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ARTICLE

Study of seed protein electrophoretic profiles in some *Erodium* (Geraniaceae) species native to Iran

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ABSTRACT *Erodium* (L.) L'Hér. with 75 species is the third largest genus in Geraniaceae. *Erodium* is distributed in all continents but it shows a great diversity in the Mediterranean region. In Flora Iranica , Schonbeck-Temesy (1970), 15 species, four subsections and three subspecies have been mentioned for *Erodium* in Iran. Electrophoretic patterns of seed storage proteins in the genus *Erodium* has not been studied extensively. In this study for the first time seed protein electrophoretic patterns of seven *Erodium* species from different regions of Iran have been investigated. Protein extracts of seeds were obtained and protein concentration was determined by the Bradford method and analyzed with SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Results were evaluated by multivariate analysis methods including cluster analysis and factor analysis. In the electrophoretic pattern of seed storage proteins, generally, 48 specific bands with molecular weight between 13-102 kDa were detected. Electrophoretic data confirmed sub-generic classification of *Erodium* (to *Plumosa* and *Erodium* sections). Species relationships are discussed.

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KEY WORDS

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Erodium is the third largest genus of Geraniaceae with 75 species of annual or biennial herbaceous rosette form (Fiz et al. 2008). These taxa are distributed in all continents except Antarctica, but most of them are found in temperate and tropical regions of the world with a great diversification in Mediterranean region (Fiz et al. 2008; Takhtajan 2009). *Erodium* species are common weeds, but are used in traditional medicine because of its astringent properties (Gohar et al. 2003). L'Heritier (1789) was the first who used *Erodium* for this genus. Boissier (1867) divided *Erodium* into two sections: *Barbata* Boiss and *Plumosa* Boiss. Knuth (1912) in his study divided *Erodium* into two sections as *Plumosa* with five species and *Barbata* with the remaining species. Schonbeck-Temesy in Flora Iranica (1970) followed Boissier system, but used *Erodium* instead of *Barbata* for section name. However in 1972, Guittonneau considered these two parts as two subgenera *Plumosa* and *Barbata* (El Hadidi et al. 1984; El Naggar 1991). All mentioned classifications were based on different mericarp characters as beak and bristles condition. In *Plumosa* section, species have deciduous beak and hairy bristle, but in *Barbata* beak is persistent and there is no feather like bristles. Janighorban (2005) pointed to 15 species without any sectional classification for this genus in Iran.

In the present study the Schonbeck-Temesy (1970) classification has been used which defined two sections in *Erodium* as section *Plumosa* Boiss and section *Erodium* (*Barbata* Boiss) with *E. oxyrrhynchum* M. Bieb. is the only species of first section present in Iran. At the same time, *Erodium* section is composed of 5 subsections as *Gruina* Willk. et Lange. including *E. gruinum* (L.) L Hér. and *E. hoefftianum* C.A. Mey., subsection *Absinthioidea* (Brumh.) Guitt. including *E. ciconium* (L.) L Hér., *E. stephanianum* Willd., and *E. dimorphom* Wendelbo, subsection *Malacoidea* Brumh. with *E. malacoides* (L.) Willd., *E. neuradifollium* Del. Ex Godr. and *E. pulverulentum* (Cav.) Willd., subsection *Bovei* (Delile) Schonbeck-Temesy including *E. laciniatum* (Cav.) Willd. and finally subsection *Cicutaria* Willk. et Lange. including *E. cicutarium* L Hér., *E. moschatum* (L.) L Hér and *E. deserti* Eig.

Over the last decades, investigation of protein electrophoretic patterns was frequently used in plant taxonomic studies. In most cases, the seed was used as protein source, because it displays a certain stage of the plant life and is less affected by environmental stress than the leaves (Crawford 1990). Concerning *Erodium* species, the only study on seed protein electrophoretic patterns of the genus was conducted by Sharawy and Badr (2008) on 12 *Erodium* species of Egypt. In our present study, the electrophoretic protein profiles of seven *Erodium* species native to Iran are investigated.

Materials and Methods

Plant material

Sampling was carried out from distribution range of the seven studied *Erodium* species in 2012 and 2013 growth period. Accession details for studied species are displayed in Table 1.

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Table 1. Voucher details of the investigated *Erodium* species.

Taxa	Locality	Herbarium number
E. cicutarium	Tehran, Vanak, Najafian & Taghipour. 1439 m. Tehran, Peykanshahr, Botanical Garden of Iran, Taghipour. 1270 m	911 918
E. ciconium	Tehran, Peykanshahr, Botanical Garden of Iran, Taghipour. 1261 m	913
E. oxyrrhynchum	Tehran, Peykanshahr, Botanical Garden of Iran, Taghipour. 1261 m	912
E. malacoides	Gilanegharb, Vizhnan, Najafian. 920 m	927
E. moschatum	Gilanegharb, Vizhnan, Najafian. 920 m	929
E. gruinum	Gilanegharb, Vizhnan, Najafian. 915 m	926
E. hoefftianum	Karaj, Kondor, Taghipour. 1897 m	9215

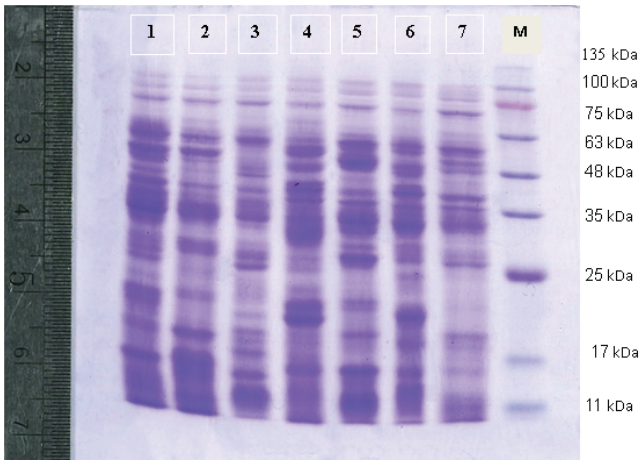

Figure 1. SDS-PAGE pattern of the examined isolates of Iranian *Erodium*. M: pre-stained protein ladder (10-170 kDa). Lane 1-7: *E. cicutrium, E. ciconium*, *E. oxyrrhynchum*, *E. moschatum*, *E. hoefftianum*, *E. malacoides*, and *E. gruinum*, respectively.

Extraction of Seed Proteins

In order to extract seed storage proteins, seeds pods were removed and 0.1 g of each seed sample was powdered and homogenized with 1 ml extraction buffer (0.2 M Tris-HCl, pH 8) in mortar in ice bath for 1 hour. Extractions were centrifuged in a cold room with 13400 rpm for 30 min. Supernatant was transferred to a clean tube and used immediately for electrophoresis (Laemmli 1970; Sharawy and Badr 2008).

Bradford method

The protein concentration was measured by Bradford (1976) method. To produce 100 ml of 0.01% Coomassie Brilliant Blue G250 solution, 10 mg of Coomassie Brilliant Blue G-250 was dissolved in 5 ml of 95% ethanol, then 10 ml 85% phosphoric acid was added and when the dye has completely dissolved, it diluted to 100 ml with distilled water, and filtered through Whatman #1 paper just before use. In this method, Bovine Serum Albumin (BSA) solution (1 g/l) in Tris-HCl buffer was prepared and used as standard protein solution. The different concentrations of BSA from 0 to 0.6 mg/ml were prepared using extraction buffer, then 5 ml of Coomassie Brilliant Blue G250 solution was added and their absorbances were read in 595 nm (Bradford 1976). A standard curve of absorbance versus mg/ml protein was prepared and the concentration of samples was determined from the curve.

SDS-polyacrylamide gel electrophoresis

Gel electrophoresis was performed with Tris-glycine running buffer (pH 8.3) at room temperature. SDS-PAGE was consisting of 4% stacking gel (pH 6.8) and 15% separating gel (pH 8.8). Electrophoresis was done in constant voltage (120 V) for 2 hours. The gel was stained with Coomassie Brilliant Blue R-250 in 50% methanol and 3.5% acetic acid for about 30 min, followed by destaining in 10% methanol and 10% acetic acid overnight or until the gel background was cleared (Laemmli 1970).

Proteins molecular weight measurement

For the preparation of a protein molecular weight standard curve a 10-170 kDa protein ladder was used. After electrophoresis of the sample protein solutions their relative mobility values (R_f) were calculated. The logarithm of the molecular weights of protein ladder against their R_fs were plotted. The molecular weights of the samples were calculated by comparison of their R_fs with the standard curve. Multivariate statistical analysis of data was performed using SPSS Software (version 18; SPSS, Chicago, IL, USA). Cluster analysis by WARD method (minimum variance) was used to illustrate branching patterns as an appropriate method for quantitative variables.

Results

For the first time, seed storage protein electrophoretic profile of *E. hoefftianum* is recorded. About 48 specific bands were observed and their molecular weights (13-102 kDa) were calculated (Fig. 1). Presence and absence of bands were considered as qualitative binary or multistate features in multivariate statistical analysis. Some bands were exclusively observed in certain species, for example six bands were only observed in *E. oxyrrhynchum,* four in *E. cicutarium* and other four bands only in *E. moschatum.* There was also one band in common in *E. moschatum*, *E. cicutarium* and also three bands in common in *E. malacoides* and *E. hoefftianum* species pairs.

Cluster analysis by WARD method (Fig. 2) showed that there are two main clusters in distance level 25. The first

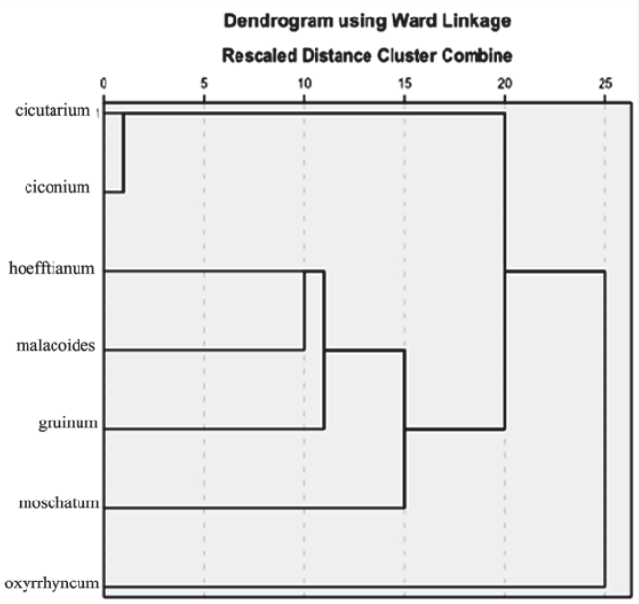

Figure 2. WARD dendrogram based on the electrophoretic data of *Erodium* seed proteins.

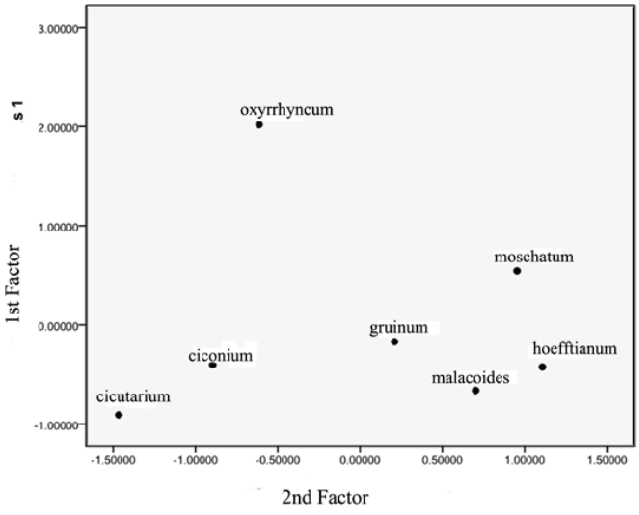

Figure 3. PCA scatter diagram based on the electrophoretic data for studied *Erodium* species. (Axis values are based on Principal Component Analysis).

cluster contains *Erodium* species of section *Erodium*. This is divided into two sub-clusters, which contain two closely related species as *E. ciconium* and *E. cicutarium*. The other sub-cluster comprises two species: *E. hoefftianum* and *E. malacoides* at a distance of about 10. The second main cluster contains *E. oxyrrhynchum* that belongs to *Plumosa* section.

In scatter diagram of Principal Component Analysis based on the first and second factors of electrophoretic data (Fig. 3), it is evident that *E. oxyrrhynchum* as the only species of *Plumosa* section, has been separated from other species (belonging to *Erodium* section).

Discussion

Comparison of the electrophoretic profiles revealed that the most common bands are observed between *E. cicutarium* and *E. ciconium* (possessed in common five bands) and also between *E. malacoides* and *E. hoefftianum* (possessed in common five bands). *E. cicutarium* and *E. ciconium,* and also *E. malacoides* and *E. hoefftianum* species pairs showed the highest similarities. According to WARD clustering pattern (Fig. 2), the two main clusters are in accordance with the Schonbeck-Temesy (1970) sectional classification (*Erodium* and *Plumosa*). In main cluster of *Erodium* section, *E. gruinum* and *E. hoefftianum* species of *Gruina* subsection, there were two near sub-clusters, which are in accordance with the Schonbeck-Temesy (1970) sub-sectional classification. The second major cluster contained *E. oxyrrhynchum* of *Plumosa* section. This is clearly separated from *Erodium* section. Cluster analysis by WARD method for *Erodium* species is somehow in accordance with Sharawy and Badr studies (2008) on Egyptian *Erodium*, especially in sectional separation, but there are some differences in species separation pattern, for example *E. cicutarium* from *Cicutaria* subsection and *E. ciconium* from *Absinthioidea* subsection are grouped in the same cluster in the present study. *E. malacoides* from *Malacoidea* subsection and *E. hoefftianum* from *Gruina* subsection are also grouped as closely related species in the same sub-cluster. *E. gruinum* and *E. hoefftianum* are elements of the same subsection, but are grouped in different sets. According to PCA graph based on electrophoretic data (Fig. 3), results clearly confirmed sub-sectional classification of *Erodium* presented by Schonbeck-Temesy (1970).

The results of seed storage protein electrophoresis for seven studied *Erodium* species of Iran showed that species separation at sectional level is in accordance with previous studies in Egypt (Sharawy and Badr 2008) and also confirmed the Schonbeck-Temesy opinion about sectional classification. The present study reinforced that electrophoretic profiles of seed storage proteins have taxonomic value in species delimitation in the genus *Erodium*.

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