TAXONOMIC VALUE OF LEAF ANATOMY IN ORYZOPSIS MICHX. S. L. (POACEAE) SPECIES OF IRAN

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The aim of this project is to find diagnostic characters from anatomical features for species separation in *Oryzopsis* s. l. in Iran. Leaf anatomical structures (dorsal epidermis and cross section at the middle of leaf) of 10 species from *Oryzopsis* s. l. were studied for the first time. In this research, 43 quantitative and qualitative characters in 23 populations were measured and evaluated. These characters were varied among species, but stable between specimens of one species. Observed variations were investigated using statistical methods (PCA, NJ, UPGMA). Factor analysis based on principle component analysis revealed that most variable characters among *Oryzopsis* s. l. species are the number and shape of short cells, long cells shape, form of long cells walls, situation of short cells, shape of bulliform cells, length and number of long cells, number of vascular bundles, radial differentiation in mesophyll, sclerification of inner bundle sheath cells, width of silica bodies, presence or absence of macrohairs in adaxial surface and shape of abaxial surface. In addition, an identification key using anatomical characters is presented for *Oryzopsis* s. l. species in Iran.

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Key words. Leaf anatomical structure, abaxial epidermis*, Poaceae, Oryzopsis,* identification key, Iran.

ارزش تاکسونومیکی ساختار تشریحی برگ در گونه‌های جنس Oryzopsis Michx. (Poaceae) در ایران

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هدف از این تحقیق، مطالعه صفات تشریحی و استفاده از آنها به منظور دستیابی به صفات تشریحی افتراقی برگ جهت تفکیک بهتر گونه‌های جنس *Oryzopsis* s. l. می‌باشد. بدین منظور، ساختار تشریحی برگ (بشره پشتی و برش عرضی) در ۱۰ گونه از جنس *Oryzopsis* s. l. در ایران برای اولین بار مورد بررسی قرار گرفت. در این تحقیق ٤٣ صفت کمی و کیفی در ۲۳ جمعیت کدگذاری و ارزیابی شدند. این صفات در میان گونه‌ها متغیر، اما در بین نمونه‌های یک گونه ثابت بودند. سپس نتایج با استفاده از روش‌های آماری PCA, NJ, UPGMA مورد بررسی قرار گرفتند. آنالیز فاکتور بر اساس تجزیه به مولفه‌های اصلی نشان داد که متغیرترین صفات در بین گونه‌ها عبارتند از: تعداد و شکل سلول‌های کوتاه، شکل سلول‌های بلند، شکل دیواره سلول‌های بلند، وضعیت سلول‌های کوتاه، شکل سلول‌های حبابی، طول و تعداد سلول‌های بلند، تعداد دستجات آوندی، تمایز شعاعی مزوفیل، اسکلرانشیمی شدن سلول‌های غلاف درونی دسته‌های آوندی، عرض سلول‌های سیلیسی، حضور یا عدم حضور کرک‌های بلند در سطح شکمی و شکل سطح پشتی می‌باشند. سپس کلید شناسایی برای گونه‌های جنس *Oryzopsis* s. l. ارائه می‌شود.

Introduction

Oryzopsis Michx. s. l. (*Poaceae*, *Pooideae*, *Stipeae*) comprised of 35 species (Watson & Dallwitz, 1992). The genus of *Oryzopsis* was named by Michaux in 1803, according to *O. aspersifolia* Michx. Some authors inserted some species of *Oryzopsis* into *Piptatherum* P. Beav. (Boissier, 1884; Freitag, 1975) and some authors did not support this insertion (Pilger, 1954; De Winter, 1965; Bor, 1970; Clayton et al, 1999). In this study the species native to Iran are considered as *Oryzopsis* sensu lato (including *Piptatherum*). According to Flora Iranica (Bor, 1970) this genus has 25 species in the Iranian plateau and 10 in Iran: *O. barbellata* (Mez.) Bor., *O. gracilis* (Mez.) Pilger, *O. holciformis* (M. B.) Hack., *O. lateralis* (Regel) Stapf, *O. microcarpa* Pilger, *O. molinioides* (Boiss.) Hack. ex. Paulsen, *O. munroi* Stapf, *O. pubiflora* Hack., *O. sphacelata* (Boiss. & Buhse) Hack., *O. virescens* (Trin.) Beck.

As *Oryzopsis* species are perennial, they produce significant amount of forage for domesticate animals. These species grow in alpine and snowy regions of Iran and are very useful in soil protection (Nasirzadeh, 2004). *O. hymenoides* (Roem. & Schult.) Richer, as a native edible species of Latin America has nutritious value. *O. miliaceae* (L.) Ascherson & Schweinf. is sometimes cultivated as an ornamental grass in European and American gardens. *O. holciformis* (M. B.) Hack. is used as poultry food in some parts of Russia (Bor, 1968).

Anatomical characters are very important and valuable in addition to morphological features in the taxonomy of *Poaceae* (Prat, 1932; Metcalfe, 1960; Clifford and Watson, 1977; Alemi et al, 2007). Raol and Desai (2009) used epidermal characters for segregation in some members of *Andropogoneae*. They suggested that presence and absence of prickles, silica bodies, papillae and stomatal pattern could help in identification of studied taxa. There are some reports that showing anatomical characters are useful in identification of species of a genus (Ghahreman et al, 2006; Alemi et al, 2007; Eslamy Jooiandeh et al, 2008; Keshavarzi et al, 2009). Ghahreman et al (2006) studied anatomical structures in some species of *Bromus* L. of Iran. Alemi et al. (2007) studied micro-morphological characters of *Bromus* species as taxonomic evidences. Eslamy Jooiandeh et al. (2008) used leaf anatomical characters in taxonomy of Iranian fine-leaved *Festuca*. Keshavarzi et al. (2009) utilized leaf anatomical structures and dorsal epidermis for segregation of *Phalaris* L. species of Iran.

A few studies have considered anatomical structures of *Oryzopsis*. Barkworth (1981) examined abaxial epidermis of basal leaves of 49 species of *Stipeae*, almost all were of North American origin, and amongst them are some species of *Oryzopsis*. Metcalfe (1960) investigated anatomical structures of three species of *Oryzopsis*. There is no evidence of *Oryzopsis* studies in Iran. The present work considers leaf anatomical characters and dorsal epidermis of *Oryzopsis* species of Iran for the first time.

Materials and methods

Accessions were gathered from 21 different locations. Careful identification were based on different Floras as Flora Iranica (Bor, 1970), Flora Orientalis (Boissier, 1884), Flora of Pakistan (Cope, 1982) and Flora of Iraq (Bor, 1968). Vouchers are deposited in Herbarium of Shahid Beheshti University (HSBU) (Table 1).

For leaf cross sections, the third upper leaf of plant during flowering period was used.

Study of dorsal epidermis. The peels were made by scraping pieces of fresh or softened dried leaves (by boiling in water) with the help of scalpel. Materials were then transferred to 10% NaOCl and stained with 1% methyl green for 1 min.

Study of leaf anatomical structure. Leaves were put in 70% ethanol for 2 days. Then hand sections were cleared in 10% NaOCl and stained with 2% Carmin (10-15 min) followed by 1% methyl green (1 min) and mounted in glycerin.

In total, 43 anatomical characters (23 quantitative and 20 qualitative) were used for phenetic analysis (tables 2 – 5).

Phenetic analysis: In order to group OTUs with anatomical similarity, clustering and ordination based on principal components analysis (PCA) were performed. Clustering methods using neighbor joining (NJ) and un-weighted paired group method with mean average (UPGMA) were used.

In order to determine the most variable characters among the species, factor analysis based on principal component analysis was performed on standardized data. For the phenetic analysis NTSYS version 2.2 (1998) software was used.

Table 1. Voucher specimens of *Oryzopsis* species.

Species	Abbreviation	Voucher specimen
O. barbellata (Mez.) Bor	b 1	Esfahan: Soh Mt., 90 km to Esfahan, Between Esfahan and Kashan. 2310 m, Yazdanbakhsh 8800019.
O. barbellata (Mez.) Bor	b 2	Fars: 10 km to Saadat Shahr, 1800 m, Yazdanbakhsh 8800040.
O. barbellata (Mez.) Bor	b 3	Yazd: Bidakhavid, 30 km to Taft, 2397 m, Yazdanbakhsh 8800022.
O. barbellata (Mez.) Bor	b 4	Yazd: Tange-Chenar, 80 km t0 Yazd, 2400 m, Yazdanbakhsh 8800021.
O. gracilis (Mez.) Pilger	g 1	Mazandaran: Taker, 1775 m, Yazdanbakhsh 8800026.
O. gracilis (Mez.) Pilger	g 2	Mazandaran: Balade, 2135 m, Yazdanbakhsh 8800027.
O.holciformis (M. B.) Hack.	o 1	Fars: Sepidan, 2790 m, Yazdanbakhsh 8800009.
O. holciformis (M. B.) Hack.	o 2	Fars: Estahban waterfall, 2300 m, Yazdanbakhsh 8800008.
O.holciformis (M. B.) Hack.	o 3	Mazandaran: Rozan, 1345 m, Yazdanbakhsh 8800015.
O. holciformis (M. B.) Hack.	o 4	Golestan: 15 km to Ramian towards Olang pasture, 678 m, Yazdanbakhsh 8800014.
O. lateralis (Regel) Stapf	L 1	Kohgilooy-e and Boyer-Ahmad, Kakan, 2350 m, Yazdanbakhsh 8800036.
O. lateralis (Regel) Stapf	L 2	Fars: Eghlid, Bel Mt., 2750 m, Yazdanbakhsh 8800038.
O. microcarpa Pilger	m 1	Khorasan: Fariman barrier, 1500 m, Yazdanbakhsh 8800001
O. microcarpa Pilger	m 2	Khorasan: Robat-Sang, Ghonchi, 1942 m, Yazdanbakhsh 8800000.
O. molinioides (Boiss.) Hack. ex. Paulsen	n 1	Kerman: Lalehzar Mt., 3800 m, Baniasadi 8800043.
O. molinioides (Boiss.) Hack. ex. Paulsen	n 2	Fars: Eghlid, Bel Mt., 2750 m, Yazdanbakhsh 8800028.
O. munroi Stapf	u 1	Khorasan: Kalat pass, 2050 m, Yazdanbakhsh 8800002
O. pubiflora Hack.	p 1	Fars: Ghader-Abad, 2325 m, Yazdanbakhsh 8800031.
O. pubiflora Hack.	p 2	Chahar-Mahal and Bakhtyari: Gandoman, 2220 m, Yazdanbakhsh 8800032.
O. sphacelata (Boiss. & Buhse) Hack.	s 1	Fars: Bazbache (1), 40 km to Eghlid, 2350 m, Yazdanbakhsh 8800048.
O. sphacelata (Boiss. & Buhse) Hack.	s 2	Fars: Bazbache (2), 42 km to Eghlid, 2340 m, Yazdanbakhsh 8800049.
O. virescens (Trin.) Beck.	v 1	Golestan: 15 km to Ramian towards Olang pasture, 700 m, Yazdanbakhsh 8800024.
O. virescens (Trin.) Beck.	v 2	Golestan: Golestan popular park, 120 km to Gorgan, 800 m, Yazdanbakhsh 8800025.

Table 2. Qualitative characters of dorsal epidermis.

Intercostal zone	
1. situation of short cells	single=1 paired=2 single or paired or ternate=3
2. short cells shape	oblong=1 not oblong=2
3. long cells shape	oblong=1 spindly=2 oblong or spindly=3
4. form of long cells wall	smooth=1 undulate=3
5. subsidiary cells shape	low-dome-shaped=1 tall-dome-shaped=2 parallel-sided=3 low-dome-shaped or triangular=4
6. silicification of short cells	silicified=1 not silicified=2
Costal zone	
7. silica bodies shape	cross-shaped=1 oblong=2 crenate=3 cross-shaped, oblong=4 cross-shaped, oblong, sinuate=5 cross-shaped and crenate=6 oblong, sinuate=7

Table 3. Quantiative characters of dorsal epidermis.

Intercostal zone	
1. short cells number*	2. prickles number*
3. stomata number*	4. number of stomata row*
5. long cells number*	6. long cells length (μm)
7. long cells width (μm)	8. stomata diameter (μm)
9. short cells width (μm)	10. short cells length (μm)
11. stomata width (μm)	12. thickness of long cells walls (μm)
Costal zone	
13. prickles number*	14. silica bodies length (μm)
15. silica bodies width (μm)	

* (Studied area=0.05 square millimeter)

Table 4. Qualitative leaf cross-section characters.

1. bulliform cells shape	regular fan shaped=1 irregular fan shaped=2
2. radiate difference of mesophyll	radial differentiation=1 no radial differentiation=2
3. presence or absence of macro-hairs in adaxial surface	presence=1 absence=2
4. size of inner sheath cells	equal=1 unequal=2
5. size of outer sheath cells	equal=1 unequal=2
6. inner sheath of central vascular bundle state	complete=1 interrupted=2
7. outer sheath of central vascular bundle state	complete=1 interrupted=2
8. furrows depth of adaxial surface	shallow=1 deep=2
9. shape of abaxial surface	smooth=1 slightly undulate=2 undulate=3
10. shape of ribs and furrows on the adaxial surface	dome-shaped=1 truncate quadrangular or dome-shaped=2 dome-shaped or acute=3 quadrangular or dome- shaped=4
11. sclerification of inner bundle sheath cells	sclerified=1 not sclerified=2
12. shape of midrib	truncate quadrangular=1 dome-shaped=2 quadrangular=3
13. presence or absence of prickles on the adaxial surface	presence=1 absence=2

Table 5. Quantitative leaf cross-section characters.

1. number of vascular bundles	2. number of bulliform cells
3. length of the largest bulliform cell (μm)	4. width of the largest bulliform cell (μm)
5. number of cells of central outer bundle sheath	6. number of cells of central inner bundle sheath
7. prickles number on the abaxial surface	8. number of the large vascular bundles

Results

Our studies showed that *Oryzopsis gracilis* and *O. virescens* are different from other species in many characters. Short cells are silicified in *O. gracilis* and *O. virescens*, while in the rest of species are not. Number of short cells in Leaf Area Unit (LAU) is abundant (more than 45) in *O. gracilis* and *O. virescens*, while in other species are 1-4. Short cells in *O. gracilis* and *O. virescens* are bell-shaped and cross-shaped while in other species are oblong. Subsidiary cells in *O. gracilis* and *O. virescens* are tall-dome-shaped, in *O. barbellata* low-dome-shaped and rarely triangular; in *O. holciformis* parallel sided and in the rest of species are low-dome-shaped. Long cells walls in *O. gracilis* and *O. virescens* are undulate and in other species are smooth.

Long cells length in *O. sphacelata*, *O. microcarpa* and *O. munroi* is more than 100 μm (111-137 μm) and in other species is 59.5-88 μm. Different species of

Oryzopsis show differences in silica bodies shape (Figs. 1 & 2).

The general characters of dorsal epidermis in different species of *Oryzopsis* are as follow:

Short cells over the veins are abundant, alternately with silica bodies. Inter- coastal short cells in most species are oblong, in a few species more and less cross-shaped, crescent, bell-shaped, single or paired, sometimes ternate. Subsidiary cells of stomata are tall or low dome-shaped or parallel sided and seldom triangular. Prickles are acute, with a flat and swollen base. Inter-costal long cells spindle or oblong shaped, with smooth walls, but in few species long cells comprised with undulate walls.

Different species of *Oryzopsis* showed some differences in cross sections at the middle of leaf. Shape of midrib in *O. gracilis* is completly quadrangle; in *O. holciformis* is truncate quadrangle and in other species is dome-shaped. Macro-hairs in *O. munroi* are lengthy and abundant (up to 34), but macro-hairs in *O. holciformis* and *O. gracilis* are not seen. In other species, number of macro- hairs is about 1-8. In all species of *Oryzopsis*, abaxial surface of leaf blade is more or less undulate, excluding *O. gracilis*, which is smooth. Merely, in *O. microcarpa* and *O. gracilis*, cells of inner bundle sheath have been sclerified and in other species, cells of inner bundle sheath have not been sclerified. Shape and number of bulliform cells are variable in different species (Figs. 3-6).

The general characters of cross sections at the middle of leaf in the species of *Oryzopsis* are as follow:

Adaxial surface has uncongenial furrows and ribs; abaxial surface is undulate, excluding *O. gracilis* which its' abaxial surface is smooth. Abaxial surface has no macro-hairs, adaxial surface may have macro-hairs or not. Vascular bundles in large, medium and small sizes, angular in outline, most vascular bundles with adaxial and abaxial T-shaped girders of scleranchyma, but a few small-sized vascular bundles with strands of scleranchyma. There is a double layered bundle-sheath with colorless cells; cells of the outer sheath are bigger than those of inner sheath, cells of inner sheath with u-shaped thickening.

Bulliform cells in fan-shaped groups, length of bulliform cells more than a quarter of the leaf blade.

Grouping of the OTUs, based on anatomical characters are presented in figures 7 and 8. Different methods of cluster analysis showed approximately similar results. In UPGMA cluster analysis, the species are well segregated. NJ cluster analysis showed that populations of *O. pubiflora* and *O. lateralis* are confused. Ordination of the species/populations from different localities based on principal components analysis (PCA) of anatomical characters is presented in Fig. 9. This graph showed that *O. gracilis* and *O. virescens* are far from other species and from each other. This confirms that, these species varied in many anatomical characters from other species and from each other, as pointed previously in this work. Remaining species are relatively near together. Principal components analysis of anatomical data showed that the first three components comprise more than 52% of total variance (Table 6). The first components comprises 27.648% of total variance in which characters such as short cells number, shape of short cells, shape of bulliform cells, form of long cell walls, situation of short cells, long cells number, number of vascular bundles and shape of leaf blade on the abaxial surface possessed the highest correlation (> 0.7). The second component comprises 13.662% of total variance in which characters such as length of long cells, sclerification of inner sheath cells, width of silica bodies and prescence or absence of macrohairs in adaxial surface possessed the highest correlation (> 0.7). The third component comprises 10.702% of total variance in which character of radiate difference of mesophyll showed the highest correlation (> 0.7) (Table7).

Discussion

In both cluster analysis (UPGMA and NJ**)** based on anatomical characters, different populations of studied species occurred near together. This event indicated that anatomical characters are suitable for separation of species and suitable for determining relationship among the species. Anatomical characters can help morphological studies.

In UPGMA cluster analysis of anatomical data, *O. lateralis* and *O. pubiflora* were occurred near together and in NJ cluster analysis and ordination based on principal components analysis (PCA) of anatomical characters, populations of these two species were confused. This evidence supported the merge of these species. Morphological studies showed that *O. lateralis* and *O. pubiflora* are synonymous and are not two distinct species, but Bor (1970) separated these species. Bor (1970) used quantitative characters like lemma length, spicklet length and plant size for separation *O. pubiflora* and *O. lateralis*. According to our morphological studies, these characters are very variable and depend upon environmental factors and there is not a constant character to segregate *O. pubiflora* from *O. lateralis*. Smith (1985) and Freitag (1975) considered that these two species as synonymous, too. Freitag (1975) believes, it is impossible to separate these two species, because they are separated by some measurements that are variable. We did not find situable anatomical characters for

Table 6. Results of principal component analysis and variance percentage based on anatomical characters.

Componenet	% of variance	% of cumulative
1	27.648	27.648
2	13.662	41.310
3	10.702	52.012

Table 7. First and second and third components based on principal components.

	Characters	Component 1	Component 2	Component 3
1	short cells number	0.956	-0.187	3.184E-03
2	prickles number* in intercostal zone	-0.464	0.397	-0.243
3	stomata number*	-0.166	0.606	-0.227
4	number of stomata row*	2.112E-02	0.648	7.816E-02
5	long cells number*	0.803	5.158E-02	2.752E-02
6	stomata diameter (µm)	-0.273	0.494	-0.005
7	long cells width (µm)	-0.695	0.166	-0.104
8	long cells length (µm)	0.125	-0.806	0.209
9	short cells width (µm)	0.157	3.188E-02	0.535
10	short cells length (µm)	-0.458	2.735E-02	0.583
11	stomata width (µm)	0.218	0.568	0.406
12	thickness of long cells walls (µm)	0.599	0.375	0.528
13	prickles number* in costal zone	-0.285	0.598	-0.032
14	silica bodies length (µm)	-0.053	-0.018	0.182
15	silica bodies width (µm)	0.389	0.741	0.257
16	silicification of short cells	0.645	-0.148	-0.088
17	short cells shape	0.937	-0.267	-0.021
18	long cells shape	-0.238	0.288	-0.259
19	form of long cells walls	0.937	-0.267	-0.021
20	subsidiary cells shape	0.589	-0.128	-0.278
21	silica bodies shape	0.395	0.241	-0.594
22	situation of short cells	0.937	0.267	2.123E-02
23	number of vascular bundles	-0.762	-0.012	-0.026
24	number of bulliform cells	0.196	0.246	-0.059
25	length of the largest bulliform cell (µm)	-0.144	-0.058	0.500
26	width of the largest bulliform cell (µm)	-0.528	-0.061	0.429
27	number of cells of central outer bundle sheath	0.592	-0.117	0.117
28	number of cells of central inner bundle sheath	0.205	-0.173	4.215E-02
29	prickles number on the abaxial surface	0.696	0.450	0.236
30	number of the large vascular bundles	-0.157	0.125	0.410
31	bulliform cells shape	0.820	0.404	0.289
32	radiate difference of mesophyll	0.356	0.185	0.755
33	presence or absence of macro-hair in adaxial surface	0.245	0.748	-0.141
34	size of inner sheath cells	0.455	-0.048	-0.478
35	size of outer sheath cells	0.476	-0.014	-0.231
36	inner sheath of central vascular bundle state	-0.189	-0.259	0.603
37	outer sheath of central vascular bundle state	-0.069	8.637E-02	-0.249
38	furrows depth of adaxial surface	0.491	8.047E-02	0.655
39	shape of abaxial surface	-0.762	-0.325	0.182
40	shape of ribs and furrows on the adaxial surface	0.142	0.482	-0.215
41	sclerification of inner sheath cells	0.653	0.748	0. 327
42	shape of midrib	0.676	-0.507	-0.144
43	presence or absence of prickle on the adaxial surface	0.546	0.551	-0.244

* (Studied area=0.05 square millimeter)

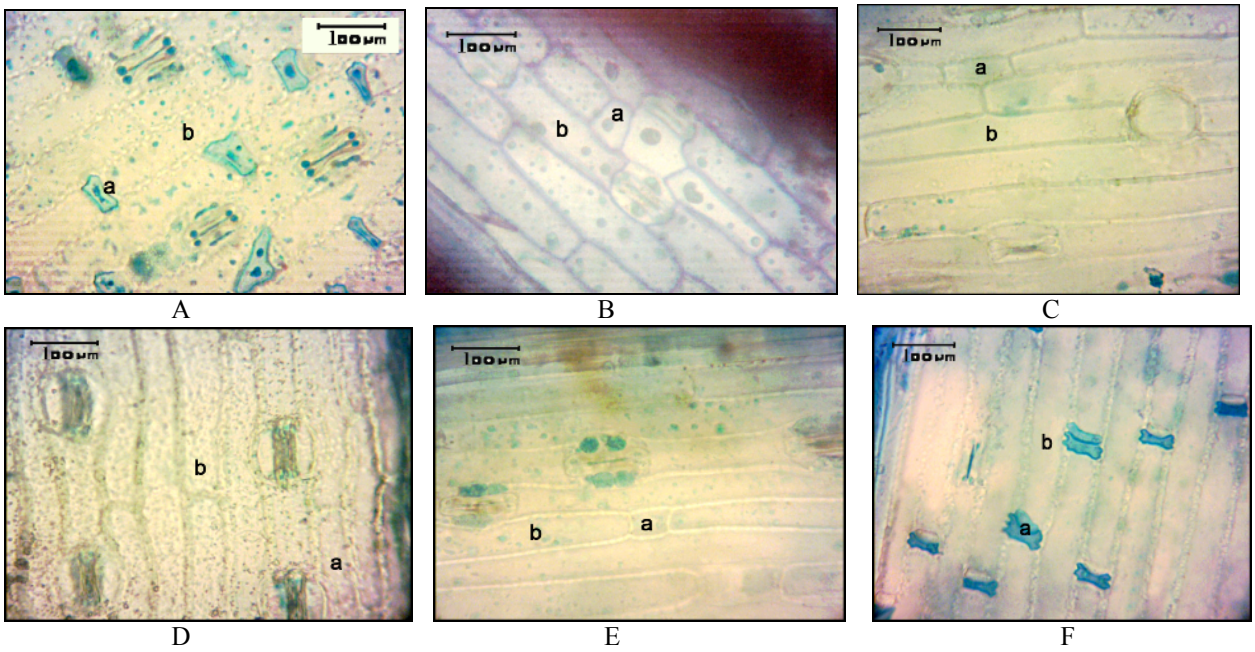

Fig. 1. Abaxial epidermis A-F: Inter-costal zone, A. *Oryzopsis gracilis*, B. *O. barbellata*, C. *O. microcarpa*, D. *O. molinioides*, E. *O. pubiflora* and F. *O. virescense*. (a. short cell, b. long cell)

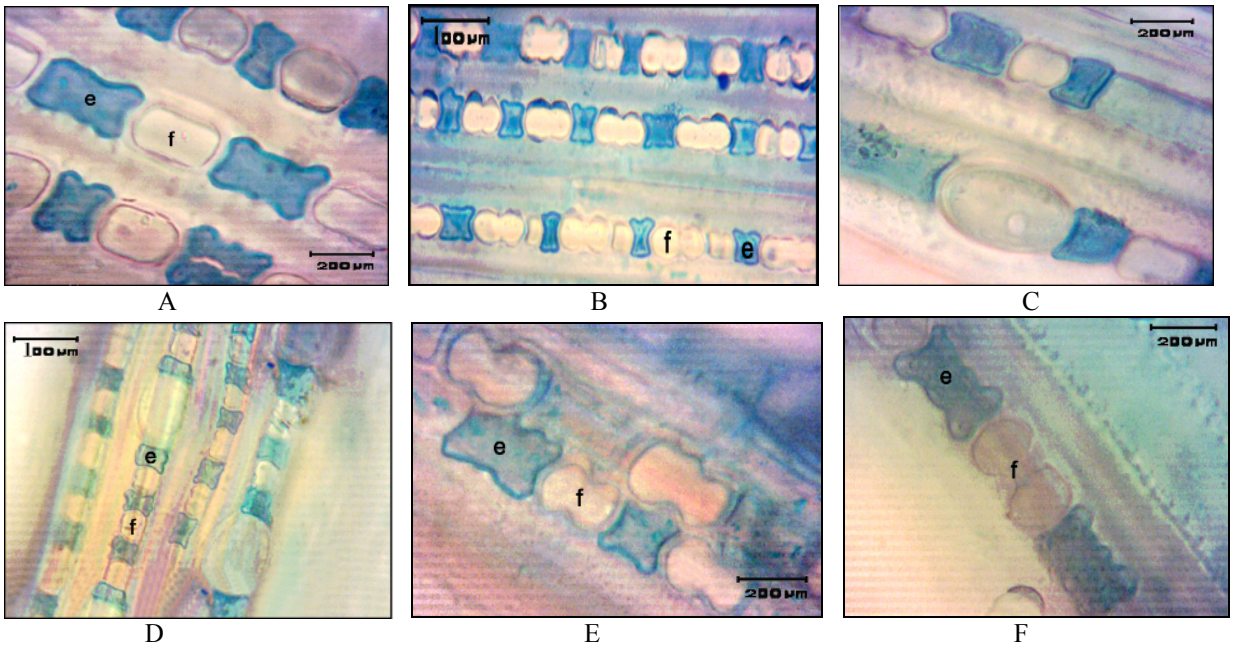

Fig. 2. Abaxial epidermis: Costal zone. A. *Oryzopsis gracilis*, B. *O. barbellata*, C. *O.pubiflora*, D. *O. microcarpa*, E. *O. molinioides* and F. *O. virescense*. (e. silica body. f. costal short cell).

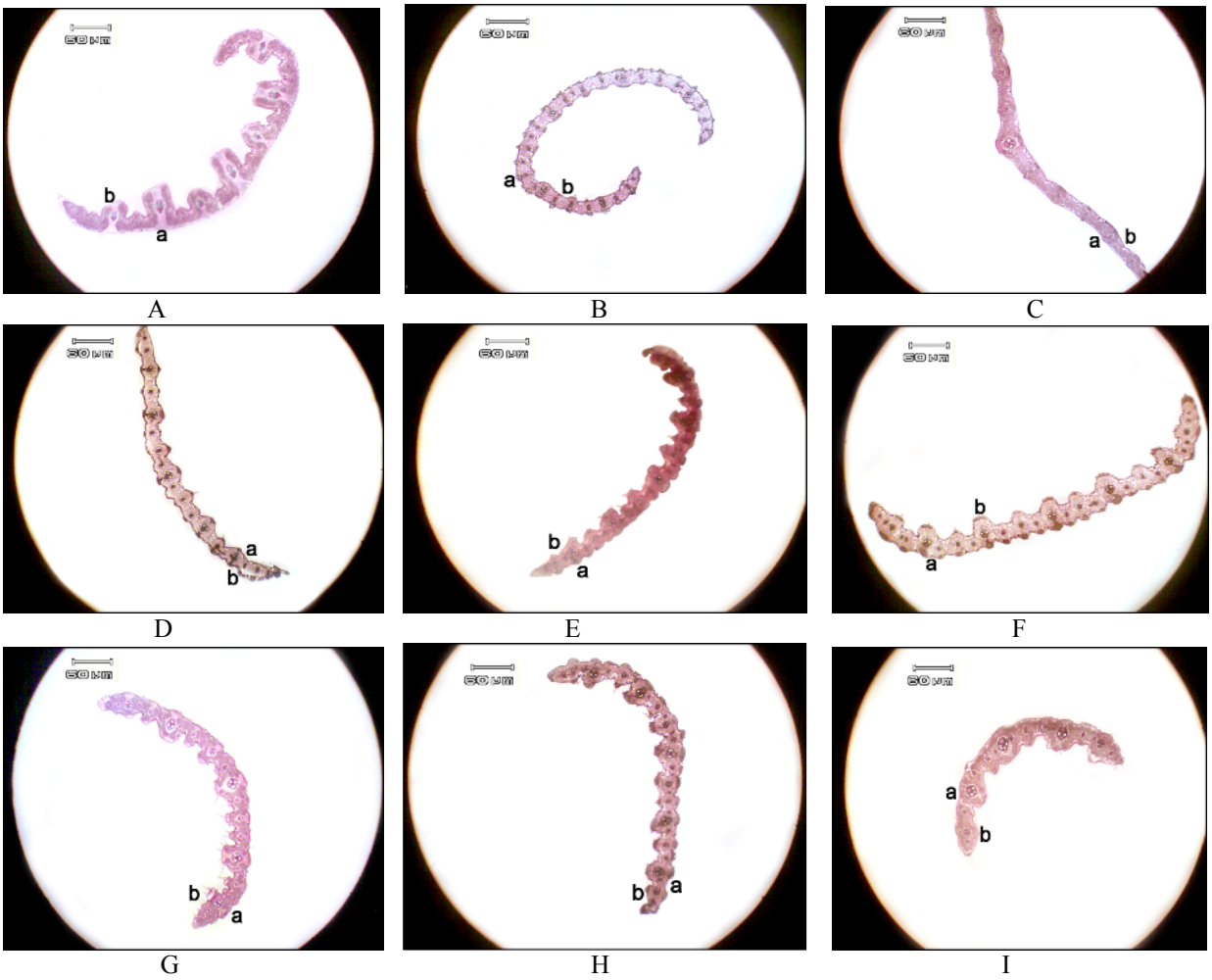

Fig. 3. General shape of leaf cross section. A. *Oryzopsis gracilis*, B. *O. barbellata*, C. *O. holciformis*, D. *O. pubiflora*, E. *O. microcarpa,* F. *O. molinioides,* G. *O. munroi,* H. *O. sphacelata* and I. *O. virescens* (a. abaxial surface, b. adaxial surface).

segregation of these species. Thus, we assume that they are not two distinct species. *O. pubiflora* is the correct name according to the priority rule of botanical nomenclature.

Bor (1975) suggested two varieties for *O. holciformis*, *O. holciformis* var. *holciformis* and *O. holciformis* var. *longiglumis* (Hausskn.) Halacsy. He separated these two varieties using of spikelet length. We studied many populations of *O. holciformis* morphologically. Our investigations showed spikelet length is an adjoining character and depends on environmental factors. Thus two varieties suggested by Bor (1970) are not accepted. In UPGMA cluster and NJ (Figs. 7 & 8) populations of *O. holciformis* formed two groups that were placed beside together. A stable anatomical character was not found to separate these two groups. *O. holciformis* Sepidan population (O1) and *O. holciformis* Estahban population (O2) are from Fars province. *O. holciformis* Rozan population (O3) and *O. holciformis* Golestan population (O4) are from north of Iran. This evidence may be showing local ecotypes. We need more study to recognize probable ecotypes (under study).

The results of morphological studies confirmed the remoteness of *O. virescens* and *O. gracilis* from the other species and from each other. There are many morphological differences between these two species and others. For example in *O. virescens* awn is subterminal and permanent, ligule is very small or absent, and lemma and palea are oblanceolate. In *O. gracilis* branches are rotate, lemma and palea entirely are covered with white and lengthy hairs, hillum is continued to half of caryopsis. These characters are not seen in the other species.

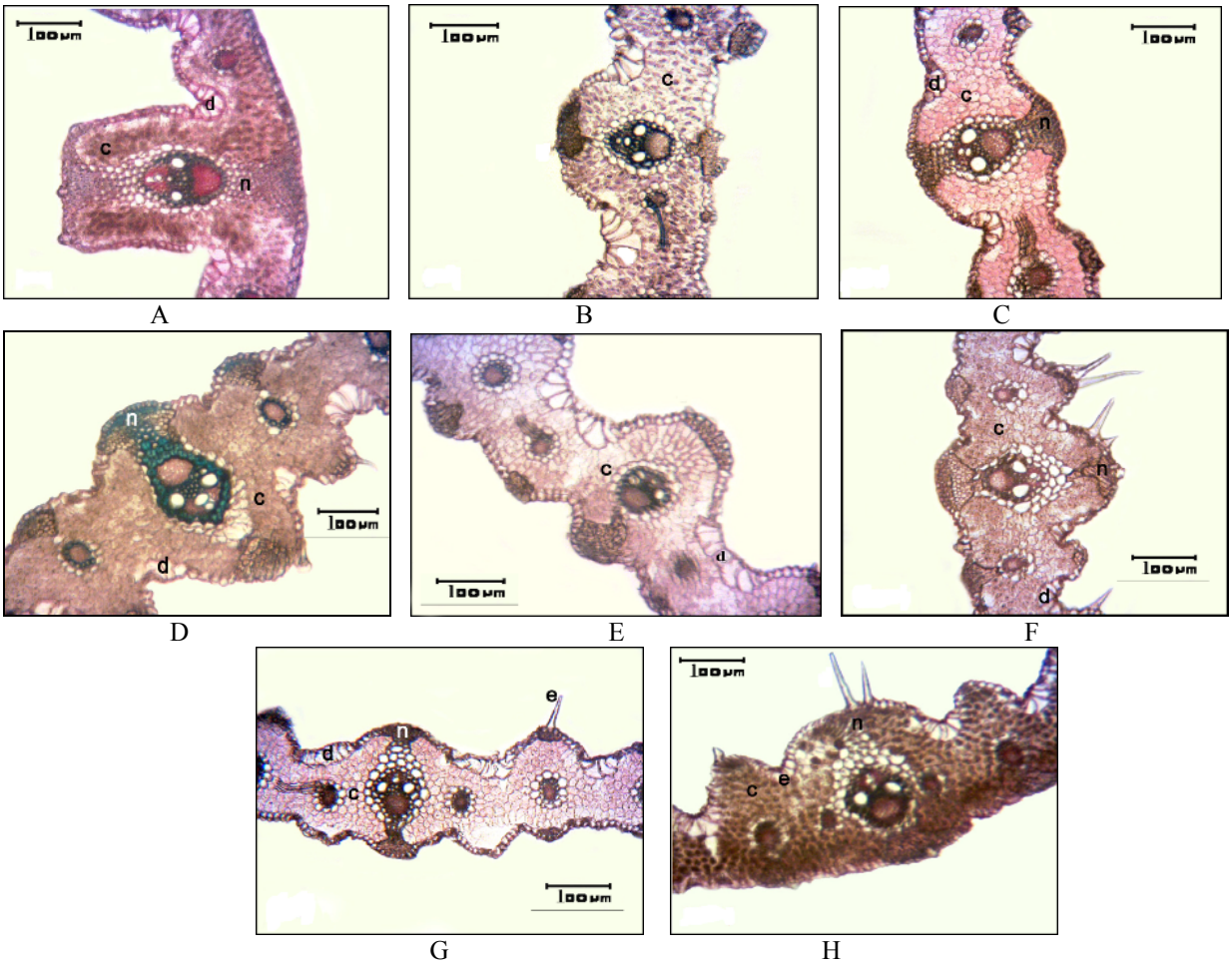

Fig. 4. General shape of leaf cross section: Keel and midrib. A. *Oryzopsis gracilis*, B. *O. barbellata,* C. *O. holciformis*, D. *O. microcarpa*, E. *O. molinioides*, F. *O. munroi*, G. *O. pubiflora* and H. *O. virescens* (c. mesophyll, d. bulliform cells, e. macrohair, n. sclerenchyma).

It seems that the study of anatomical characters of reproductive organs as lemma and palea is useful for segregation of species of *Oryzopsis*.

According to statistical analysis and specification of anatomical characters value, the identification key was provided.

1.a. Abaxial surface of leaf blade smooth, bulliform cells irregular fan-shaped groups in deep furrows, number of vascular bundles less than 10 *O. gracilis* (Mez.) Pilger

b. Abaxial surface of leaf blade more or less undulate, bulliform cells regular fan-shaped groups in shallow furrows, number of vascular bundles more than 10 2

2.a. Long cell walls undulate, short cells more than 45, not oblong *O. virescens* (Trin.) Beck.

b. Long cell walls smooth, short cells less than 45, oblong 3

3.a. Adaxial surface of leaf blade without macro-hairs 4

b. Adaxial surface of leaf blade with macro-hairs 5

4.a. Cross section of leaf v-shaped with an angle about 180°, abaxial surface of leaf blade slightly undulate, furrows of adaxial surface of leaf blade shallow, midrib truncate quadrangular, subsidiary cells of stomata parallel-sided *O. holciformis* (M. B.) Hack.

b. Cross section of leaf linear with inverse edges to inside, abaxial surface of leaf blade undulate, furrows of adaxial surface of leaf blade deep, midrib dome-shaped, subsidiary cells of stomata low-dome-shaped *O. molinioides* (Boiss.) Hack.

5.a. Cross section of leaf u-shaped with an obtuse angle, abaxial surface of leaf blade slightly undulate 6

b. Cross section of leaf linear, abaxial surface of leaf blade undulate 7

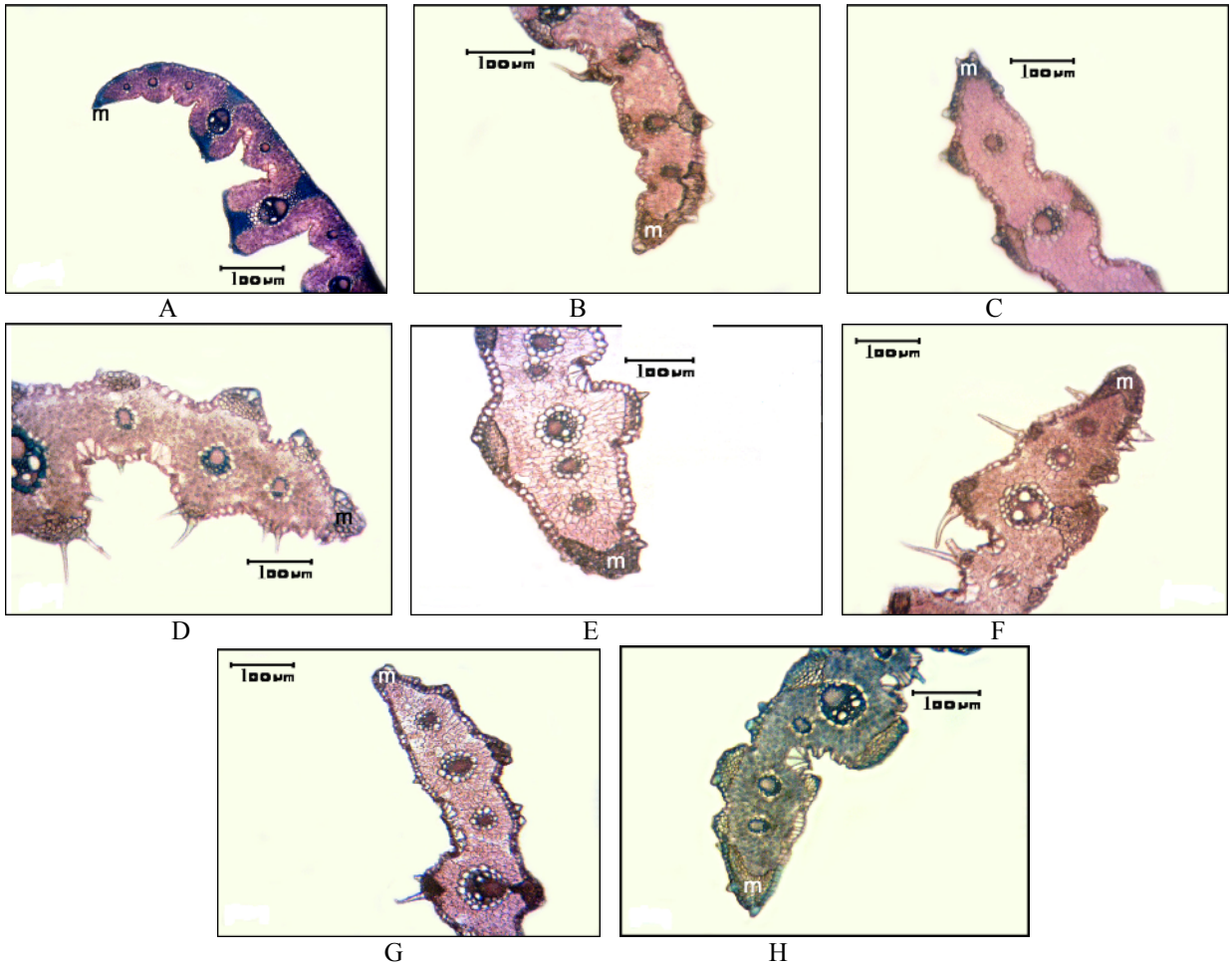

Fig. 5. Leaf margin. A. *Oryzopsis gracilis*, B. *O. barbellata*, C. *O. holciformis*, D. *O. microcarpa*, E. *O. molinioides*, F. *O. munroi*, G. *O. pubiflora* and H. *O. sphacelata* (m. sclerenchyma).

6.a. Furrows of adaxial surface deep, outer sheath of central vascular interrupted, macro-hairs of adaxial surface abundance and long *O. munroi* Stapf
b. Furrows of adaxial surface shallow, outer sheath of central vascular complete, macro-hairs of adaxial surface a few and short *O. barbellata* (Mez.) Bor
7.a. Mesophyll not radial differentiation, cells of inner bundle sheath sclerified *O. microcarpa* Pilger
b. Mesophyll having radial differentiation, cells of inner bundle sheath not sclerified 8
8.a. Furrows of adaxial surface deep, ribs dome-shaped and truncate quadrangular, midrib truncate quadrangular *O. sphacelata* (Boiss. & Buhse) Hack.
b. Furrows of adaxial surface shallow, ribs both dome-shaped and acute, midrib dome-shaped
O. pubiflora Hack.

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References
Alemi, M., Attar, F. & Hamzeh`ee, B. 2007: Micro-morphological studies of indumentum in Bromus L. (Poaceae) as taxonomical evidence. -Iran. J. Bot. 13 (2): 129-137.

Barkworth, M. E., 1981: Foliar epidermis and taxonomy of North American Stipeae (Gramineae). -Systematic Botany. 6 (2): 136-152.

Boissier, E., 1884: Oryzopsis in Flora Orientalis. 5: 506-509. -Genevae et Basiliae.

Bor, N. L., 1968. Oryzopsis in Townsend, C. C., Guest, E. & Al-Rawi, A. (eds.) Flora of Iraq 9: 410-418. -Minstry of Agriculture of the Republic of Iraq, Baghdad.

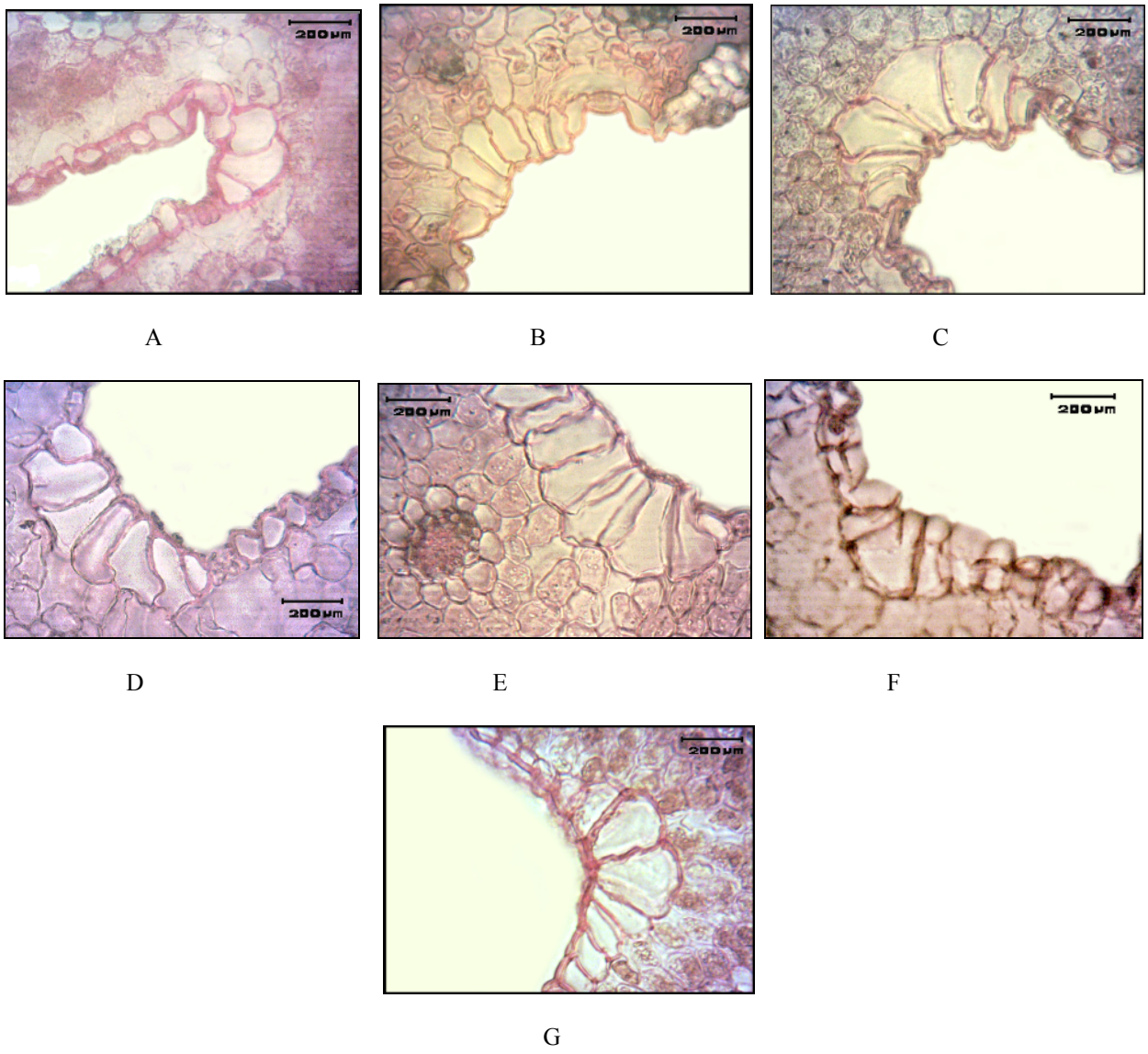

Fig. 6. Bulliform cells. A. *Oryzopsis gracilis*, B. *O. babellata*, C. *O. microcarpa*, D. *O. molinioides*, E. *O. pubiflora*, F. *O. sphacelata* and G. *O. virescens*.

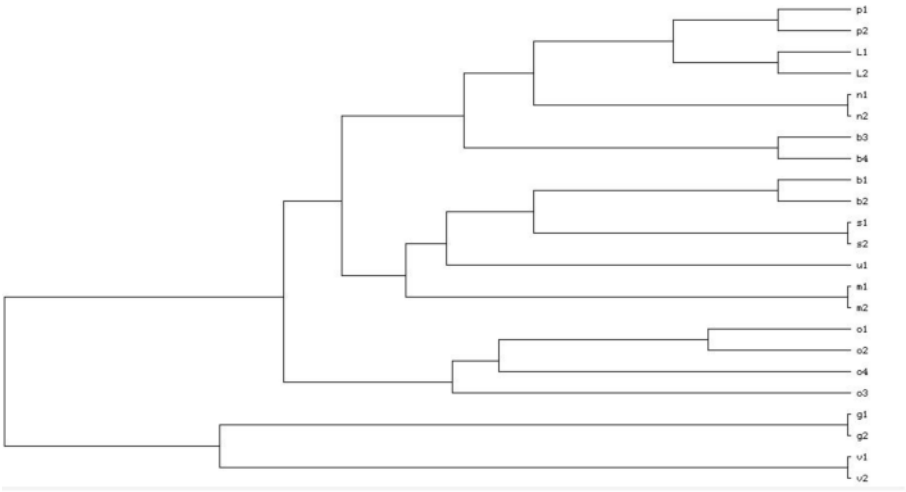

Fig 7. UPGMA cluster analysis of anatomical characters. Species/populations code as in table 1.

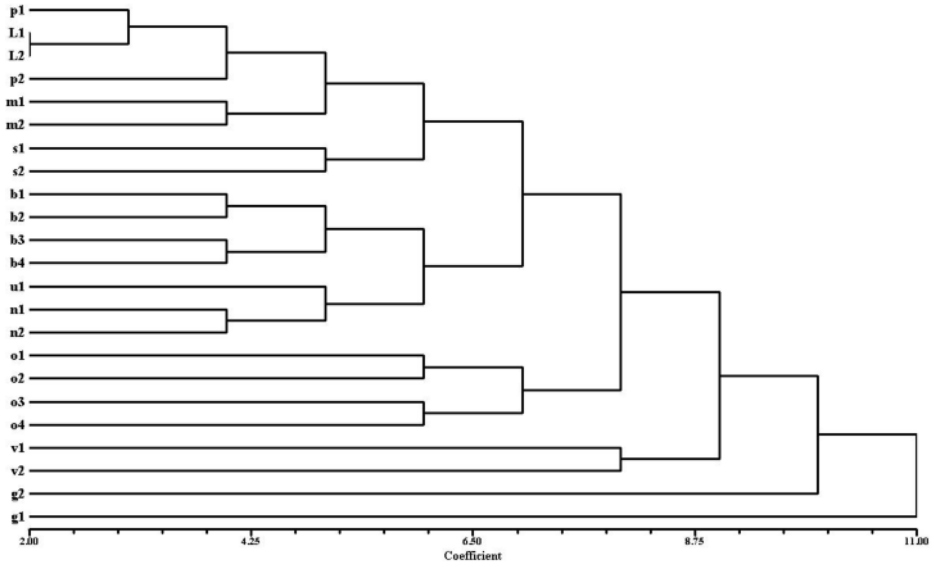

Fig. 8. Neighbor joining cluster analysis of anatomical characters. Species/populations code as in table 1.

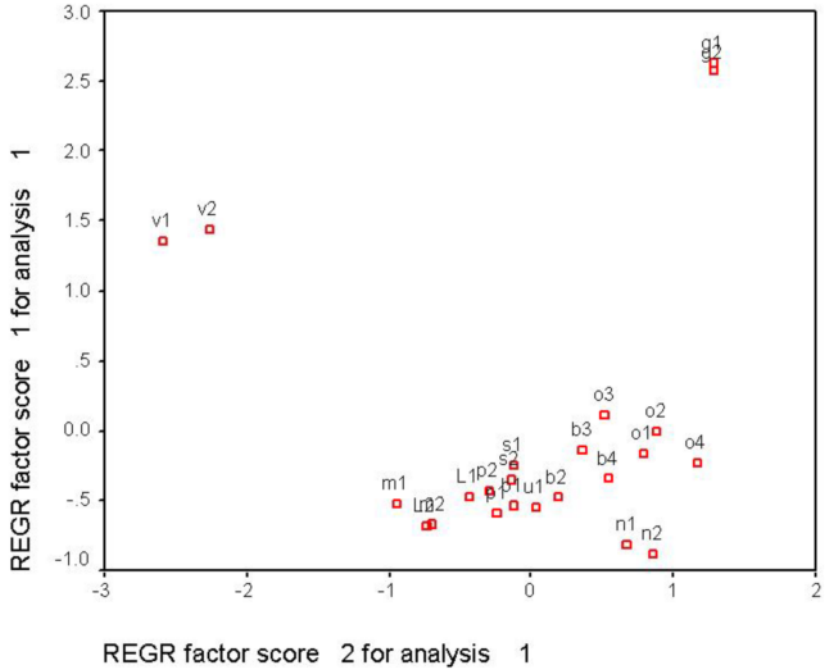

Fig 9. Ordination of taxa based on PCA of anatomical characters anatomical characters. Species/populations code as in table 1.

Bor, N. L., 1970*:* Oryzopsis in Rechinger, K. H. (ed.). Flora Iranica 70: 400-413. -Akademische Drick-U. Verlagsantal. Graz. Austria.

Clayton, W., Harman, K. T. Williamson, H., 1999: Onwards. Grass base-The online world grass Flora. ver 28th , Jan. 2008.

Clifford, H. T. & Watson, L., 1977: Identifying grasses: data, methods and illustrations. -Univ. Queensland Press, St. Lucia, Queensland, Australia.

Cope, T. A., 1982: Piptatherum in Nasir. E. & Ali. S. I. (eds.) Flora of Pakistan 143: 547-554. -Herbarium Royal Botanic Gardens Kew. London.

De Winter, B., 1965: The South African Stipeae and Aristideae (Gramineae). -Bothalia 8: 201-204.

Eslamy Jooiandeh, N., Zarrin-Kamar, F. & Assadi, M., 2008: Taxonomic value of leaf anatomy in Iranian fine-leaved Festuca. -Rostaniha.9 (2): 166-177.

Freitag, H., 1975: The genus Piptatherum (Gramineae) in southwest Asia. -Notes. Roy. Bot. Gard. Edinburgh 33: 341-408.

Ghahreman, A., Alemi, M., Attar, F., Hamzeh`ee, B. & Columbus, J. T. 2006: Anatomical studies in some species of Bromus L. (Poaceae) in Iran. -Iran. J. Bot. 12 (1): 1-14.

Keshavarzi, M., Khaksar, M. & Saifali, M., 2009: Studying leaf anatomical variation in Phalaris (Poaceae) species in Iran. -Shahid Chamran Univ., J. Sci., Section B., 23: 116-128.

Metcalfe, C. R., 1960: Anatomy of the Monocotyledons. I- Gramineae. -Oxford University Press, London. 1-711.

Nasirzadeh, A., 2004: Cytomorphological studies on Oryzopsis species in Fars province, Project report no. 26692. -Research Institute of Forests and Rangelands, Tehran, Iran.

Pilger, R., 1954: Das system der Gramineae. -Bot. Jahrb. Syst. 76: 281-384.

Prat, H. 1932: L`epidermis des Graminee etude anatomique et systematique . -Ann. Sci. Nat. Bot. (Serie X) 14: 117-324.

Raol, V. M. & Desai, R. J. 2009: Epidermal studies in some members of Andropogoneae (Poaceae). -Not. Bot. Hort. Agrobot. Cluj. 37: 59-64.

Smith, P. M., 1985: Piptatherum in Davis, P. H. (ed.) Flora of Turkey and the East Aegean Islands 9: 553-559. -Edinburgh at the University Press.

Watson, L. & Dallwitz, M. J. 1992: Onwards. The grass genera of the world: descriptions, illustrations, identification and information retrieval; including synonyms, morphology, anatomy, physiology, phytochemistry, cytology, classification, pathogens, world and local distribution, and references. Ver.28th, Nov. 2005.