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Biosystematic study of *Phalaris* L. species (Poaceae) in Iran

Maryam Keshavarzi *, Mahnaz Khaksar and Parinaz Ghadam

Department of Biology, Faculty of Sciences, Alzahra University, Tehran, Iran

Abstract
This study dealt with banding patterns of seed storage proteins using sodium dodecylsulphate-polyacrylamide gel electrophoresis (SDS-PAGE) with extract of bulked seeds of *Phalaris* L. species of Iran. The results showed that two varieties of *P. paradoxa* L. as var. *praemorsa* and var. *paradoxa* L. were closely related. A close relationship and high protein similarity (J=0.583) were found between *P. arundinacea* L. and *P. brachystachys* Link.. Electophoretic results were compared with previous anatomical and morphological studies.
Key words: *Phalaris*, Taxon relationships, SDS-PAGE

Introduction

The genus *Phalaris* L. has had a complicated taxonomic and nomenclatural history (Baldini, 1993, 1995). It comprises 22 species of annual and perennial grasses in temperate regions throughout the world. These are commonly adventives species of open habitats. There are 4 species and 5 taxa of *Phalaris* in Iran (Bor, 1970): *P. minor* Retz., *P. brachystachys* Link., *P. paradoxa* L. (with 2 varieties) and *P. arundinacea* L.. These species are distributed in various regions of Iran. They are among important forage plants. Members of the genus *Phalaris* display many variations on the standard structure of the inflorescence (Bor, 1968). There has been no report of systematic study on *Phalaris* species of Iran.

Many authors demonstrated that sodium dodecylsulphate-polyacrylamide gel electrophoresis (SDS-PAGE) of proteins extracted from seeds produces a composite pattern for the phenotypes in the analyzed population (Yusaf *et al.*, 2006; Sheidai *et al.*, 2008). SDS-PAGE can be used to characterize the seed protein banding profiles of species and cultivars in several grass genera, compare the cultivars of different geographical origin and provide taxonomically useful descriptors that are substantially free from environmental influence. This procedure has provided useful data for many grasses as *Lolium* L. and *Festuca* L. complex (Aiken *et al.*, 1992), *Dactylis* L. and *Leucopoa* Griseb. (Aiken *et al.*, 1998). There was no report of SDS-PAGE in *Phalaris* species but limited variation was found out by means of Isozymes in annual *Phalaris* species (Hucle and Matus, 1999).

Seed protein banding profiles have proved to provide informative supplementary data for morphological features in resolving problems in the grass taxonomy. In this research, we tried to resolve *Phalaris* species relationship by using SDS-PAGE as supplementary data for

* Corresponding Author: keshavarm@alzahra.ac.ir

morphology in Iran. The objective of this study was to assess the level of seed electrophoretic patterns of *Phalaris* taxa in Iran. We tried to reveal the degree of coincidence between morphological variations and SDS-PAGE profiles for *Phalaris* native to Iran for the first time.

Materials and Methods

In this study, five populations were chosen to study electrophoretic pattern of seed storage proteins. Seed samples were obtained from the sources indicated in Table 1. 1 gr of seed was used from each accession. Voucher specimens for this study were collected from the wild and all have been deposited at the Herbarium of Alzahra University, Tehran.

Table 1. Origin of seed samples of *Phalaris* species native to Iran

Taxon	Voucher details
P. minor	Tehran, Vanak 1700 m, Keshavarzi, 83m19.
P. brachystachys	Tehran, Karaj, Mardabad, 1267 m, Keshavarzi, 1383b8.
P. paradoxa var. *paradoxa*	Khuzestan, 15 km to Izae, 827 m, Nanaii, 85pp3.
P. paradoxa var. *praemorsa*	Mazandaran, Sari, Sameskandeh, 100 m, Khaksar, 85pp7.
P. arundinacea	Tehran to Chalous, Dizin, 2700 m, Khaksar, 85a1.

The final extract was loaded on SDS PAGE and stained by coomassic brilliant blue (Lammeli, 1970). We used Jaccard similarity coefficient. In statistical analysis, presence or absence of each band was considered as a qualitative feature. Then, the dendrogram was constructed using WARD hierarchical and UPGMA clustering by SPSS software ver. 11. In order to find the most variable protein band in studied taxa, principal component analysis was done. Standard proteins (β-galactosisase, Ovalbumin, Lactate dehydrogenase, lactoglobulin-β, Lysozyme and Bovine serum albumin) were used to evaluate the molecular weight of the unknown proteins. The protein density was determined by Bradford Protocol. Banding patterns were studied and R.F. values were measured. We used Jaccard similarity coefficient.

Results and Discussion

SDS-Page electrophoretic data were analyzed (Table 2). Jaccard similarity index was evaluated (Table 3). Totally 25 bands were observed for these taxa. The 4th, 5th, 6th, 20th and 25th bands were common in studied taxa. While band numbers 11, 13, 14, 15, 17, 21 and 24 were only observed in *P. paradoxa* var. *paradoxa* and *P. paradoxa*. Band number 9 was exclusively observed in *P. paradoxa* var. *praemorsa*. Merely in *P. arundinacea* the band number 12 was shown. Band number 19 was found only in *P. minor*. All of the studied taxa had band number one but not *P. brachystachys*. The highest numbers of bands were observed in *P. paradoxa* var. *paradoxa* and the least one in *P. brachystachys* (Figure 1).

In order to find most variable protein band in the studied taxa, principal component analysis was implemented. Primitive analysis showed that three first factors were responsible for the 95% of total studied variation in taxa. In the first factor with almost 61% of the total variation, bands number 1, 7, 10, 11, 14, 15, 16, 17, 18, 21 and 24 had the highest positive correlations. Bands number 8, 12 and 22 had the highest negative correlation. In the second factor with near 20% of observed variation, band number 8, had the highest positive correlation and band number 19 had the highest negative one. In the third factor with 14.16% of total variation, bands number one to three had the highest positive correlations (Table 4).

Table 2. Seed storage protein banding profiles of seed samples of *Phalaris* species native to Iran (1- band is present in the seed sample, 0- band is absent in the seed sample).

Taxon / Band and R.F.	*P. paradoxa* var. *praemorsa*	*P. paradoxa* var. *paradoxa*	*P. minor*	*P. brachystachys*	*P. arundinacea*
1- 0.26	1	1	1	0	1
2 -0.27	0	1	0	0	1
3- 0.29	0	1	0	0	1
4 -0.31	1	1	1	1	1
5- 0.33	1	1	1	1	1
6-0.35	1	1	1	1	1
7- 0.36	1	1	1	0	0
8- 0.37	0	0	0	1	1
9-0.38	1	0	0	0	0
10-0.39	1	1	1	0	0
11-0.40	1	1	0	0	0
12-0.41	0	0	0	0	1
13-0.42	1	1	0	0	0
14-0.43	1	1	0	0	0
15-0.45	1	1	0	0	0
16-0.47	1	1	1	0	0
17-0.48	1	1	0	0	0
18-0.51	1	1	1	0	0
19-0.53	0	0	1	0	0
20-0.55	1	1	1	1	1
21-0.57	1	1	0	0	0
22-0.58	0	0	1	1	1
23-0.60	1	1	0	1	0
24-0.62	1	1	0	0	0
25-0.65	1	1	1	1	1

Table 3. Jaccard similarity index based on electrophoretic data of seed storage protein in *Phalaris* taxa native to Iran

Case	Jaccard Measure				
	1:M	2:B	3:A	4:P.P	5:P.R
1:M *P. minor*	-	0.429	0.438	0.455	0.476
2:B *P. brachystachys*	0.429	-	0.583	0.273	0.286
3:A *P. arundinacea*	0.438	0.583	-	0.348	0.250
4:P.P *P. paradoxa* var. *paradoxa*	0.455	0.273	0.348	-	0.857
5:P.R *P. paradoxa* var. *praemorsa*	0.476	0.286	0.250	0.857	-

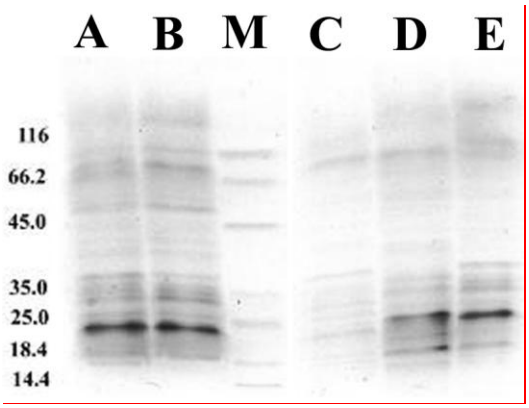

Figure 1: Seed protein banding profile of wild *Phalaris* species of Iran. A) *P. paradoxa* var. *paradoxa*; B) *P. paradoxa* var. *praemorsa*; C) *P. brachystachys*; D) *P. arundinacea*; D) *P. minor*; M) marker.

Table 4. Factor analysis results based on SDS-PAGE electrophoretic characters for *Phalaris* species of Iran

Band no.	1st factor	2nd factor	3rd factor
1	--	--	0.79
2	--	--	0.70
3	--	--	0.70
7	0.84	--	--
8	--	5.10	--
10	0.84	--	--
11	0.96	--	--
13	0.96	--	--
14	0.96	--	--
15	0.96	--	--
16	0.84	--	--
17	0.96	--	--
18	0.84	--	--
21	0.96	--	--
24	0.96	--	--

Cluster analysis result is shown in WARD dendrogram (Figure 2). UPGMA dendrogram was similar to WARD one. The taxa are clearly separated based on electrophoretic data of seed storage proteins. Results revealed that two varieties of *P. paradoxa* as var. *praemorsa* and var. *paradoxa* were closely related. High similarity index is a reflex of genomic identity (J=0.857). Dendrogram showed close relationship and high protein similarity (J=0.583) between *P. arundinacea* and *P. brachystachys*. On the other hand, *P. minor* comprised a separate cluster itself. Ordination of studied taxa based on PCA (Figure 3), showed that there was a concordance with cluster analysis.

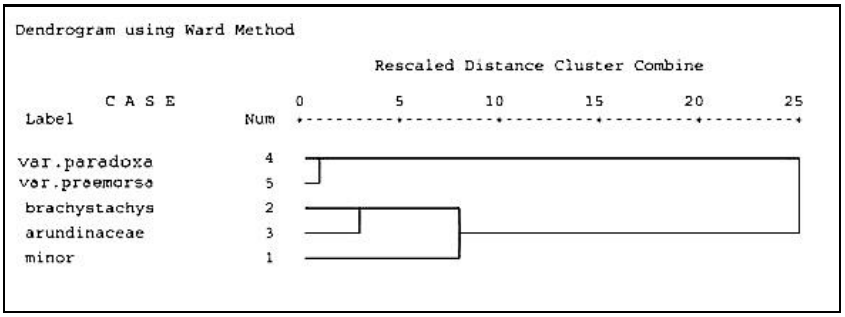

Figure 2. Dendrogram depicting clustering by WARD method of Taxa of *Phalaris*, by cluster analysis of seed storage protein

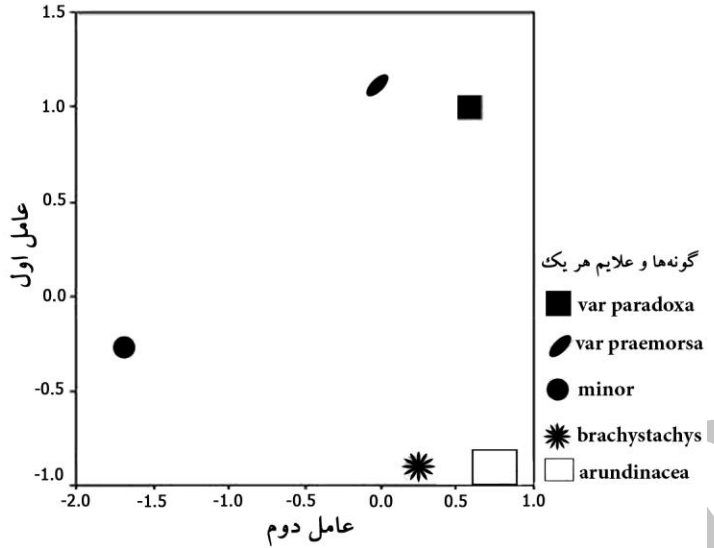

Figure 3. PCA ordination of the *Phalaris* species based on SDS-PAGE characters

Authors are convinced of existence of two centers of variation for this taxon in the whole world: Mediterranean region with 11 species and South West of USA with 4 species. There are four species of this genus in Iran, all belonging to the Old World species group (Baldini, 1993). *P. minor* has a vast distribution region in Irano-Touranian and Saharo-Sindian phytogeographic region (Keshavarzi *et al.*, 2007). *P. paradoxa* has a high morphological variability. Morphological studies in Iran revealed that two variations of *P. paradoxa* (*P. paradoxa* var. *praemorsa* and *P. paradoxa* var. *paradoxa*) make a closely related group also with *P. minor*. *P. brachystachys* is related to these taxa at the level 10. *P. arundinacea* which is the only perennial species of these taxa in Iran which makes a separate cluster and it is far from the other species.

Phalaris species of Iran showed differences in leaf anatomical structure and leaf dorsal epidermis (Keshavarzi *et al.*, 2009). Main differences were observed in hair type and frequency, stomata number in dorsal leaf area, stomata size and general outline of leaf cross sections. These were of diagnostic value and an identification key was made based on these features. Anatomical studies revealed that two varieties of *P. paradoxa* were distingusihed from each other while morphological studies (Keshavarzi *et al.*, 2011) indicated a close relationship between these two. Main morphological diagnostic features for these varities, which are used in identification keys, are the shape of rudimentary spikelets and ligule surface. In *P. paradoxa* var. *praemorsa* rudimentary spikelets were club like and ligule surface lacked hair while *P. paradoxa* var. *paradoxa* showed no club like rudimentaries and had hairy ligule.

In the meantime, anatomical and morphological studies both confirmed the separation of the studied taxa and the present result of SDS-PAGE was in accordance with previous results although there were some differences in clustering patterns.

Electrophoretic data also confirmed a close relationship and identity between the two variations of *P. paradoxa*. *P. arundinacea* was very different from the other species of this genus morphologically, but it was located near them according SDS-PAGE data. Hucle and Matus (1999) stated that *P. minor*, being an auto-tetraploid taxon, is a highly variable species within this genus according results of studying its enzyme electrophoretic patterns. We found out high morphological variability in different accessions of this species as well

(Keshavarzi *et al.*, 2007). As this species is tetraploid with 2*n*=28 (Baldini, 1995) it is not surprising. Sometimes this variability is related to soil conditions. Rich or poor soils cause difference of morphological features of *P. minor* individuals. Despite high variation of this species *P. brachystachys* (2*n*=12) and *P. paradoxa* (2*n*=14) are diploids with more similar populations (Baldini, 1995). As *P. arundinacea* is out of selection it was supposed to show more variability. We thought that low variability could be due to insufficient sampling from localities. A vast field study and new collections from different species of *Phalaris* is recommended. As enzyme electrophoresis are capable presenting inter- and intra- specific variations and also some morpho-geographic subspecies could be separated.

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بررسی بیوسیستماتیک گونه‌های *Phalaris* L. (خانواده غلات) در ایران

مریم کشاورزی *، مهناز خاکسار و پریناز قدم
گروه زیست‌شناسی، دانشکده علوم، دانشگاه الزهرا (س)، تهران، ایران

چکیده
الگوی باندینگ پروتیئن ذخیره‌ای بذر بر اساس الکتروفورز SDS-PAGE با استخراج عصاره پروتئینی گونه‌های *Phalaris* در ایران بررسی شد. نتایج نشان داد که دو واریته *P. Paradoxa* L. یعنی *Praemorsa* و *Paradoxa* ارتباط بسیار نزدیکی دارند. ارتباط بسیار نزدیکی نیز بر مبنای ضریب شباهت بالای پروتئینی (J=۰/۳۸۵)بین دو گونه *P. Arundinacea* L. و *P. brachystachys* Link. مشاهده شد. نتایج الکتروفورزی با نتایج بررسی‌های پیشین در مورد ساختمان تشریحی و تنوع ریختی مقایسه شده است.

واژه‌های کلیدی: *Phalaris*، روابط تاکسون‌ها، SDS-PAGE

* keshavarzim@alzahra.ac.ir