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. akhanii, F. elwendiana, F. heterophylla, F. sulcata, F. valesiaca, F. arundinacea, F. gigantean* and *F. drymeia*) and leaf anatomy structure of three species of *Festuca* (*F. arundinacea, F. gigantean* 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. akhanii*. 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 Poae which occur in polar, temperate, and alpine regions of both hemispheres (Tzvelev, 1976).

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; Borrell, 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. akhanii Tsvelev and F. drymeia Mertens et Koch (Table 1). The specimens were diagnosed using Flora Iranica (Bor, 1970), Flora Orientalis (Boisser, 1875), Flora of Iran, Flora of Iraq (Bor, 1968, 1970), the illustrated Flora of Golestan National Park, Iran (Akhanii, 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.

<table>
<thead>
<tr>
<th>Species</th>
<th>Morphological studied (Morphology and anatomy)</th>
<th>Voucher specimen</th>
</tr>
</thead>
<tbody>
<tr>
<td>F. akhanii</td>
<td>Morphology</td>
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<td>F. arundinacea</td>
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<td>Yazd, 2700-2900 m, Mozaffarian (TARI, 77545)</td>
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<td>F. drymia</td>
<td>Morphology and Anatomy</td>
<td>Gorgan, Loo forest, Bagheri (TARI, 90267)</td>
</tr>
<tr>
<td>F. drymia</td>
<td>Morphology and Anatomy</td>
<td>Mazandaran, Noshahr, 500 m (TARI, 33471)</td>
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<td>Morphology</td>
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<tr>
<td>F. elwendiana</td>
<td>Morphology</td>
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<td>Morphology and Anatomy</td>
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<td>Morphology</td>
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<td>F. valesiaca</td>
<td>Morphology</td>
<td>Golestan, Golestan Forest, Sharlagh, 2200 m, Bagheri (TARI, 90263)</td>
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</table>
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. akhanii*, 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 ¼ 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: *C. 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 epidermis: 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.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Characters</th>
<th>F. drymia</th>
<th>F. drymia</th>
<th>F. gigantea</th>
<th>F. arundinacea</th>
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<td>2</td>
<td>3</td>
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<tr>
<td></td>
<td>Shape furrow</td>
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</table>

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).

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 buliform cells could be also valuable for taxonomic application.
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The PCA plot suggested that the position of *F. akhanii* was between the fined 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 Schedorous, *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.

**References**


آناتومی و ریز ریخت‌شناسی برگ جنس Festuca و کاربرد تاکسونومی آن

فاطمه زرین کمر و نسرین اسلامی جوینده
گروه علم کیاهی، دانشکده علوم زیستی، دانشگاه تربیت مدرس، تهران، ایران

چکیده
در این تحقیق، ساختار ریز ریخت‌شناسی برگ گونه از جنس Festuca با نام‌های F. arundinacea, F. akhani, F. valesiaca و F. sulcata و ساختار تشريحي برگ در F. valesiaca و F. sulcata و F. gigantea و F. elwendiana و F. drymeia به تکرار های متقارن و پرویز قرار گرفت. حدود 40 صفحه عناصر با کیفیت و کمیت متفاوت، ترتیب آمیخته درآیل، آرایش دستی‌ها و تعداد دستی‌های بایستی برای اسکلت‌شناسی است. داده‌های حاصل از مطالعات ریزماتوری و تشريحي برگ‌ها مورد تجزیه و تحلیل آماری قرار گرفت. نتایج آماری حاصل از روشن‌بندی متقارن و پرویزی دستی‌نامیده (Between Groups و Single Linkage) مشابه بوده و دو جفت گونه از گونه‌ها F. arundinacea و F. gigantean با F. akhani و F. sulcata و F. valesiaca (F. elwendiana و F. drymeia) و F. valesiaca (F. arundinacea و F. gigantean و F. drymeia) با نشان داد. واژه کلیدی: آناتومی، ایران، ریز ریخت‌شناسی، اسکلت‌شناسی

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