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RESEARCH ARTICLE

Morphometric analysis and zymogram patterns of peroxidase and superoxide dismutase enzymes in populations of mosses in north of Iran

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ABSTRACT: The Hyrcanian forests have a remarkable variety of moss species which research on their taxonomy is of great importance. Since *Forsstroemia remotifolia*, *Homalia besseri* and *Pseudoleskeella catenulata* are exclusive and native mosses species of Hyrcanian forests, so in the current study, fourteen populations from three provinces in the north of Iran including Golestan, Mazandaran and Guilan were collected at the same altitudes in autumn 2017. In order to reveal the relationships among these species and populations, a cluster analysis based on numerical taxonomy and zymogram patterns of peroxidases and superoxide dismutase with Euclidean distances was performed. Numerical taxonomy analysis showed plant length, marginal laminal cell length and middle laminal cell length are appropriate traits to distinguish the species of *F. remotifolia*, *H. besseri* and *P. catenulata* from each other as well as their populations. The zymogram analysis showed genetic variability among species and also within populations of *F. remotifolia*, *H. besseri* and *P. catenulata*. Accordingly, the isozyme banding pattern of peroxidases showed a total of 6, 7 and 5 bands for *F. remotifolia*, *H. besseri* and *P. catenulata*, respectively. However, 4 isozyme bands were detected for superoxide dismutase for all three species. Furthermore, the morphological analyses in some populations was not matched with the isoenzyme banding pattern of enzymes in the current study. In conclusion, the biosystematics studies (morphometry and zymogram patterns of peroxidase and superoxide dismutase) indicate the close relationship between *F. remotifolia* and *P.* catenulata.

KEYWORDS: Biosystematics, Hyrcanian forests, Numerical taxonomy, Zymogram

INTRODUCTION

The moss species have high distribution in Hyrcanian forests which were surrounded by the Caspian Sea in the north of Iran [1]. However, there is not much information about the taxonomy of these plants in Iran, yet alive [2]. The remarkable diversity of moss species can be attributed to the propagation method and remarkable dynamics found in some moss populations. Sexual reproduction in mosses can lead to the production and release of copious amounts of spores, but the establishment of spores is extremely difficult. Therefore, the distribution of mosses is highly dependent on asexual propagation to maintain populations in many species. Asexual propagation may play a role in maintaining genetic diversity. Besides, it can be a protection strategy to limit the local extinction of genes [3].

About 16 orders, 107 families, and approximately 880 genera and 12,000 mosses species have been recognized world widely [4]. The bryoflora of mosses in Iran consists

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of 438 mosses species [1, 2, 5-20]. The Hyrcanian forests are the refuge of many species including *Forsstroemia remotifolia*, *Homalia besseri*, and *Pseudoleskeella catenulata*. *F. remotifolia* and *H. besseri* are widely distributed in Golestan, Mazandaran and Guilan provinces in the north of Iran, but *P. catenulata* is only presented in Mazandaran province [21].

About half of all moss species in the world are dioecious. Mosses are divided into five groups in terms of growth platform and establishment, including terricolous, saxicolous, lignicolous, aquatic, and epiphytic [22]. *F. remotifolia*, *H. besseri* and *P. catenulata* remain exclusive and native species of Hyrcanian forests. These species are epiphytic, dioecious and produce sporophytes several times in their life span [23].

Isozyme patterns of different enzymes and numerical taxonomy method are functional tools for the study of diversity within and between plant species and their populations[24]. In the past, the classification was based on morphological characteristics which were highly accepted, but numerous studies on classification systems have been effective in including and excluding new genera in the family and other levels [25]. The numerical taxonomy method is thoroughly accurate because, in addition to qualitative traits, quantitative traits are also examined, so it is a convenient method for the classification of many plants [26]. The genetic variation can be explained by the banding patterns of one or several antioxidant enzymes [27]. Antioxidant enzymes are recognized as a defense mechanism that is presented in most of plant species [28, 29]. Given that the isoenzyme banding pattern exhibits much natural genetic variation, therefore antioxidant enzymes are preferred for biosystematics study over the other enzyme systems due to their important role in physiological processes and plentiful allozymes [30].

The purpose of this study was to investigate numerical taxonomy and electrophoretic banding patterns of antioxidant isoenzymes like peroxidase (POD) and superoxide dismutase (SOD) of species including *F. remotifolia*, *H. besseri*, and *P. catenulata* and also their population in the north of Iran.

MATERIALS AND METHODS

Sampling

In our study, six populations of *F. remotifolia*, six populations of *H. besseri*, and two populations of *P. catenulata* were collected in the 2017 autumn in three northern provinces of Iran including Guilan, Mazandaran, and Golestan (Figure 1) and plants were transferred to the laboratory at low temperature (4°C) for identification, morphometric analysis and electrophoretic analysis. However, for electrophoretic analysis first the fresh weight of the plant samples was measured then transported to -20˚ C until enzyme extraction. Population name (abbreviation), locations, and altitude for each population are shown in Table 1.

After collection, the plant specimens were placed into paper envelopes and transported to the lab for identification, morphometric analysis. In addition, some samples for electrophoretic analysis were transferred to the laboratory at 4˚C, their fresh weight was measured and transported to -20˚ C until enzyme extraction.

Morphometric evaluations

The morphometric analysis was performed using thirty quantitative and qualitative characters that shown in Table 2. Separate analyses were carried out on the populations. The range of each character was compared for all populations with box plots. A principal component analysis (PCA) based on the correlation matrix was performed. The morphometric study was conducted by the ward method using the SPSS software package V.25.

Zymogram analysis

Peroxidase (POD) isozymes

The plants were carefully cleaned under a dissecting microscope. The sample was extracted using a 1:10 (W/V) ratio of 0.1M potassium phosphate buffer pH 7.5 and subsequently centrifuged at 4°C for 25 min at 9,000 g. The supernatant was stored at -80°C for electrophoresis

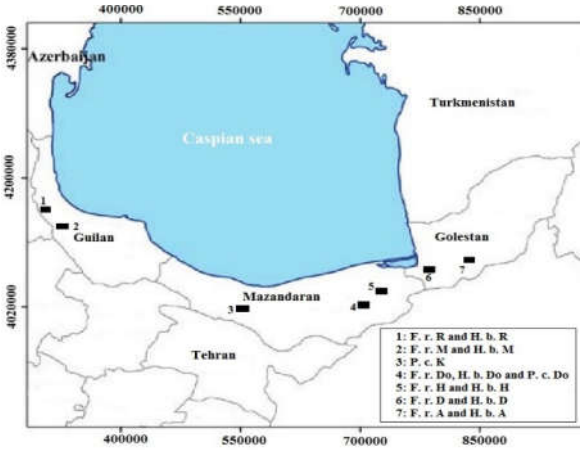

Figure 1. The collection location of *F. remotifolia*, *H. besseri*, *P. catenulata* populations.

Table 1. Population's name (abbreviation) and Geographical indicators of sampling locations (Latitude, Longitude and altitude) of *F. remotifolia*, *H. besseri* and *P. catenulata* in north of Iran.

Species	City	Population	Latitude Longitude	Altitude (m)
Fosstroemia remotifolia	Rezvanshahr	F. r. R	37°31'52.50"N 48°51'19.30"E	700
	Masal	F. r. M	37°18'26.48"N 49° 00' 7.46"E	709
	Dodangeh	F. r. Do	36°13'40.70"N 53°15' 53.6" E	720
	Hezarjerib	F. r. H	36°33'43.60"N 53°34'30.26"E	712
	Derazno	F. r. D	36°42'48.48"N 54° 9'16.40" E	718
	Aliabad	F. r. A	36°50'42.74"N 54°57'4.42"E	704
Homalia besseri	Rezvanshahr	H. b. R	37°31'52.50"N 48°51'19.30"E	700
	Masal	H. b. M	37°19'0.10"N 48°59'36.50"E	663
	Dodangeh	H. b. Do	36°13'39.10"N 53°15'54.00"E	730
	Hezarjerib	H. b. H	36°33'43.60"N 53°34'30.26"E	712
	Derazno	H. b. D	36°43'2.50"N 54° 6'36.20"E	635
	Aliabad	H. b. A	36°49'21.20"N 55° 0'3.80"E	620
Pseudoleskeella catenulate	Nowshahr	P. c. K	36°20'30.70"N 51°24'22.10"E	1386
	Dodangeh	P. c. Do	36°20'30.70"N 51°24'22.10"E	720

analysis [31]. The POD isoenzymes, detected with 15% polyacrylamide gel electrophoresis (PAGE). Then the gel was flushed with 200 ml sodium acetate buffer containing 2mM benzidine and the reaction was initiated by addition of H_2O_2 [32].

Superoxide dismutase (SOD) isozymes

Similar to POD extraction, the plants were carefully cleaned under a dissecting microscope. The plant sample was extracted in the ratio of 1:3 (W/V) with 50 mM Tris-HCl buffer pH 7.5, containing 1mM DTT, 2mM EDTA, 50mM NaCl, 0.5mM phenylmethylsulfonyl fluoride (PMSF) and 1% (w/v) polyvinylpyrrolidone (PVP), and centrifuged at 4°C for 15 min at 3,500 g. The supernatant was used to perform SOD isoenzymes [33]. The SOD isoenzymes detected with the sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). A Tris-glycine system (1.5M Tris / 0.191M Glycine, pH 8.3) was used as the running buffer. The SOD isoforms were detected by using riboflavin-NBT (Beauchamp and Fridovich, 1971). The gel was incubated in a staining buffer (0.24 mM NBT with 28 µM riboflavin, 28 mM TEMED, 0.5 M EDTA and 50 mM Monosodium phosphate) for 60 minutes at room temperature, under dark conditions. After staining, the gel was exposed to two fluorescent lamps (20W each) until the SOD bands became visible.

Data analysis

Band frequencies were calculated for each population and each enzyme system (Table 3). The intra-population variation was estimated by the Shannon diversity index (H) [34] (Table 4). The affinities and divergence among populations were examined by cluster analysis and Ward method operating based on the band frequencies matrix. The software SPSS V.25 and also UPGMA method using the software NTSYS-pc V.2.02k which was utilized based on Euclidean distances (Table 5).

Table 2. Characteristics used in morphometric analysis

No.	Character	No.	Character	No.	Character
1	Plant length	11	Costa length	21	Marginal cell width
2	Leaf length	12	Costa width	22	Average of upper marginal laminal cell length
3	Upper leaf width	13	The number of costa cells	23	Average of upper marginal laminal cell width
4	Middle leaf width	14	Costa distance to Marginal cell	24	Basal marginal laminal cell length
5	End of leaf width	15	Costa shape	25	Average of middle marginal laminal cell length
6	Leaf apex length	16	Thickness costa	26	Average of middle marginal laminal cell width
7	Leaf apex width	17	Upper laminal cell width	27	The number of Marginal laminal cell
8	The number of leaf apex cells	18	Middle Laminal cell length	28	Leaf marginal shape
9	Allarcell length	19	Middle Laminal cell width	29	Plant color
10	Allarcell width	20	Length of the first branch	30	Laminal cell shape

Table 3. Band frequencies of POD and SOD enzyme in 14 populations.

Population		P. c. Do	P. c. K	F. r. A	F. r. D	F. r. Do	F. r. H	F. r. M	F. r. R	H. b. A	H. b. D	H. b. Do	H. b. H	H. b. M	H. b. R
Isozymes	Allozymes														
POD	A	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	B	0	0	0.33	1	1	0.67	0.33	1	1	0.33	0.67	1	1	1
	C	0.67	0.67	0	0	0	0	0	0	0	0	0	0	0	0
	D	0	0	0	0.33	0.67	0	0	0	0	0	0	0	0	0
	E	0.33	0.67	0.33	1	1	0.5	0.33	0.33	0.5	0.67	0.67	0	0.33	0
	F	0.67	0.67	0.67	0.33	0.67	1	1	1	1	1	1	1	0.33	0.33
	G	0	0	0.67	1	1	0.67	0.67	0.67	0	0	0	0	0	0
	H	0.33	1	1	1	0.67	1	1	1	0.33	1	1	1	1	1
	I	1	0.33	0.33	0	0	0	0	0	0	0.33	0	0.33	0	0
	J	0	0	0	0	0	0	0	0	0.33	1	0	1	0.5	0.67
	K	0	0	0	0	0	0	0	0	0.5	1	0.5	1	0.5	0.5
	L	0	0	0.5	1	0.67	0.67	0.67	1	1	0.67	0	1	1	1
	M	0	0	0	0	0	0	0	0	0	0	1	0	0	0
	N	0	0	0	0	0	0	0	0	0	0	0.67	0	0	0
SOD	A	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	B	0.67	0.67	0	0	0	0	0	0	0	0	0	0	0	0
	C	0	0	1	1	1	1	1	1	0	0	0	0	0	0
	D	0	0	0	0	0.17	0	0	0	0.67	0.67	1	0.67	0.67	0.67
	E	0	0	0	0	0	0	0	0	1	1	1	1	1	0.67
	F	0	0	0.67	1	1	1	1	0.67	0	0	0	0	0	0
	G	0.67	1	0	0	0	0	0	0	0	0	0	0	0	0
	H	1	1	1	1	1	1	1	1	1	1	1	1	1	1

P. c. Do: *P. catenulata* Dodangeh; P. c. K: *P. catenulata* Kikoh; F. r. A: *F. remotifolia* Aliabad; F. r. D: *F. remotifolia* Derazno; F. r. Do: *F. remotifolia* Dodangeh; F. r. H: *F. remotifolia* Hezarjerib; F. r. M: *F. remotifolia* Masal; F. r. R: *F. remotifolia* Rezvanshahr. H. r. A: *H. besseri* Aliabad; H. r. D: *H. besseri* Derazno; H. r. Do: *H. besseri* Dodangeh; H. r. H: *H. besseri* Hezarjerib; H. r. M: *H. besseri* Masal and H. r. R: *H. besseri* Rezvanshahr.

Table 4. The Shannon (H) diversity index for POD and SOD enzyme in 14 populations.

Population	P. c. Do	P. c. K	F. r. A	F. r. D	F. r. Do	F. r. H	F. r. M	F. r. R	H. b. A	H. b. D	H. b. Do	H. b. H	H. b. M	H. b. R	Mean
Isozymes															
POD	1.703	1.737	1.984	2.000	2.059	1.915	1.863	1.897	1.985	2.127	2.050	2.041	1.985	1.883	1.945
SOD	1.366	1.373	1.373	1.386	1.500	1.386	1.386	1.373	1.373	1.373	1.386	1.373	1.373	1.366	1.385
Mean	1.535	1.555	1.679	1.693	1.780	1.651	1.625	1.635	1.679	1.750	1.718	1.707	1.679	1.625	1.665

RESULTS

Morphometric analysis

In our study, we employed the cluster analysis to produce the dendrogram (Figure 3) and the principal component analysis (PCA) to produce scree plots and scatter plots (Figures 5 and 6). Thirty characters (Table 2) were used to construct the box plot showing variation (Figure 2).

According to the dendrogram (Figure 3), the 14 specimens could be divided into two clusters: cluster 1 includes eight specimens (F. r. Do, F. r. D, F. r. A, F. r. M, F. r. R, F. r. H, P. c. K, P. c. Do) and cluster 2 includes the other six specimens (H. b. R, H. b. M, H. b. Do, H. b. H, H. b. D and H. b. A). The dendrogram of 14 specimens revealed that the main branch at the 25th level is divided into two branches (1 and 2), which covers about 75 percent similarity of traits.

PCA reveals that 74.99% of the indicated variation is explained by the four main factors (40.411, 13.4, 11.238, and 9.945). The first factor was recognized as the most influential factor, which includes the following characters: upper leaf width, Middle leaf width, End of leaf width, Leaf tip width, Upper laminal cell width, Middle Laminal cell length, Middle Laminal cell width, Average of upper marginal laminal cell length, Average of upper marginal laminal cell width, Basal marginal laminal cell length, Average of middle marginal laminal cell length, Costa distance to Marginal cell, Allarcell length, Allarcell width, Leaf marginal shape, Costa shape,

Table 5. Euclidean distances; pair-wise comparisons of *F. remotifolia*, *H. besseri* and *P. catenulata* populations based on Isozyme diversities.

Population	Euclidean Distance													
	P. c. Do	P. c. K	F. r. A	F. r. D	F. r. Do	F. r. H	F. r. M	F. r. R	H. b. A	H. b. D	H. b. Do	H. b. H	H. b. M	H. b. R
P. c. Do	0.000													
P. c. K	1.414	.000												
F. r. A	3.000	2.646	.000											
F. r. D	3.317	3.000	1.414	.000										
F. r. Do	3.317	3.317	2.000	1.414	.000									
F. r. H	3.000	2.646	.000	1.414	2.000	.000								
F. r. M	3.000	2.646	.000	1.414	2.000	.000	.000							
F. r. R	3.000	2.646	.000	1.414	2.000	.000	.000	.000						
H. b. A	3.162	3.162	2.646	3.000	3.000	2.646	2.646	2.646	.000					
H. b. D	3.464	3.162	2.646	2.646	3.000	2.646	2.646	2.646	1.414	.000				
H. b. Do	3.464	3.162	3.000	3.317	3.606	3.000	3.000	3.000	2.449	2.449	.000			
H. b. H	3.742	3.464	3.000	2.646	3.000	3.000	3.000	3.000	2.000	1.414	2.449	.000		
H. b. M	3.742	3.464	3.000	2.646	3.000	3.000	3.000	3.000	2.000	1.414	2.449	.000	.000	
H. b. R	3.742	3.464	3.000	2.646	3.000	3.000	3.000	3.000	2.000	1.414	2.449	.000	.000	.000

P. c. Do: *P. catenulata* Dodangeh; P. c. K: *P. catenulata* Kikoh; F. r. A: *F. remotifolia* Aliabad; F. r. D: *F. remotifolia* Derazno; F. r. Do: *F. remotifolia* Dodangeh; F. r. H: *F. remotifolia* Hezarjerib; F. r. M: *F. remotifolia* Masal; F. r. R: *F. remotifolia* Rezvanshahr. H. r. A: *H. besseri* Aliabad; H. r. D: *H. besseri* Derazno; H. r. Do: *H. besseri* Dodangeh; H. r. H: *H. besseri* Hezarjerib; H. r. M: *H. besseri* Masal and H. r. R: *H. besseri* Rezvanshahr.

Plant color and Laminal cell shape. The scatter plot (Figures 5 A and B) showed these two clusters are completely separated from each other. The scatterplot of the 14 specimens for the first and second PCA (Figure 5 A) reveals two groups. The first group contains the specimens determined as *F. remotifolia*, mixed with specimens of *P. catenulata* and the second group includes all populations of *H. besseri*. The scatter plot of the first component and the third (Figure 5B) grouping is repeated.

Zymogram analysis

The results of zymogram analysis are presented in Figures 6 and 7. A total of 14 peroxidases and eight superoxide dismutase isozyme bands were detected (Figure 6 and 7). According to the banding pattern of peroxidase enzyme, the population of *F. remotifolia*, *H. besseri* and *P. catenulata* consisted of six, seven and five isozyme bands, respectively (Figure 6).

Band A in isozyme peroxidase was common to all populations including P. c. Do, P. c. K, F. r. A, F. r. D, F. r. Do, F. r. H, F. r. M, F. r. R, H. b. A, H. b. D, H. b. Do, H. b. H, H. b. M and H. b. R. Some bands were common for *F. remotifolia* and *H. besseri* and *P. catenulata*, such as the band B shared between all populations of *F. remotifolia* and *H. besseri.* The band C was seen only in the populations of P. c. Do and P. c. K. The band D and I was seen only in the populations of, F. r. Do, P. c. Do, respectively. In addition, the band E was present in all populations except in the H. b. Do, H. b. H, H. b. M, H. b. R. The band F was found in P. c. Do, P. c. K, F. r. A, F. r. H, F. r. M, F. r. R, H. b. A and H. b. Do populations. The band G has moderately stained in populations F. r. D, F. r. Do, H. b. H, H. b. M, and H. b. R but were absent in all the remaining populations. The band H was particularly evident in populations P. c. K, F. r. A, F. r. D, F. r. H, F. r. M, F. r. R, H. b. D, H. b. Do, H. b. H, H. b. M and H. b. R, whereas these bands were absent in P. c. Do, F. r. Do and H. b. A population. The band J was observed in all populations of *H. besseri* except the H. b. Do population. The band K was seen in all populations of *H. besseri*. The band L was observed in all populations except the H. b. Do population. The band M and N were seen only in the population of H. b. Do (Table 3).

Isozyme banding patterns of superoxide dismutase enzyme showed the band number 1 and 8 was seen in all populations. The band number 2 and 7 were seen only in the populations of P. c. Do and P. c. K. The band number 3 and 6 was observed in all populations of *F. remotifolia.* The band number 4 and 5 was present in all population of *H. besseri* (Figure 7).

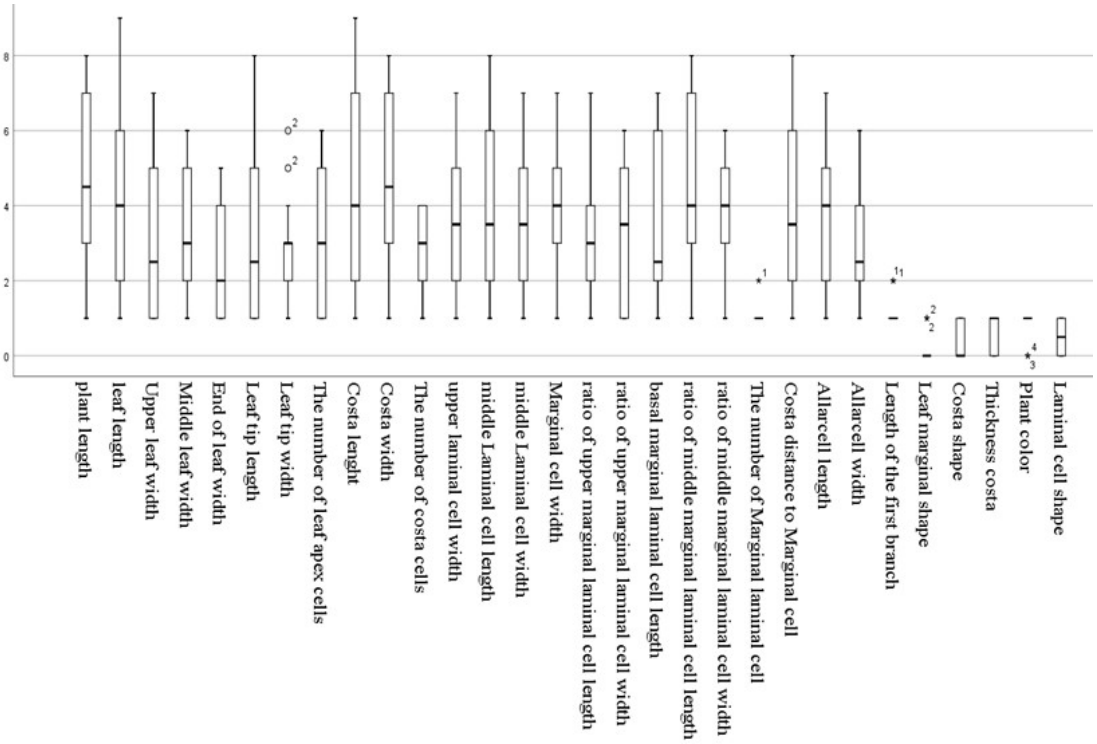

Figure 2. Box plot of quantitative and qualitative characters screened for cluster analysis.

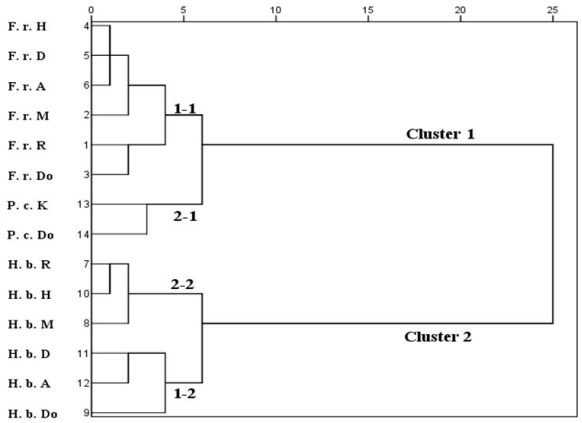

Figure 3. Dendrogram obtained from Ward analysis of Euclidean distance values based on morphological variation.

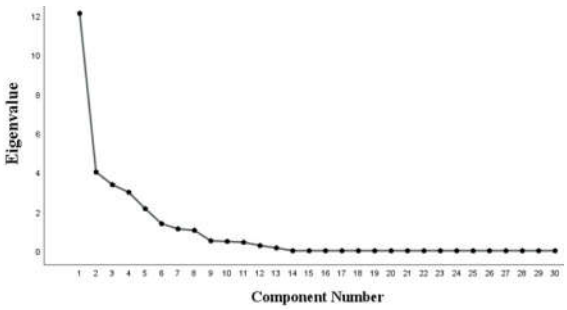

Figure 4. Scree plot for the thirty morphological characters used in the morphometric traits.

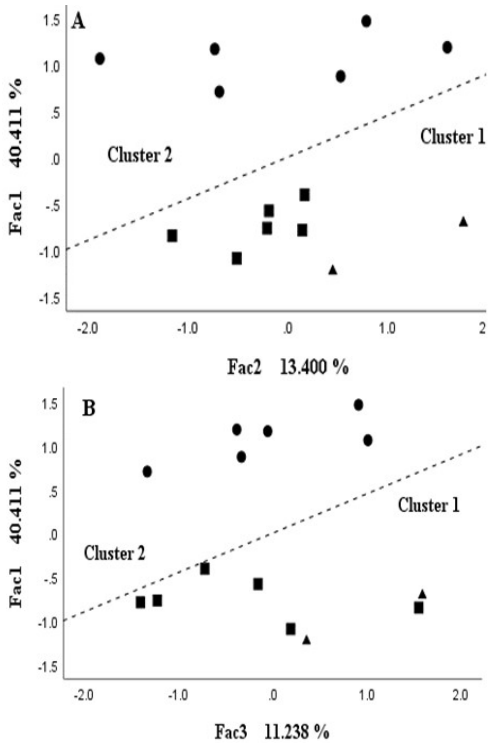

Figure 5. A) scatterplot of the first and second factors of the principal component analysis (PCA) into species studied. **B)** scatterplot of the first and third factors of the PCA into species studied. Circle, *H. besseri*; square, *F. remotifolia*; triangle, *P. catenulata*.

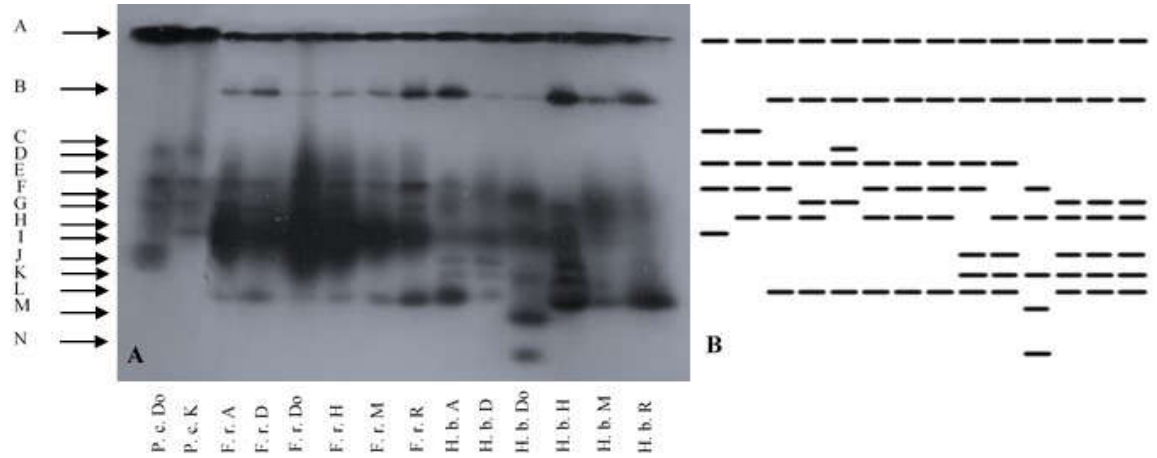

Figure 6. A) Isozyme banding patterns and **B**) zymograms of POD enzyme in different populations of *F. remotifolia*, *H. besseri* and *P. catenulata*.

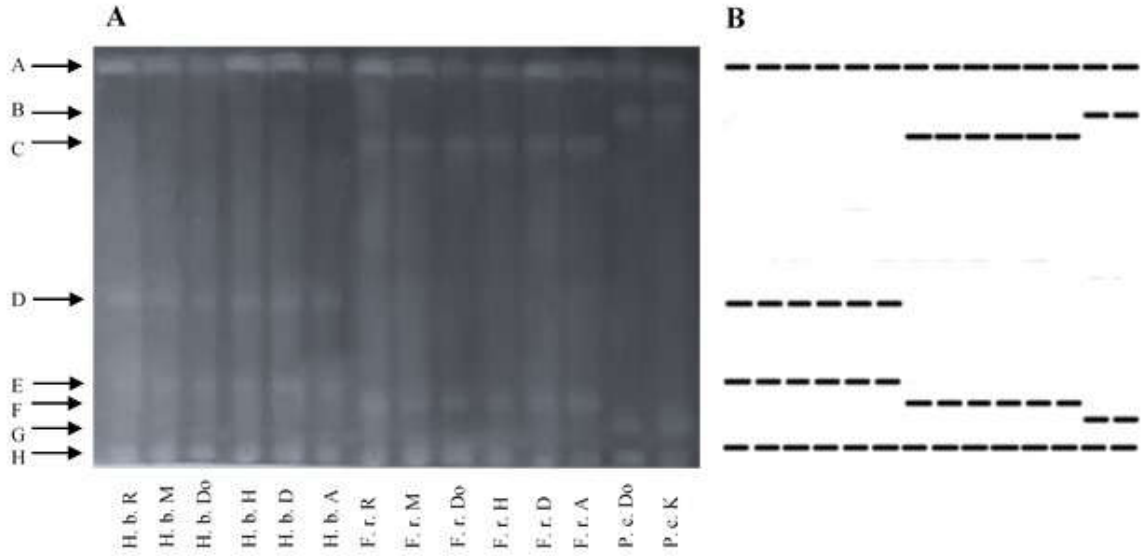

Figure 7. A) Isozyme banding patterns and **B**) zymograms of SOD enzyme in different populations of *F. remotifolia*, *H. Besseri* and *P. catenulata*.

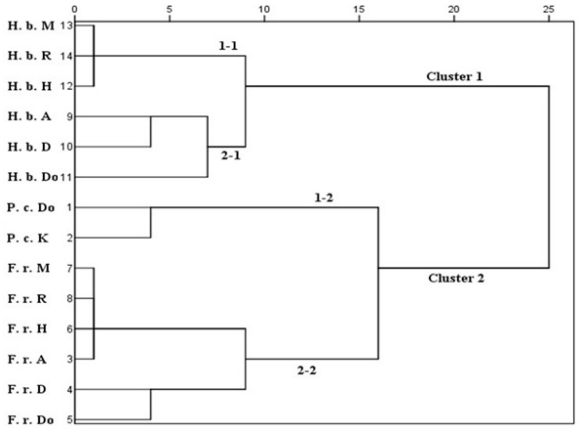

Figure 8. Dendrogram obtained from Ward analysis of Euclidean distance values based on Isozyme variations patterns.

The frequency values ranged from 0 to 1. The average Shannon (H) diversity for each enzyme system ranged from 1.385 (SOD) to 1.945 (POD) (Table 4). The matrix Euclidean distance was calculated for all populations. The minimum Euclidean distance was observed between the populations 'P. c. Do' and 'P. c. K' equaling 1.414. Based on the dendrogram produced by Ward method (Figure 8) Two Cluster can be seen that each cluster divided tow sub-cluster.

The dendrogram of isozyme revealed that the local branch at the 25th level is divided into two branches 1 and 2. Branch 1 consists of two subbranches 1-1 and 1-2. Subbranch 1-1 includes the populations of H. b. M, H. b. R and H. b. H and subbranch 1-2 includes H. b. A, H. b.

D and H. b. Do. Branch 2 consists of two subbranches 2-1 (P. c. Do and P. c. K) and 2-2 (F. r. M, F. r. R, F. r. H, F. r. A, F. r. D, F. r. Do; Figure 7).

DISCUSSION

The dendrogram obtained from numerical taxonomy in the current study showed that the populations of H. b. M and H. b. H belong to *H. besseri* are closer to each other than H. b. R to H. b. M while these two last populations stayed in more close geographical distance. Also, the populations of F. r. H and F. r. Do belong to the *F. remotifolia* with low geographical distance were identified in branches with more far distant. The separation of H. b. M and H. b. R populations in far sub-branches could be due to differences in upper leaf width and leaf tip width. Also, in the case of populations F. r. H and F. r. Do, differences in traits such as leaf length, leaf tip length, marginal cell width, and the number of marginal laminal cells may lead to the separation of these two populations. It has been observed [35] that the length of cauloid and the number of leaves distinguish the population of *Anacolia webbii* species. Also, the differences among populations of *Macromitrium blumei* were mainly based on leaf length, length of excurrent costal awn, and the ratio of leaf length to leaf width [36]. Therefore, it seems that the quantitative traits related to leaves are important in the separation of mosses′s populations, too.

The results of zymogram analysis showed that mosses maintain a certain amount of genetic variability within populations [37-41]. The average value of the Shannon diversity index (H) estimated for each population ranged from 1.535, in P. c. Do, to 1.780 in F. r. Do (Table 4). The high genetic diversity within populations can be due to the reproduction by sexual diaspores or caused by spores that are big and barely dispersed by wind [42, 43]. According to our previous study the spore size has been reported to be medium and large in all populations (Seyed Mousavi et al., in press).

Morphological variation as detected by PCA was mainly determined by quantitative characters such as leaf length, upper leaf width, middle leaf width, leaf tip width. In the present study, plant length, marginal laminal cell length and middle laminal cell length traits distinguished *F. remotifolia* from species of *H. besseri* and *P. catenulata*. Appelgren and Cronberg [44] stated cell length at leaf apex, leaf length, angle of leaf apex and seta length present distinctive traits between genotypes of *Neckera oligocarpa*. Also, it has been shown that the revolute margin and upper leaf cells represent diagnostic traits that allow differentiation between *Braunia andrieuxii* and *Braunia secunda* [45]. Therefore, it seems that quantitative traits are more effective than qualitative traits in separation of moss species and populations as well [46].

Euclidean distance showed a genetic variation between the population of *F. remotifolia* and *H. besseri*. The Euclidean distance among populations of *F. remotifolia* ranged from 1.414 to 2.449 in *H. besseri*. This distance among the populations of *F. remotifolia* and *P. catenulata* was less than that between the populations of *F. remotifolia* and *H. besseri*. Euclidean distance between populations F. r. H and F. r. Do belong to *F. remotifolia* was amount 2.000 but this value in F. r. H and F. r. M populations have reached 0.000. However, the population of F. r. H and F. r. Do have less geographically distant from each other. The Euclidean distance showed a low genetic distance between populations H. b. H and H. b. M while they have a high geographical distance.

Grundmann et al. [47] stated, genetic differences among populations are not always affected by geographical distances, but may also be attributed to the founder effects.

Several studies have shown that the POD isoenzyme banding pattern has been effective in separating of species [48, 49]. However, in the current study we observed that the zymogram banding pattern of both antioxidant enzyme systems (POD and SOD) were efficient to discriminate among species. In addition, in the current study a variation among the molecular size of POD isozymeś s bands has been observed which may show high genetic differences between populations. While the molecular sizes of SOD isozymes′s bands were hardly changed among the populations [50].

Furthermore, the morphological analyses in some populations was not matched with isoenzyme banding pattern of enzymes in current study. Previous studies have shown some mosses may genetically different but they have similar morphology [40, 51].

In addition, the exact systematic position of *F. remotifolia* is a matter of discussion since a long time ago. This species previously belonged to the Leskeaceae family and its old name was *Leskea catenulata* subsp. *remotifolia* [52]. In addition, Townsend [53] showed that *Leskea catenulata* subsp. *remotifolia* was the same species as *Pseudoleskeella laxiramea* (Schiffn.) Broth. It has been

revealed that based on morphological data *P. laxiramea* is different from other *Pseudoleskeella* species and is more similar to *Forsstroemia*, therefore it was renamed *F. remotifolia* [52]. However, as an interesting issue in this study it can be mentioned that the result of cluster analysis in both methods (morphometric and isoenzyme) shows that *F. remotifolia* is closer to *P. catenulata* than *H. besseri*.

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آنالیز مورفومتریک و الگوهای زایموگرام آنزیم‌های پراکسیداز و سوپراکسید دیسموتاز در جمعیت‌های خزه های شمال ایران

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چکیده

جنگل‌های هیرکانی دارای تنوع بالایی از خزه‌ها است و تحقیق در رابطه با طبقه بندی آن‌ها از اهمیت بالایی برخوردار است. از آنجائیکه سه گونه *Forsstroemia remotifolia*، *Homalia besser*i و *Pseudoleskeella catenulata* ازگونه‌های انحصاری و بومی این جنگل‌ها هستند، لذا در پژوهش حاضر، ۱۴ جمعیت از سه استان گیلان، مازندران و گلستان در ارتفاعات تقریبا یکسان در پاییز سال ۱۳۹۶ جمع‌آوری و مورد بررسی قرار گرفتند. به‌منظور آشکار کردن روابط بین این جمعیت‌ها، تجزیه و تحلیل خوشه‌ای بر اساس تاکسونومی عددی و الگوهای زایموگرام آنزیم‌های پراکسیداز و سوپراکسید دیسموتاز با مسافت اقلیدسی انجام شد. تجزیه و تحلیل داده‌های تاکسونومی عددی نشان داد که ارتفاع گیاه، طول سلول‌های حاشیه لامینال و طول میانی سول‌های لامینال صفات مناسبی جهت تمایز بین گونه های *F. remotifolia*، *H. besseri* و *P. catenulata* و همچنین جمعیت آن‌ها از یکدیگر است. تجزیه و تحلیل داده‌های زایموگرام تنوع ژنتیکی را در بین گونه‌ها و همچنین در درون و میان جمعیت‌های *F. remotifolia*، *H. besseri* و *P. catenulata* نشان داد. بر این اساس، الگوی باندی ایزوآنزیم پراکسیداز به ترتیب ۶، ۷ و ۵ باند برای *F. remotifolia*، *H. besseri* و *P. catenulata* نشان داد. همچنین ۴ باند ایزوآنزیمی برای سوپراکسید دیسموتاز برای هر سه گونه شناسایی شد. علاوه بر این در مطالعه حاضر، تجزیه و تحلیل های مورفولوژیکی در برخی از جمعیت ها با الگوی باندی ایزوآنزیم‌ها مطابقت ندارد. به عنوان نتیجه‌گیری کلی می‌توان بیان کرد که مطالعات بیوسیستماتیکی (مورفومتری و ایزوآنزیم های پراکسیداز و سوپراکسید دیسموتاز) *F. remotifolia* و *P. catenulata* رابطه نزدیک بین آن‌ها را نشان می‌دهد

کلمات کلیدی: بیوسیستماتیک، جنگل‌های هیرکانی، تاکسونومی عددی، زایموگرام