

Foliar anatomy and micromorphology of *Festuca* L. and its taxonomic applications

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Abstract

In this study, leaf micromorphological structure of eight species of *Festuca* (*F. akhaniai*, *F. elwendiana*, *F. heterophylla*, *F. sulcata*, *F. valesiaca*, *F. arundinacea*, *F. gigantea* and *F. drymeia*) and leaf anatomy structure of three species of *Festuca* (*F. arundinacea*, *F. gigantea* and *F. drymeia*) belonging to the four subgenera were examined with different repetitions. About 40 quantitative and qualitative anatomical features of the leaves were statistically analyzed for several times from superficial view and on transversal section. These characters included observation of ribs and furrows in epidermis, the density of stomata and trichome, and the arrangement of vascular bundles. Sclerenchyma and bulliform cells were studied and their taxonomic value was verified in order to classify different species. The micromorphology data and anatomy characteristics of the species were used for multivariate analysis, which partly supported the taxonomic treatment of the genus *Festuca* in the flora of Iran. In order to group the species studied on the basis of similarity in their anatomical features as well as their micro morphological characteristics, different clustering methods of Between Groups, Single Linkage and WARD were observed. The first cluster composed of *F. sulcata*, *F. valesiaca*, *F. elwendiana*, *F. heterophylla* and *F. akhaniai*. The species of *F. arundinacea*, *F. gigantea* and *F. drymeia* were positioned in clusters two.

Key words: Anatomy, *Festuca*, Iran, Micro morphology, Sclerenchyma

Introduction

The genus *Festuca* L. contains an estimated 450 species (Clayton and Renvoize, 1986) and is a large and ancient group and one of the main evolutionary lines in the tribe Poeae which occur in polar, temperate, and alpine regions of both hemispheres (Tzvelev, 1976).

More than a century, foliar anatomy has been used in the taxonomy of the *Festuca*. Hackel (1882) published the first monograph of the genus *Festuca* relating to Europea He illustrated characteristics of 37 taxa using block diagrams. Metcalfe (1960) gave diagnostic generic characters and detailed information on the anatomy of several species of *Festuca*. Tzvelev (1976) in U.S.S.R. and Alexeev (1982) of North America made considerable use of leaf cross section as a taxonomic tool. In flora European Markgraf-Dannenberg (1980)

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using leaf cross section characters such as the number of veins and the distribution of sclerenchyma succeeded in identifying the species in this genus. The available literature from the other parts of the world dealing with anatomical study of *Festuca* supported such an assumption (Holmen, 1964; Badoux 1971; Borrill, 1972; Frederickson, 1977; Connor, 1960; Howarth, 1924, 1925; Snait-Yeves, 1925; Aiken *et al.*, 1984, 1985, 1995; Sawicki *et al.*, 2001; Aryavand and Panahi, 2003; Namaganda and Lye, 2008, 2009; Zarinkamar, 2008). However, anatomical study of the genus *Festuca* is insufficient for the species growing wild in Iran.

The present study considers anatomy and micromorphology of eight *Festuca* species occurring in Iran with the aim of providing some basic anatomical data for the country, and its taxonomic applications.

Materials and Methods

Micromorphological studies were performed on eight populations of eight *Festuca* species, namely *F. sulcata*, *F. arundinacea* Schreb, *F. valesiaca* s.l. Schleich. ex Gaudin, *F. gigantea* (L.) Vill. *F. elwendiana* Markgr.-Dann., *F. heterophylla* Lam., *F. akhania* Tsvelev and *F. drymeia* Mertens et Koch (Table 1). The specimens were diagnosed using Flora Iranica (Bor, 1970), Flora Orientalis (Boissier, 1875), Flora of Iran, Flora of Iraq (Bor, 1968, 1970), the illustrated Flora of Golestan National Park, Iran (Akhania, 2005) and Grasses of the Soviet Union (Tzvelev, 1976). The voucher specimens are deposited in the Herbarium of TARI (Table 1).

Table 1: Voucher specimen of *Festuca* specimens. Abbreviations: TARI, Research Institute of Forests and Rangelands Herbarium.

Species	Morphological studied (Morphology and anatomy)	Voucher specimen
<i>F. akhania</i>	Morphology	Golestan, Golestan Forest, 1600 m, Bagheri (TARI, 90264)
<i>F. arundinacea</i>	Micromorphology and Anatomy	Yazd, 2700-2900 m, Mozaffarian (TARI, 77545)
<i>F. drymia</i>	Morphology and Anatomy	Gorgan, Loo forest, Bagheri (TARI, 90267)
<i>F. drymia</i>	Morphology and Anatomy	Mazandaran, Noshahr, 500 m (TARI, 33471)
<i>F. elwendiana</i>	Morphology	Hamedan, Alvand, 2300 m, Kelovande & Baghere (TARI, 90265)
<i>F. elwendiana</i>	Morphology	Hamedan, Ganjnameh, 2100 m, Assadi & Mozaffarian (TARI, 36729)
<i>F. gigantea</i>	Morphology and Anatomy	Mazandaran, Tonekabon, 1400 m, Hamzehee & Asree (TARI, 71073)
<i>F. heterophylla</i>	Morphology	Tehran, Lar, 2420-2550 m, Wendelbo & Assadi (TARI, 13348)
<i>F. heterophylla</i>	Morphology	Azərbayjan, 45 KM Sabalan, 2900 m, Mozaffarian (TARI, 9319)
<i>F. sulcata</i>	Morphology	Azərbayjan, Arasbaran, 2500 m, Assadi (TARI, 23910)
<i>F. sulcata</i>	Morphology	Azərbayjan, Arasbaran, Dooghrool, 2600-2800 m, Assadi & Sardabi (TARI, 23997)
<i>F. valesiaca</i>	Morphology	Golestan, Yakhte kalan, 2100 m, Bagheri (TARI, 90262)
<i>F. valesiaca</i>	Morphology	Golestan, Golestan Forest, Sharlagh, 2200 m, Bagheri (TARI, 90263)

Methods

Anatomical analyses of leaves were done on permanent slides, prepared by the standard method for light microscopy (Strittmatter, 1973). Cross-sections of the external leaves from vegetative shoots were cut on a Reichert sliding microtome and by hand cutting. Sections were prepared from the middle region between one quarter and one half of the total length of blade, cleared in sodium hypochlorite and stained with carmine-vest (1% w/v in 50% ethanol) and methyl green (1% w/v, aqueous) then mounted in gelatin.

Epidermal surface were studied with Scanning Electron Microscope (SEM) for which the samples was covered by gold.

All morpho-anatomical measurements were done and the data processed in the statistical package. For each of the quantitative characters, 65 leaf samples were obtained from different individuals belonging to each of the eight populations analyzed. 37 quantitative and 40 qualitative anatomical characters were statistically analyzed in superficial view and on transversal section.

Statistical Analyses

In order to group the species studied based on similarities in their anatomical features as well as micromorphological characteristics, different clustering methods of Between Groups, Single Linkage and WARD (minimum spherical cluster method) were used. To obtain value of characters, PCA (principle component analysis) was employed.

Results

Festuca L. is divided into two groups based on lamina width: fine-leaved and broad-leaved. In the fine-leaved, abaxial leaf surface was coated by silica and there were single prickles on veins and leaf margin. In abaxial surface of *F. akhaniai*, trichomes were distributed with different orientations and some outgrowths (Figure 1, A and B). In *F. elwendiana*, trichomes had different sizes (Figure 1, C). In *F. heterophylla*, prickles were only seen on veins and leaf margin of abaxial surface (Figure 1, E and F). There were ribs and furrows on adaxial surface which stomata frequently presented in furrows (Figure 1, D and J). The ribs were pubescent (Figure 1, E).

In the broad-leaved species, external surface and stomata were covered by silica (Figure 2, A and C). Trichomes were present on leaf margins (Figure 2, F). In *F. drymeia* there were no ribs and furrows and stomata were distributed on both sides of leaf surfaces. In *F. gigantea* and *F. arundinacea* there were ribs and furrows and stomata were frequently present on furrows.

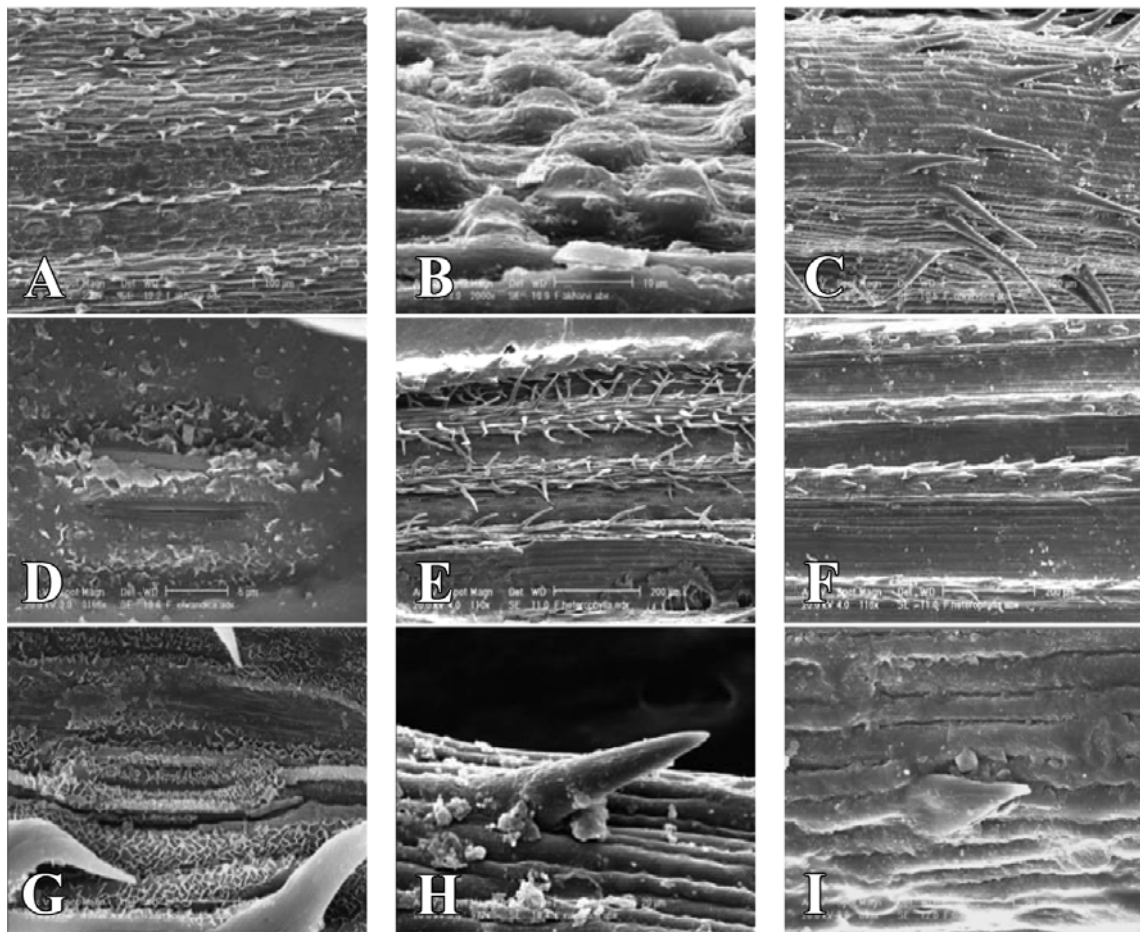


Figure 1: Superficial view of the fine-leaved species. A and B: *F. akhanii* (223X), (2000X); C and D: *F. elwendiana* (239X), (3155X); E and F: *F. heterophylla* (110X), (116X); J and H: *F. valesiaca* (1673X), (972X); I: *F. sulcata* (839X).

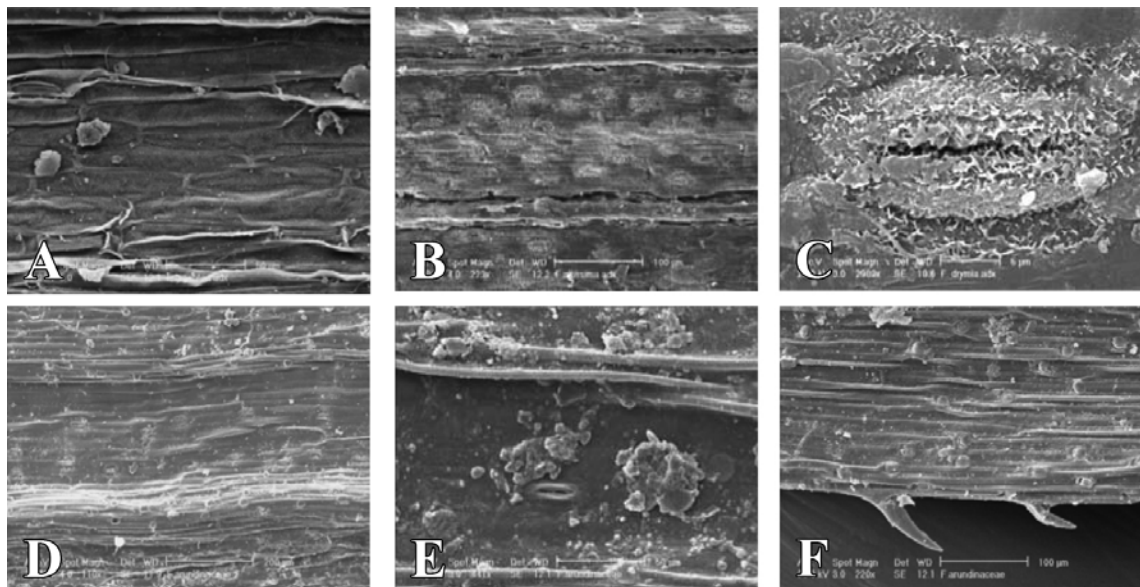


Figure 2: Superficial view of broad-leaved. A: *F. gigantea*; B and C: *F. drymeia*; D and F: *F. arundinacea*.

Leaf cross section: Fine-leaved of *Festuca* species were ≤ 2 mm wide because they were more or less tightly rolled or folded, often with leaf margin overlapping. A previous study on fine-leaved of *Festuca* species, demonstrated that some characters as number of ribs and furrows in adaxial surface, number of vascular bundles and number of sclerenchyma bundles could distinguished species (Eslami *et al.*, 1387).

Broad-leaved species included *F. arundinacea*, *F. gigantea* and *F. drymeia*. Epidermal cells were different in size and type. Leaves were or appeared to be, ≤ 4 mm wide.

The characters are suitable for distinguishing the species as follows: Adaxial surface in *F. drymeia* is smooth (Figure 2, D, E and F), whereas in *F. arundinacea* (Figure 3, G, H and I) and *F. gigantea* (Figure 3, A, B and C) are observed as rounded rib over each vein. Bulliform cells occupy $\frac{1}{4}$ of the leaf thickness on the adaxial epidermal cells or at the base of the ribs (Figure 3, F). In the above-mentioned species, minute sclerenchyma tissues were observed opposite middle and as large vascular bundles. Sometimes sclerenchyma girders were extended from vascular bundles to both adaxial and abaxial epidermis (Figure 3, D, E, F, G, H and I).

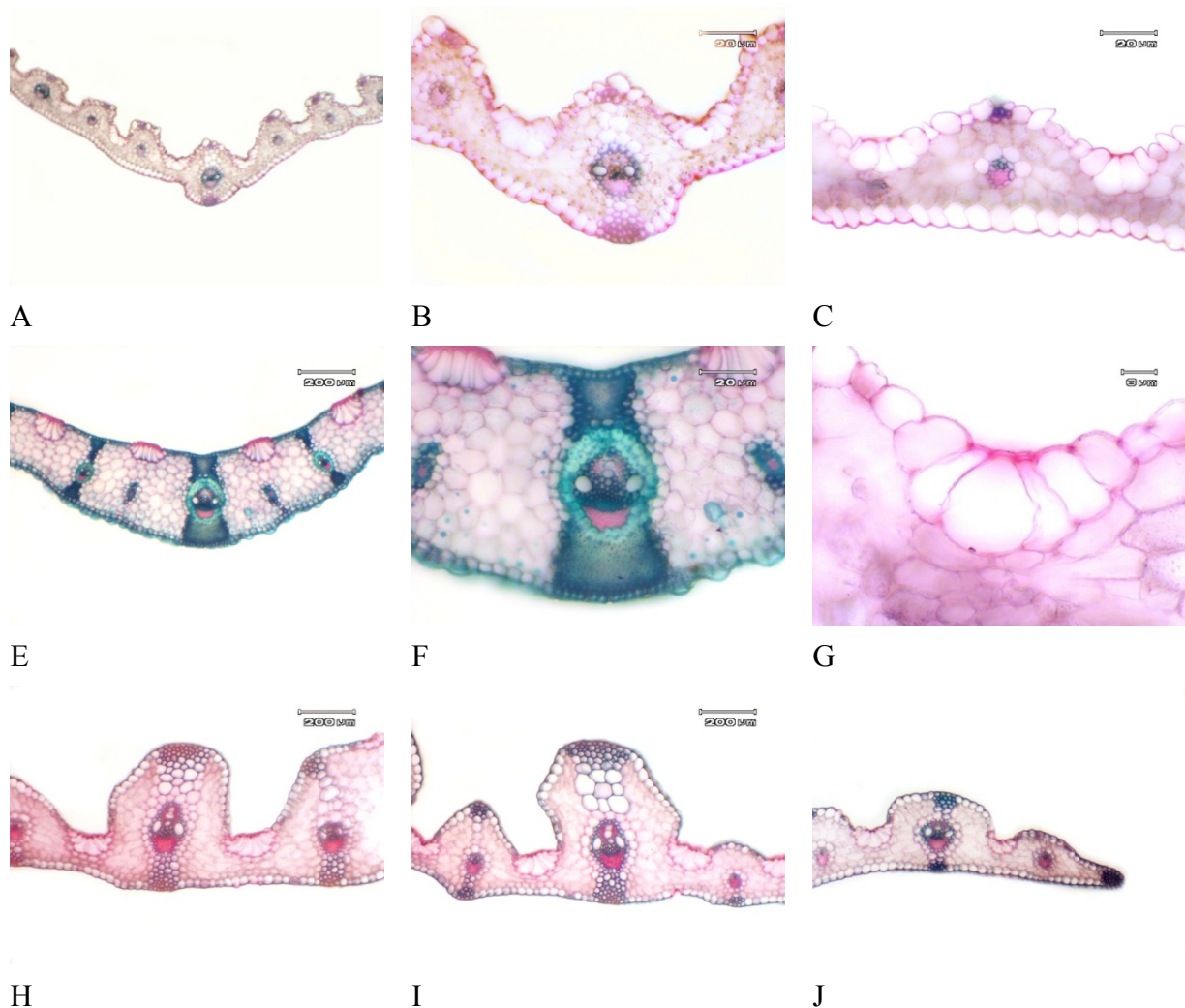


Figure 3: Transversal section of broad-leaved species. A and B: *F. gigantea*; D, E and F: *F. drymeia*, G, H and I: *F. arundinacea*.

Table 2: Useful anatomical characters for distinguish species. Relation the sclerenchyma tissue of Midrib and Adaxial epidermal: 1, attachment; 2, apart. Shape furrow: 1; V-shape between 45°; 2, V-shape between 90° and 180°. Epidermal cell shape: 1, oblong; 2, oblong- elliptic. Stomata type: 1, present, 2, absent.

Characters	Taxon			
	<i>F. drymia</i>	<i>F. drymia</i>	<i>F. gigantea</i>	<i>F. arundinaceae</i>
Relation the sclerenchyma tissue of midrib and adaxial epidermis	1	1	2	2
Number of large vascular bundle	3	3	2	1
Number of middle vascular bundle	6	6	2	3
Number of small vascular bundle	4	1	3	3
Shape furrow	0	0	1	2
Adaxial epidermal cell shape	2	2	1	2
Stomata type on adaxial surface	3	2	3	2
Buliform cells shape	1	1	4	4
Abaxial epidermal cell shape	1	1	3	2
Stomata type on abaxial surface	3	2	3	2
Number of adaxil furrow	0	0	13	13
Number of adaxil ribs	0	0	5	14
Number of buliform cells on adaxial surface	0	0	30	24
Number of sclerenchym bundle	26	23	8	16
Number of sclerenchym layer in tip leaf	6	4	2	7
Number of sclerenchym layer under midrib	10	6	3	7

Different clustering methods like Between Groups, Single Linkage and WARD produced similar results whereas Between Groups possessed the highest correlation. In analyses, two clusters were observed. The first cluster was composed of *F. sulcata*, *F. valesiaca*, *F. elwendiana*, *F. heterophylla* and *F. akhanii*. The species of *F. arundinacea*, *F. gigantea* and *F. drymeia* were positioned in cluster two (Figure 4).

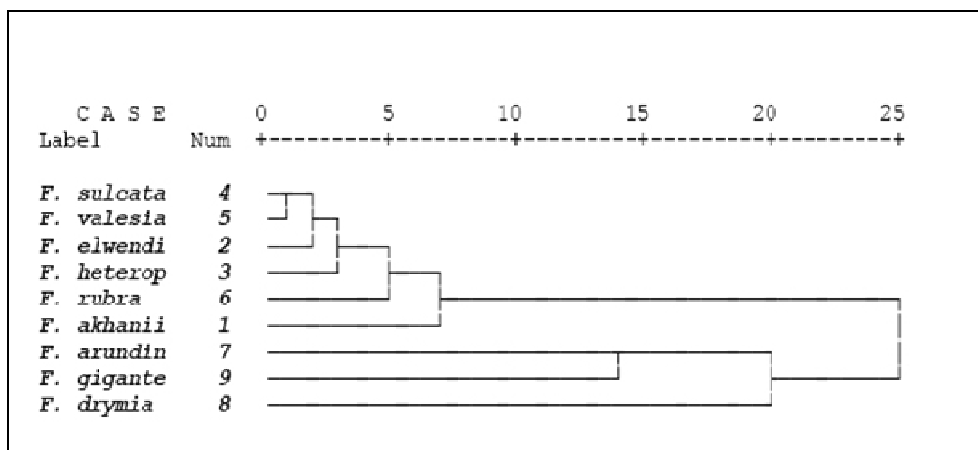


Figure 4: Ward method clustering of the *Festuca* species studied

The PCA data demonstrated the most important characters for anatomy as follow: width and thickness of leaf cross section, shape and number of veins and arrangement of vascular bundles. Moreover, the position and degree of scleranchyma development, number of ribs in adaxial surface, density of crystal, observation of stomata in abaxial surface, form of epidermal cells and number of bulliform cells could be also valuable for taxonomic application.

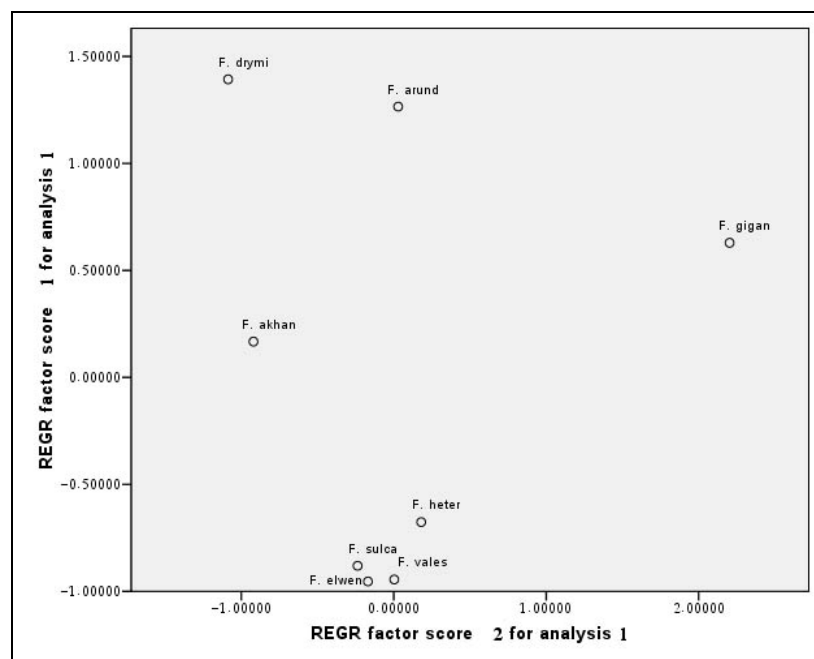


Figure 5: PCA plot of the *Festuca* species studied

The PCA plot suggested that the position of *F. akhanii* was between the fine and broad-leaved species of *Festuca* (Figure 5).

Discussion

The anatomical characters in cross section of leaf are important in identification of the taxa. Broad-leaved species such as *F. arundinacea* from the subgenus Schedorus, *F. drymeia* from the subgenus Drymanthele and *F. gigantea* from the subgenus Drymoneates have linear leaves with 2mm width and sheaths that divided into two groups based on ribs and furrows but in *F. drymeia* there are not ribs and furrows on both surface linear and have 2 mm width.

In general, bulliform cells are dispersed between veins on the adaxial surface. Papilla or prickle are scattered on both surfaces. There is one extension of sclerenchyma tissue from the abaxial to adaxial surface in midrib and the middle sized veins, this structure is actually absent in small veins. These species have no auricle in leaf sheath.

These furrows on the adaxial surface of *F. arundinacea* are distinguished from smooth adaxial surface of *F. gigantea*.

Stomata are present on both sides but more dense on adaxial furrows, and prickles are just present on the leaf margin. Another significant anatomical feature of *F. arundinacea* is the presence of a big vascular bundle on both sides of midrib.

F. gigantea and *F. arundinacea* are morphologically distinguished from other broad-leaved species by the presence of auricle leaf sheath.

Fine-leaved species includes *F. elwendiana*, *F. valesiaca*, *F. sulcata*, *F. akhanii* and *F. heterophylla* from the subgenus *Festuca*.

The species of *F. akhanii* is distinguished by sclerenchyma strand, presence of furrows on both sides of the midrib and papilla, while other species of the subgenus *Festuca* has more than two furrows on adaxial surface and no sclerenchyma strand.

Another important and stable anatomical character is the number of sclerenchyma tissues on the adaxial surface which is useful character to distinguish other species of the

subgenus *Festuca*. There are three groups of sclerenchyma tissues with different layers in *F. valesiaca*, *F. sulcata* and *F. elwendiana* on abaxial surface. The sclerenchyma tissues are completely separated in *F. valesiaca* and *F. sulcata* sometimes tends to continue in *F. elwendiana*. *F. elwendiana* is morphologically characterized by the presence of variable sized of trichomes on both leaf surfaces, pubescence sheathes and glabrous ovary. *F. sulcata* is morphologically and anatomically similar to *F. valesiaca* which is distinguished by group of four bulliform cells, complexes with wavy walls and furrows on adaxial surface near midrib.

There are five to seven sclerenchyma tissue groups with different layers in *F. heterophylla*. Sclerenchyma tissue forms discrete strands opposite to the large vascular bundles.

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آناتومی و ریز ریخت‌شناسی برگ جنس *Festuca* و کاربرد تاکسونومی آن

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چکیده

در این تحقیق، ساختار ریز ریخت‌شناسی برگ ۸ گونه از جنس *Festuca* با نام‌های *F. arundinacea*، *F. akhaniai*، *F. valesiaca* و *F. sulcata*، *F. heterophylla*، *F. gigantea*، *F. elwendiana*، *F. drymeia* و *F. gigantea* و *F. drymeia*، *F. arundinacea* سه گونه *F. gigantea* با تکرارهای متفاوت مورد بررسی قرار گرفت. حدود ۴۰ صفت معنی‌دار کمی و کیفی به منظور آنالیز آماری چند متغیره در نظر گرفته شد. برخی از این صفات شامل وجود یا عدم وجود فرورفتگی و برآمدگی در اپیدرم، تراکم روزنه‌ها و گُرک‌ها در سطح، آرایش دستجات آوندی و تعداد دستجات بافت‌های اسکلرانشیمی است. داده‌های حاصل از مطالعات ریز ساختاری و تشریحی برگ‌ها مورد تجزیه و تحلیل آماری قرار گرفت. نتایج آماری حاصل از روش‌های دسته‌بندی متفاوت مانند *Between Groups*، *Single Linkage* و *WARD* مشابه بوده، دو خوشه جداگانه شامل گونه‌های ریز برگ (*F. elwendiana*، *F. heterophylla*، *F. sulcata*، *F. valesiaca*) و *F. akhaniai* و *F. drymeia*، *F. gigantea* و *F. arundinacea* را نشان داد.

واژه‌های کلیدی: آناتومی، ایران، ریز ریخت‌شناسی، *Festuca*، اسکلرانشیم