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# Seed Protein Study on Some Populations of *Salvia* (Lamiaceae) using Electrophoresis Technique in North-East of Iran

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**Abstract:** *Salvia* L. belongs to Lamiaceae family, have 900 species around the world. Seven of them distributed in Mashhad (North-East of Iran) which are following: *S. spinosa* L., *S. staminae* Montbr and Auch, *S. sclarea* L., *S. chloroleuca* Rech and Aell., *S. virgata* Jacq, *S. chorassanica* Bung. and *S. nemorosa* L. In present research seed proteins of *Salvia sclarea* L., *S. spinosa* L. and *S. chloroleuca* Rech and Aell. studied by sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) method. The aim of present study was identifying the variation between seed protein in populations and relationship with morphological characters and geographical distance. Analysis showed 22 bands from which some of them were specific for a population. Cluster analysis of populations were carried out. The variation bands have correspondence to geographical distance and morphological characters.

**Key words:** *Salvia sclarea*, *S. spinosa*, *S. chloroleuca*, SDS-PAGE electrophoresis, Iran

## INTRODUCTION

*Salvia* L. from Lamiaceae family, Stachyoideae subfamily, Salvieae tribe having numerous variety of species, it is expanded from Italy to Iraq, Iran, Pakistan and Afghanistan. In some of references *Salvia* belongs to Mentheae tribe (Jay, 2004, 2007). This genus has aromatic essential oil and antimicrobial effects and medicinal usage (Bruna *et al*., 2006; Jafari and Nikian, 2008). The essential oil or the infusion of the aerial part of the plant is used against Colds, coughs, tooth-ache, throat-ache, stomach-ache, diabetes, rheumatism and skin diseases (Fragaki, 1969).

Aqueous extracts from *Salvia officinale* leaves displayed potent anti- HIV-1 activity by increasing the virion density (Geuenich *et al*., 2008). Also, this species extract had hepatoprotective effects (Amin and Hamza, 2005). *Salvia* L. has 51 species in Iran, 7 species of which are found in Mashhad (North-East of Iran) and are following: *S. sclarea* L., *S. spinosa* L., *S. chloroleuca* Rech and Aell, *S. staminae*. Montbr and Auch, *S. virgata* Jacq, *S. chorassanica* Bung. and *S. nemorosa* L. (Rechinger, 1982). For present research, seed protein electrophoresis and morphological studying carried out on *S. sclarea* L., *S. spinosa* L. and *S. chloroleuca* Rech and Aell. The aim of selection of these species was its high diversity in Mashhad and distribution in different localities with different distances. This study was done for first time. There are some reports of molecular analysis of these species from other countries (Skoula *et al*., 1999). But we didn't find any report about seed protein electrophoresis of *Salvia* species.

## MATERIALS AND METHODS

The populations of *Salvia* L. collected for seed protein analysis from different localities in Mashhad (North-East of Iran) in May 2008 (Table 1). For extraction of seed protein, (20-25 seeds) were homogenized to obtain a fine powder. Protein were extracted in pre-cooled mortar with a 100 mM Tris phosphate buffer (pH 6.8), 0.5 mM EDTA (pH 8) and Mercaptoetanol. The resulting mixture

Table 1: The locality and studied populations of *Salvia* L.

| Species | Date of collection (2008) | Locality | Altitude (m) | No. of populations | The average of raining in collection months (mm) |
|---|---|---|---|---|---|
| *Salvia spinosa* | May 13 | Torogh | 1350 | 1 | 47 |
| *Salvia chloroleuca* | May 24 | Moghan | 1400 | 1 | 40 |
| *Salvia chloroleuca* | May 23 | Zoshk | 2200 | 1 | 60 |
| *Salvia chloroleuca* | May 23 | Kang to Zoshk | 2300 | 2 | 70 |
| *Salvia sclarea* | June 19 | Kang | 2000 | 1 | 68 |

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was centrifuged at 12000 g for 20 min. The crude extracts were boiled for 5 min in 0.5 M Tris-HCl (pH 6.8),10% Mercaptoethanol and 3% glycerol (Sanches-Yelamo *et al*., 1995; Sammour, 1999). Protein electrophoresed by SDS-PAGE used 20 mg of protein in each lane. Vertical slab gels 1 mm thick were electrophoresed at a constant current of 30 mA for 4 h. Coomasie Brilliant Blue R-250 was used for overnight gel staining follow by trichloroacetic acid as fixative. Cluster analysis was carried out on the basis of seed protein data. Each protein band was considered as a qualitative character and coded as 1 (presence) versus 0 (absence) (Carreras *et al*., 1997). For statistical analysis used MINITAB ver.11.

## RESULTS AND DISCUSSION

The seed protein analysis carried out for first time. The results of SDS-PAGE analysis showed, 22 bands. Bands 12 and 22 were presented in all studied populations. Bands 1, 8, 9 and 16 were observed in *Salvia sclarea*. Bands 7 and 17 existed in *S. chloroleuca* (Kang 2) and only band 15 observed in *S. chloroleuca* (Moghan) with RF = 0.629. Also, band 19 only existed in *Salvia spinosa* (Torogh) with RF = 0.833, which bands were specific for these populations.

In Torogh population observed 9 bands which only band 10 with RF = 0.509 existed in this population and Kang 2 population had maximum bands (13). In Zoshk population existed 8 bands without specific band with spmw = 734000. In Kang 1 observed 10 bands which 5 of them were specific for this population with RF = 0.407 and spmw = 935000. In *S. sclarea* existed 9 bands which only bands 1, 8, 9 and 16 were specific for this population with spmw = 847500. The most number of bands were 13 band in Kang 2 and the least of them was 6 in Moghan (Fig. 1a, b).

Cluster analysis of seed protein data were presented on the absence or presence of bands. Cluster analysis of protein data showed: Moghan and Kang 1 populations of *S. chloroleuca* had similarity 78.94 because their locality are mountainous with stony slopes. Also, the population of Zoshk and Kang 2 from *S. chloroleuca* were similar because they growing in country. The minimum similarity observed between *S. spinosa* and *S. chloroleuca* with distance level 19.362 and similarity 35.90 (Fig. 2). Morphologically confirmed the difference between *S. spinosa* and another species by having different calyx shape and symmetry, calyx denth shape and calyx and leave hairs. For example *S. spinosa* had regular and tubular calyx but *S. chloroleuca*, had irregular and campanulate calyx. Cluster analysis confirmed the similarity between *S. sclarea* and *S. chloroleuca* because having the same calyx shape but

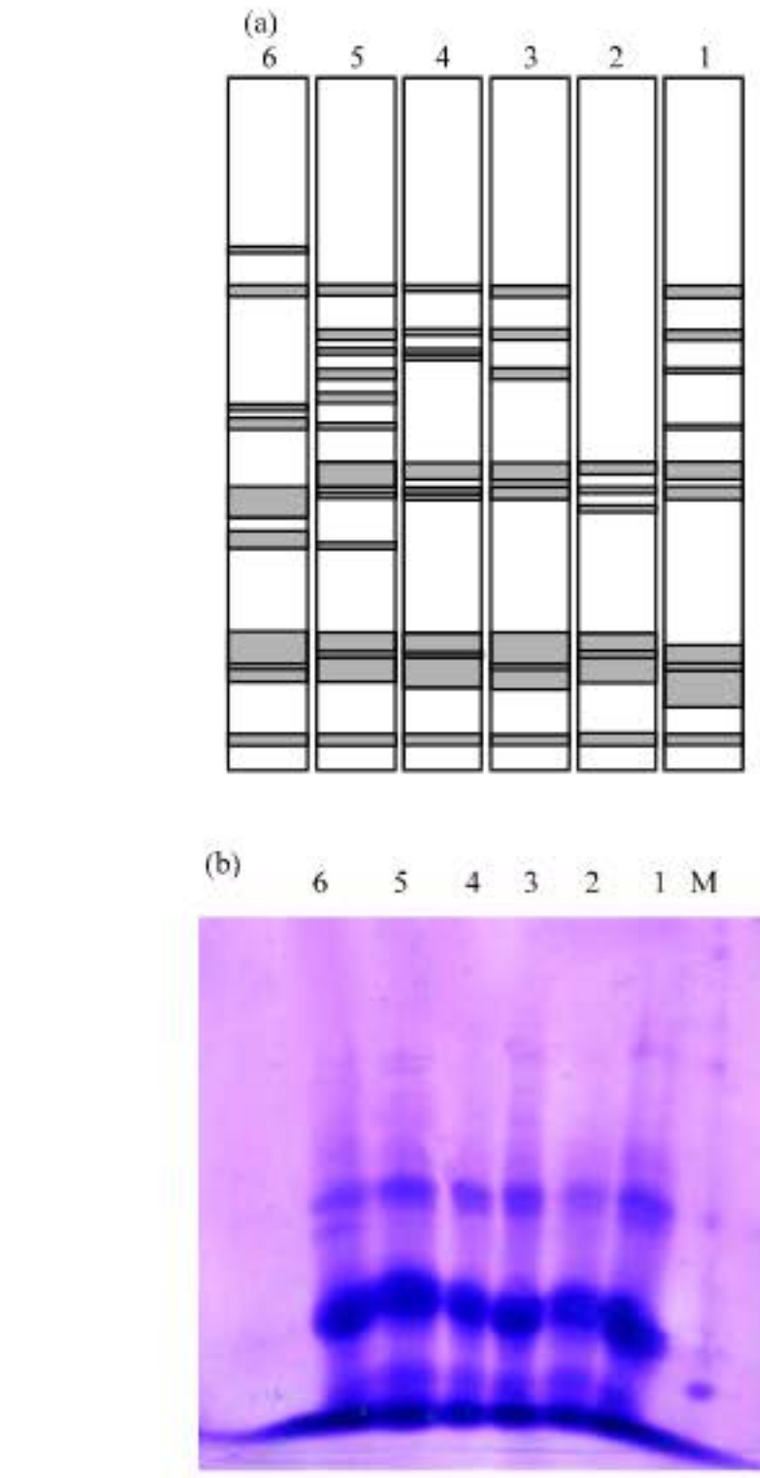


Fig. 1: Seed protein bands: (a) Diagram (b) Electrophoregram of seed protein bands of 1: *Salvia spinosa* (Torogh), 2: *S. chloroleuca* (Moghan), 3: Zoshk, 4: Kang 1, 5: Kang 2, 6: *S. sclarea* ( Kang)

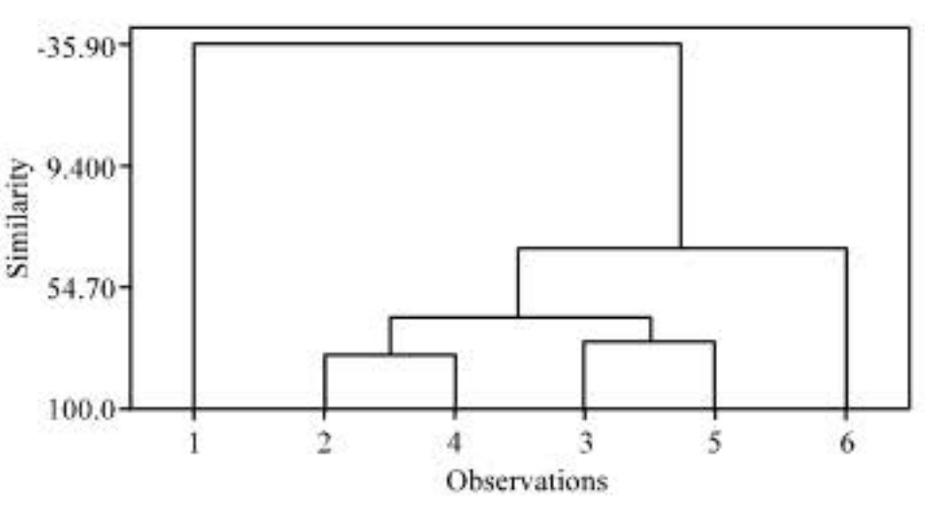


Fig. 2: Dendrogram of cluster analysis of *Salvia* populations on the basis of seed protein bands. The dendrogram showing similarity level between clusters (population). 1: *Salvia spinosa* (Torogh), 2: *S. chloroleuca* (Moghan), 3: Zoshk, 4: Kang 1, 5: Kang 2, 6: S. *sclarea* ( Kang)

Table 2: Hierarch cluster analysis of observations euclidean distance, ward linkage and amalgamation steps

| Step | No. of clusters | Similarity level | Distance level | Clusters joined | | New cluster | No. of observation in new cluster |
|---|---|---|---|---|---|---|---|
| 1 | 5 | 78.94 | 3.000 | 2 | 4 | 2 | 2 |
| 2 | 4 | 74.69 | 3.606 | 3 | 5 | 3 | 2 |
| 3 | 3 | 65.07 | 4.977 | 2 | 3 | 2 | 4 |
| 4 | 2 | 39.26 | 8.654 | 2 | 6 | 2 | 5 |
| 5 | 1 | -35.90 | 19.362 | 1 | 2 | 1 | 6 |

*S. sclarea* calyx had dense hairs. So, difference between morphology and seed protein of *S. spinosa* and *S. chololeuca* is observed. Also, variation of band in population Kang 1 and Kang 2 due different ecological conditions. Hierarch cluster analysis of observations euclidean distance, ward linkage and amalgamation steps are shown in Table 2.

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