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Anatomical modifications in some *Bambusa* Schreb. species to combat ecological constraints

Mansoor Hameed[1*], Sana Fatima[2], Farooq Ahmad[1], Sadia Noureen Zafar[1], M. Sajid Aqeel Ahmad[1], Ummar Iqbal[1], Safura Bibi[1], Iftikhar Ahmad[3]

[1]Department of Botany, University of Agriculture, Faisalabad, Pakistan
[2]Department of Botany, The Islamia University of Bahawalpur, Bahawalpur, Pakistan
[3]Department of Botany, University of Sargodha, Sargodha, Pakistan

Abstract
Five selected species of genus *Bambusa* considered for comparative anatomical characterization, were collected from the different areas of Punjab, Pakistan. The magnitude of variation was immensely high in *Bambusa* spp. relating to their root, culm, and leaf anatomical characters. These species were specific as well as representative of specific environmental adaptations. In different environmental stresses, *Bambusa vulgaris* found to be the most tolerant. Distinctive anatomical adaptations in this species were dense sclerification in root and stem (on inner side of epidermis and in vascular region), stem and adaxial leaf surface with thick epidermis, dense hairy stem and abaxial leaf surface, and bulliform cells with a peculiar arrangement in adaxial leaf surface. Overall, a strong relation of understudy features with plant anatomy was recorded, and therefore, these traits can be employed as a tool for studying the taxonomy of genus *Bambusa*. Moreover, by using these structural modifications their tolerance level to environmental stresses can be easily assessed.

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Bambusa;
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Abbreviations:

Species: BBA- *B. bambos*, **BBL-** *B. blumeana*, **BGL-** *B. glaucescens*, **BVU-** *B. vulgaris*, **BVV-** *B. vulgaris* 'Vittata'; **Anatomical parameters: Root: RExCA-**Root exodermis cell area, **RCCA-**Root cortical cell area, **REnCA-**Root endodermis cell area, **RPCA-**Root pericycle cell area, **RVRT-**Root vascular region thickness, **RMA-**Root metaxyelm area, **RPCA-**Root pith cell area, **RPA-**Root pith area; **Stem: SEpT-**Stem epidermal thickness, **SEpCA-**Stem epidermal cell area, **SSCA-**Stem sclerenchyma cell area, **SPhA-**Stem phloem area, **SMtA-**Stem metaxylem area, **SVBA-**Stem vascular bundle area, **SCCA-**Stem cortical cell area; **Leaf:** MrT-Midrib thickness, **LMT-**Lamina thickness, **AbECA-**Abaxial epidermal cell area, **AdECA-**Adaxial epidermal cell area, **SCA-**Sclerenchyma cell area, **BA-**Bulliform area, **VBA-**Vascular bundles area, **MtA-**Metaxylem area, **PhA**-Phloem area, **TrL-**Trichome length**.**

Introduction

The tribe Bambusoideae (Family Poaceae) comprises three sub-tribes based on their specific vegetative characters and distributional range. The sub-tribe Bambuseae has tropical woody bamboos, while woody bamboos of temperate region belong to tribe Arundinarieae. Non-woody species with soft herbaceous culm fall in sub-tribe Olyreae (Aliscioni et al., 2012; Kelchner et al., 2013). Bambusoideae is a large tribe having about 80 genera and 1500 species (Wysocki et al., 2015).

Bamboos have prime economic importance as about 20 million tonnes annual utilization is for commercial and ornamental purposes. In industries, its major use is in construction work, paper, chipboard and cardboard manufacturing, and for furniture, and decoration pieces preparation (Choudhury, 2012). Main bamboos producing countries are China and India that contribute over 60% of the total world bamboo production (Nongdam, 2014).

***CONTACT** Mansoor Hameed, hameedmansoor@yahoo.com, Department of Botany, University of Agriculture, Faisalabad, Pakistan

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Naturally grown populations of bamboos have ability to survive in different environmental conditions, with a wide distributional range. This is the reason of their ecological success. Their domination is more towards cooler climates like high mountainous ranges and cold humid rainforests (Bystriakova et al., 2003; Liua et al., 2016). Bamboos have an ecological importance as they are used for checking soil erosion and as soil binders (Shiau et al., 2017).

Environmental conditions, particularly abiotic stresses are depicted and indicated by different anatomical features (Hameed et al., 2009). In the current investigations, some *Bambusa* species from Faisalabad and Lahore regions (Punjab province), Pakistan, were examined to observe their structural modifications in response to different habitats and explore the possibility of using some key anatomical characters to assess the adaptability potential and ecological success of different species of *Bambusa*.

Materials and methods

A survey was conducted of different areas of Faisalabad and Lahore to study the distribution and anatomical modifications in different *Bambusa* species. Five species of genus *Bambusa* namely *B. bambos, B. blumeana, B. glaucescens, B. vulgaris and B. vulgaris* 'Vittata' were collected from ecologically different areas of these regions (details presented in **Table 1; Figure 1**). Plant specimens were preserved and deposited in the Herbarium of Department of Botany, University of Agriculture, Faisalabad, for future correspondence.

The free hand sectioning technique was used for the preparation of permanent slides of leaf, stem and root transverse sections of five *Bambusa* species. For root anatomy, a 2-cm piece from the main junction of the thickest root, and for leaves, 1-cm piece from the leaf middle portion along the midrib were taken. The material was immediately preserved in FAA (formalin-acetic-alcohol) solution containing v/v formalin 5%, acetic acid 10%, ethanol 50% and distilled water 35% for a limited time. After that, the samples were transferred into vials containing acetic alcohol solution (v/v 25% acetic acid and 75% ethanol) for longer period. The sections were dehydrated with a series of ethanol (30%, 50%, 70%, 90% and 100%), stained with safranin (1%) and fast green (1%), and mounted on slides using Canada balsam as described by Ruzin (1999). Data were recorded by an ocular micrometer and photographs were taken with a digital camera-fitted compound microscope.

Statistical analysis

Data were recorded for dermal, parenchymatous, mechanical and vascular tissues of leaf, stem, and root, and were subjected to one-way analysis of variance (Steel et al., 1997). The anatomical data were also subjected to the multivariate (cluster) analysis using Minitab statistical software (Vern. 20) to assess the relationship between anatomical traits and species. The significance of anatomical characteristics in distributional pattern and taxonomy of the species of Family Poaceae were recorded from the Punjab province. The Principal Correspondence Analysis (PCA) technique was also applied on different anatomical parameters to determine the degree of association between species and parameters studied using the XLSTAT software (Vern. 14).

Results

Root anatomy

In *Bambusa vulgaris,* a prominent sclerenchyma layer was observed just below the root exodermis, while in all other species there was a pronounced disintegration in root exodermis along with cortical region (**Table 2, Figure 2**). A little variation was observed in the cortical cell area of all species, except in *B. blumeana* in which cortical cells were much smaller compared with those of the other species, while pith area was maximum in *B. vulgaris.*

Bambusa blumeana and *B. glaucescens* had endodermis of large size while small in other species; there was less variation in endodermis cell area among all the species. More thickening was observed on the inner side of the endodermis of *B. glaucescens*, however, the sidewalls of the endodermis in *B. vulgaris* were also thick (**Figure 2**). The pericycle cells were of very small size in *B. blumeana* and *B. vulgaris.* In *B. glaucescens*, vascular tissues were considerably large sized, while a reduced vascular region was observed in *B. vulgaris* and its cultivar. The metaxylem area was maximal in *B. glaucescens,* and metaxylem vessels had a circular pattern, however, that recorded in *B. glaucescens* was diffused and the vessels of different size were arranged in an alternate pattern.

Stem anatomy

In *B. vulgaris,* a very thick layered epidermis with large sized cells was recorded (**Figure 3**), and epidermal cells had more length rather than width. The same trend was followed by *Bambusa blumeana,*

Table 1. Details of *Bambusa* species collected from Faisalabad and Lahore regions

Species	Collection site	Ecology/Nativity
Bambusa bambos (L.) Voss (Giant thorny bamboo)	Botanic Garden, U.A., Faisalabad Coordinates: 31°.35'; 43.96"	Native to India and widely cultivated in the eastern side of river Ravi (Cope, 1982). It has high economic value (Kumar et al., 2005) and relatively high biomass production potential (Scurlock et al., 2000).
Bambusa blumeana Schult. (Thorny bamboo)	Changa Manga Forest plantation Coordinates: 73°.93'; 43.25"	Economically an important bamboo (Bodner and Gereau, 1988), found wild and cultivated in many countries of Asia (Zu, 2006). It is widely distributed and forms dense clumps along river banks and hillsides up to 1000 m altitude (Smith, 1979).
Bambusa glaucescens (Willd.) Sieb. ex Munro (Hedge bamboo)	Botanic Garden, U.A., Faisalabad Coordinates: 31°.35'; 43.96"	A hardy plant, native to China and Japan. It is cultivated as a hedge plant and in gardens throughout the Punjab (Cope, 1982).
Bambusa vulgaris Schult. ex Wend. (Common bamboo)	Changa Manga Forest plantation Coordinates: 73°.93'; 43.25"	Native to tropical Asia, but introduced in many tropical and sub-tropical countries including many islands (Quatrocchi, 2006). Widely distributed at low altitudes (1000 m) in humid moist habitats like river banks, roadsides, wetlands and open areas. It can tolerate unfavourable environmental conditions like dry seasons and low temperatures, and a wide range of soil types (Ohrnberger, 1999). It forms exclusive monospecific stands along streams into forests (Blundell et al., 2003).
Bambusa vulgaris Schult. ex Wend. 'Vittata' (Painted bamboo)	Botanic Garden, U.A., Faisalabad Coordinates: 31°.35'; 43.96"	A large tender clumping bamboo, widely cultivated in tropical and subtropical countries of the world (Ohrnberger, 1999).

U.A. Faisalabad: University of Agriculture, Faisalabad

Table 2. Anatomical characteristics of some *Bambusa* species from Faisalabad and Lahore regions

Characteristics	***Bambusa bambos***	***Bambusa blumeana***	***Bambusa glaucescens***	***Bambusa vulgaris***	***B. vulgaris* 'Vittata'**
			Root		
Exodermis cell area (μm²)	Absent	Absent	Absent	209.78a	Absent
Cortical cell area (μm²)	666.39a	409.78c	646.82b	664.31a	636.32b
Endodermis cell area (μm²)	472.01b	509.78a	514.67a	452.44c	479.70b
Pericycle cell area (μm²)	314.67b	209.78d	479.70a	252.44c	269.92c
Vascular region thickness (μm)	277.78c	345.86b	473.03a	177.01d	264.16c
Metaxylem area (μm²)	15156.77d	11450.61e	2412.49c	4580.24a	4405.42b
Pith cell area (μm²)	839.12c	1153.80b	1311.13a	699.27d	457.33e
Pith area (μm²)	101534.67e	122530.31c	11341.80d	38232.82a	20296.43b
			Stem		
Epidermal thickness (μm)	7.29d	14.34b	10.72c	17.67a	17.67a
Epidermal cell area (μm²)	64.47b	52.44c	52.44c	104.89a	64.47b
Sclerenchyma cell area (μm²)	24.51c	32.68b	24.51c	46.34a	32.68b
Phloem area (μm²)	1839.12e	3468.47c	4311.13a	3776.08b	2472.01d
Metaxylem area (μm²)	2202.71e	4720.10b	5048.91a	3356.51c	3048.91d
Vascular bundle area (μm²)	11013.57e	25331.21b	26922.81a	23128.49c	20279.33d
Cortical cell area (μm²)	1573.36e	2573.36a	1649.27d	2202.71b	2048.91c
			Leaf		
Midrib thickness (μm)	441.18a	220.59c	253.27b	204.25e	212.42d
Lamina thickness (μm)	318.63a	147.06b	106.21d	122.55c	81.72e
Abaxial epidermal cell area (μm²)	75.86a	52.44c	75.86a	64.74b	52.44c
Adaxial epidermal cell area (μm²)	52.44d	74.79b	75.86b	104.36a	64.45c
Sclerenchyma cell area (μm²)	57.19a	32.68b	24.51c	32.68b	32.68b
Bulliform area (μm²)	40961.12a	2097.82c	1936.95d	2569.83b	2888.04b
Vascular bundle area (μm²)	73109.12d	8810.85b	8810.85b	9545.09a	7552.16c
Metaxylem area (μm²)	3356.51a	839.12b	839.12b	209.78c	209.78c
Phloem area (μm²)	2472.01a	629.34d	1888.04b	839.12c	472.01e
Trichome length (μm)	Absent	Absent	Absent	114.38a	57.19b

Means sharing similar letters are statistically non-significant in each row but the size of epidermal cells was relatively smaller. Dense sclerenchymatous cells with a maximum area were prominent in *B. vulgaris,* and also conspicuous in *B. blumeana,* while *B. bambos* and *B. vulgaris* 'Vittata' had reduced sclerification. In *B. glaucescens* vascular bundles were almost round shaped, particularly those present deeply and with maximum vascular bundles and metaxylem area. However, in *B. blumeana,* vascular bundles were somewhat elliptic in shape. In *B. bambos,* vascular bundle area was found to be the smallest. *B. glaucescens* showed maximum phloem area, while presence of stem hairs was recorded only in *B. vulgaris,* but not observed in other species.

Leaf anatomy

Leaf thickness was maximum in *Bambusa bambos,* while abaxial leaf epidermis cell area showed the maximum value in *B. bambos* and *B. glaucescens,* but maximum adaxial leaf epidermal cell area was recorded in *B. vulgaris*. In *B. bambos,* sclerenchyma cells area showed the maximum value, while minimum in *B. glaucescens*. Bulliform area was found to be maximum in *B. bambos* and the minimum in *B. glaucescens*. A clear-cut difference was observed in shape and arrangement of bulliform cells, particularly in *B. bambos*. In this species, the cells were found in fan shaped groups. Vascular tissues were highly developed in *B. bambos,* with a maximum area of vascular bundles, metaxylem vessels and phloem in leaf. Long trichomes were present in *B. vulgaris* and small in *B. vulgaris* 'Vittata, while absent in other species.

Multivariate analysis

The dendrogram of root anatomical characteristics showed very specific clustering of the species studied (**Figure 4**). BGL was different from rest of the species with regard to root characteristics that showed an peculiar behaviour. Other species were clustered into two groups, each containing two species, in which first comprising BVU and BVV, while the second showed clustering of BBA and BBL. In case of stem anatomical characteristics, there was a totally different behaviour of the species, as three species namely BBA, BGL and BVU showed independent clustering than the other species. On the other hand, the species BBL formed a cluster with BVV. The dendrogram for leaf anatomical characteristics showed isolated behaviour for BBA species, whereas other four species were arranged in three clusters, in which BGL and BVU were independently clustered, and BBL showed clustering with BVV.

The PCA biplot of root and stem anatomical characteristics showed a strong association with species (**Figure 5A &B**). SET, SCCA, RexCA and SECA showed a close association with BVU, while RPCA, REnCA and RVRT exhibited a very close relation with BBL and BGL. On the other hand, RMA showed closeness to BBA and RCCA with BVV. Among the relationships of leaf anatomical characters with species, AdECA and TrL showed a strong relationship with BVU and BGL, whereas VBA, LMT, BA, MrT, SCA and MtA were closely associated with BBA.

Discussion

In the present investigation, *Bambusa* species and its cultivars growing in different habitats of the Punjab plains were studied. *Bambusa bambos* generally requires a large quantity of water to grow and develop normally (Cope, 1982; Piouceaua et al., 2014). On the other hand, *Bambusa vulgaris* can tolerate harsh conditions, i.e., extreme temperatures and drought (Ohrnberger, 1999; Roy et al., 2014). *Bambusa vulgaris* 'Vittata' and *B. glaucescens* cultivated in the Punjab plains on a large scale (Cope, 1982), showed relatively more sensitivity to drought stress. *Bambusa blumeana* with a broad distributional range has high potential to adapt to a diversity of habitats (Smith, 1979; Helander et al., 2013).

All *Bambusa* species showed variation regarding root, culm and leaf anatomy, indicating their adaptations to heterogeneous environments. Adaptive components in the form of anatomical features have been reported by Helander et al. (2013) in family Asteraceae, Olsen et al. (2013) in *Andropogon gerardii,* and, Tomás et al. (2013) in 15 herbaceous dicots. Very specific tissue architecture was shown by all species and cultivars, specifically sclerification in roots, stem and leaves, presence of trichomes on leaf and stem, and pattern and orientation of vascular tissues.

In monocotyledonous plants succulence is not very common (Flowers and Colmer, 2008), but *B. bambos* was found to have high levels of succulence in leaf lamina. Hameed et al. (2009) related drought and salinity tolerance with foliar succulence, but in the present case, this seems to be not relevant, as *B. bambos* requires relatively more water than the other species. Deposition of thick cuticle layer and large sized epidermis are specific characteristics of plants growing in dry habitats they help reduce transpiration (Ristic and Jenks, 2002). Large cells on the adaxial epidermis of leaves and dense hairiness on abaxial leaf epidermis in *Bambusa vulgaris* show better adaptability potential of this species to survive and tolerate water scarce environments like highly saline and drought affected areas. Presence of hairs on leaves is indispensable for not only to minimize or prevent water loss, but also to control leaf temperature by protecting leaves from direct solar radiations. Nature of bulliform cells (size and type) is an important adaptation, and is considered to be a significant transformation against drought and salinity stresses (Bahaji et al., 2002). Epidermal cell size and nature of bulliform cells have been related to abiotic stress tolerance, which can be used as important indicators having considerable taxonomic value (Balsamo et al., 2003). Rolling of leaf is effectual to avoid transpiration from foliar surface in many members of Poaceae (Abernethy et al., 1998). *Bamboo bambos* is characterized by having large sized bulliform cells grouped in continuous rows under the upper leaf epidermis acting as a storage. In contrast, *B. blumeana* and *B. vulgaris* have bulliform cells of irregular size and shape, which could be effective for rolling of leaves.

The species that are adapted to water deficit conditions (xerophytes and halophytes) have thick epidermal layer that is most effective against water loss when water is a scarce commodity (YuJing et al., 2000). The thick epidermal layer on the upper surface of leaves in *B. vulgaris* is an effective tool to tolerate environmental stresses.

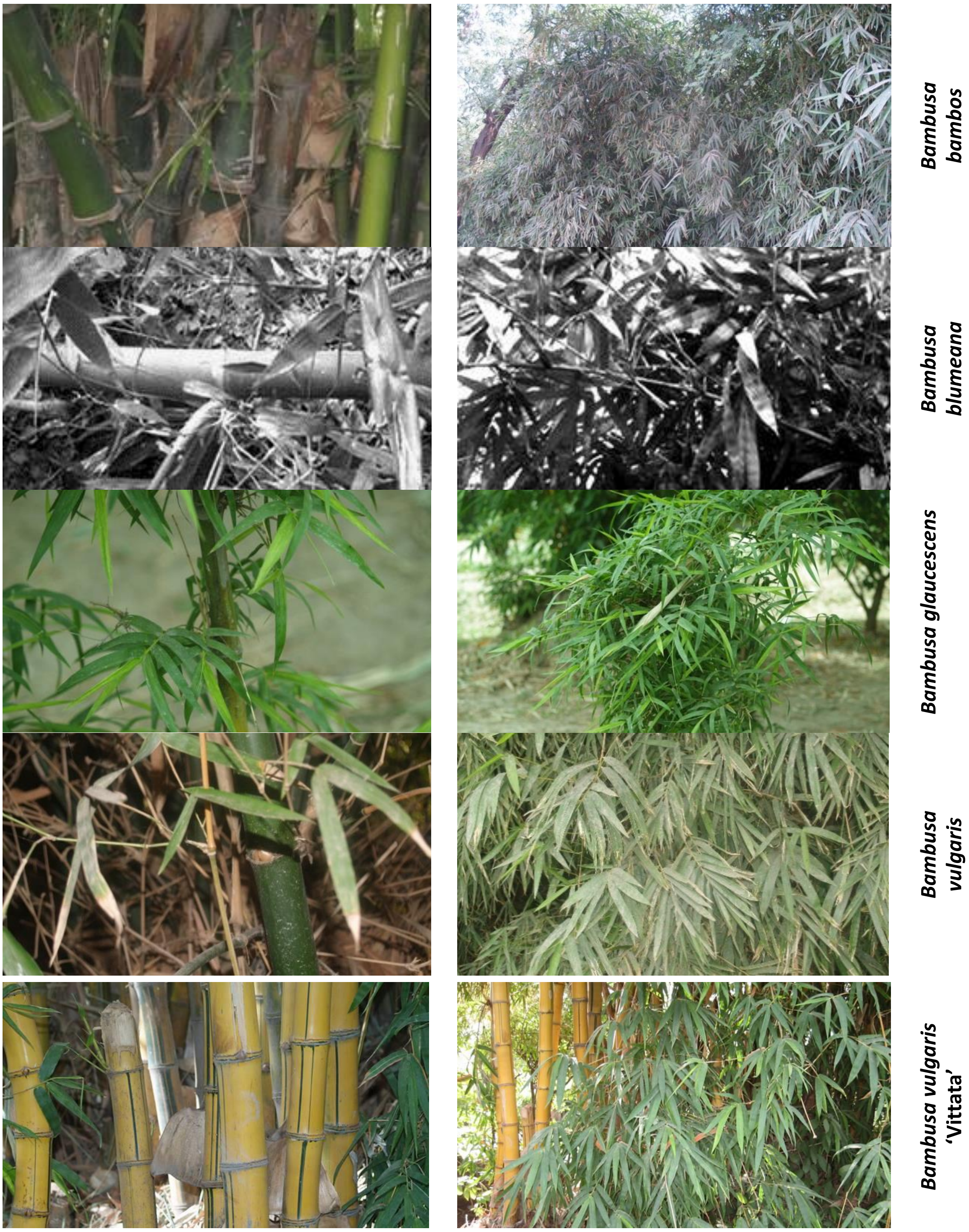

Figure 1. Some species of genus *Bambusa* from Faisalabad and Lahore regions

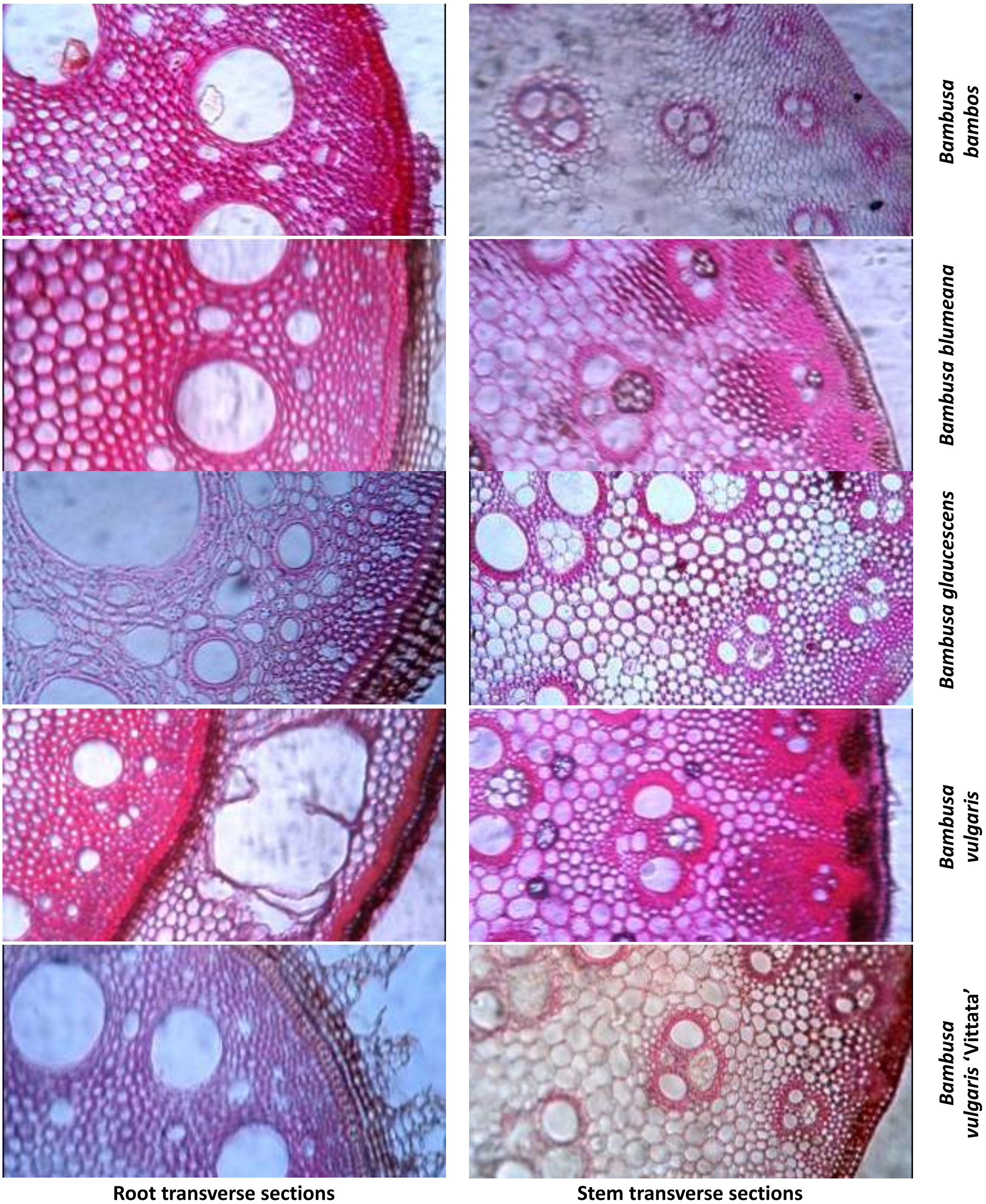

Figure 2. Root and stem transverse sections of some *Bambusa* species from Faisalabad and Lahore regions

Bambusa bambos is adapted to areas with more available water, but it has less tolerance to environmental stresses. In roots, there are specific structural modifications, i.e., sclerification is on the inner side of the endodermis and pericycle has sinuous arrangement; stem is not sclerified, and vascular bundles are oval shaped. These alterations are helpful in providing rigidity to roots, especially when there is a limited availability of moisture content in soil (Freschet et al., 2021). It has thick leaves (lamina) with vascular bundles of large size, and bulliform cells arranged in a specific pattern, i.e., arranged in continuous rows and covering most of the surface along the adaxial epidermis. This is again an adaptive characteristic of the species to reduce the undue water loss under dry weather conditions. It is believed that large bulliform cells develop only in grasses to unroll their leaves under water stress conditions (Matschi et al., 2020).

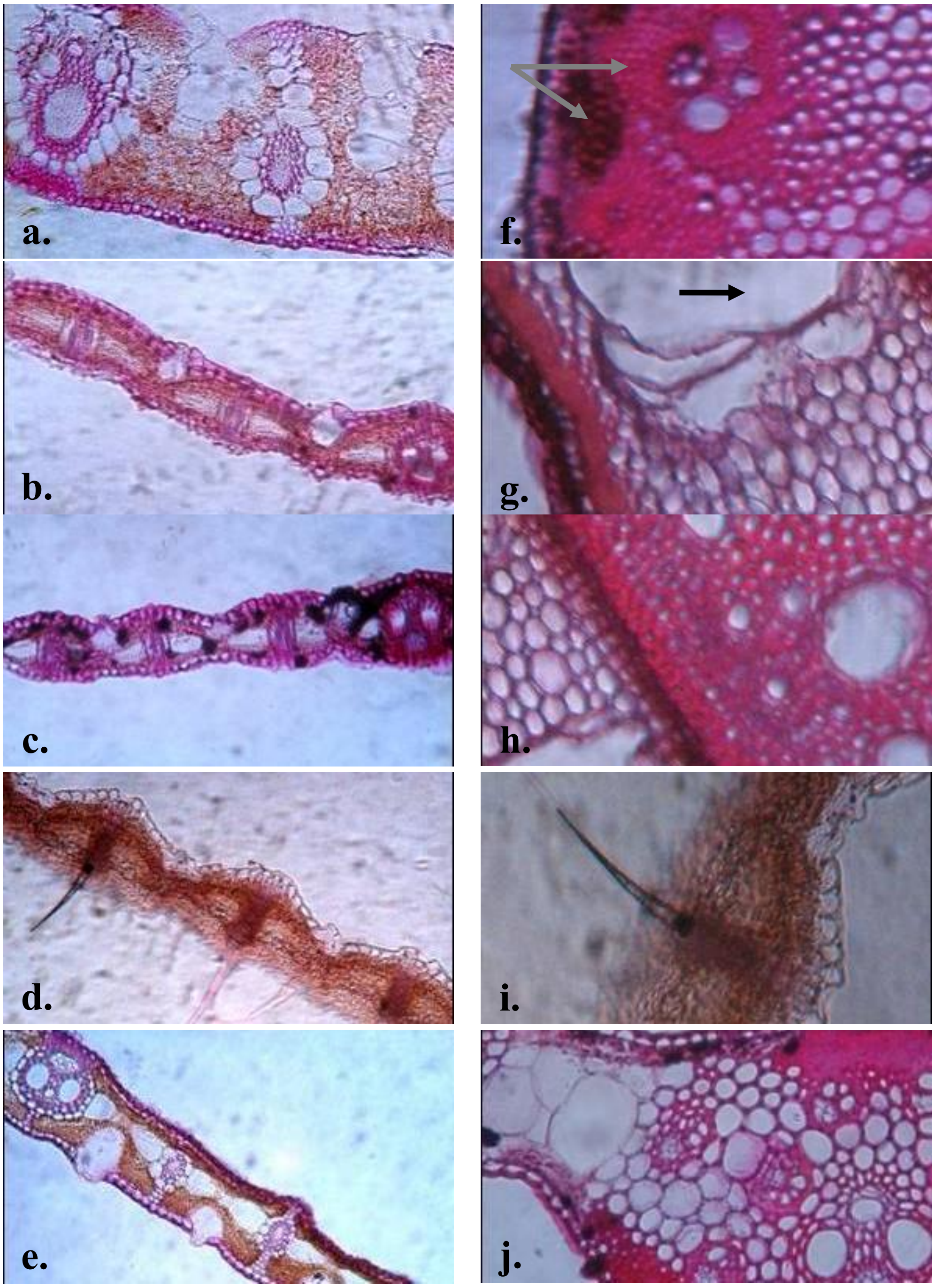

Figure 3. Transverse sections of some *Bambusa* species from Faisalabad and Lahore regions: a. Leaf of *B. bambos;* b. Leaf of *B. blumeana;* c. Leaf of *B. glaucescens;* d. Leaf of *B. vulgaris*; e. Leaf of *B. vulgaris* 'Vittata', f. Stem of *B. bambos* (intensive sclerification in hypodermal and vascular region; g. Root of *B. vulgaris* (large aerenchyma in cortical parenchyma); h. Root of *B. vulgaris* (intensively sclerified endodermis and pericycle), i. Leaf of *B. vulgaris* (dense long trichomes on abaxial surface), and j. Leaf of *B. glaucescens* (intensive sclerification in both leaf side in midrib region)

Bambusa blumeana with a wide distribution in the plains of Punjab has a potential to cope with different types of abiotic stresses. This species also exhibits specific adaptive features, i.e., in root, inner side of the epidermis is densely sclerified. In stem, surroundings of the peripheral vascular bundles are also heavily sclerified and metaxylem vessels are enlarged. Leaf lamina is thin-layered with bulliform cells of large size. All these modifications are vital under extreme environmental conditions. These are not only critical for efficient water and mineral translocation, but also helpful in mechanical strength of soft tissues by preventing them from collapse under desiccation (Fatima et al., 2018; Zhang et al., 2021).

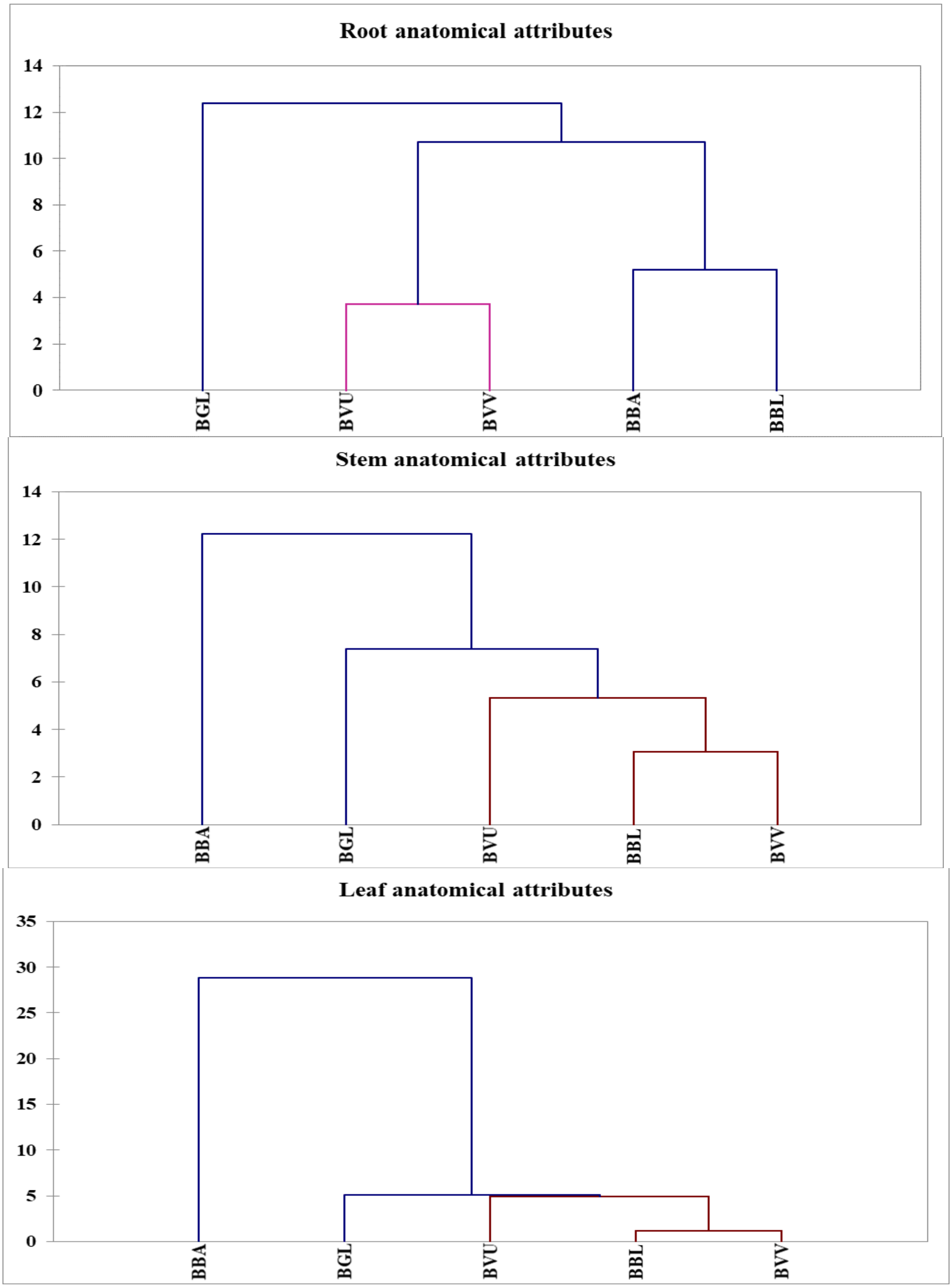

Figure 4. A dendrogram of anatomical attributes (root, stem and leaves) of some *Bambusa* species from Faisalabad and Lahore regions

Bambusa glaucescens is cultivated on a large scale and is grown for making hedges or in gardens as an ornamental plant throughout the Punjab province. Metaxylem vessels of large size in the root and stem are slightly sclerified with metaxylem of large size and lacunar cavities. Lamina is thin-layered with epidermal cells of large size, particularly at the abaxial foliar surface. Large parenchyma cells with enlarged vacuole are a critical strategy for storage of additional water, especially in arid and hot climatic conditions (De Micco and Aronne, 2012). Moreover, development of large and highly sclerified metaxylem vessels and lacunar cavities allow maximum water uptake and gaseous exchange, that may ensure their success in variable environmental conditions (Fernandes et al., 2018).

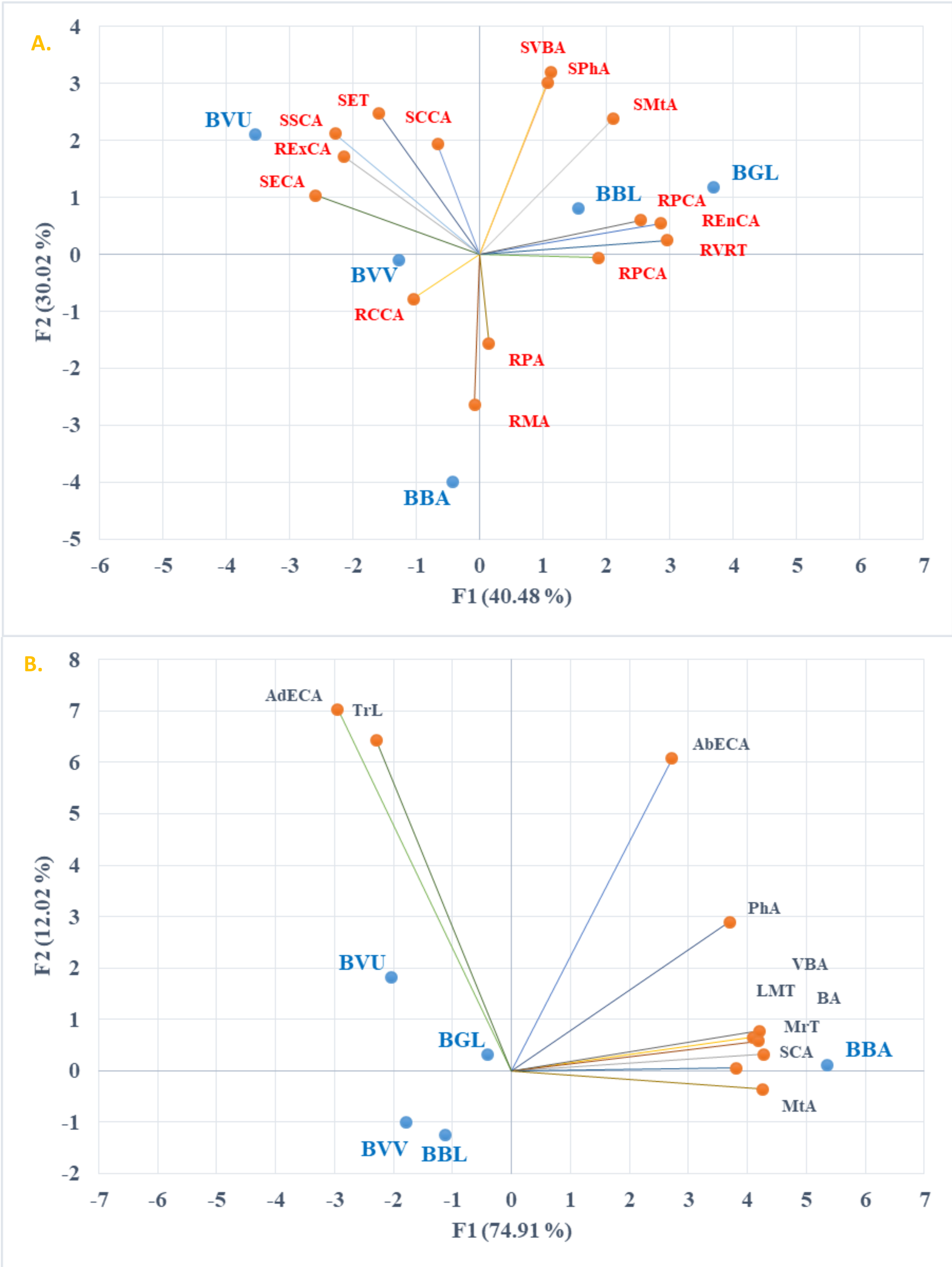

Figure 5. PCA (Principal Correspondence Analysis) showing biplot of - A.) species with root and stem, and B.) species with leaf anatomical attributes of some *Bambusa* species from Faisalabad and Lahore regions

In the present study, *Bambusa vulgaris* was found to have maximum potential to tolerate extreme drought and saline conditions. Specific adaptations were observed in its root, stem and foliar anatomy. Vascular tissues were densely sclerified, but the inner side of root epidermis was also heavily sclerified and metaxylem vessels of small size. Dense sclerification around the vascular bundles was observed and epidermis cells of large size were covered by thick deposition of cuticle and with a dense hairy stem. Adaxial leaf surface has large epidermal cells and densely covered large macrohairs were present on the abaxial leaf surface. Leaves are quite responsive to multiple abiotic stresses, which depict the harshness of prevailing environmental conditions in the form of altered structural and growth attributes (Soliveres et al., 2014). Leaf thickness, intensive sclerification in leaves and other organs of plants, multi-layered epidermis, size and shape of bulliform cells, stomatal orientation on both leaf surfaces, size and intensity of sclerification in vascular region, mainly around the metaxylem vessels (Mansoor et al., 2019), nature and density of pubescence, hairs/trichomes, and excretory structures (Keshavarzi, 2021) are good indicators of environmental stresses like salinity and drought.

In the root and stem of *Bambusa vulgaris* ‘Vittata’ (a cultivated species) a slight sclerification was present. Leaf lamina was thin layered and abaxial leaf surface was with densely covered short hairs and with well-developed bulliform cells. Leaf blade with large and deeply grooved bulliform cells, and roughness of leaf surface by hairiness might have enabled it to fold immediately upon a little shift in turgidity of bulliform cells. Leaf folding under a tight cylinder is a very effective phenomenon in grasses which protect the leaves from direct exposure to sun rays, aridity, wind speed and greenhouse gases, causing reduced transpirational rate (Ahmad et al., 2020), and improving the water use efficiency (WUE) of plants (Kadioglu et al., 2012).

Conclusion

Diverse ecological adaptations were found to be indicated by many different anatomical features. Xerophytic adaptations, i.e., densely pubescent leaves, with large size bulliform cells, densely sclerified hypodermis and vascular region in stem, and sclerification in the endodermal region of root, can be used as taxonomic markers to identify the degree of drought and salinity tolerance in different bamboo species.

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