Acta Biologica Szegediensis

Volume 66(1):63-68, 2022
Acta Biologica Szegediensis
http://abs.bibl.u-szeged.hu/index.php/abs
DOI:10.14232/abs.2022.1.63-68

ARTICLE

The culm and glume anatomical study on *Lolium* (Poaceae) species in Iran

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ABSTRACT *Lolium* (Poaceae) is composed of five species in Iran that are of forage and weed importance. Culm and glume anatomy of grasses is inadequately explored. In the present study culm and glume anatomical structures of 17 population of four *Lolium* species is considered by use of 16 qualitative and quantitative features to distinguish *Lolium* species in Iran and to determine their diagnostic value. The culm cross-section showed multilayered hypodermis, ribbed or un-ribbed culm cross-sections, cuticles with different thicknesses, and the cortex with different tissues. Vascular bundles are arranged in two to more rings in the culm anatomy. Glume anatomical observations showed differences in girder attachment to vascular bundles, the outline of the cross-section, and the cortex tissue composition. The multi-variate statistical method based on culm and glume anatomical features showed that *L. perenne* and *L. multiflorum* are grouped near each other but there is more mixture between *L. persicum* and *L. rigidum*. Our findings are in agreement with the grouping pattern achieved by previous researches on leaf anatomy and lemma and palea micro-morphology of the same species. The molecular study by ISSR also provides support for the present study. The culm and glume anatomy of species studied provides valuable data for taxonomic purposes.
Acta Biol Szeged 66(1):63-68 (2022)

KEY WORDS
culm
glume
Iran
Lolium
similarity

ARTICLE INFORMATION
Submitted
03 May 2022
Accepted
28 August 2022
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Introduction

Lolium L. (Poaceae, Pooideae, Poeae) is a genus of the Eurasian indigenous range of distribution. Poeae is a tribe with 121 genera and around 2500 species worldwide (Soreng et al. 2007) and is characterized by simple panicles with spikelets disarticulating above the glumes. Their glumes are shorter than the adjacent lemmas which are either un-awned or with terminal or nearly terminal awns (Soreng et al. 2007).

In Iran, five species are recorded for *Lolium* genus as *L. multiflorum*, *L. persicum*, *L. perenne*, *L. temulentum* and *L. rigidum* (POWO 2019). *Lolium* species possess C3 Photosynthesis pathway and in their leaf blade, anatomical structure non-radiate chlorenchyma is present. The prominent adaxial ribs and midrib with one vascular bundle are observed in *Lolium* species (Keshavarzi et al. 2014). There are fan-shaped bulliform cells in the adaxial leaf epidermis. Sclerenchyma girders accompanied the leaf blade vascular bundles (Keshavarzi et al. 2014).

Various studies have examined the morphology (Nataj et al. 2010), micromorphology (Keshavarzi et al., 2021), leaf anatomy (Keshavarzi et al. 2014), and genetic variation (Raeisi Chehrazi et al. 2015; Tabaripour and Keshavarzi 2021) of the genus, however, culm and glume anatomical studies are not favored.

Jarvie and Barkworth (1992) studied some perennial Triticeae. In their study, they considered leaf and glume anatomical features to assess the correlation between anatomical characteristics and genomic constitution. They found that monogenomic species showed distinct glume and leaf blade anatomical characters while heterogenomic ones illustrated some features of each genome present. They used features such as the width of glume, depth of keel, depth of mid-glume between vascular bundles, number of vascular bundles in glume halfway along the length, number of nodular crystals in the epidermis, the abundance of rhomboidal crystals in the epidermis, the abundance of sclerified epidermal cells, number of parenchyma layers between chlorenchyma and adaxial epidermis and type of parenchyma cells between chlorenchyma and adaxial epidermis in their glume anatomical study. They found similarities between the glume anatomical structure and the genomic constitution of the taxa studied.

Safaiipour (2001) considered glume cross-sections of *Aegilops* species in Iran and found that features such as glume width, midrib diameter, parenchyma layers, number of vascular bundles, and silica-bodies frequency are of diagnostic importance. Safaiipour and Keshavarzi (2000)

Table 1. Voucher details of studied populations.

Species	Locality
L. perenne	Mazandaran, Ghaemshahr to Sari, Mazandaran ANRRTC, 36º 28' 33.43" N., 52º 52' 54.63" E., 40 m.
	Mazandaran, Sari, Zoghalchal, 36º 33' 57.84" N., 53º 06' 42.30" E., 38 m.
	Tehran, Vanak, Alzahra University Campus, 35º 46' 07.54" N., 51º 23' 33.88" E., 1504 m.
	Khuzestan, Behbahan, 30º 36' 42.94" N., 50º 15'11.69" E., 318 m.
L. multiflorum	Gilan, Astaneh-ye Ashrafiyeh, 37º 15' 52.88" N., 49º 57'30.41" E., -12 m.
	Gilan, 33 km North of RostamAbad, -, 35 m.
L. persicum	Gilan, 3 km E. Lahijan, 36º 28' 33.43" N., 52º 52' 54.63" E., 40 m.
	Mazandaran, Babolsar, Babolroud, 36º 27' 41.47" N., 52º 41' 39.05" E., 13 m.
	Mazandaran, 18 km E. Behshar, Between Tileh Now & Khorshid Kola, 36º 43' 24.63" N., 53º 46'17.29" E., 2 m.
	Gilan, Rasht, 37º 14' 11.04" N., 49º 35'23.31" E., 8 m.
	Mazandaran, 5 km W. Ramsar, 36º 56' 45.96" N., 50º 38'08.87" E., -20 m.
L. rigidum	Mazandaran, Babolsar, Behnamir, 36º 39' 45.54" N., 52º 45' 50.36" E., -15 m.
	Lurestan, Badrabad, 33º 25' 11.72" N., 48º 17' 20.26" E., 1200 m.
	Gilan, Rasht, near Rice Research Institute of Iran, 37º 12' 19.34" N., 49º 38' 46.40" E., 22 m.
	Kerman, Kerman. Artificial forest, 1755 m.
	Isfahan, Ghamsar, 33º 45' 09.84" N., 51º 25' 44.25" E., 1862 m.
	Khuzestan, Masjed Soleyman, 31º 58' 16.52" N., 49º 17' 08.29" E., 247 m.

studied the glume epidermis of *Aegilops* species and they pointed out that the type and number of silica bodies and stomata showed variation in the species studied.

Leaf anatomy is an appropriate tool in grass systematics studies and in many phylogenetic studies, there are some points of using leaf anatomy of taxa to infer phylogenies (Soreng et al. 2017). Culm anatomy in grasses has been inadequately explored in grass systematic and phylogenetic studies. Ramos et al. (2002) stated that the most important variations in the culm anatomy are the cortical and medullar parenchyma, the development and position of the vascular bundles, and the associated sclerenchyma appendages.

Previous investigations have reported that some characters of culm anatomy are less useful for determining relationships in Poaceae. However, the culm anatomy study in *Bouteloua* Lag. and Bamboo species showed that the culm can provide some diagnostic features (Siqueiros-Delgado 2007).

Culm sections general outline, the outer sheath of vascular bundles, the distribution pattern of vascular bundles and associated sclerenchyma tissue, and the bulliform shape and position (Traiperm et al. 2011). Culm inter-nodal anatomy of the tribe Oryzeae was studied by Sumanon et al. (2018). They found that some features as the presence and the arrangement of intercellular cavities, the presence of collapsed cells, the number of parenchyma cell layers in the cortex between the innermost vascular bundle ring and the hollow center, the number of vascular bundle rings, the presence of a sclerenchyma ring, and the number of fiber cell layers in the sclerenchyma ring are of diagnostic importance in elements of Oryzeae.

There is no study of the culm and glume anatomy for the *Lolium* species. *Lolium* species show morphological similarities so the present study aims to investigate the culm and glume anatomy as an attempt to distinguish *Lolium* species in Iran and to determine their value in phylogenetics.

Materials and methods

Plant collection and sample preparation

Samples were collected from natural habitats throughout Iran (Table 1). Vouchers are deposited at ALUH. Totally 17 accessions of four Lolium species from different geographical regions were surveyed. Cross-sections of mature flowering culms were obtained from the middle part of the first internode below the inflorescence and the first glume of the lowermost spikelets. Sections were made from the mid-glume region.

Microscopy

Samples were fixed in a fixative made of glycerin, alcohol and water (1:1:1). The tissues were sectioned by a razor blade and stained by Congo red and Methylene Blue or Toluidine blue O. sections were photographed under light microscopes with a digital camera (Olympus DP12) and described using grass terminology of Metcalfe (1960). The studied features for each part are shown in Tables 2 and 3.

Table 2. Qualitative culm and glume cross-section anatomical features and states of characters.

State of character	Feature
Culm	
Culm outline	Ribbed (1), un-ribbed (2)
Uniformity of vascular bundles	Present (1), Absent (2)
Bundle sheath	1-layered (1), 2-layered (2)
Hypodermis	Present (1), absent (2)
Girder appendage	Present (1), absent (2)
Glume	
Girder appendage	Present (1), absent (2)
Abaxial surface	Wavy (1), Smooth (2)
Vascular bundle size	Same size (1), not so (2)

Table 3. Quantitative features measured in culm and glume cross sections.

Culm
Epidermis thickness
Number of vascular bundles rings in cortex
Number of sclerenchyma mass
Glume
Number of vascular bundles in glume
Epidermis thickness
Glume thickness

In order to detect significant differences in studied characteristics of each species analysis of variance (ANOVA) was performed. To reveal the species similarity based on culm and glume anatomical features, cluster analysis and principal component analysis (PCA) were done. For multivariate analysis the mean of quantitative characteristics is used but for qualitative features these were encoded as binary/multi-state features. Variables were standardized for multivariate statistical analyses. The average taxonomic distances and squared Euclidean distances were used as the dissimilarity coefficient in cluster analysis of selected set of anatomical data. To prepare the dendrogram based on selected set of anatomical characters, PAST ver. 2.17C software was used.

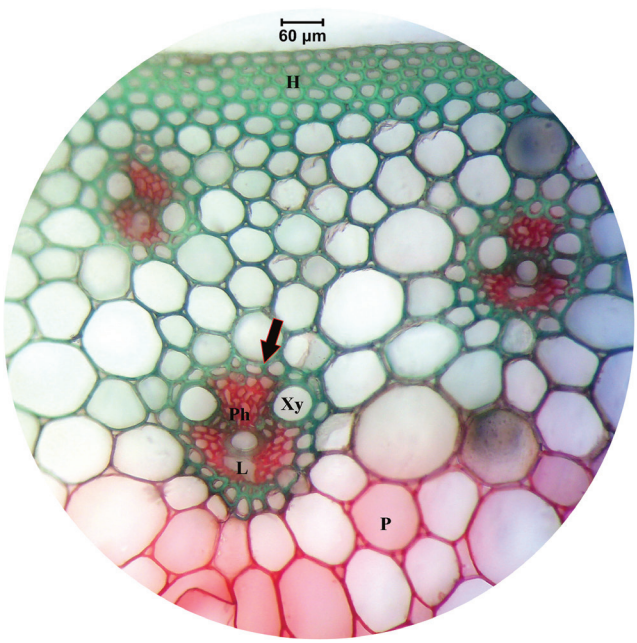

Figure 1. Culm cross section of *Lolium rigidum* (Masjed Soleyman population). Xy: xylem; Ph: phloem; H: hypodermis; L: lysogenic duct; P: parenchyma. Arrow shows the sclerenchyma fibers.

Results

Culm anatomical observation

The culm cross-section showed multilayered hypodermis (Fig. 1). Sclerenchyma is associated with vascular bundles in culm anatomical structure. Different sized vascular bundles are observed, and each bundle has normal phloem and xylem and a lysogenic duct (Fig. 1). Culm parenchyma became sclerenchymatous in some parts while toward the central parts of culm parenchyma shows normal intercellular air spaces. In each vascular bundle, meta- and proto-xylem elements are distinct.

Different species of *Lolium* show minor variation in their culm anatomy (Fig. 2). In *L. multiflorum* (Fig. 2.a and b) culm is ribbed with sclerenchymatous mass at the ribs. There are two rings of vascular bundles in the culm anatomy of this species. One ring with large bundles and the other with very small vascular bundles. There is a layer epidermis and multilayered hypodermis. Sclerenchyma surrounded the vascular bundle.

In *L. persicum* (Fig. 2.c and d) vascular bundles are observed in three different sizes. Two tissues parenchyma and sclerenchyma are evident in the culm cortex. Sclerenchyma forms a continuous layer in the culm anatomy of *L. persicum*. Culm is not ribbed. In *L. perenne* (Fig. 2.e and f) a thick cuticle is observed, and the girder is formed that the sclerenchyma is fused with the vascular bundle. Different sizes of vascular bundles were observed. A bundle sheath is evident around the vascular bundle. In *L. rigidum* (Fig. 2.g and h) there are collenchyma and sclerenchyma in the cortex. Girder is present. Culm is ribbed but not as much as *L. multiflorum*.

Glume anatomical observation

The anatomical structure of the glume (Fig. 3) showed

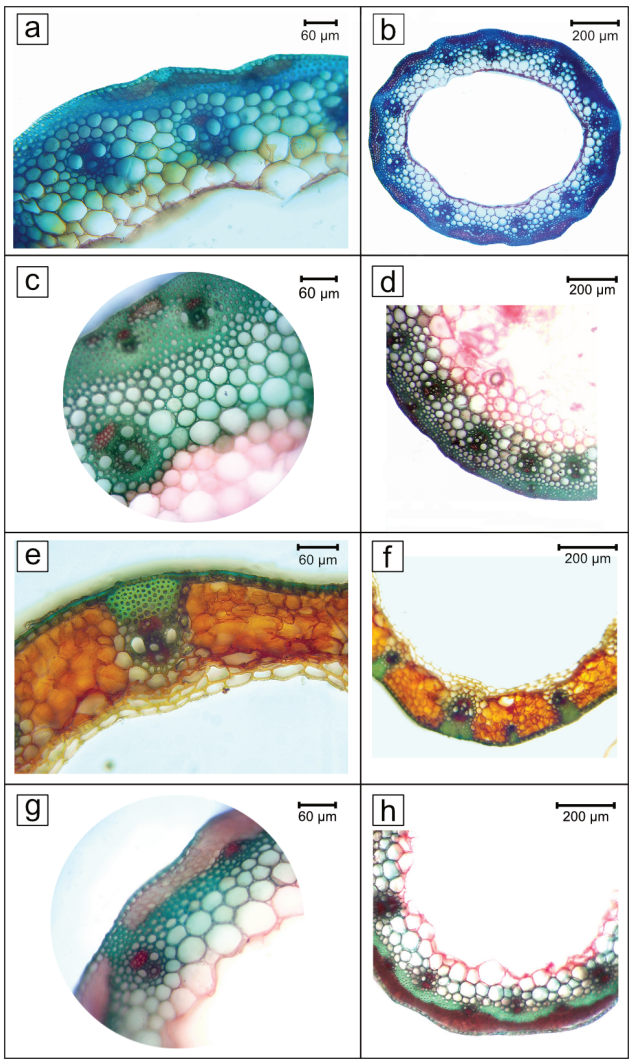

Figure 2. Culm cross sections in *Lolium* species. *L. multiflorum* (Rostam Abad population) (a and b). *L. persicum* (Rasht population) (c and d). *L. perenne* (Zoghalchal population) (e and f). *L. rigidum* (Ghamsar population) (g). *L. rigidum* (Kerman population) (h).

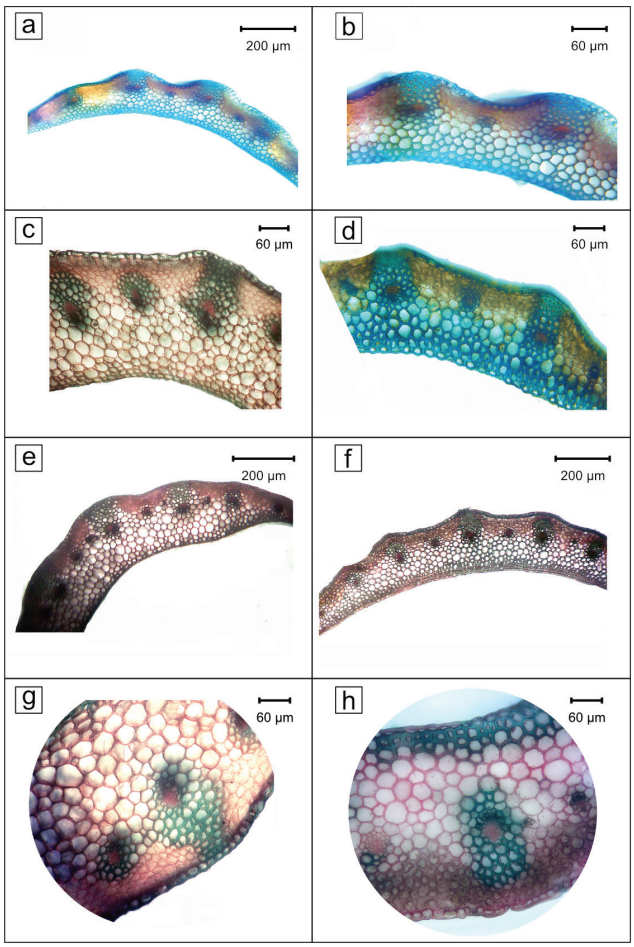

Figure 3. Glume cross sections in *Lolium* species. *L. multiflorum* (Astaneh-ye Ashrafiyeh population) (a and b). *L. persicum* (Ramsar population) (c). *L. perenne* (Tehran population) (d). *L. rigidum* (Badr Abad population) (e and f). *L. persicum* (Babolroud population) (g). *L. persicum* (Rasht population) (h).

variation in the species studied. In *L. multiflorum* (Fig. 3.a and b) glume is ribbed with girder attachment to vascular bundles. Vascular bundles are of two different sizes. The vascular bundles are surrounded by sclerenchyma fibers. The main parts of the glume cortex are sclerenchymatous while some parts are collenchymatous. In *L. persicum* (Fig. 3.c and d) ribs are evident with shallow depth. There are girders appendage in some parts of the glume structure and two sizes of vascular bundles.

In glume anatomy, the general shape of the cross-section shows more variation, but the inner structure shows more similarity. In *L. rigidum* (Fig. 3.e and f) ribs are present and vascular bundles are accompanied by girders. Cortex is composed of sclerenchyma and collenchyma tissues. In *L. persicum* (Fig. 3.g and h) same structures are present with sclerenchyma and collenchyma while in some samples girder is not attached to the vascular bundle yet and the cortex is parenchymatous.

Data analysis

By considering paired-group pattern of species studied based on culm and glume anatomical observations, we find that the *L. multiflorum* accessions makes a separate cluster (Fig. 4). *L. persicum* provide clusters which are somehow scattered. The Ramsar population of this species is grouped in *L. rigidum* clusters. The *L. perenne* composes a group while Sari population makes a separate cluster (Fig. 4).

In dendrogram *L. perenne* and *L. multiflorum* are

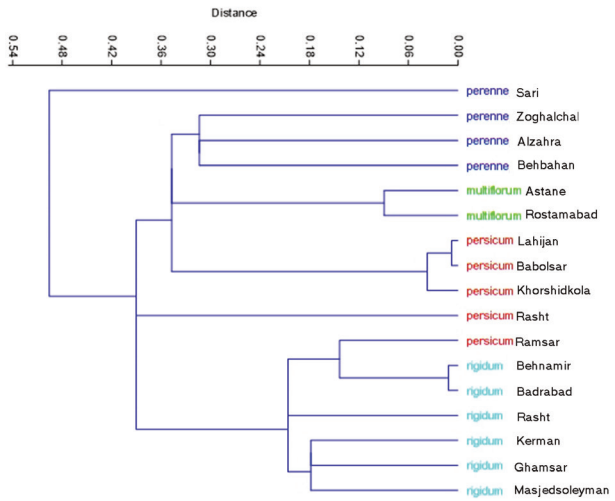

Figure 4. Paired group dendrogram of the species studied based on the culm and glume anatomical data for the *Lolium* species.

grouped near each other but there is more overlapping between *L. persicum* and *L. rigidum*. Even with linkage or WARD methods, dendrograms show same groupings. The scatter diagram of PCA (Fig. 5) agrees with these groupings. *L. perenne* are more scattered than other species studied. Also accessions of *L. persicum* are distributed in different parts (near *L. rigidum* or near *L. multiflorum*).

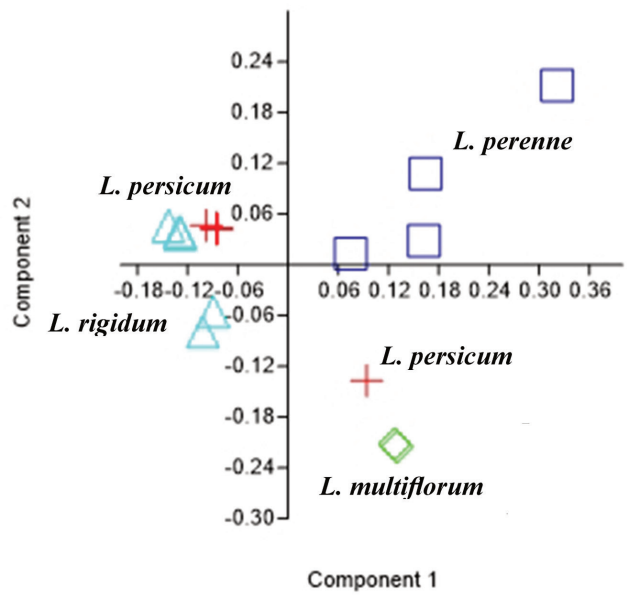

Figure 5. Principal component analysis scatter plot of the species studied based on the culm and glume anatomical data.

Discussion

The present study provides information about culm and glume anatomy of four *Lolium* species in Iran. No record is available for the culm variation in species studied. Although leaf anatomical variation in *Lolium* species in Iran show more variation (Keshavarzi et al. 2014) than culm anatomy, but it provides some diagnostic features as the general shape of culm cross-section, girder presence, number of vascular bundles' ring, etc. Almost glume features are considered in morphological or micro-morphological studies (Safaiipour and Keshavarzi 2000; El-Gazzar et al. 2013) but no data was available about the glume anatomical structure.

The present study on culm and glume anatomical variation supports more similarity of *L. multiflorum* and *L. perenne*. Anatomical study of leaf in same species agrees with present findings (Keshavarzi et al. 2014). This finding is in concordance with the molecular results of Tabaripour et al. (2021) that used ISSR markers in their study. In their study the *L. persicum* accessions were grouped separately. The micro-morphological data of lemma and palea features of *Lolium* species Tabaripour et al. (2022) showed that more similarity is evident between *L. multiflorum* and *L. perenne*. A close similarity was recorded for leaf anatomical structure of *L. persicum* and *L. rigidum* (Keshavarzi et al. 2014) and by lemma and palea micro-morphological study (Tabaripour et al. 2022) that agree with the findings of present study of culm and glume anatomy. In result we pointed to the scattering of *L. perenne* populations same pattern was present in molecular findings of Tabaripour et al. (2021).

The culm and glume anatomy of *Lolium* species was described and some characters were recognized as valuable for taxonomic purposes. These features might be useful in breeding programs of *Lolium*.

Acknowledgments

Vice Chancellor for research and technology of Alzahra University financially supported this study. We would also acknowledge anonymous reviewers for their suggestions and comments.

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