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Hybrid Approach for Electrophoresis Pattern of Seed Storage Proteins and Soil Study (Pedology); a Study on Intra Specific Diversity of *Stachys inflata* Benth

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Authors' contributions

This work was carried out in collaboration between both authors. Author EJ did laboratory experiments and performed data analysis and also wrote the first draft of the manuscript. Author ACR designed the study and wrote the proposal and managed the project. Both authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: For determination of levels and types of intraspecific diversity in the identified groups, electrophoresis studies and soil studies were employed.
Study Design: This study has designed base on accumulation of species and laboratory works.
Place and Duration of Study: Department of biology, plant laboratory of BASU University in Hamedan and the duration was between September 2009 and September 2010.
Methodology: *Stachys*, a genus of the Lamiaceae family, with more than 300 species is wildly distributed throughout the world. DSS method (Determination of Special Station) is the method of choice for this study which has established itself as an efficient method to study intraspecific diversity. 13 special stations for *Stachys inflata* were selected where 72 plant species are distinguished as associated species of these special stations. Floristic-ecologic data analyzed by

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Anaphyto software using F.C.A (Factorielle Correspondance Analysis) method.
Results: Comparison of the results led to determination of 9 groups for this species which reveals the existence of intraspecific diversity in *Stachys inflata.* The electrophoretic profiles of seed storage proteins are in accordance with floristic- ecologic and soil study groupings. Results obtained from the analysis of electrophoresis of seed storage proteins by MVSP (Multi Variante Statistical Package) and NTSYS software were also in accordance with floristic-ecological groupings. The number of bands and their density varied in this species, indicating their intraspecific diversity in the populations of *Stachys inflata* Benth.
Conclusion: Therefore, in this species, the grouping by floristic marker was confirmed by electrophoresis as well as soil data. As a result by using D.S.S method, the number of groups and their members were completely identical and utilizing ecological data such as soil caused to detect intraspecific diversity in this species.

Keywords: DSS method; electrophoresis; intraspecific diversity; soil data; Stachys inflate.

NOMENCLATURES

DSS : Determination of Special Station
UPGMA : Unweighted Pair Group Method with Arithmetic Average
FCA : Factorielle Correspondance Analysis
MVSP : Multi Variante Statistical Package
SDS-PAGE : Sodium dodecyl sulfate polyacrylamide gel electrophoresis
EC : Electrical conductivity
OC : Organic carbon

1. INTRODUCTION

Inter and intraspecific diversity is the most important factors that led to biodiversity [1]. The connection between diversity in protein and plant species and also their mechanisms are vital and basic resource [2]. DSS method means Determination of Special Station (Special Station is an area of vegetation that is homogeneous view point of floristic-ecologic) [3]. This method examined frequently and clarified that for plant diversity studies is a proper method [4,3,5-10].

Stachys from family Lamiaceae, and Sub family Laminoideae is one of the largest genera of this family and distributed extensively in the tropical and subtropical countries.

Previous studies in the genus *Astragalus* performed in our lab. Therefore, we did an analysis like *Astragalus* in *Stachys* sect.

According to Chehregani [7], Atri [8] and Yavari [11] studies about electrophoresis pattern and seed protein storage, showed variance in number and density of the protein bands indicating existence of intraspecific diversity in the populations of *Artemisia incana*. Also for proving DSS method, a study by [10] on *Artemisia scoparia* was done to show the intraspecific diversity by DSS method in the west of Iran.

However, no electrophoresis studies was documented in the Iranian *S. inflata* populations, therefore using (SDS-PAGE) for storage protein seems most potential, grow plant to maturity. Our aims in this paper are to contribute to the general knowledge of electrophoresis pattern of seed storage proteins in different Iranian populations of *S. inflata* that grow in different ecological conditions and also describe the variation patterns among different populations and species of *Stachys inflata.*

D.S.S method is achieved by following steps:

1. Choosing Ubiquist taxa (with various habitats).
2. Determining the distribution (Localities) of species by referring to the flora, resources and systematic expers.
3. Referring to the region and identify common habitats of species.
4. Determination of Special Station (D.S.S) in the individual of species in each of the habitats, based on presence of individuals and using the minimum level.
5. Collecting floristic- ecological data from each of the special stations.
6. Analyzing data based on species composition of each special stations (as a marker of floristic) using appropriate software.
7. Classification of special stations based on the analysis of data to determine inter and intraspecies diversity.
8. Identifying the distinguished species for each groups.

9. Determining the type and level of diversity (ecophene, chemo type, ecotype, ecocline, etc.) by using morphometric, anatomy, photochemistry and carpological techniques.
10. Demonstrating the effect of ecological factors by using proper software to determine the intra and interspecie diversity [12].

D.S.S method is used as a proper method for demonstrating diversity in plant species [10,3,6,7,13,8,9]. Thus, we decided to perform such an analysis for *Stachys* sect. According to [7,8,11] studies about electrophoresis pattern and seed protein storage, showed variance in number and density of the protein bands that led to depicting intraspecific diversity in the populations of *Artemisia incana*. Also for proving D.S.S method, a study by [10] on *Artemisia scoparia* was done to show the intraspecific diversity by D.S.S method in the west of Iran. Also according to the findings that obtained from Chromosome counts in different populations of *Stachys inflata* in Hamedan province, D.S.S method showed Chromosome number variation in 9 groupings and The results indicated that the intraspecific polyploidy was obtained as the most significant evolutionary trend within this species [13]. The present work aims is determination of intraspecific diversity in *Stachys inflata* by D.S.S method in Iran. This study is among the unique studies on the topic for the selected species.

2. MATERIALS AND METHODS

2.1 Plant Materials

To demonstrate existing intraspecific diversity, different stations of *Stachys inflata* were determined in Hamedan province by using the flora, reference, herbarium and available information [14]. Then in growth seasonal, different stations in studied area determined. Location of each special station signaled base on existence of individual species in our study. Then for determination of special station (DSS) of individual study species, minimal area detected by using the area-species method .All floristic–ecologic data including longitude, latitude, altitude (in meters), soil elements like EC, pH, OC and texture were collected from each special station [11]. To determine physical and chemical properties of soil in each populations, soil samples were taken from the 30-0 cm depth and

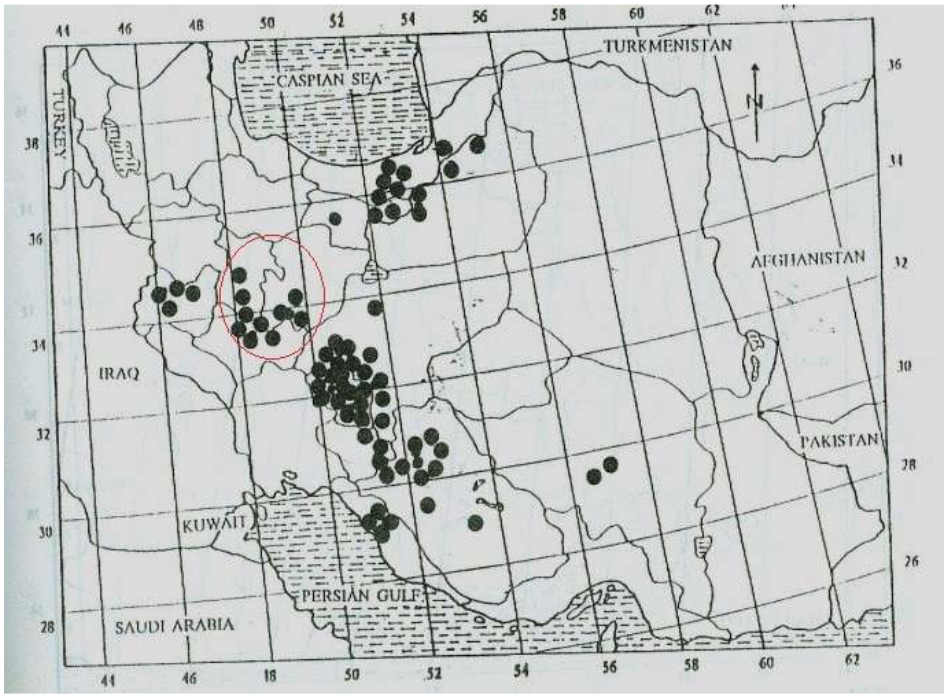

Fig. 1. Distribution map of *Stachys* species in Iran and *Stachys inflat* collected in this study [15]

in terms of characteristics such as texture, electrical conductivity (EC), acidity (pH) and organic carbon (OC). Then in special laboratory which its scope is about soil, water and plant in Malayer city were evaluated.

According to Fig. 1, several origins and distribution of *Stachys* in Iran provided, as it observed the most distribution is in west and southwest. The red cycle depicts *Stachys infalat* collected for this project in Hamedan province.

Total 13 special stations were determined. 72 plant species distinguished as associated species in these special stations. The collected species were kept in the herbarium of Bu Ali Sina University [13]. The information about population of *Stachys inflata* consist of location, geographical charecters, altitude, collector and date of collection mentioned in Table 1.

2.2 Extraction of Seed Storage Protein Studies

For electrophoresis studies in Stachys inflata, mature seeds were harvested from 13 populations in Hamedan province. "Water soluble seed storage protein extraction of normal and treated plants was carried out separately at 4℃ in Tris–HCl buffer (pH7.6). Based on Laemmli [16] method, 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was applied for all proteins [14]. Also in sample buffer (0.1% bromphenol blue dye, 10% β-mercaptoethanol, 20% glycerol, 4% SDS, 0.125 M Tris–HCl, pH6.8), that was heated about 3 or 4 miniute at 100℃ (before loading), soluble proteins were obtained. For each channel, content of protein was 10 µg and complete current was 14 mA. Tris- glysine buffer (pH 8.3) with 0.1% SDS was a location for running gel and also calibrated with a protein marker standard" (Sigma, St.Louis, Mo) [17].

Table 1. The populations of *Stachys inflata* that were subjected for electrophoresis and ecological studies

	Locations	Geographical characters	Altitude (m)	Herbaium numbers	Date of collection
1	Hamedan, Arak road, Protective area of Lashkardar, back of soldier garrison	N: 34°08' 28.5" E: 48°13' 03.2"	1918	19611	2 July 2009
2	Hamedan, Arak road, Lashkardar, back of soldier garrison, first groove after garrison.	N: 34°08' 28.5" E: 48°13' 03.2"	1926	19619	2 July 2009
3	Hamedan, Protective area of Khangormaz	N: 34°46.3' 49" E: 48°07' 06"	2198	19627	1 July 2009
4	Hamedan, Kabudarahang, 3 Km. to airy radar.	N: 35°11.5' 12" E: 48°16.1' 17"	2324	19635	1 July 2009
5	Hamedan, Razan, Boghati mountain	N: 35°32.8' 29" E: 48°43.1' 38"	2365	19653	1 July 2009
6	Hamedan, Abbasabad mountain	N: 34°22' 33" E: 48°34' 12"	2049	19663	30 June 2009
7	Hamedan, Abbasabad to Ganjnameh	N: 34°22' 34" E: 48°23' 12	2066	19673	30 June 2009
8	Hamedan, Nahavand, Gian forest, 200 m higher than mirage	N: 34°47' 29.2" E: 48°29' 19"	1737	19675	2 July 2009
9	Hamedan, Nahavand, Gian forest. not arrived to rocks, right side of valley	N: 34°08' 03.5" E: 48°13' 05.2"	1737	19677	2 July 2009
10	Hamedan, after Tajabad village, right side of valley	N: 35°06.6' 16" E: 98°47' 54"	2119	19679	9 July 2009
11	Hamedan, Almagholagh mountain	N: 35°06.6' 16" E: 98°47.5' 04"	2104	19681	9 July 2009
12	Hamedan, Road of Malayer to Arak, 5Km , Sardehkouh	N: 34°08' 28.5" E: 48°13' 03.2"	1822	19682	2 July 2009
13	Hamedan, Asadabad to Hamedan	N: 34°47'.7 55" E: 48°07' 40"	1800	19683	1 July 2009

3. RESULTS

3.1 Results of Ecologic Factors

Different populations of *Stachys inflata* were selected in different localities of Hamedan province, 13 special stations were recognized for each population and also seed proteins of these special stations were studied.

If we centralize *Stachys inflata* in different stations, we can collect the associated species in a level that is monotonous from floristic- ecologic point of view. Therefore, 72 plant species distinguished as associated species in these special stations as it depicts by following details:

List of plants in special station number 1:

Stachys inflata Benth, *Euphorbia macroclada* Boiss & Buhse, *Astragalus verus*, - Cousinia bijarensis, *Aegilops triuncialis* L, *Stipa barbata* Desf *Tragopagon maturates* Boriss, *Centaurea virgata* Lam

List of plants in special station number 2:

Stachys inflata Benth, *Dendrostellera lessertii, Euphorbia macroclada, Astragalus verus, Tragopogon maturates* Boriss, *Aegilops triuncialis (*Wikstrom*)* Van Tiger, *Trigonella spicata* Sibth. & Sm., *Silene aucheriana* Boiss.

List of plants in special station number 3:

Stachys inflata Benth, *Trichodesma incanum* (Bge.) A.DC, *Cousinia bijarensis* Rech. f, *Euphorbia macroclada* Boiss & Buhse, *Astragalus glaucacathus* Fisch, *Poa bulbosa* L, *Bromus tectorum* L, *Teucrium polium* L.

List of plants in special station number 4:

Stachys inflata Benth, *Bromus tectorum* L, *Eremopoa persica* (Trin.)Roshev, *Taeniatherum crinitum* (Schreb.), *Bromus danthoniae* Trin, *Aegilops triuncialis* L, *Cerastium inflatum* Link ex Desf, *Arenaria leptocladus* (Reichenb.), *Ferula oopoda (*Boiss & Buhse) Boiss, *Euphorbia macroclada, Amygdalus lycioides* Spach, *Linum album* Ky ex Boiss., *Scrophularia striata* Boiss, *Onosma sericeum* Willd, *Anthemis hyalina* DC, *Eryngium noeanum* Boiss.

List of plants in special station number 5:

Stachys inflata Benth, *Poa bulbosa* L, *Bromus danthoniae* Trin, *Eremopoa persica* (Trin.) Roshev, *Taeniatherum crinitum* (Schreb.), *Crucianella gilanica* Trin, *Euphorbia spinidens* Bornm. ex Prokh, *Acanthophyllum microcephalum* Boiss, *Onosma Sericeum* Willd.

List of plants in special station number 6:

Stachys inflata Benth, *Carthamus oxyacantha* M. B, *Phlomis olivieri* Benth, *Eryngium billardieri* F. Delaroche, *Picris strigosa* M. B, *Minuartia meyeri* (Boiss.) Bornm, *Stipa barbata* Desf, *Gundelia tournefortii* L., *Centaurea virgata* Lam., *Taeniatherum crinitum* (Schreb.).

List of plants in special station number 7:

Stachys inflata Benth, *Astragalus verus, Cousinia elwendensis* Bornm, *Gundelia tournefortii* L, *Onosma microcarpum* DC.

List of plants in special station number 8:

Stachys inflata Benth, *Phlomis bruguiere* Desf, *Eryngium billardieri* F, *Onopordon heteracanthum* C. A. Mey, *Scabiosa flavida* Boiss. & Hausskn, *Astragalus verus, Teucrium polium* L, *Alyssum minus* (L.) Rothm, *Ziziphora tenuir* L, *Glaucium corniculatum* (L) Rudolph, *Gaudinopsis macra* (M. B.).

List of plants in special station number 9:

Stachys inflata Benth, *Sophora alopecuroides* L *Crupina crupinastrum* (Moris*)* Vis, *Festuca ovina* L, *Acantholimon olivieri* (Jaub. & Spach) Boiss, *Consolida anthoridea* (Boiss.), *Erysimum crassipes* Fisch. & C. A. Mey, *Allium stamineum* Boiss, *Minuartia meyeri* (Boiss.) Bornm, *Xeranthemum squarrosum* Boiss, *Taeniatherum crinitum* (Schreb.)

List of plants in special station number 10:

Stachys inflata Benth, *Acantholimon olivieri* (Jaub. & Spach) Boiss, *Astragalus verus, Festuca ovina* L, *Ceratium dichotomum, Elymus hispidus* (Opiz), *Stipa barbata* Desf, *Scabiosa flavida* Boiss. & Hausskn.

List of plants in special station number 11:

Stachys inflata Benth, *Scariola orientalis* (Boiss), *Picris strigosa* M. B, *Taeniatherum crinitum* (Schreb.), *Bromus danthoniae* Trin, *Acinus graveolens* M. B, *Cephalaia dichaetophora* Boiss, *Minuartia meyeri* (Boiss.), *Lens orientalis* Boiss, *Bromus tectorum* L.

List of plants in special station number 12:

Stachys inflata Benth, *Astragalus verus*, *Festuca ovina* L, *Stipa barbata* Desf, *Salsola boissieri* Botsch, *Verbascum speciosum*, *Elaeostica nodosa* Boiss, *Buffonia macrocarpa Ser*, *Phlomis olivieri* Benth, *Noaea mucronata* (Forsk.) Aschers. et Schweinf, *Tanacetum polycephalum* Schultz-Bip.

List of plants in special station number 13:

Stachys inflata Benth, *Agropyrum cristatum* (L.) Gaert, *Centaurea virgata* Lam, *Euphorbia spinidens* Bornm. ex Prokh, *Minuartia meyeri* (Boiss.), *Papaver dubium* L, *Camelina rumelica* Velen, *Alyssum szowitsianum* Fisch. & C. A. Mey, *Ephedra major*, *Alyssum minus.*

3.2 Results of Protein Studies

Seed storage proteins were extracted and carried out on SDS-PAGE after preparation. According to Fig. 1 results showed that the protein band pattern and numbers of bands were the same in all groups and there were not any differences between them. Consequences of electrophoresis studies with 0 and 1 codes are as follow in Table 2.

According to Table 2 and Fig. 2, totally 10 protein bands were observed in different special stations. The least number of bands was observed in station number 8 that has 3 bands.

Special stations 1, 2, 6, 7 and 11 all have 4 bands, special station 3 has 6 band, special stations 4, 5 and 12 have 5 bands. Special stations 9 and 10 have 7 bands, and finally special station 13 has 10 bands.

So the most bands observed in special station 13. Band 2 was common in all of the special stations that probably this band is related to the special proteins of *Stachys inflata*.

Table 1. 1 and 0 Data about protein bands of populations in *S. inflate*

	Station no	1	2	3	4	5	6	7	8	9	10	11	12	13
	1	0	0	1	0	0	0	0	0	0	0	0	1	0
	2	1	1	1	1	1	1	1	1	1	1	1	1	1
	3	1	1	0	1	1	1	1	1	1	1	1	1	1
	4	0	0	1	1	1	0	0	0	0	0	1	1	0
Band	5	1	1	0	0	0	0	0	0	0	0	0	0	0
no	6	0	0	0	0	0	0	0	0	0	0	0	0	1
	7	0	0	0	1	1	0	0	0	1	1	0	0	1
	8	1	1	1	0	0	0	0	0	0	0	0	0	1
	9	0	0	1	1	1	1	1	1	1	1	1	1	1
	10	0	0	1	0	0	0	0	0	1	1	0	0	0

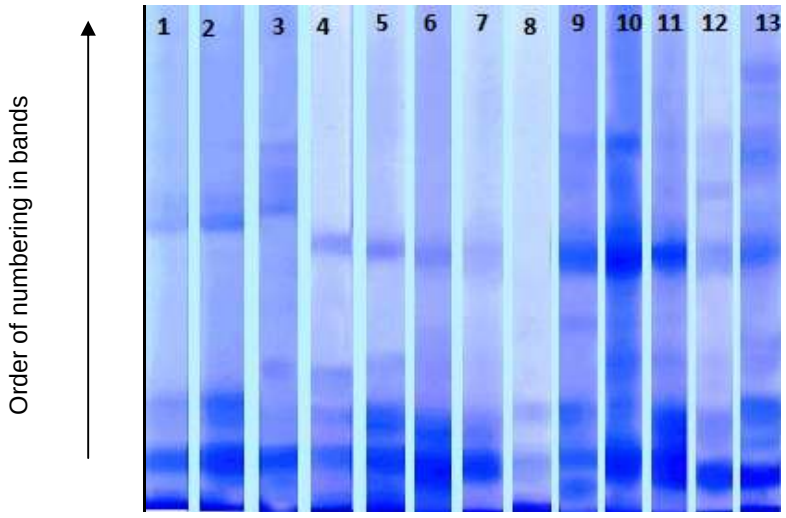

Fig. 2. Seed protein bands in populations of *Stachys inflata* by SDS-PAGES (Sodium dodecyl sulfate polyacrylamide gel electrophoresis) patterns

Location of each special station signaled base on existence of one individual species in our study (*Stachys inflata*) Then for determination of special station (DSS) of individual study species, minimal area detected by using the area-species method, and base of this fact electrophoresis data analysis performed. According to Figs. 3, 4 and 5, using MVSP software with complete method, MVSP software with P.C.O method and MVSP software with UPGMA method showed 9 groups contains: group A: special stations 1 and 2, B: special station 3, C: special stations 4 and 5, D: special stations 6 and 7, E: special station 8, F: special stations 9 and 10, G: special station 11, H: special station 12, I: special station 13 and it is the same in P.C.O and UPGMA method.

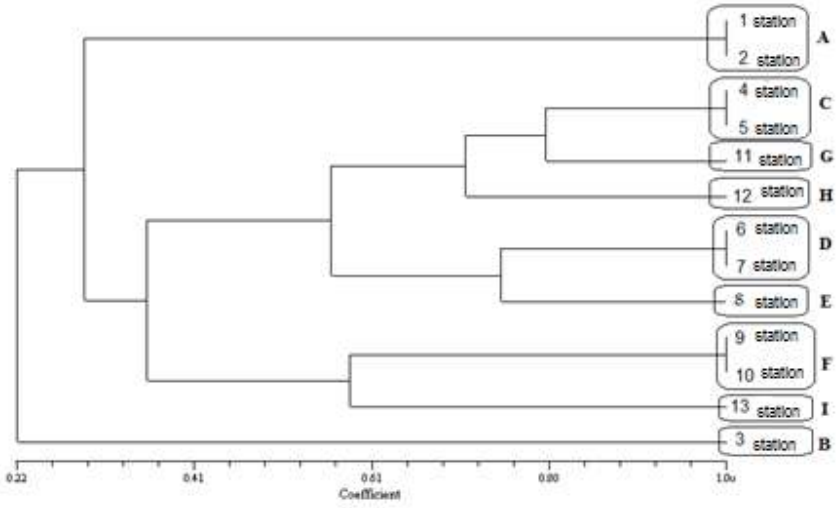

Fig. 3. Results of classification by electrophoresis data analysis in special stations by MVSP (Multi Variante Statistical Package) software with complete method

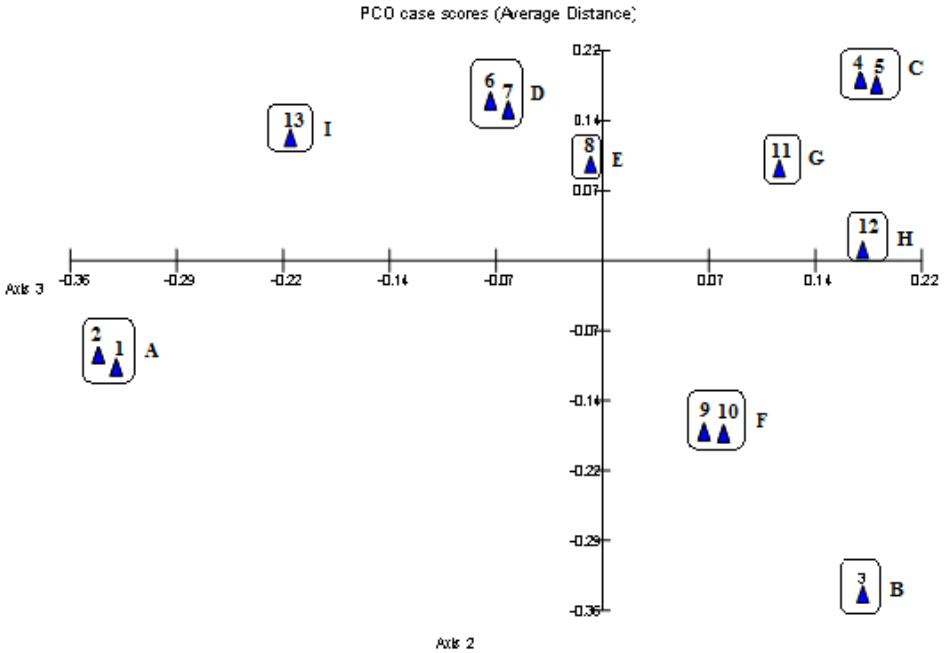

Fig. 4. Results of classification by electrophoresis data analysis in special stations by MVSP (Multi Variante Statistical Package) software with P.C.O method

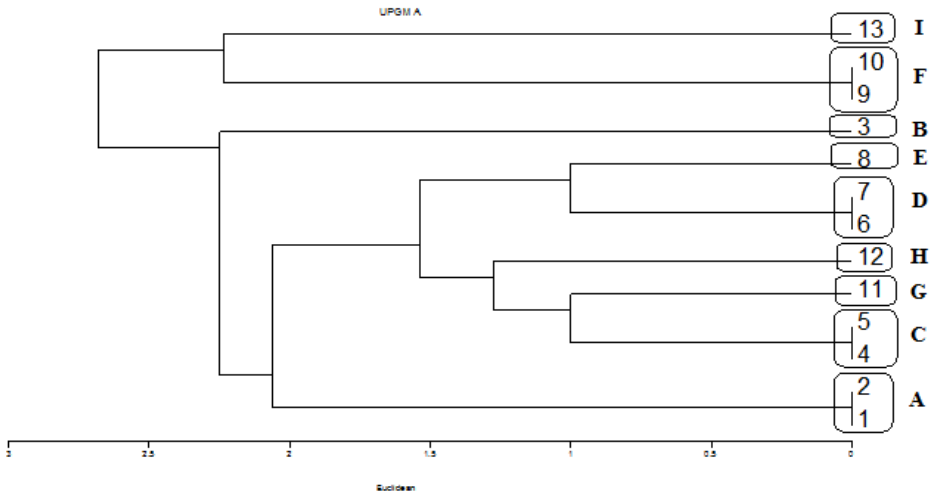

Fig. 5. Results of classification by electrophoresis data analysis in special stations by MVSP software with UPGMA (Unweighted Pair Group Method with Arithmetic Average) method

3.3 Diversity Results Obtained by Pedology (Soil Study) Data

In this study, we considered some factors like height, ramp orientation and soil in every special stations, and they analyzed by MVSP software with P.C.O, UPGMA and complete method.

Comparing the results shows that according to the floristic-ecologic marker, 9 groups have been obtained and it clarify the intraspecific diversity in *Stachys inflata* in Hamedan province.

According to Table 3 about ecologic data based on pedology (soil study), populations of *Stachys inflata* are found in PH range of 7/26 – 7/75. It could be concluded that soil PH of special stations in this species is in neutral range. In addition, there was no significant difference among their PH. It means all groups were in a same range of PH. Then the average value for each element (PH, EC, OC and soil texture) calculated that it was 7.51 and also standard deviation quantified and it was 0.15.

Due to the Table 3, EC (electrical conductivity) of all individuals of *Stachys inflata* are under 1. The least EC is for stations 6 and 7 (0/23), these 2 stations have clay bed and also they are in the same height, these factors cause they place in a same group. Also the most EC is for station 8 (0/36).

In the study of OC (organic carbon), It concludes that the least OC is for station 7 (0/1) and the most OC is for station 12 which significantly revealed different amount (8/99), it could be due to being in a separate group.

Soil texture of special stations for *Stachys inflata* is often Loamy (L) means mix of clay, silt and sand in different amounts.

Finally, according to Table 3 it is concluded that PH upper than 7, EC under 1, OC in vicinity of 1, soil texture L and Si-L, direction of incline eastern and western, soil bed stone and rocky and altitude upper than 2000m are the best ecological condition for growth and development in *Stachys inflata* in Hamedan province. Populations of *Stachys inflata* present in stone, rocky and slither beds, and they grow in dry and far from the water places.

Fig. 6 demonstrates ecological factors like altitude, type of bed, soil texture, PH, EC (electrical conduct) and OC (organic carbon) by C.C.A method. Each triangle indicates one special station and each arrow represents one ecological factor. The size of arrows display the quality and amount of effects about each ecological factors for each special station of *Stachys inflata.*

On the base of Fig. 6, it inferred that among studied ecological factors, soil texture in special stations 1, 2, 5, 6, 7; direction of incline on stations 3 and 4; EC and direction of inclination on special stations 8 and 9; soil texture and OC on special station 12; altitude on station 13 and finally altitude and PH on special stations 10 and 11 are the most effective ecological factors.

Table 3. Ecologic data based on soil study (pedology) in 13 special stations of *S. inflate*

Special station number	PH	EC (ds/m)	OC	Soil texture			
				% Si	%S	% Cl	Texture
Special station 1	7.50	0.26	0.89	32	58	10	S.L
Special station 2	7.52	0.24	0.78	38	50	12	L
Special station 3	7.30	0.32	1.11	46	40	14	L
Special station 4	7.36	0.32	1.17	62	24	14	Si.L
Special station 5	7.38	0.25	1.60	66	20	14	Si.L
Special station 6	7.58	0.23	0.29	36	52	12	S.L
Special station 7	7.58	0.23	0.1	46	40	14	L
Special station 8	7.26	0.36	1.11	50	38	12	L
Special station 9	7.69	0.33	1.50	60	30	10	Si.L
Special station 10	7.67	0.27	1.66	40	50	10	L
Special station 11	7.66	0.31	1.11	66	18	16	Si.L
Special station 12	7.45	0.34	8.99	50	32	18	L
Special station 13	7.75	0.32	2.34	42	46	12	L
Mean value	7.51	0.29	1.74	48.76	38.30	12.92	
STD Dev	0.15	0.043	2.16	11.08	12.324	2.30	

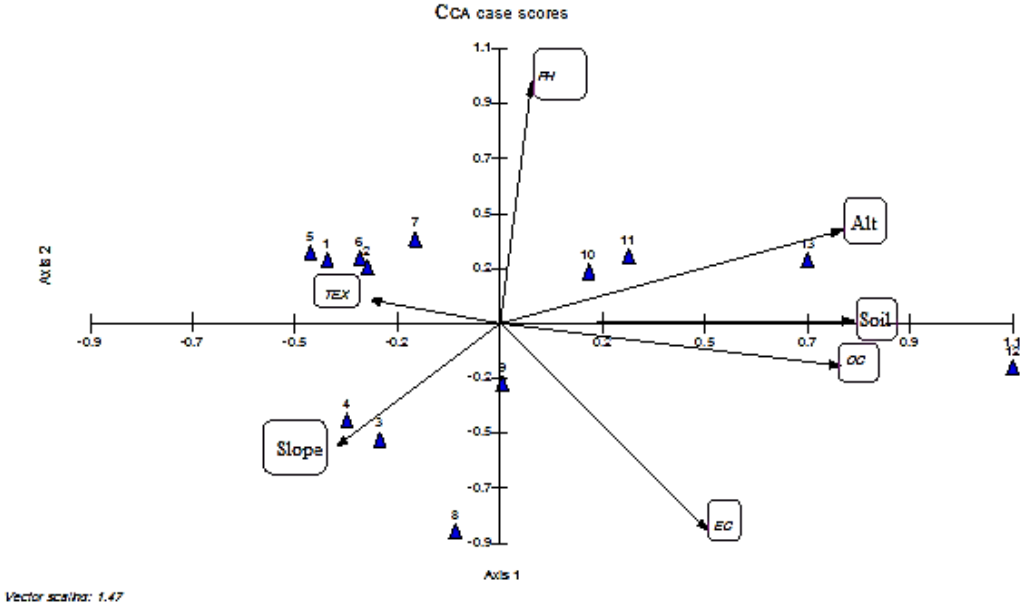

Fig. 6. The outcomes of analyzing the ecological data by MVSP (Multi Variante Statistical Package) software with C.C.A method

4. DISCUSSION

This research is the first report about the electrophoresis of seed storage proteins in *inflata Stachys* in Iran. Our findings indicate that the electrophoretic profiles are in accordance with floristic- ecologic groupings. Results obtained from the analysis of electrophoresis of seed storage proteins by MVSP and NTSYS software were also in accordance with floristic- ecologic groupings. As it observed, different number of bands and their density of the protein bands, varied in this species and showed intraspecific diversity in the populations of *Stachys inflata*. So according to the differences in protein bands they classified in different groups. Therefore, the whole 9 groups of *Stachys inflata* (revealed by floristic marker) are also confirmed with ecological and electrophoresis markers.

Generally there are some investigations that prove intraspecific diversity, for instance the survey of electrophoresis pattern, showed the differences regarding number and density of the

protein bands indicating the intraspecific diversity in the populations of *Artemisia incana* [7,8]. Therefore in this species, the grouping according to floristic marker, confirmed with ecological and electrophoresis markers. Study by [10] on *Artemisia scoparia* was carried out to determine intraspecific diversity by D.S.S method in the west of Iran [11].

This investigation is the first article about seed storage protein electrophoresis and pedology in *Stachys inflata* and there is no reports about these features in this species until now. By comparing the results and tables or figures, this fact can be depicted that seed storage protein electrophoresis is an useful method in level of intraspecies or interspecies populations to prove diversity among 13 special stations, and patterns of several bands can be analyzed easily too, These bands have been compared and according to existence or absence of each bands, taxonomic characters can be determined. And also as D.S.S method illustrates intraspecies diversity in our previous articles and studies in several cases such as chromosome counting, morphometric studies, ecological factors, in this survey again this approach was really helpful.

Also according to the findings that obtained from Chromosome counts in different populations of *Stachys inflata in Hamedan* province, DSS method showed Chromosome number variation in 9 groupings and the results indicated that the intraspecific polyploidy was confirmed in this species [13].

A research about floristic-ecologic variation of *Stachys inflata* depicted that there is clear connection between the floristic compositions of 13 studied populations, the ecological characteristics of the habitat and their associated taxa and exactly showed intra specific diversity. [12].

5. CONCLUSION

This study provided this fact that there is a relationship between seed protein diversity in 13 populations, ecological attitudes specially soil factors and also habitat of *Stachys inflata*. As a matter of fact, the members of ecological- floristic and electrophoretic groups were identical. The grouping by floristic marker, was confirmed by electrophoresis as well as soil data. In this study by electrophoresis method 9 groups obtained and as soil is a kind of ecological factor, according to Fig. 6, grouping showed 9 groups. So the number of groups in classification, results of seed storage proteins by MVSP and NTSYS software and associated taxa, are perfect evidences to prove intraspecific diversity.

Totally based on this study and other studies that done by DSS method until now, the high efficiency of it in determination of intraspecific diversity existence is shown. As a result electrophoresis pattern of seed storage protein can be an adequate evidence for proving DSS method, and this method is an affordable way to obtain more results in intraspecific diversity and taxonomic studied.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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