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Numerical Taxonomy of *Phalaris* (Poaceae) Species Based on Morphological Characteristics

Maryam Keshavarzi^{1*}, Mahnaz Khaksar², Mahvash Seifali¹, Parinaz Ghadam¹

1- Department of Biology, Faculty of Science, Alzahra University, Vanak, Tehran, Iran.

2- Graduated M.Sc. Student of Plant systematic, Department of Biology, Faculty of Science, Alzahra University, Vanak, Tehran, Iran.

Abstract

Phalaris is a grass which is distributed throughout the temperate regions of the world. There are many taxonomic problems in this genus. In order to clarify the taxonomy and the interrelationships among Iranian *Phalaris* taxa, including *Ph. minor*, *Ph. arundinaceae*, *Ph. brachystachys* and *Ph. paradoxa* 70 qualitative and quantitative morphological characteristics were examined. Numerical taxonomy was performed on 36 accessions of 5 taxa, concerning intra- and inter-populations variations as well as inter-specific relationships. The most variable morphological characters in the species delimitation were also determined. The species differed significantly in most of chosen qualitative characters. Results show a clear similarity between two varieties of *Ph. paradoxa*. Despite the wide range of habitats where the accessions were gathered, they showed four definite spots in their PCA ordination graphs. This showed the efficiency of chosen morphological characters in this study. An identification key based on studied features was provided and the species relationships were discussed.

Keywords: Cluster analysis, Ordination, *Phalaris*, relationship

تاکسونومی عددی گونه‌های *Phalaris* از تیره غلات بر اساس صفات ریخت‌شناسی

مریم کشاورزی^{۱*}، مهناز خاکسار^۲، مهوش سیفعلی^۱، پریناز قدم^۱
۱- گروه زیست‌شناسی، دانشکده علوم پایه، دانشگاه الزهراء، ونک، تهران، ایران.

۲- دانش‌آموخته کارشناسی ارشد سیستماتیک گیاهی، گروه زیست‌شناسی،
دانشکده علوم پایه، دانشگاه الزهراء، ونک، تهران، ایران.

چکیده

جنس *Phalaris* واجد گیاهانی علفی با توزیعی گسترده در مناطق معتدله دنیاست. مشکلات تاکسونومیکی متعددی در این جنس وجود دارد. به منظور روشن ساختن وضعیت تاکسونومی و روابط میان تاکسون‌های *Phalaris* در ایران، مشتمل بر *Ph. minor*، *Ph. arundinaceae*، *Ph. brachystachys* و *Ph. paradoxa* در مجموع ۷۰ صفت کمی و کیفی ریخت‌شناختی مورد بررسی واقع شد. تاکسونومی عددی بر روی ۳۶ واحد جمعیتی از ۵ تاکسون با در نظر گرفتن تنوع درون جمعیتی، بین جمعیتی و بین گونه‌ای انجام شد. متغیرترین صفات ریختی در تعیین حدود گونه‌ها مشخص شدند. گونه‌ها تفاوت معنی‌داری در غالب صفات کیفی انتخاب شده نشان دادند. نتایج مویید شباهت بالایی بین دو وارسته *Ph. Paradoxa* است. علی‌رغم تنوع زیاد در زیستگاه‌های واحدهای جمعیتی مورد بررسی، آن‌ها چهار توده مشخص را در نمودار رسته‌بندی مشخص ساختند. این امر کارآیی صفات ریختی انتخاب شده را در این بررسی نشان می‌دهد. کلید شناسایی بر مبنای صفات مورد بررسی ارائه شده و روابط بین گونه‌ها مورد بحث قرار گرفته است.

کلمات کلیدی: تجزیه خوشه‌ای، رسته بندی، *Phalaris* و قرابت‌ها.

* Corresponding author. E-mail Address: neshat112000@yahoo.com

Introduction

The genus *phalaris* L. has had a complicated taxonomic and nomenclatural history (Baldini, 1993 & 1995). *Phalaris* comprises 22 species of annual and perennial grasses in temperate regions throughout the world. It is commonly adventives species of open habitats. *Phalaris* species are distributed throughout various regions of Iran and are among important forage plants of Iran. There are four species and 5 taxa of *Phalaris* in Iran (Bor, 1970): *Ph. minor* Retz., *Ph. brachystachys* Link., *Ph. paradoxa* L. (with 2 varieties) and *Ph. arundinaceae* L. Members of the genus *Phalaris* display many variations on the standard structure of the inflorescence (Bor, 1968).

There is no report of a systematic study on the *Phalaris* species of Iran. The objective of this study was to assess the level of morphological variation of 36 accessions of four *Phalaris* species. We have tried to reveal the inter-population morphological variations and inter-specific relationships for the first time.

Materials and Methods

Morphometric studies performed on 36 populations of 4 species (5 taxa) native to Iran (Table 1). Voucher specimens were deposited in the herbarium of Alzahra University. We studied 70 quantitative and qualitative

morphological characteristics (Tables 2 and 3) selected on the basis of the studies of Bor (1968 & 1970), Baldini (1993 & 1995) and our own field observations. For each population, 10 individuals were studied for their qualitative and quantitative morphological characteristics.

In order to detect significant differences in studied characteristics among populations of each species and also among different species, analysis of variance (ANOVA) followed by the least significant differences (LSD) tests, were performed.

To reveal species relationships cluster analysis and principal component analysis (PCA) was used (Ingrouille, 1986). For multivariate analysis the mean of quantitative characteristics was used while qualitative characters were coded as binary/multi-state characteristics. Standardized variables used for multivariate statistical analysis. The average taxonomic distances and squared Euclidean distances were used as the dissimilarity coefficient in cluster analysis of morphological data. In order to determine the most variable morphological characters among the studied species, factor analysis based on principal components analysis performed. We used SPSS ver. 9 (1998) software for statistical analysis.

Table 1- Voucher details of *Phalaris* sampled in this study.

Taxon	Locality, Height from S.L and collector	Herb. No.
<i>Ph. minor</i>	Yazd, Ardakan, 1400 m, Keshavarzi.	83m8
	Khozestan, Masjed Soleiman, Boromandi.	68m12
	Tehran, Chitgar, Keshavarzi.	80m16
	Yazd, Meyboud, 1400 m, Keshavarzi.	83m14
	Tehran, Saeii Park, 1600 m, Keshavarzi.	66m22
	Qoum, Vejdani.	71m17
	Markazi, Mahalat, Vejdani.	70m18
	Khozestan, Ramhormouz, Keshavarzi.	83m18
	Fars, Shiraz, 1500 m, Keshavarzi.	84m10
	Khozestan, Molasani, Farasat.	83m5
	Markazi, Saveh, Yalabad, Keshavarzi.	83m3
	Lorestan, Khoramabad, Kew Park, Keshavarzi.	83m7
	Khozestan, Haftapeh, Choghazanbil, Keshavarzi.	83m13
	Khozestan, Behbahan, Keshavarzi.	83m6
	Tehran, Vanak 1700 m, Keshavarzi.	83m19
	Mazandaran, Sari, Komeilidost.	82m15
	Fars, Kazeroun, Baghenazar, 900 m , Keshavarzi.	84m1
	Khozestan, Ahvaz, Keshavarzi.	83m4
	Golestan, Gorgan, Khaksar.	83m23

Continue Table 1

Taxon	Locality, Height from S.L and collector	Herb. No.
<i>Ph. brachystachys</i>	Mazandaran, Sari, Dashtenaz, Khaksar	1385b2
	Khozestan, Ramhormouz, Bayman arayez Village, Farasat.	1385b1
	Mazandaran, Sari, Sameskandeh Khaksar.	1385b3
	Tehran, Khaksar.	1366b6
	Khozestan, Izae, Eshkafe Soleiman, Keshavarzi.	1383b7
	Khozestan, Molasani, Farasat.	138b4
	Tehran, Karaj, Mardabad, Keshavarzi.	1383b8
<i>Ph. paradoxa</i> var. <i>paradoxa</i>	Khozestan, Molasani, Farasat.	85pp1
	Khozestan, 15 km to Izae, Nanaii.	85pp3
	Khozestan, Ramhormouz, Bayman Arayez Village, Farasat.	85pp2
<i>Ph. paradoxa</i> var. <i>praemorsa</i>	Mazandaran, Sari, Bayekola, 15 km Nesa, Khaksar.	85pps1
	Mazandaran, Savadkooh, zir Abe joush Village, Khaksar.	85pps1
	Mazandaran, Sari, Sameskandeh, Khaksar.	85pp7
	Golestan, Gorgan, Khaksar.	85pps6
	Mazandaran, Behshahr, Khaksar.	85pps9
	Mazandaran, Dashtenaz, Khaksar.	85pps5
<i>Ph. arundinaceae</i>	Tehran to Chalous, Dizin, Khaksar.	85a1

Table 2 - Quantitative morphological characteristics in the *Phalaris* species studied. All scales are in mm.

Character	Character
Plant height	Length of lowermost Glume
Palae width	Width of lowermost Glume
Length of longest internode	Length of Lemma
Caryopsis length	Width of Lemma
Caryopsis width	Length of palae
Leaf number	Length/width of caryopsis
Leaf width	Plant height/length of longest internode
Leaf length	Length/width of leaf blade
Ligule Length	Length/width of spike
Spikelet Length	Length /width of spikelet
Spikelet width	Length/width of lowermost glume
Spike length	Length/width of uppermost Glume
Spike width	Length/width of lemma
Floret number	Length/width of palae
Length of uppermost Glume	Length of spike to length of spikelet
Width of uppermost Glume	Width of Spike to Width of spikelet

Table 3 - Qualitative morphological characteristics
In the *Phalaris* species studied.

No	Character	State of Character
1	Plant habit	Annual (0), Perennial (1)
2	Culms direction	Erect(0), Curved (1)
3	Inflorescence compactness	Dense(0), Lax (1)
4	Fertility of Spikelets	All fertile (0), Fertile and Sterile (1)
5	Inflorescence Rachis	Fragile (0), Tough (1)
6	Spike Shape	Ovoid(0), elliptic (1), Oblanceolate (2)
7	Spike covering	Dense (0), sparse (1)
8	Spike compactness	Compact (0), Lax(1)
9	Leaf surface	Smooth (0), Rough (1)
10	Leaf Color	Pale Green (0), Dark Green (1)
11	Leaf sheath	Covering spike(0), Not so (1)
12	Shape of Ligule apex	Truncate (0), Rounded (1), Fringed (2), Cylindrical(3)
13	Ligule covering	Smooth(0), Hairy (1)
14	Spikelet Surface	Hairy (0), Smooth (1)
15	Sterile Spikelets	Uniform (0), Some club like (1)
16	Rudimentary spikelets	Present (0), Absent (1)
17	Glume Color	Pale Green (0), Dark Green (1)
18	Glume Apex	Longley pointed (0), Shortly pointed (1)
19	Glume Covering	Smooth(0), Hairy (1)
20	Glume Hair Density	Dense (0), sparse (1)
21	Glume nerves	Convergent (0), Not so (1)
22	Winged Glume	Present (0), Absent (1)
23	Glume Wing	Tooth like (0), Not so (1)
24	Glume Wing Margins	Smooth (0), Undulate (1)
25	Glume Wing Texture	Membranous(0), non-membranous (1)
26	Shape of lemma Apex	Pointed (0), rounded (1)
27	Lemma Texture	Membranous(0), non-membranous (1)
28	Lemma shape	Ovoid (0), elliptic – oblong (1)
29	Lemma nerves	Present (0), absent (1)
30	Lemma Nerves	Convergent (0), not so (1)
31	Lemma Hairs	Dense (0), sparse (1)
32	Lemma hairs Length	Long (0), Short (1)
33	Palae Texture	Nerved (0), not so (1)
34	Palae hairs	Present (0), Absent (1)
35	Palae hair position	At margins (0), distributed (1)
36	Palae hairs Length	Long (0), Short (1)
37	Caryopsis color	Brown (0), Yellow (1), Green (2)
38	Caryopsis Shape	Ovoid (0), Elliptic (1), Lanceolate (2)

Results

The analyses showed that the range of quantitative characters is somehow different from what is given in different flora and species descriptions. The species studied also differed significantly in most of the quantitative characteristics as revealed by ANOVA and LSD tests. The mean of such quantitative characters may be useful for species delimitation. This is supported by the clustering (Fig 1.) of the studied species based on merely quantitative morphological characteristics. Figure 1 show that *Ph. arundinaceae* makes a separate cluster and populations of *Ph. brachystachys* make separate ones.

The cluster analysis and PCA ordination of *Phalaris* species of Iran, based on both quantitative

and qualitative characteristics produced similar results (Fig 2). In the overall morphological analysis, two major clusters are formed. The first major cluster is comprised of two sub-clusters, in the first of which populations belonging to *Ph. brachystachys*. The other sub-cluster is comprised of two sub-clusters itself, one of which contains *Ph. minor* while *Ph. paradoxa* is placed in the other. *Ph. arundinaceae* is placed in a separate cluster. Thus it is evident *Ph. minor* and *Ph. paradoxa* are closely related which, in turn, are both related to *Ph. brachystachys*. *Ph. minor* and *Ph. arundinaceae* were considered to be closely related to each other based on having merely fertile spikelets in their inflorescences in the Flora Iranica (Bor, 1970). He also considered a separate place for *Ph. arundinaceae* due to its perennial habit.

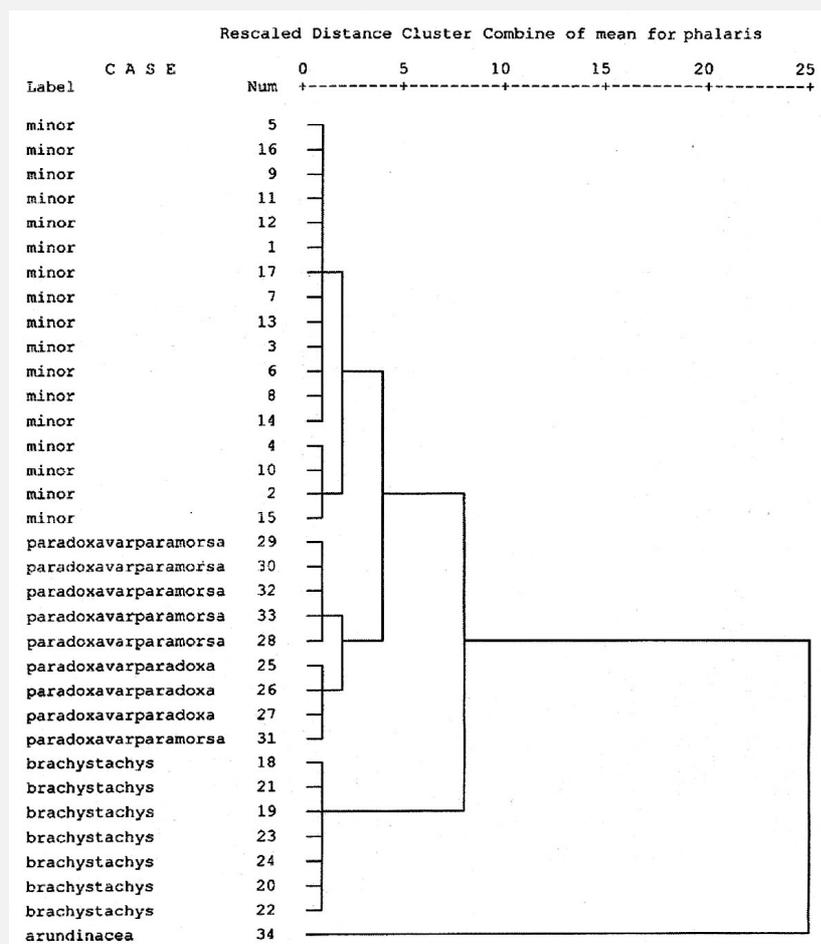


Figure 1- WARD clustering of *Phalaris* species based on the mean of quantitative morphological characters.

In order to determine the most variable characters among the studied species, factor analysis based on PCA was performed revealing that the first two factors comprise about 77% of total variation. In the first factor with about 58% of total variation (Table 4), characteristics such as spike width, length of first inter-node and spikelet, width of leaf blade and leaf number, spike length, width of upper glume, length and width of lemma, width and length of lower glume possessed the highest positive correlation (≥ 0.7).

Table 4 - Factor analysis results based on the morphological characteristics of *Phalaris* populations of Iran.

Character	Factor 1	Factor 2
Spike Width	0.97	
Length of first internode	0.96	
Length of spikelet	0.96	
Width of leaf blade	0.95	
Leaf number	0.95	
Spike length	0.95	
Width of upper glume	0.95	
Length of lemma	0.93	
Width of lower glume	0.91	
Lemma width	0.90	
Length of lower glume	0.72	
Length of upper glume		0.85
Caryopsis width		0.77
Caryopsis length		0.72

In the second factor with about 19.4% of total variation, characteristics such as the length of the upper glume and caryopsis width and length possessed the highest positive correlations. Therefore, these are the most variable morphological characters among *Phalaris* species of Iran.

In assessing the inter-specific affinities and intra-specific variability of a complex group of species, the multivariate analysis methods had been shown to be very helpful. Such methods were used by Ng *et al.* (1981) in their studies of rice species to clarify the inter-specific relationships and to distinguish species

or geographical forms. The use of similar methods in this present study has indicated that, two varieties of *Ph. paradoxa* (*praemorsa* and *Paradoxa*) make a sister group (Fig. 1). These two show the nearest relationship with *Ph. minor*. *Ph. brachystachys* in level 10 and show a relationship with these clusters. *Ph. arundinaceae* which is the only perennial species in this genus of Iran, forms a separate cluster (level 25). Cluster analysis of the anatomical features of dorsal epidermis and leaf trans-sections by the WARD method provide further evidence for such a relationship (Keshavarzi *et al.*, 2009).

Considering the results of factor analysis here we provide an identification key for Iranian species of *Phalaris*:

- 1a- Plant annual, Spike compact, glume winged 2
- b- Plant perennial, spike loose, glume without wing
- Ph.arundinaceae*
- 2a - All spikelets fertile, glumes' wing dentate, ligule fringed and wide
- Ph.minor*
- b-Spikelets fertile and sterile, glumes' wing not dentate, ligule tip rounded or truncate 3
- 3a- Rudimentary spikelets present, glumes' wing tooth like 4
- b-Rudimentary spikelets absent, glumes' wing complete
- Ph.brachystachys*
- 4a- Rudimentary spikelets club like, ligule surface without hair
- Ph. paradoxa* var. *praemorsa*
- b- Rudimentary spikelets not club shaped, ligule surface hairy
- Ph. paradoxa* var. *paradoxa*

Discussion

The most polymorphic species in Iran is *P. minor*. Our results are in accordance with those of Hucle and Matus (1999). Their vast variation could be related to the auto-tetraploid condition of this species. There also some edaphic conditions which are able to affect *ph. minor* populations in different places. We found that individual size is related to soil conditions. In rich soils of fields *Ph. minor* could reach up to 100 cm and bear up to 6 cm spikes, while in poor soils *Ph. minor* individuals will be 10-15 cm height and bear only 1.5 cm spikes (Keshavarzi *et al.*, 2007 b).

Anderson (1961) clarified the genetic relationships of annual species of *phalaris*. He believed that four types of variations occurred in sterile florets of this

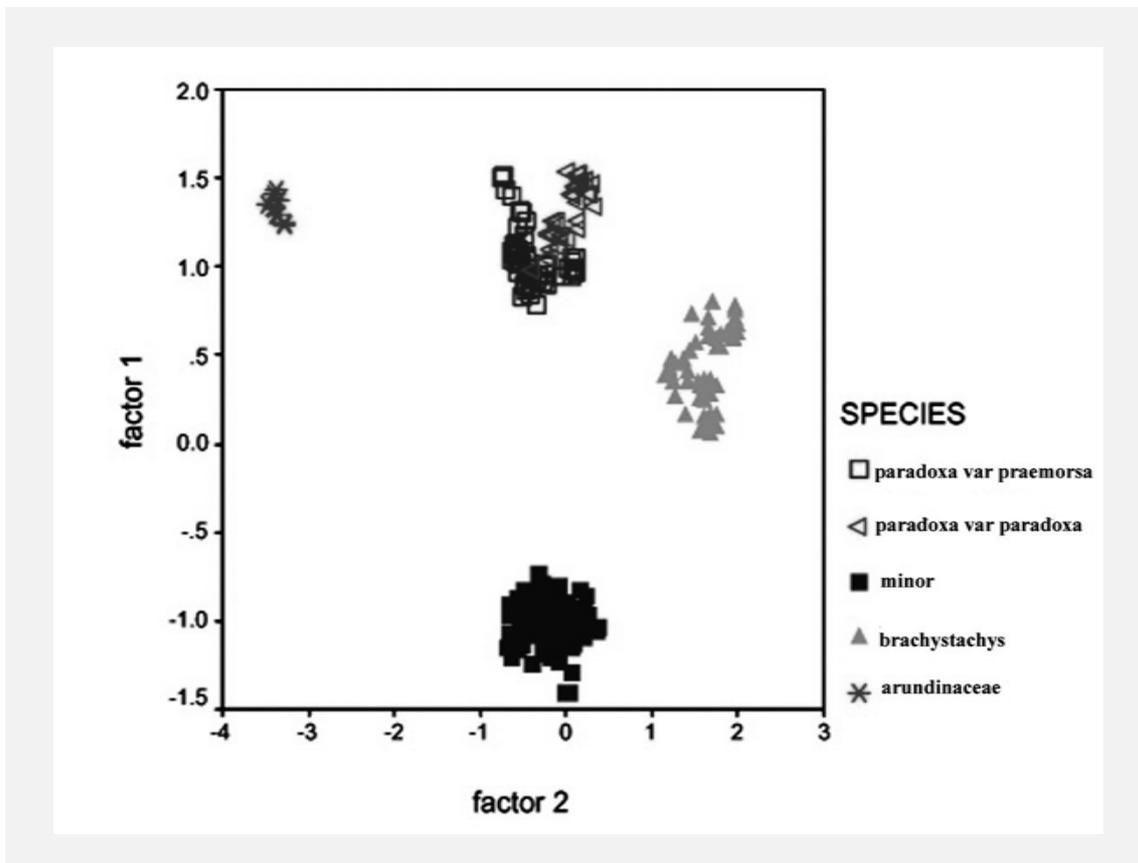


Figure 2- PCA ordination of the *Phalaris* species based on morphological characteristics.

genus. He defined four definite lineages in this genus: *Ph. paradoxa*, *Ph. brachystachys*, *Ph. arundinaceae* and ten other species containing *Ph. minor*. He separated the last group by their geographical distribution pattern to old world (eg: *Ph. minor*) and new world. Baldini (1995) believed that there are two centers of diversification for these taxa, one of which is Mediterranean area and the other is south western USA. Iranian species of *Phalaris* are of the old world group. Baldini believed that *Ph. arundinaceae* is the only species that connects new and old world species. As the *Phalaris* migration from the Mediterranean area occurred during the tertiary period, we conclude that *Phalaris* species of Iran have experienced a relatively long period of divergence and adaptations to local conditions in their habitats in Iran.

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