



Genetic variation of some Iranian *Hyoscyamus* Landraces based on seed storage protein

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Article information	Abstract
<p>Article history: Received: 23 Jun. 2014 Accepted: 2 Sep. 2014 Available online: 15 Sep. 2014 EPP 2014; 1 (2):14-22</p> <p>Keywords: <i>Hyoscyamus spp.</i> Jaccards' similarity coefficient SDS-PAGE</p> <p>*Corresponding author: Assistant Professor, Department of Medicinal Plant, Higher Education Center of Shahid bakeri of Miandoab, Urmia University, Urmia, Iran. E-mail: f.nejadhabibvash@urmia.ac.ir</p>	<p>The genus <i>Hyoscyamus</i> belongs to the tribe Hyoscyameae Miers of Solanaceae family. Variation in protein bands elaborates the relationship among the collections from various geographical regions. In this study the seed storage protein diversity of 19 accessions of <i>Hyoscyamus</i> (<i>H. niger</i>, <i>H. reticulatus</i> and <i>H. pusillus</i>) from West Azerbaijan (Iran) was investigated according to the Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) method. Seed protein was analyzed through slab type SDS-PAGE using 15% polyacrylamide gels. The similarity matrix was computed by using Jaccard's similarity coefficients, based on polymorphic bands, and dendrogram established through UPGMA cluster analysis. Genetic similarity ranged from 0.07 to 0.87, which indicates high genetic distance at interspecies level. The plotted dendrogram revealed two major clusters. The result of this study indicates that the accessions of <i>Hyoscyamus</i> could effectively be differentiated on the basis of polymorphism, detected by protein bands pattern. Therefore, seed protein profiles seem to be rapid and reliable method to detect inter and intra-specific variation in <i>Hyoscyamus</i> landraces in relation to geographical origin for future breeding programs.</p> <p>Copyright © 2014 Kerman Graduate University of Advanced Technology. All rights reserved.</p>

Introduction

The genus *Hyoscyamus* belongs to the tribe Hyoscyameae Miers of Solanaceae family with 18 species all over the world (Yousaf et al., 2008) and 13 species in Iran (Khatamsaz., 1998). *Hyoscyamus* is one of the desert medicinal plants of family Solanaceae. The plant produces some important compounds as secondary metabolites, mainly hyoscyamine and scopolamine, which exhibits a wide range of pharmacological and toxic activity. The seeds of this plant are used as an analgesic, anti-inflammatory, anti-spasmodic, and sedative. Health problems such as stomach cramps, heavy coughs, neuralgia, Parkinson, asthma, colic and diarrhea could be treated by seeds of this genus (Sengupta et al., 1998). Most species display a complex of genetic variations along their range of distribution (Miller & Schaal., 2006). It seems that Iran is an important diversification center of this genus with seven endemic species (Khatamsaz., 1998). For landraces, this is a function of species characteristics, such as breeding system, migration and dispersal mechanisms

which determine the movement of genes among population (Herlihy & Eckert., 2004). Furthermore, biotic pressure such as competition, predation, local anthropogenic influence, and biotic selection intensities are determined by location (Ferguson et al., 1998). Genetic conservation strategies are initially concerned with understanding of the genetic variation within species, and geographical distribution of species (Sanchez- Yelamo et al., 1995). A major goal of genetic resource conservation is to conserve as wide as possible of genetic variations in target taxa. This is irrespective of the relative frequency of any gene or linked gene complex in germplasm. Satisfying this objective depends on the efficiency of selection of species and location for the sampling (Sanchez- Yelamo et al., 1995). The identification of *Hyoscyamus* is very complex, difficult, and often confused (Khatamsaz & Zangirian., 1998). Hence, the introduction of biochemical technique could be more accurate evaluation of genetic diversity. Seed proteins are used as genetic markers in the study of

genetic variation. Proteins are the primary products of structural genes. Any change in the coding sequences of a gene generally reflects the corresponding change in the primary structure of protein (Srivalli et al., 1999). Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) is an economical, simple and extensively used biochemical technique for polymorphism of seed storage protein diversity of crop germplasm (Fufa et al., 2005). However, the seed storage proteins are also used for identification of varieties of Rangeland plants (Ferguson et al., 1998). It is also known that variation in protein bands elaborate the relationship among the collections from various geographical regions (Gafoor et al., 2002). To the best of our knowledge, no report has been recorded on seed storage protein variability of *H. niger*, *H. reticulatus*, and *H. pusillus*

accessions in West Azerbaijan in association with geographical distribution. In this study, we hired SDS-PAGE technique to detect inter and intra-specific variation of the seed storage proteins in *Hyoscyamus* landraces related with geographical origin. The results will help the plant breeders to be succeeded in breeding programs.

Materials and Methods

• Plant material

Nineteen accessions belonging to genus *Hyoscyamus* were collected for seed protein analysis from various agro-ecological regions in West Azerbaijan (Iran) in Jul 2010 (Table1 and Fig 1).

Table 1: *Hyoscyamus* accessions and their collection sites.

No.	<i>Hyoscyamus</i> accessions	collection site	Altitude(m)	Latitude/Longitude
1	<i>Hyoscyamus niger</i>	30 km of Oshnavieh: Lolkan village: h1	1870	37°12' N 45°7' E
2	<i>H.niger</i>	Bazargan: Village of Emamkandi: h2	2300	39°35'N 44°12' E
3	<i>H.niger</i>	Salmas road: Abgarm:h3	1485	38°3' N 44° 57' E
4	<i>H.niger</i>	Urmia: Village of Neichalan: h4	1480	37°41' N 44°45'E
5	<i>H.niger</i>	Urmia: 20 km of Urmia to Mahabad: h5	1500	36°5' N40°15' E
6	<i>H. niger</i>	Khoy: h6	1526	37°48' N 45°3' E
7	<i>H. niger</i>	Oshnavieh :40 km to Oshnavieh: h7	1537	37°15' N 45° 7'E
8	<i>H.niger1</i>	Nagadeh: Soltanyagoob: h8	1389	36°40'N 45°7' E
9	<i>H.niger2</i>	Urmia: Marmisho : h9	1545	37°35' N44°52'E
10	<i>H.niger3</i>	Urmia: Jabaljeiran: h10	1282	37°25' N 45°13'E
11	<i>H.niger4</i>	Silvana road : Razhan: h11	1537	37°10' N 45°8' E
12	<i>H.reticulatus</i>	50 Km Piranshahr to Sardasht: h12	1313	36°40'N 46°7' E
13	<i>H.pusillus</i>	3 km of Nagadeh to Piranshahr: h13	1330	36°56' N 45°20'E
14	<i>H.pusillus</i>	Urmia: Rikan village: h14	1283	37°30' N45° 11'E
15	<i>H.reticulatus</i>	3 km of Nagadeh to Piranshahr: h15	1330	36°56' N 45°20'E
16	<i>H.reticulatus</i>	35 km of Oshnavieh: h16	1641	37°11' N 45°9' E
17	<i>H.reticulatus</i>	Piranshahr: h17	1522	37°52' N 45°24'E
18	<i>H.pusillus</i>	Shahindezh: h18	1463	36°55' N46°24' E
19	<i>H.reticulatus</i>	Gooshchi mountain: h19	1314	37°12' N 44°7' E



Figure1. Sources and geographical localities of *Hyoscyamus* accessions tested. The red circles are representative of collection sites.

• Protein extraction

For the extraction of proteins 19 accessions belong to *H. niger*, *H. reticulatus* and *H. pusillus* (Table 1). 200 mg seeds of each sample were homogenized to obtain a fine powder. Proteins were extracted in a pre-cooled mortar, and pestle over ice with a 0.39 M Tris - Phosphate buffer (pH 8.3). The resulting mixture was centrifuged at 15000g for 10 min. The crude extracts were boiled for 3 min in 77mM Tris - Hcl (pH 6.8), 4% Sodium Dodecyl Sulphate (SDS), 10% 2-mercaptoethanol and 3% glycerol (Sanchez-Yelamo et al., 1995).

• SDS-Polyacrylamide Gel Electrophoresis

SDS-PAGE of total seed protein was carried out following the method of Laemmli (1970). 100 µg of each extract was loaded in lanes with micropipette. A protein molecular weight marker (Fermentas, Cat No. FM 0431) was also incorporated into the gel (as marker lane) as reference to detect molecular weights of the bands.

Seed protein was analyzed through slab type SDS-PAGE using 15% polyacrylamide gels; A current of 20mA was applied; Protein electrophoresis (SDS-PAGE) was carried out using 20µl of protein in each lane.

• Fixing and staining

Immediately upon the completion of the electrophoresis, the gels were removed and immersed in the fixative solution containing 100 ml of 45.4% methanol and 9.2% acetic acid then the gels were stained overnight in 0.02% (w/v) Coomassie Brilliant Blue, 25% isopropanol and 10% acetic acid.

• De-staining

Gels were de-staining in methanol and acetic acid for 60 minutes. The gels were further de-stained until the background was clear enough for bands scoring. The banding profile was photographed and scored.

• Data analysis

The gels were read on gel scanner; Molecular weights of protein bands and their relative mobility (R_m), were estimated with respect to the marker using Carestream Computer Software. Bands were scored as presence (1) or absence (0) and pair wise similarities between the accessions calculated using Jacquard's coefficient (Sneath & Sokal., 1973). Cluster analysis was performed on the similarity matrix by UPGMA method. All computations were performed using NTSYS-pc2.02 software (Rohlf., 1998).

Results

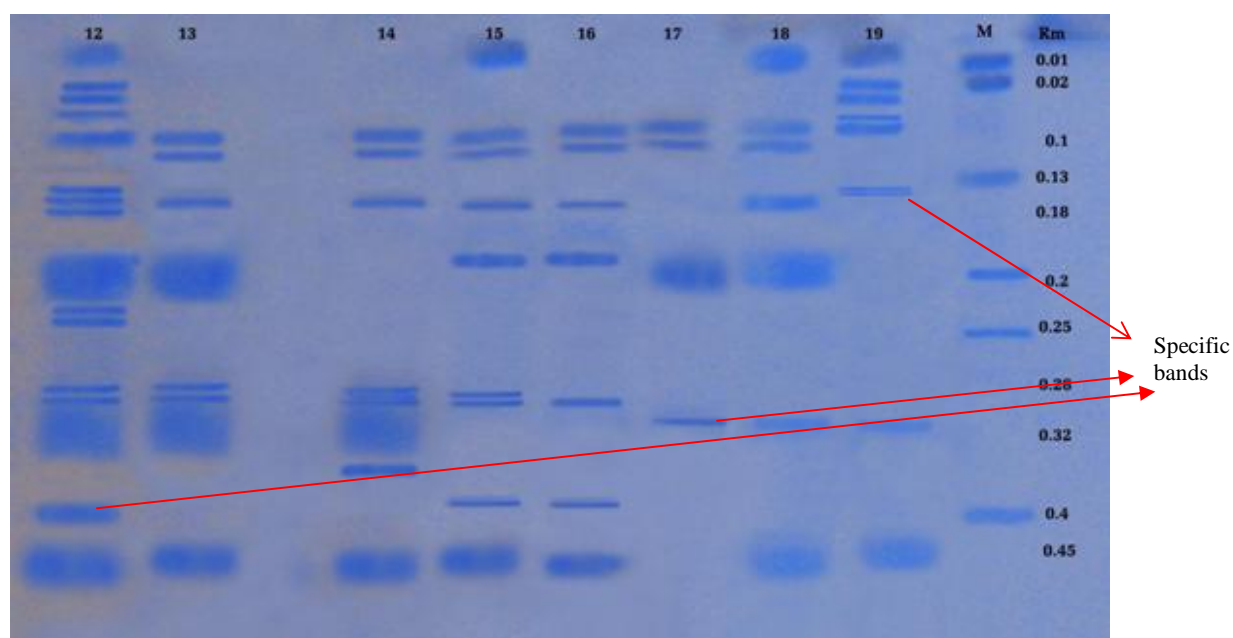
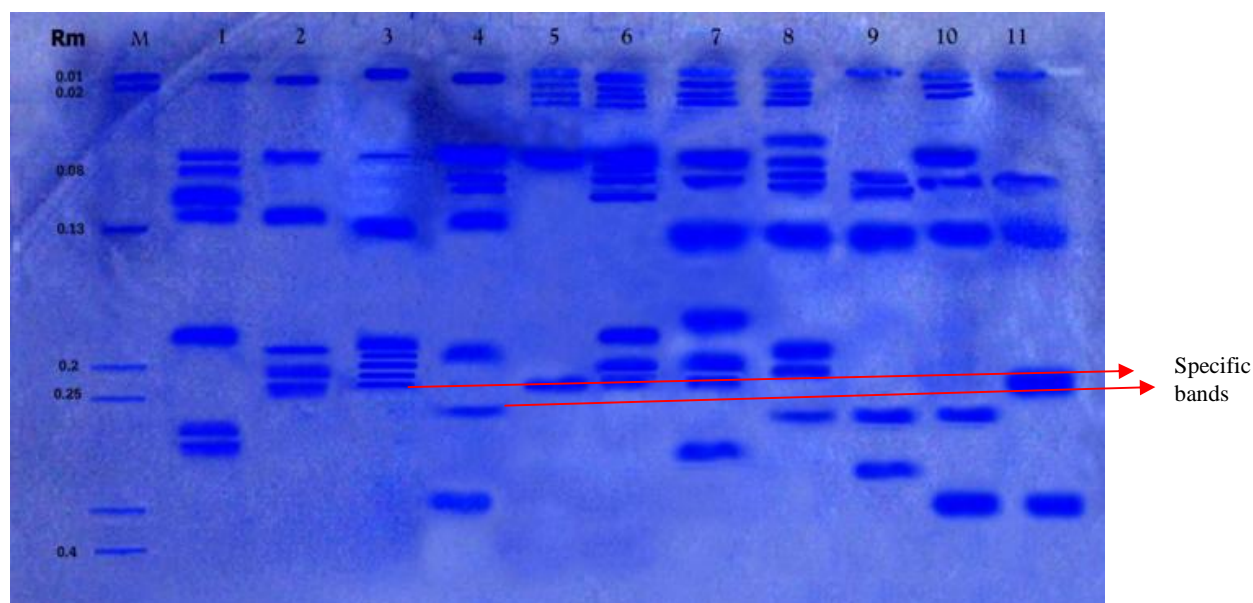
Electrophoresis analysis of nineteen *Hyoscyamus* accessions showed inter and intra specific variations in terms of band number and molecular weight (Table 2). Each protein band was considered as a single locus / allele. Alleles were scored as present (1) or absent (0). Bivariate (0, 1) data matrix was generated (Table 2). Totally 34 alleles were scored in 19 accessions giving an average of 1.79 alleles per accession. The polypeptide bands of diverse molecular weights ranged from 13.15 KDa to 79.79 KDa (Table2).

Table 2: Relative mobility (Rm) values, molecular weights(MW) and band presence or absence in 19 accessions of *Hyoscyamus*.

Band	Rm	MW(K Da)	h 1	h 2	h 3	h 4	h 5	h 6	h 7	h 8	h 9	h 10	h 11	h 12	h 13	h 14	h 15	h 16	h 17	h 18	h 19
1	0.01	79.79	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	0	0	0	1
2	0.02	75.85	0	0	0	0	1	1	1	1	0	1	0	0	0	0	0	0	0	0	0
3	0.03	73.55	0	0	0	0	1	1	1	1	0	1	0	0	0	0	0	0	0	0	0
4	0.04	70.59	0	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0
5	0.05	67.76	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1
6	0.06	65.04	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	1
7	0.07	62.43	1	1	1	1	1	1	1	1	0	1	0	1	0	0	0	0	0	0	0
8	0.08	59.92	1	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0
9	0.09	57.51	0	0	0	1	0	1	1	1	1	1	1	0	0	0	0	0	0	0	1
10	0.10	55.2	0	0	0	1	0	0	0	0	1	0	0	1	1	1	1	1	1	1	1
11	0.11	52.99	1	0	0	0	0	1	0	0	0	0	0	0	1	1	1	1	1	1	0
12	0.12	50.86	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	0.13	48.82	0	0	1	0	0	0	1	1	1	1	1	0	0	0	0	0	0	0	0
14	0.15	44.97	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
15	0.16	43.17	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1
16	0.17	41.43	0	0	0	0	0	0	1	0	0	0	0	1	1	1	1	1	0	1	0
17	0.18	39.77	1	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0
18	0.19	38.17	0	1	1	0	0	0	0	1	0	0	0	0	0	0	1	1	0	0	0
19	0.20	36.64	0	0	0	1	0	1	1	1	0	0	0	1	1	0	0	0	1	1	0
20	0.21	35.17	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21	0.22	33.75	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
22	0.23	32.4	0	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
23	0.24	31.1	0	0	1	0	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0
24	0.25	29.85	0	1	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0
25	0.26	28.65	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
26	0.28	26.39	1	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0
27	0.29	25.33	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0
28	0.30	24.32	0	0	0	0	0	0	1	0	0	0	0	1	1	1	0	0	0	0	0
29	0.31	23.34	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
30	0.32	22.4	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	0	1	1
31	0.35	19.81	0	0	0	1	0	0	0	0	1	1	1	0	0	1	0	0	0	0	0
32	0.39	16.81	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0
33	0.40	16.14	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
34	0.45	13.15	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	0	1	1
Total number of bands			8	6	8	8	6	11	11	12	6	8	5	15	8	9	9	8	4	7	10

Figure 2 is representative of protein band pattern belongs to 19 accessions of *Hyoscyamus*. Among the accessions h17 showed minimum (4) resolved protein bands while, the maximum (15) was observed in h12 accession. Some accessions possessed bands which

were unique. Bands with 16.14, 23.34, 28.65, 35.17, and 44.97 KDa molecular weights were specific to h12, h17, h4, h3 and h19 genotypes, respectively. Genetic variability among the genotypes was estimated using Jaccard similarity coefficient.



Figures 2: Seed storage protein pattern of 19 *Hyoscyamus* accessions. Lanes 1, 2, 3... are representative of h1, h2, h3 ... and h19, respectively. M and Rm represent protein Ladder and relative mobility, respectively.

The range of genetic similarity was obtained between 0.00 to 0.87 among various comparisons (Table 3).

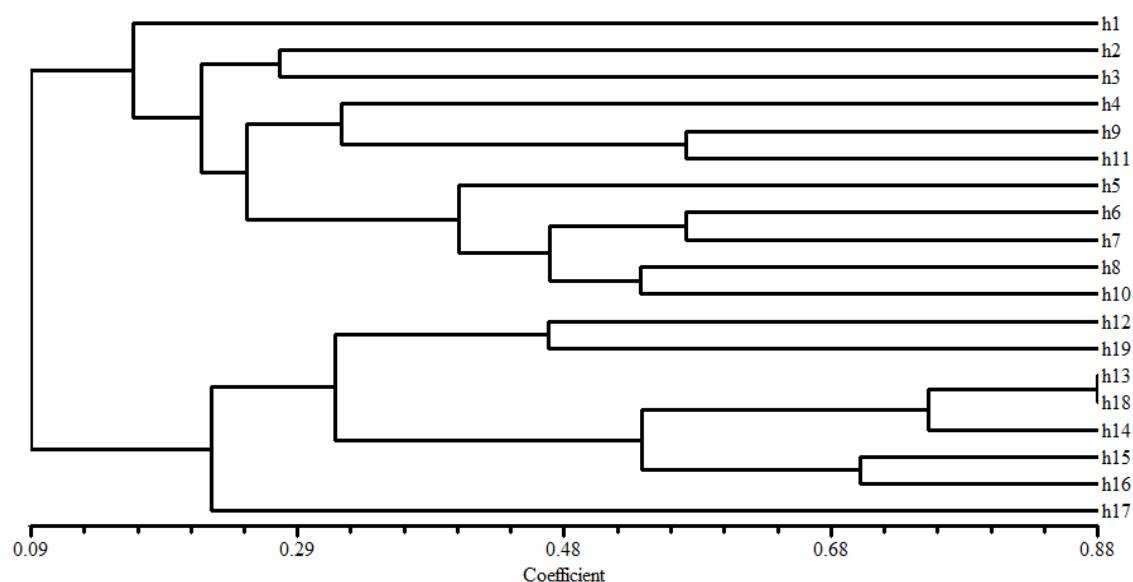
The highest similarity percentage (0.87) was obtained between h13 and h18.

Table 3: Genetic similarity values among 19 *Hyoscyamus* accessions derived from seed storage protein data.

	h1	h2	h3	h4	h5	h6	h7	h8	h9	h10	h11	h12	h13	h14	h15	h16	h17	h18	h19	
h1	1.00																			
h2	0.25	1.00																		
h3	0.06	0.27	1.00																	
h4	0.28	0.25	0.13	1.00																
h5	0.15	0.20	0.16	0.15	1.00															
h6	0.33	0.21	0.18	0.25	0.41	1.00														
h7	0.11	0.21	0.26	0.25	0.41	0.57	1.00													
h8	0.16	0.28	0.25	0.23	0.38	0.53	0.53	1.00												
h9	0.07	0.20	0.16	0.36	0.09	0.13	0.21	0.28	1.00											
h10	0.13	0.27	0.23	0.30	0.40	0.35	0.46	0.53	0.50	1.00										
h11	0.07	0.10	0.30	0.27	0.22	0.14	0.23	0.21	0.50	0.40	1.00									
h12	0.14	0.10	0.15	0.20	0.16	0.13	0.23	0.17	0.10	0.09	0.10	1.00								
h13	0.13	0.00	0.00	0.13	0.00	0.11	0.18	0.05	0.07	0.00	0.00	0.43	1.00							
h14	0.11	0.00	0.00	0.18	0.00	0.10	0.16	0.04	0.10	0.05	0.07	0.38	0.80	1.00						
h15	0.20	0.15	0.06	0.12	0.07	0.11	0.11	0.10	0.10	0.06	0.07	0.26	0.40	0.40	1.00					
h16	0.06	0.07	0.06	0.06	0.00	0.05	0.11	0.05	0.07	0.00	0.00	0.27	0.60	0.63	0.70	1.00				
h17	0.08	0.00	0.00	0.30	0.00	0.15	0.07	0.06	0.10	0.00	0.00	0.11	0.30	0.27	0.18	0.20	1.00			
h18	0.06	0.00	0.00	0.14	0.00	0.12	0.20	0.05	0.08	0.00	0.00	0.37	0.87	0.70	0.45	0.66	0.37	1.00		
h19	0.05	0.00	0.05	0.18	0.06	0.10	0.16	0.15	0.23	0.12	0.15	0.47	0.20	0.25	0.26	0.28	0.07	0.30	1.00	

The tree-cluster analysis illustrated the distribution of accessions in two main clusters (Fig 3). Cluster I which was divided into two sub-clusters; the accession h1 was grouped in the first sub-cluster at the similarity of 0.17. Second sub-cluster consisted of accessions labeled as h2, h3, h4, h5, h6, h7, h8, h9, h10 and h11. Therefore, all accessions of *H. niger* were collected from different locations placed into one cluster. This cluster is separated from second cluster at the similarity of 0.09. Cluster II comprised of four sub-clusters. The sub-cluster one consisted of

accessions h12 and h19 at the similarity of 0.48. The h13, h14 and h18 (accessions of *H. pusillus*) were grouped in second sub cluster at the similarity of 0.76. The third sub-cluster was comprised of h15 and h16 (accessions of *H. reticulatus*) at the similarity of 0.72. The accession h17 was placed into last sub cluster at the similarity of 0.2. Therefore, our seed storage protein data reveals a high correlation between genetic diversity and geographical origin.

**Figure 3.** UPGMA dendrogram based on Jaccard' similarity coefficient among *Hyoscyamus* accessions.

The highest cophenetic correlation coefficient ($r = 0.90$) was obtained with Jacquard similarity coefficient. Cophenetic correlation coefficient, indicating a good fit between the dendrogram clusters, and the similarity matrices. Principal component analysis (PCA) was performed to

visualize the association among accessions in more detail (Fig 4). The results show that the first principal coordinate explain 50% of the total variation, and PCA results correspond largely to the results obtained through cluster analysis.

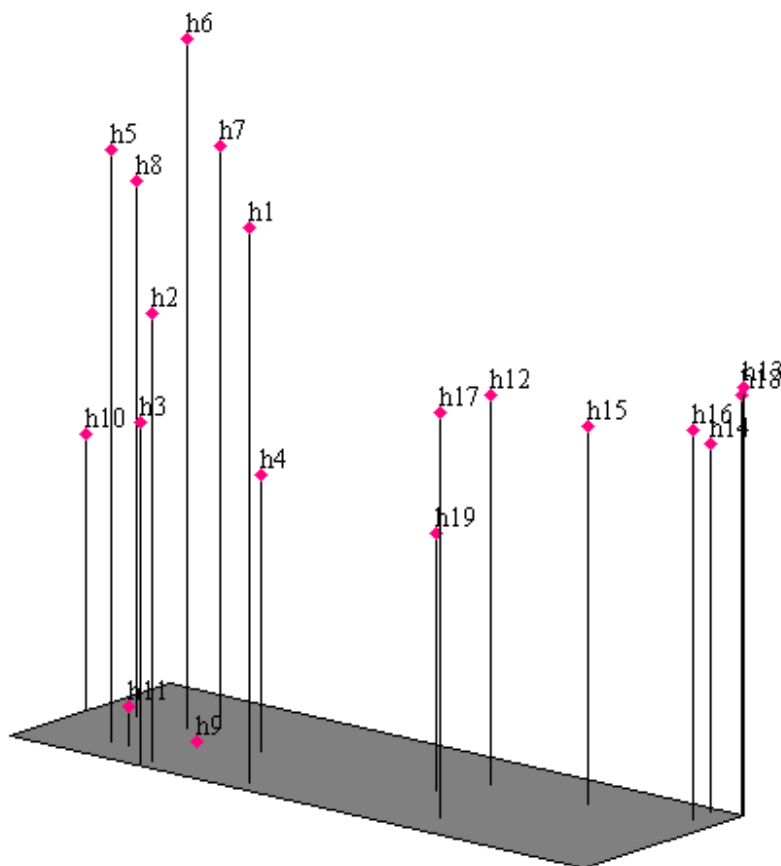


Figure 4. Pattern of relationship among 19 *Hyoscyamus* accessions revealed by PCA based on seed storage protein data.

Discussion

Seed storage protein showed distinct polymorphism in electrophoretic banding pattern which led to detection of 34 polypeptides of diverse molecular weights ranging from 13.15 KDa to 79.79 KDa. The accessions showed considerable variation in number of bands and density indicating their genetic differences. The presence of specific bands indicated the occurrence of specific genes in the species due to species diversification. Such specific protein bands may be used in *Hyoscyamus* species identification. From Jaccard's similarity matrix, the similarity coefficient values seem to be rather low among accessions which suggest a relatively broad genetic base for accessions evaluated in the present study. Plants such as soybean with a narrow genetic base, protein markers may not be sufficient for characterization, and study of genetic diversity

(Nikolić *et al.*, 2005). However, grouping the accessions of *H. niger* in one cluster despite of the different environmental conditions reflects a genetic basis of the plant. It is evident from the dendrogram that ten genotypes h2, h3, h5, h10, h11, h13, h14, h17, h18 and h19 are most distantly related. Hence, it is recommended these genotypes could be used in future breeding programs to create higher amounts of genetic variability in Iranian germplasm of *Hyoscyamus*. The results of this research showed that *H. pusillus* was close to *H. reticulatus* at the similarity of 88%. However, 10 accessions of *H. niger* separated from the *H. reticulatus* and *H. pusillus* accessions at the similarity of 9%. Electrophoresis of seed proteins was previously used for cultivars identification of many plants; such as *Capsicum* L. (Kummar & Tata., 2010) and wheat

(Fufa et al., 2005). It was also performed for studying genetic diversity and addressing taxonomic relationships in *Ocimum* (Mustafa & Badr., 2006), *Glycine* (Malik et al., 2009), *Festuca pratensis* Huds (Stonya & Boller., 2010) and red clover (Nikolić et al., 2010). Similarly, interrelationships in *Solanum* subgenus *Leptostemonum* (Solanaceae) revealed by the seed protein studies (Karihaloo et al., 2002). Munazza and co-workers (2009) studied the electrophoresis characterization in different genotypes of oilseed *Brassica* based on analysis of seed storage proteins to assess the protein polymorphisms within and different cultivated species. They clarified the genetic nature of polymorphic bands can differentiate the yellow and brown seeded varieties of *Brassica*. Also, biochemical characterization of eight *Lycopersicon esculentum* L. were carried out based on seed storage proteins electrophoresis marker. SDS-protein marker analysis indicated a considerable amount of genetic diversity among different varieties of *Lycopersicon esculentum* L (Elham et al., 2010). The high stability of protein profile makes protein electrophoresis a powerful tool in elucidating the origin and the evolution of plants. The intensity of the protein band varied among species of *Ipomoea* (Convolvulaceae). Hence, use of SDS-PAGE profile draws interrelatedness between the species of *Ipomoea* (Parameshwar & Sreenath., 2013). Seed protein profiles of 47 accessions belonging to eleven species, and four tribes of grain legumes were studied, by extracting the total proteins from single seeds in each accession, and performing SDS-Polyacrylamide gel electrophoresis. All eleven species were clearly recognizable from their protein banding patterns (Valizadeh., 2001). Sheidai and co-workers (2000) investigated five species of *Hyoscyamus* based on seed proteins. They recorded more similarities between *H. niger* and *H. reticulatus* (collection of Tehran & East Azerbaijan provinces) which is not supported in the present study. However, in agreement with mentioned researchers, SDS-PAGE analysis could be applied as a useful tool for identifying *Hyoscyamus* species. In general, the dendrogram showed large inter and intra-specific differences among *Hyoscyamus* genotypes. SDS-PAGE clearly presented differences among *H. niger*, *H. reticulatus* and *H. pusillus*. We suggest application of more molecular studies to improve the classification of these accessions .

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