761	1126 b	18.47 bc	71.2 ab	340.33 a	120.3 a	35.24 ab	1015 b	1090 c
25962	296.1c	11.01 def	26.55 bc	25.27 b	8.6 c	34.38 abc	1013 b	1124 bc
25976	608.9 bc	14.63 b-f	59.53 abc	95.07 b	27.33 b	28.62 b-f	962 c	1073 c
26041	585.2 bc	16.61 bcd	63.14 abc	98.07 b	32.69 b	33.33 abc	780 d	1073 c
26044	445.4 bc	15.25 b-f	59.19 abc	68.4 b	19.27 b	28.16 b-f	962 c	1073 c

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

Table 5. Pearson's Correlation coefficients between agro-morphological traits of *Anthemis haussknechtii.*

	plant canopy	height	flower number	fresh weight	dry weight	dry matter percentage	GDD flowering
height	0.93**						
flower number	0.86**	0.81**					
fresh weight	0.93**	0.83**	0.71**				
dry weight	0.91**	0.81**	0.64**	0.99**			
dry matter percentage	0.54**	0.51*	0.3	0.54**	0.65**		
GDD flowering	-0.01	0.01	-0.13	0.07	0.11	-0.02	
GDD maturity	0.08	0.09	0.24	-0.08	-0.07	-0.1	0.48*

**,* Significant difference at 1% and 5% respectively.

Anthemis altissima

Result of ANOVA suggested significant differences among *Anthemis altissima* populations for flower number, fresh weight, dry weight and dry matter percentage (Table 8), but The result of Duncan's Multiple Range Test (DMRT) (Table 9) showed that there were no significant different between populations in most cases except plant canopy . Population 12790 from Hamadan had the highest flower number and population 3119 from Yazd had the highest amount for plant canopy. Population 8835 from Lorestan with the highest GDD to flowering represents a late season population.

Discussion

Result of ANOVA showed significant differences among the populations of *A. haussknechtii* for all agro-morphological traits. Solouki *et al.* (2008) and Khamooshy (2012) reported comparable results, showing a wide range of variation in *Anthemis* species populations for agro-morphological traits. Results showed low diversity in *A. altissima* populations. According to ANOVA and DMRT results (table 8) *A. altissima* populations showed low diversity. Although Wide range of distribution is one of a number of factors that help species to have high levels of genetic variation (Schlichting, 1986; Hamrick and Godt, 1989), *and A. altissima* populations were collected from 5 different provinces, they showed low diversity. May be this is the characteristic of the species, however this requires more research.

Table 6. Means of the agro-morphological traits of *Anthemis haussknechtii* forming 4 clusters.

cluster	plant canopy (cm^2)	height (cm)	flower number	fresh weight (gr)	dry weight (gr)	dry matter percentage	GDD Flowering (°*C*)	GDD maturity (°*C*)
1	559.74 b	15.39 b	56.27 b	80.4 b	23.03 b	28.91 ab	721 b	1058 b
2	830.39 b	21.25 ab	93.28 a	77.3 b	18.3 b	23.9 a	1073 a	1362 a
3	309.99 b	12.03 b	28.95 c	43 c	10.33 b	26.03 ab	987 ab	1094 b
4	1656.2 a	27.37 a	81.04 ab	317.7 a	130.15 a	37.94 a	1015 a	1090 b

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

Table 7. Eigenvalue, Variance percentage, Cumulative percentage and Structure of first three component for agro-morphological traits of *Anthemis haussknechtii.*

	Component1	Component2	Component3
plant canopy	<u>0.38</u>	0.02	0.08-
height	<u>0.37</u>	0.04	0.1-
flower number	0.33	0.09	<u>0.47-</u>
fresh weight	<u>0.37</u>	0.03-	0.16
dry weight	<u>0.36</u>	0.03-	0.29
dry matter percentage	0.24	0.15-	<u>0.55</u>
GDD flowering	0.01	<u>0.67</u>	0.51
GDD maturity	0.02	<u>0.72</u>	0.28-
Eigen value	6.66	1.5	0.93
Variance percentage	0.67	0.15	0.09
Cumulative percentage	0.67	0.82	0.91

Result of PCA analysis in our experiment showed that the two first components explained 82% of total variability of the data. The first component explained 67% of total variation and includes plant canopy, height, fresh and dry weight and second component explained 15% of total variation, and GDD to flowering and GDD to maturity are main parts of this component. Similarly Pirkhezri *et al.* (2008) have taken first two components of PCA contributed 58.9 per cent of the total variance amongst *Anthemis and Matricaria* wild populations studied. The cluster and PCA analysis grouped populations 24761 and 13818 and 17018 of A. *haussknechtii*, separately (fig.1 and 2), 24761 and 13818 are divided by the aspects of morphological characteristics, a grouping which is provided by component one in PCA analysis, And population 17018 is divided by the aspects of phenological characteristics (GDD to flowering), a grouping which is provided by component two. In the investigations, the candidate *A. hausknechtii* population for highest flower amount was grouped under Cluster 2 but late matured, while early matured populations were grouped under Cluster 1, it is suggested that cross can be made between the populations of cluster 1 and cluster 2 to get early matured variety with high flower yield.

Table 8. Results from the ANOVA on 8 Agro-morphological traits of *Anthemis altissima*.

Source of variation	df	ms							
		plant canopy	height	flower number	fresh weight	dry weight	dry matter percentage	GDD flowering	GDD maturity
species	4	183207	33.61	1589.3**	9029.2**	338.21**	32.411**	17844	1794
rep	2	3487	11.79	1733.7	1590	44.93	4.631	6155	455
Error	8	97921	26.12	211	650.2	33.87	3.291	15070	4208

**Significant at 1% probability levels.

Plant canopy in *A. haussknechtii* was varied from 173.2 in population 17886 (from Fars) to 2186 cm² in population 13818 (from Golestan) and flower number was varied from 90.87 to 15.4. *A. altissima* Populations had considerably larger plants with more flowers. This wide range of variations suggests good potential for selection in breeding programs.

Table 9. Means of the *agro*-morphological traits of 5 populations of *Anthemis altissima*.

Population	Plant canopy (cm^2)	Height (cm)	flower number	fresh weight (gr)	dry weight (gr)	Dry matter percentage	GDD Flowering (°C)	GDD Maturity (°C)
3119	1904.15 a	31.67a	101.11ab	37.67b	11.13b	29.55a	1073b	1386a
8835	1559.77 b	31.9a	103.97ab	137.8a	31.27a	22.7b	1220a	1425.5a
9885	1502.31b	32.33a	114.81a	183.13a	37.93a	20.75b	1017.67b	1398.83a
12790	1728.65 a	28.69a	136.63a	154.6a	35.8a	23.68b	1073b	1362a
29610	1246.82 c	37.9a	73.21c	121.6a	29.87a	24.67ab	1135ab	1374a

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

Results of correlation analysis showed the significant correlation between height, fresh weight and flower number in A. *haussknechtii* (Table 5). The result differ from Salamon (1992) studies that reported weight and height of the plant did not affected number of flowers in chamomile, but had similarity with the observation of Pirkhezri *et al.* (2010).

Populations with more flowers are desired ones because flowers and mostly tubular florets are the main source of active substances in *Anthemis* (Vuckovic *et al.*, 2006; Williams *et al.*, 2001; Sajjadi *et al.*, 2013), and positive correlation between flower number and amount of essential oil in different *Anthemis* species of Iran have been reported (Golparvar and Ghasemi Pirbalouti, 2010; Pirkhezry *et al.*, 2008). populations with larger plants are superior too because they can produce more flowers, however populations like 26041(from kordestan) and 26044 (from kordestan), with smaller plant and high flower number, have potential to produce higher amount of essential oil, because it is possible to have more plants per unit area.

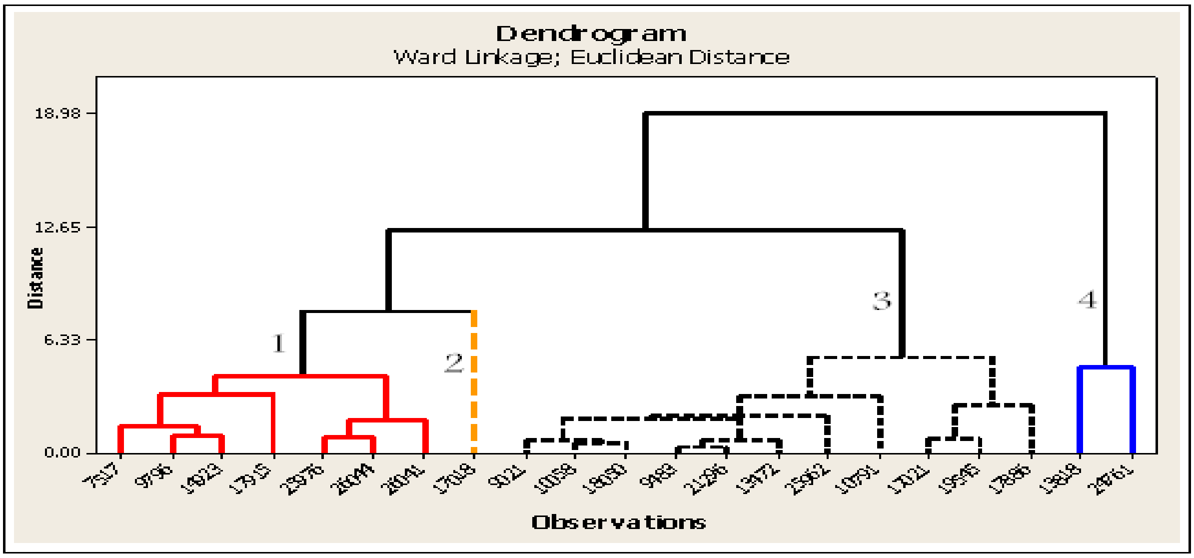

Fig. 1. Dendrogram showing genetic relationship of 21 populations of *A. haussknechtii* based on 8 agro-morphological traits.

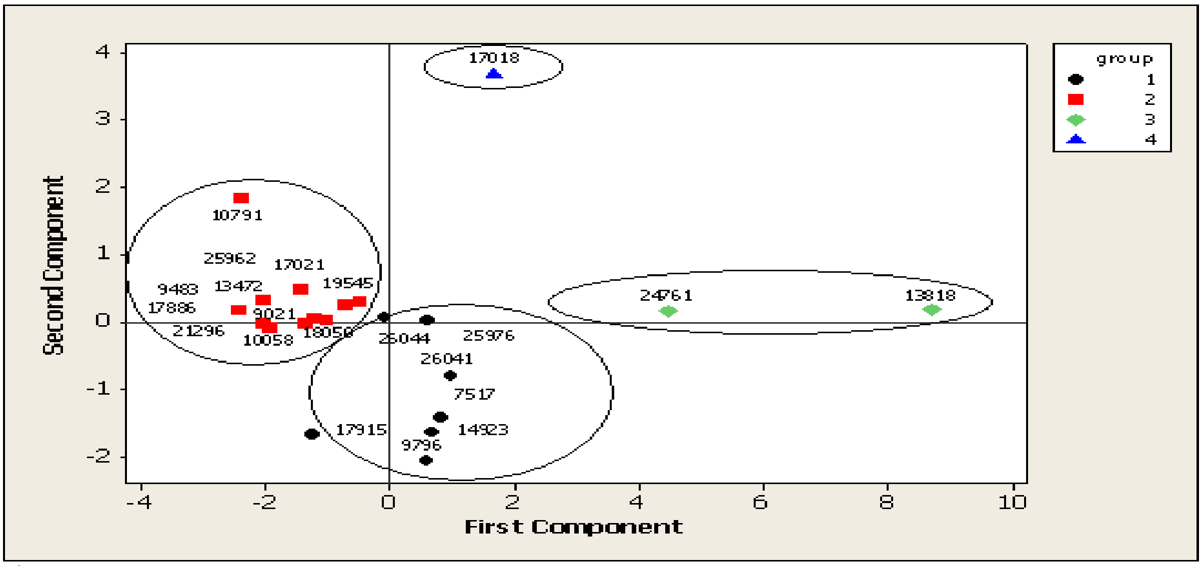

Fig. 2. Principal component diagram from first two components of PCA analysis showing the agro-morphological variation of 21 A. *haussknechtii* populations. Principal component axes 1 and 2 explain 67% and 15% of the total variance respectively.

Conclusion

Although morphology cannot be directly related to genotype, it has a strong genotypic basis (Schlichting 2002). The objectives of the research were to evaluate the diversity of *A. altissima* and *A. haussknechtii* populations, at agro-morphological level by multivariate techniques. Classifying genotypes and populations according to their agronomic traits with sophisticated multivariate techniques can reduce the cost of time and money in crop improvement. The findings will facilitate efficient breeding programs to produce varieties with the desired traits. Wide range of variations among populations of these two valuable medicinal species suggests good potential for selection in breeding programs.

Acknowledgement

We would like to thank Prof. Dr. A. Jafary from Research Institute of Forests and Rangelands (RIFR), for his valuable remarks during field studies and statistical analysis.

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