Electrophoretic study of seed storage proteins in the genus *Stellaria* (*Caryophyllaceae*) and its closest relatives in Iran

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Abstract. This study deals with banding patterns of seed storage proteins by means of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) with extract of bulked seeds of *Stellaria* L. species and its closest related genera in Iran, such as: *Stellaria media, S. pallida, S. holostea, S. persica, S. graminea, S. alsinoides, Mesostemma kotschyanum,* and *Myosoton aquaticum*. The results show that *S. persica* and *S. graminea* are closely related. A close relationship and high protein similarity (J=0.72) are found between *S. media* and *S. pallida*. Electophoretic results are compared with earlier anatomical and morphological studies. The results show that despite morphological similarities between *Myosoton aquaticum*, *Stellaria media* and *S. pallida,* they are not closely related judging by the obtained SDS-PAGE profiles. Separation of the studied taxa is obvious in the gel with 2-mercaptoethanol.

Key words: SDS-PAGE, species relationships, *Stellaria*

Introduction

Stellaria L. is a large, morphologically variable and cosmopolitan genus of the *Caryophyllaceae*, with about 120 species. Its main centers of diversifications are in the temperate and sub-temperate regions of Europe, Asia and North America, but representative species also occur in the montane floras of the tropics (Morton 2005). In Iran, this genus has nine species grouped in two sections, but some species such as *S. blatteri* Mattf., *S. scaturiginella* Rech.f. and *S. sarcophylla* Rech.f. have an uncertain section (Rechinger 1988). According to *Flora Iranica,* the *Stellaria* sections in Iran include: *Stellaria* L. with two annual species – *S. media* (L.)Vill. and *S. pallida* (Dumort.) Pire – and four perennial species, which grow in the mountain areas, including: *S. holostea* L., *S. persica* Boiss., *S. graminea* L., and *S. nemorum* L. The section *Pseudalsine* Boiss. consists of only one annual species (*S. alsinoides* Boiss. & Buhse) growing in the mountains of Iran. *Mesostemma kotschyanum* (Fenzl ex Boiss.) Vved. is the basionym of *Stellaria kotschyanum* Fenzl ex Boiss., but Rechinger in 1988 considered this taxon as *Mesostemma* Vved. *Myosoton aquaticum* (L.) Moench. is occasionally treated as *Stellaria aquaticum* (L.) Scop.

SDS-PAGE is a useful method in taxonomy as an additional approach to access the species relationships. The seed protein gel profiles reflect the genetic affinities within a taxon and even between different biological entities (Shechter 1975; Ladizinsky 1979). Uniformity of the gel profile and its additive nature make seed protein electrophoresis a powerful tool in the studies concerning the origin and evolution of polyploid plants (Johnson 1972; Ladizinsky & Hymowitz 1979). The only report of SDS-PAGE in the *Stellaria* species belongs to Gifford & Chinnappa (1986) and deals with seed proteins of *Stellaria longipes* s.l. They had found one and the same general pattern in all studied genotypes and cytotypes. In the present study, the authors have attempted to resolve the relationship

between *Stellaria* species in Iran, using SDS-PAGE for supplementary data in terms of morphology. The objective of this study was to assess the level of seed electrophoretic patterns of the *Stellaria* taxa in Iran.

Material and methods

Plant material

In the present study, eight populations of six *Stellaria* species and the two closest related genera in Iran were collected in the field: *Stellaria media, S. pallida, S. holostea, S. persica, S. graminea, S. alsinoides, Mesostemma kotschyanum,* and *Myosoton aquaticum.* Details of the voucher specimens and their localities are given in Table 1. All voucher specimens are deposited at the Herbarium of Alzahra University (AUH).

Table 1. Voucher details of the *Stellaria* species and relative genera from Iran.

Species	Locality
Stellaria media (L.) Vill.	Tehran: Vanak, 1461m, Esfandani 993
S. pallida (Dumort.) Pire	Golestan: Gorgan, Ramian, 200 m, Esfandani 1030
S. holostea L.	Tehran: Karaj, Chalus road, Siah Bisheh, 2200 m, Esfandani 1032
S. persica Boiss..	Tehran: Karaj, Chalus road, Kandovan Tunnel north slopes, 2750–2800 m, Esfandani 1035
S. graminea L.	Tehran: Karaj, Chalus road, Kandovan north slopes, 2750–2800 m, Esfandani 1036
S. alsinoides Boiss. & Buhse	Mazandaran: Haraz road, Emam Zad-e-Hashem, 2700–2800 m, Esfandani 1038
Myosoton aquaticum (L.) Moench	Gilan: Rezvanshahr, Khoshabireh, 50 m, Esfandani 1040
Mesostemma kotschyanum (Fenzl ex Boiss.) Vved. subsp. *kotschyanum*	Tehran: Mountain of Tuchal, near fifth station of Tuchal Telecabin, 2900 m, Esfandani 1041

Protein extraction and electrophoretic analysis

For protein preparation a modified method of Gifford & Chinnappa (1986) was applied. Mature seeds were ground in a mortar in 0.05 M sodium phosphate buffer (pH 7.5), in a ratio of 0.1 g fresh weight to 1ml extraction buffer. The homogenate was centrifuged for 45 min. The supernatant containing the soluble proteins was removed, the pellet was re-extracted three times and the supernatants were mixed (all procedures were carried out at 4 °C). Concentration of the final extract was determined by the Bradford protocol and subsequently it was loaded on SDS-PAGE and stained by Coomassie brilliant blue R250 (Laemmli 1970).

We have used two types of sample buffers: a gel including 2-Mercaptoethanol (+2ME) and another one without 2-Mercaptoethanol (-2ME).

Jaccard Similarity Coefficient was used to evaluate the degree of similarity between the species. In the 5statistical analysis, presence or absence of each band was considered as a qualitative feature. A dendrogram was constructed by WARD hierarchical clustering with SPSS software, ver. 16. In order to find out the most variable protein band in the studied taxa, Principal Component Analysis was applied. Standard proteins (b-galactosisase, Ovalbumin, lactate dehydrogenase, lactoglobulinb, Lysozyme and bovine serum albumin) were used to evaluate molecular weight of the unknown proteins.

Results

SDS-PAGE electrophoretic data were analyzed with the help of Jaccard Similarity Index (Tables 2 and 3).

Table 2. Jaccard Similarity Index based on electrophoretic data of seed storage proteins in the *Stellaria* species and +2ME gel relative genera from Iran.

Taxa	*S. media*	*S. pallida*	*Myosoton aquaticum*	*S.graminea*	*S. persica*	*S. alsinoides*
S. media		0.72	0.75	0.53	0.54	0.44
S. pallida	0.72		0.85	0.58	0.55	0.33
Myosoton aquaticum	0.75	0.85		0.55	0.70	0.50
S. graminea	0.53	0.58	0.55		0.41	0.30
S. persica	0.54	0.55	0.70	0.41		0.33
S. alsinoides	0.44	0.33	0.50	0.30	0.33	

Table 3. Jaccard Similarity Index based on electrophoretic data of seed storage proteins in the *Stellaria* species and +2ME gel relative genera from Iran.

Taxa	*S. media*	*S. pallida*	*S. graminea*	*Myosoton aquaticum*	*S. persica*	*S. alsinoides*
S. media		0.36	0.70	0.30	0.16	0.50
S. pallid	0.36		0.60	0.20	0.21	0.30
S. graminea	0.70	0.60		0.42	0.12	0.91
Myosoton aquaticum	0.30	0.20	0.42		0.15	0.42
S. persica	0.16	0.21	0.12	0.15		0.46
S. alsinoides	0.50	0.30	0.91	0.4 2	0.46	

Altogether, 47 bands were observed for these taxa. Bands 22.54, 88.14, 97.16 and 130.11 KD were observed only in *S. alsinoides;* bands 13.85, 24.84, 29.22 and 55.94 KD were observed exclusively in *Myosoton*

aquaticum. Bands 11.77 and 28.29 KD were found only in *S. persica*. Bands 35.51 and 63.70 KD were observed exclusively in *S. media*. Band 61.67 KD was found only in *S. pallida.* All studied taxa had 41.77 KD, except for *S. pallida*. The highest number of bands was observed in *S. persica* and the lowest in *S. media* (Fig.1).

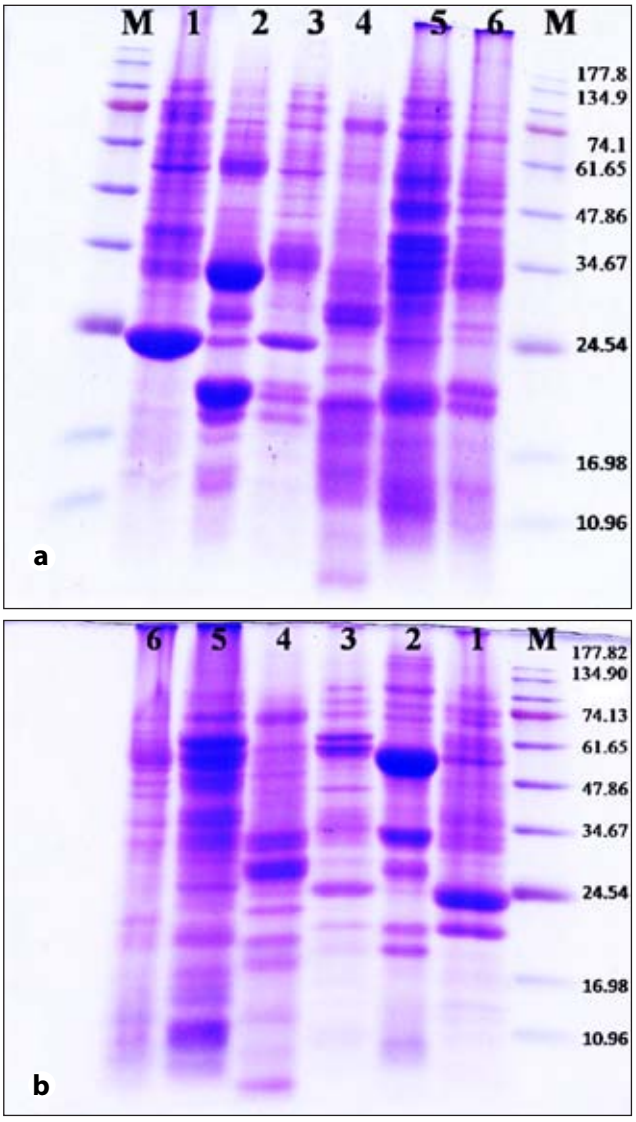

Fig. 1. Seed protein banding profile of the *Stellaria* species and relative genera from Iran examined in this study. **1**) *S. media*, **2**) *S. pallida* , **3**) *S. graminea*, **4**) *Myosoton aquaticum*, **5**) *S. persica*, **6**) *S. alsinoides*, **M**) Marker, **a**) +2ME, **b**) -2ME.

In order to find out the most variable protein bands in the studied taxa, a Principal Component Analysis was implemented. Primitive analysis showed that four factors were responsible for 86.39% of total studied variation in the taxa. In the first factor, with almost 27.60% of the total variation, bands 13.85, 15.77, 17.38, 19.79, 24.84, 29.22, 55.94, and 67.98 KD had the highest correlation. In the second factor, with about 21.34% of the observed variation, bands 25.66, 30.19, 41.77 and 61.67 KD had the highest positive correlation. In the third factor, with 20.40% of the total variation, bands 22.54, 88.14, 97.16 and 130.13 KD had the highest correlation. In the fourth factor, with 17.03% of the total variation, bands 35.51, 59.70, 63.70, 70.22, 72.54, 74.93, and 77.41, KD had the highest correlation. Results of the cluster analysis are shown in the WARD dendrogram (Fig. 2).

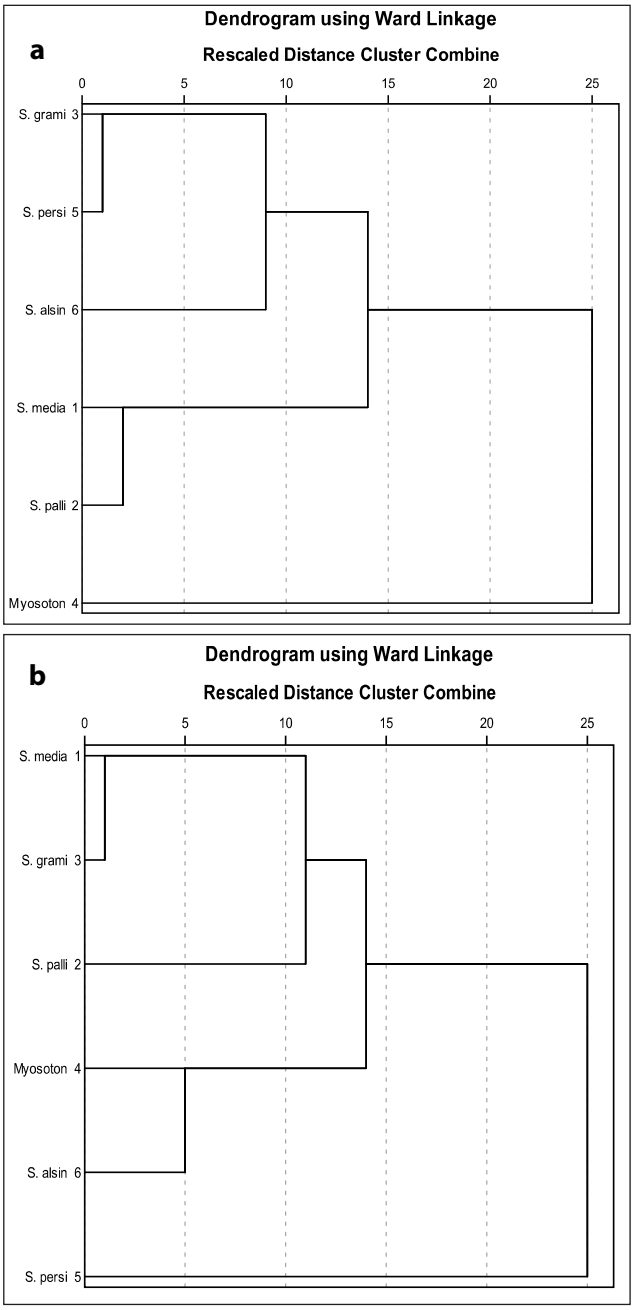

Fig. 2. Dendrogram depicting clustering by the WARD method of the *Stellaria* species and relative genera from Iran examined in this study by cluster analysis of the seed storage proteins **a**) +2ME, **b**) -2ME.

The taxa are clearly separated on the basis of electrophoretic data of seed storage proteins. The results have revealed that *S. persica* and *S. graminea* were closely related. A high Similarity Index is a reflex of genomic identity (J=0.41). The dendrogram showed close relationship and high protein similarity (J=0.72) between *S. media* and *S. pallida*. On the other hand, *S. alsinoides* itself formed a separate cluster. Ordination of the studied taxa based on PCA (Fig. 3) showed agreement with the cluster analysis.

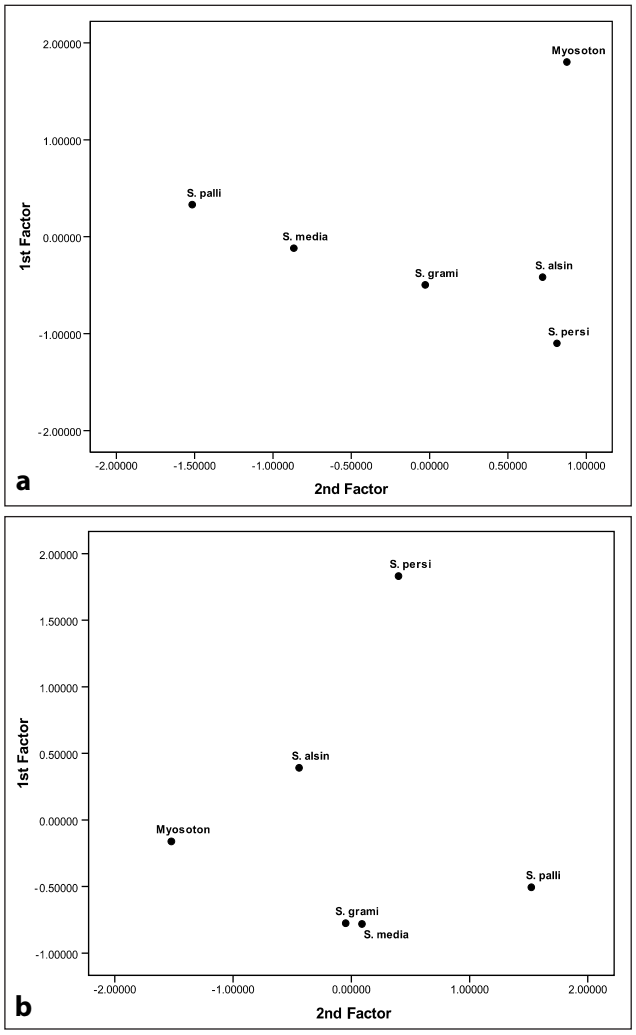

Fig. 3. PCA ordination of the *Stellaria* species and relative genera from Iran examined in this study on the basis of SDS-PAGE characters **a**) +2ME, **b**) -2ME.

Discussion

Electrophoretic data of the seed storage proteins presented in this study have shown that *S. holostea* and *Mesostemma kotschyanum* do not have soluble proteins in 0.05 M phosphate buffer pH 7.5, which sets them apart from the other studied species. Despite the morphological similarities between the paired species of *S. pallida* and *S. media* and *S. graminea* and *S. persica*, the protein profiles of these species are related, but not identical and are efficient in species separation.

The dendrogram obtained from electrophoretic data has shown that a +2ME gel of the studied species is capable of the species recognition. The species separation pattern corresponds to the morphological and anatomical ones (Esfandani 2013) and presents a proper method for determining the species relationships. The protein profiles of -2ME gel have shown differences with +2ME gel. This is due to the disulfide bond in some of these proteins.

The obtained results indicate that *Stellaria alsinoides* is grouped inside genus *Stellaria* and should not be considered as *Tytthostemma alsinoides,* as mentioned before. Despite its macro and micromorphological similarities to *S. media* and *S. pallida, Myosoton aquaticum* is clearly recognized as a separate taxon and should not be merged with the genus *Stellaria* (Esfandani 2013).

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