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Comparative Anatomical Studies of Four Accessions of *Bambusa vulgaris* Schrad. ex J.C. Wendl. Collected in Ile-Ife, Osun State, Nigeria

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A comparative anatomical study was carried out on four accessions of *Bambusa vulgaris* to identify characters of taxonomic purposes to better understand the relationship between the four accessions and also to fill the knowledge gap in the understanding of their taxonomy. Transverse sections, tangential longitudinal sections and radial longitudinal sections of mature leaf, stem and roots were cut at 8-15 μm on the Reichert sliding microtome. Some sections were done by hand for preliminary studies. Each section was stored in 50% alcohol for anatomical studies. The sections were stained in Safranin-O for three minutes, rinsed in water to remove excess stain and counterstained in Alcian blue for three minutes. Later, the sections were rinsed in water to remove excess stain and then treated with serial grades of alcohol and mounted in dilute glycerol for study. Photomicrographs were taken with the aid of 3013 ACCU-SCOPE Trinocular Microscope with Digital Camera. The qualitative investigations of the four accessions revealed that they were similar. However, accession 1 forms a sister clade with accession 3 and accession 4. This research concluded that quantitative stem and root anatomical characters are important in delineating species accessions and should be employed in separating closely related species as well as different accessions of the same species.



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Introduction

Bambusa vulgaris Schrad. ex J.C. Wendl., commonly known as bamboo in English and ‘Oparun’ in Yoruba in the Southwestern part of Nigeria, belongs to the *Poaceae* family, genus of the tribe Bambuseae and comprises typical bamboos that are woody grasses native to the warmer parts of Asia, Africa and South America [1]. They can be distinguished from other grasses by their woody culms or stems branching complex aerial and underground branching systems, stalked leaf blades, specialized sheaths on the young stem and specialized leaf anatomy. *Bambusa* is closely tufted with hollow thick walls and is a glabrous large genus of clumping bamboo. Most species of *Bambusa* are rather large, with numerous branches emerging from the nodes, and one or two much larger than the rest. The branches can be as long as 11 m (35ft) [1]. It grows widely in tropical and subtropical regions throughout the world. It has lemon-yellow culms (stems) with green stripes and dark green leaves. The culms are straight or flexuous (bent alternatively in different directions) drooping at the tip.

Among the species, *B. vulgaris* is one of the largest and most easily recognized and has 75 genera and 1250 species that occur in the world. The phytochemical screening of *B. vulgaris* showed the presence of alkaloids, terpenoids, flavonoids, saponin, tannins, phytates and oxalates while the mineral content revealed the presence of calcium and iron. The main constituents of the culms are holocellulose, pentosans, hemicellulose and lignin, and minor constituents like resins, tannins, waxes and inorganic salts. *B. vulgaris* also contains glucose, fructose and sucrose. *B. vulgaris* had been reported to contain low fat but a high proportion of carbohydrates, dietary fibers, potassium and vitamins. *B. vulgaris* shoots could be consumed raw, fermented, boiled, canned and frozen. It has been reported also to have anti-diabetic, anti-inflammatory, antimicrobial and anti-fatigue properties. In Nigeria, the decoction of this plant is used for treating diseases like gonorrhoea, diarrhea, inflammation, wounds, fever, worm and ulcers. The processes of wound healing and the orderly scheme are disturbed as a result of bacteria and bacterial products like endotoxins and metalloproteinase [1].

Several researchers have published accounts of the taxonomy of grasses in different parts of the world, in particular Nigeria [2-5]. Classes of grasses are distinct from other classes of plants in their morphological characteristics that a different

descriptive terminology has been created to identify group members. The grass family, *Poaceae*, is noted for its vast diversity which has posed many problems to taxonomists using the traditional gross morphology-based approach [6]. Despite all these ethnopharmacological properties, little is known about the anatomy of *B. vulgaris*; hence, this study aims at investigating the anatomy of the leaves, stem and root of four accessions of *B. vulgaris*.

Materials and Methods

Plant collections

Four accessions of healthy *B. vulgaris* were collected from different areas (Botanical Garden: BV1, Chemical Engineering Department: BV2, Conference Center: BV3 and Road 7 in Obafemi Awolowo University, Ile-Ife: BV4, in Osun state, Nigeria (Table 1). Samples were collected from each of the accessions for leaf, stem and root anatomical investigations, they were authenticated in the Obafemi Awolowo University, Herbarium (IFE), Ile-Ife, Osun State, Nigeria. The samples were preserved in 50% Alcohol before sectioning.

Preparation of slides

Transverse sections (TS), tangential longitudinal section (TLS) and radial longitudinal section (RLS) of mature leaves, stems and roots of the accessions were cut at 8-15 μm with Reichert Sliding Microtome (Reichert Austria Nr. 367 019). Each section was preserved in 50% alcohol before anatomical studies. Sections were later stained in Safranin-O for three minutes, rinsed in changes of water to remove excess stain and then counterstained in Alcian blue for three minutes. These were also rinsed in changes of water to remove excess stains and then passed through serial grades of alcohol and mounted in dilute glycerol for anatomical studies. Quantitative stem and root anatomical parameters such as vessel lengths and widths were taken as 25 measurements per parameter ($n=25$), as well as qualitative leaf, stem and root anatomical parameters were taken with the aid of a linear ocular micrometer inserted in the eyepiece of Leica binocular microscope. Photomicrographs were taken with the aid of 3013 ACCU-SCOPE Trinocular Microscope with a digital camera.

Data analysis

Quantitative data generated on the dimension of cells and tissue structures of the four accessions were subjected to one-way analysis of variance (ANOVA)

Table I Sites for the collection of *Bambusa vulgaris* (BV) species, including location coordinates and habitat descriptions.

Accession	Location	Coordinates	Habitat description
BV1	Botanical Garden, OAU Ile-Ife	N07°31.248, E004°31.593'	Expanse of a large area of land associated with other grass species
BV2	Behind Chemical Engineering Department	N07°31.151, E004°31.693'	Expanse with close communities of some grasses
BV3	Behind Conference Centre	N07°31.420, E004°31.837'	Water-logged area with the proximity of some grasses
BV4	Farm along Road 7	N07°30.789, E004°32.923'	Ruderal location, associated with some species of Asteraceae

and significant differences variables were evaluated using Duncan multiple range test.

Results

Morphologically, the culms were glossy, bright green, erect below and arching above and had an average height between 12-22 m. Internodes were 24–36 cm long and had an average diameter of 4–9 cm. Wall thickness ranged between 8-16 mm. Nodes were prominent, of which the lower ones were often with a narrow ring of roots and covered with brown hairs. Branches formed several clusters with 1-4 larger dominant branches and usually occurred from mid-culm to top. Narrow leaves were on average 16–26 cm long and 2–5 cm wide.

Anatomical study of BV1

Transverse section of leaf

The epidermis was uniseriate. Both epidermal surfaces were covered with a thick layer of the cuticle which was also covered with cuticular papillae. The cells of the epidermis were rectangular, oval, or squared with straight to undulating periclinal walls with abundant fibers (Fig. 1A and B).

Transverse section of stem

The cuticle was uniseriate. The epidermal layer comprised of two to four layers of collenchyma cells that were of varying sizes and shapes. The pith was occupied with many vascular bundles. The vessel elements were surrounded by bundles of parenchyma cells, that was, paratracheal. The phloem cells were situated in between the xylem cells at an angle to the metaxylem vessels. Bundles of fiber elements were seen attached to the vascular bundle. Few starch grains or granules were found littered within the parenchyma cells of the pith (Fig. 1C and D).

Tangential longitudinal section of the stem

The tangential longitudinal section of the stem was rayless (Fig. 1E).

Radial longitudinal section of the stem

Rays were clustered, irregular in arrangement and heterocellular (majority upright bar-shaped cells, occasionally squared cells and rarely procumbent) (Fig. 1F).

Transverse section of the root

The epidermis and cortex were not distinguishable and were laden with biseriate cells of sclerenchyma tissues called brachysclereid. The xylem cells were arranged in a ring pattern across the entire transverse section of the root. Paratrachea type of parenchyma cells was encountered. Sheaths of fiber elements surround the parenchyma cells. Starch grains were found within the parenchyma cells that occupied the pith (Fig. 1G and H).

Anatomical study of BV2

Transverse section of leaf

The uniseriate epidermis on both surfaces was covered by thick layers of cuticle. The epidermis was uniseriate with majorly rectangular cells, occasionally squared or oval with straight to undulating periclinal walls. There was the presence of starch grains and abundant fiber in the vascular and cortical regions (Fig. 2A and B).

Transverse section of stem

The cuticle was uniseriate. The epidermal layer comprised of two to four layer of collenchyma cells that are of varying sizes and shapes. The pith was occupied with many vascular bundles. The vessel elements were surrounded by bundles of parenchyma cells, that were paratracheal in nature. The phloem cells were situated in between the xylem cells at an

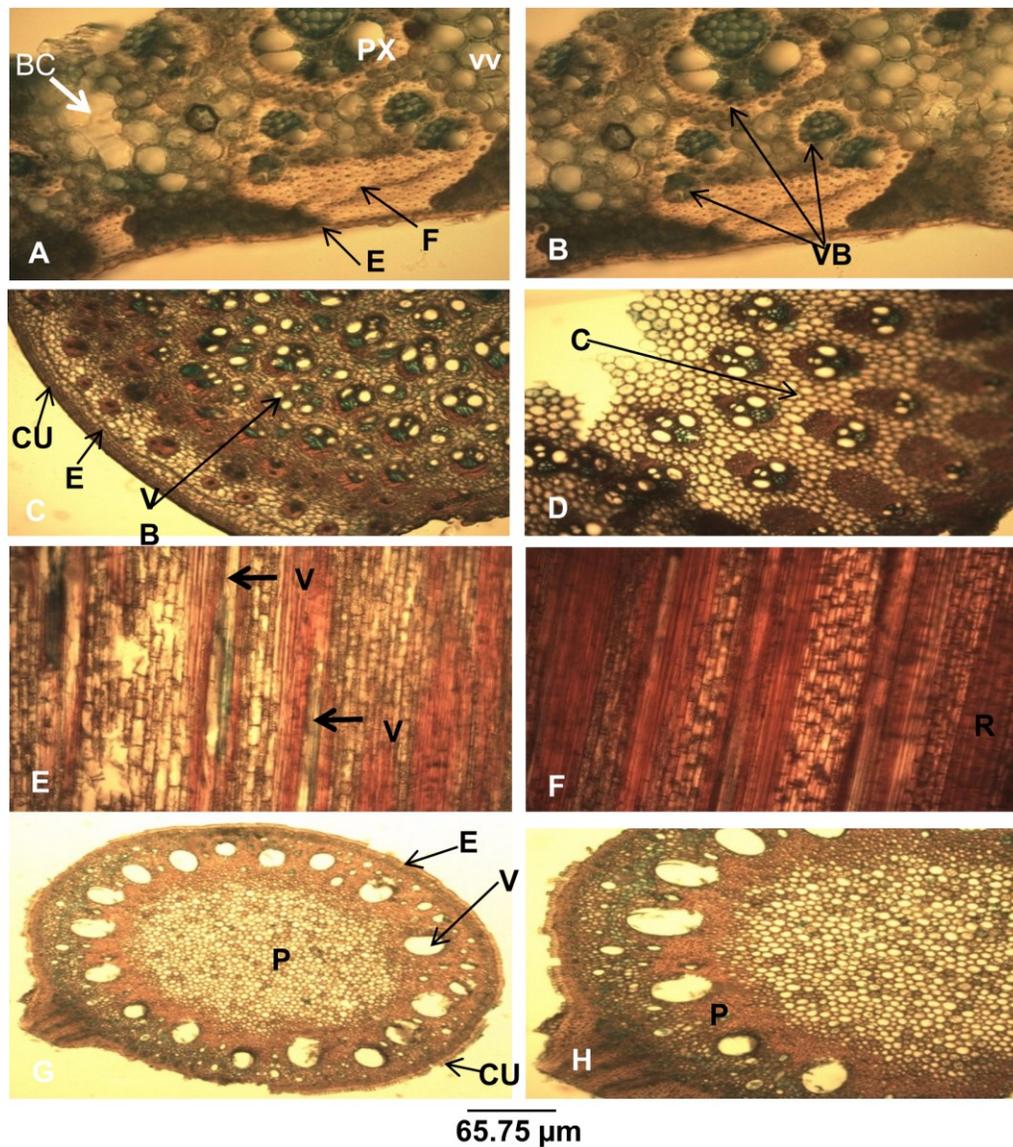


Fig. 1 Photomicrographs of *Bambusa vulgaris* accession BV1. A and B = transverse section of the leaf; C and D = transverse section of the stem; E = tangential longitudinal section of the stem; F = radial longitudinal section of the stem; G and H = transverse section of the root. BC = bulliform cell, VV = vascular vessel, E = epidermis, P = phloem, PX = protoxylem, F = fiber, CU = cuticle, VB = vascular Bundle, C = cortex, R = ray, V = vessel, ST = sieve tube.

angle to the metaxylem. Bundles of fiber elements were seen attached to the vascular bundle. Few starch grains or granules were found littered within the parenchyma cells of the pith (Fig. 2C and D).

Tangential longitudinal section of the stem

The tangential longitudinal section of the stem was rayless (Fig. 2E).

Radial longitudinal section of the stem

Rays were clustered, irregular in arrangement and

consisted of heterocellular cells (majorly upright bar-shaped cells, occasionally squared cells) (Fig. 2F).

Transverse section of root

The epidermis and cortex were not distinguishable and were laden with biseriate cells of sclerenchyma tissues called brachysclereid. The xylem cells were arranged in a ring pattern across the entire transverse section of the root. Paratracheal type of parenchyma cells were encountered. Sheaths of fiber elements surround the parenchyma cells. Starch grains were

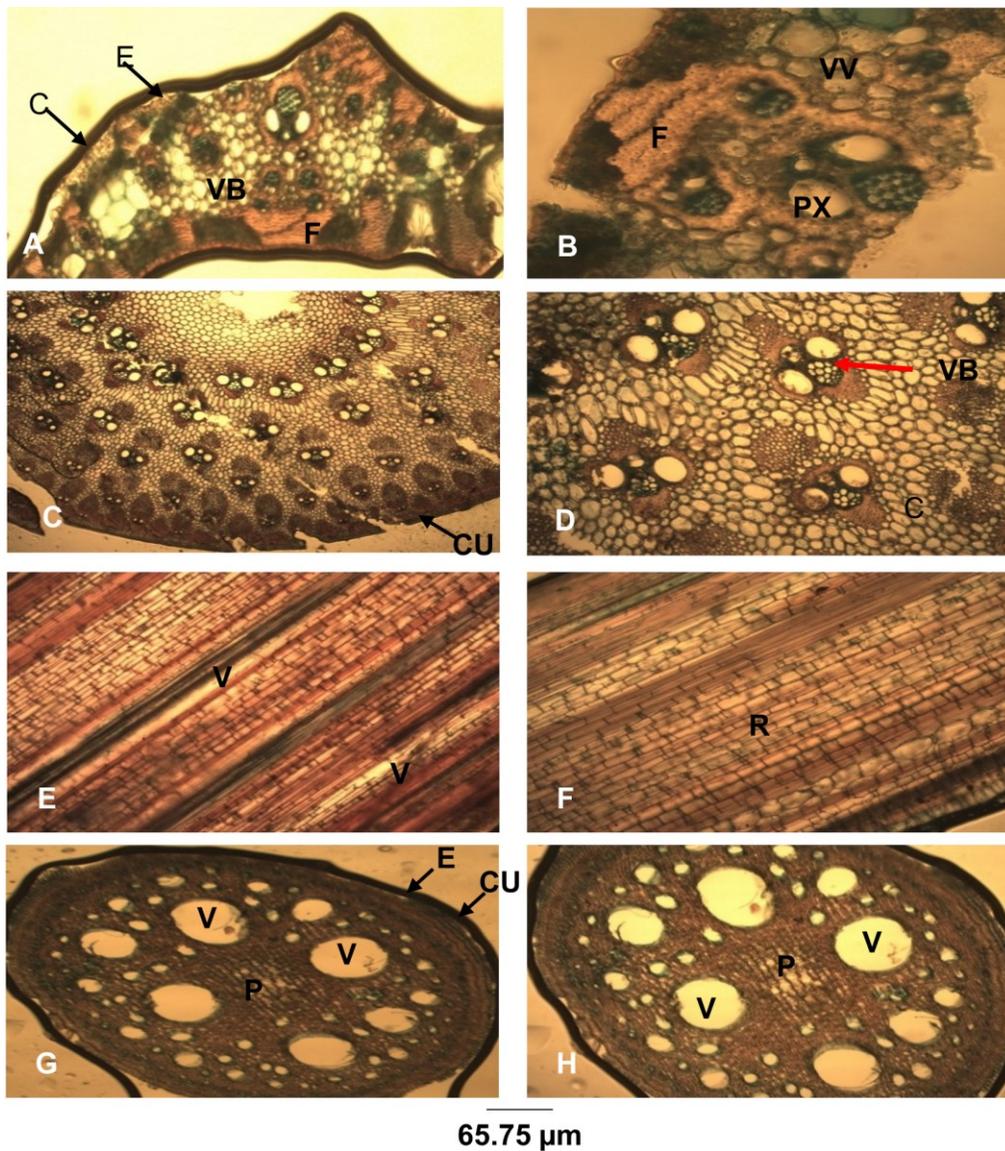


Fig. 2 Photomicrographs of *Bambusa vulgaris* accession BV2. A and B = transverse section of the leaf; C and D = transverse section of the stem; E = tangential longitudinal section of the stem; F = radial longitudinal section of the stem; G and H = transverse section of the root. BC = bulliform cell, VV = vascular vessel, E = epidermis, P = phloem, PX = protoxylem, F = fiber, CU = cuticle, VB = vascular Bundle, C = cortex, R = ray, V = vessel, ST = sieve tube.

found within the parenchyma cells that occupied the pith (Fig. 2G and H).

Anatomical study of BV3

Transverse section of leaf

The uniseriate epidermis on both surfaces was covered by thick layers of cuticle. The epidermis was uniseriate with majorly rectangular cells, occasionally squared or oval with straight to undulating periclinal walls. There was the presence of starch grains and few fibers in the vascular region,

and in the cortical region. (Fig. 3A and B).

Transverse section of stem

The cuticle was uniseriate. The epidermal layer comprised of two to four layers of collenchyma cells that were of varying sizes and shapes. The pith was occupied with many vascular bundles of different sizes both similar arrangements. The vessel elements were surrounded by bundles of parenchyma cells, that were paratracheal in the arrangement. The phloem cells were situated in between the xylem cells at an angle to metaxylem. Bundles of fiber elements were

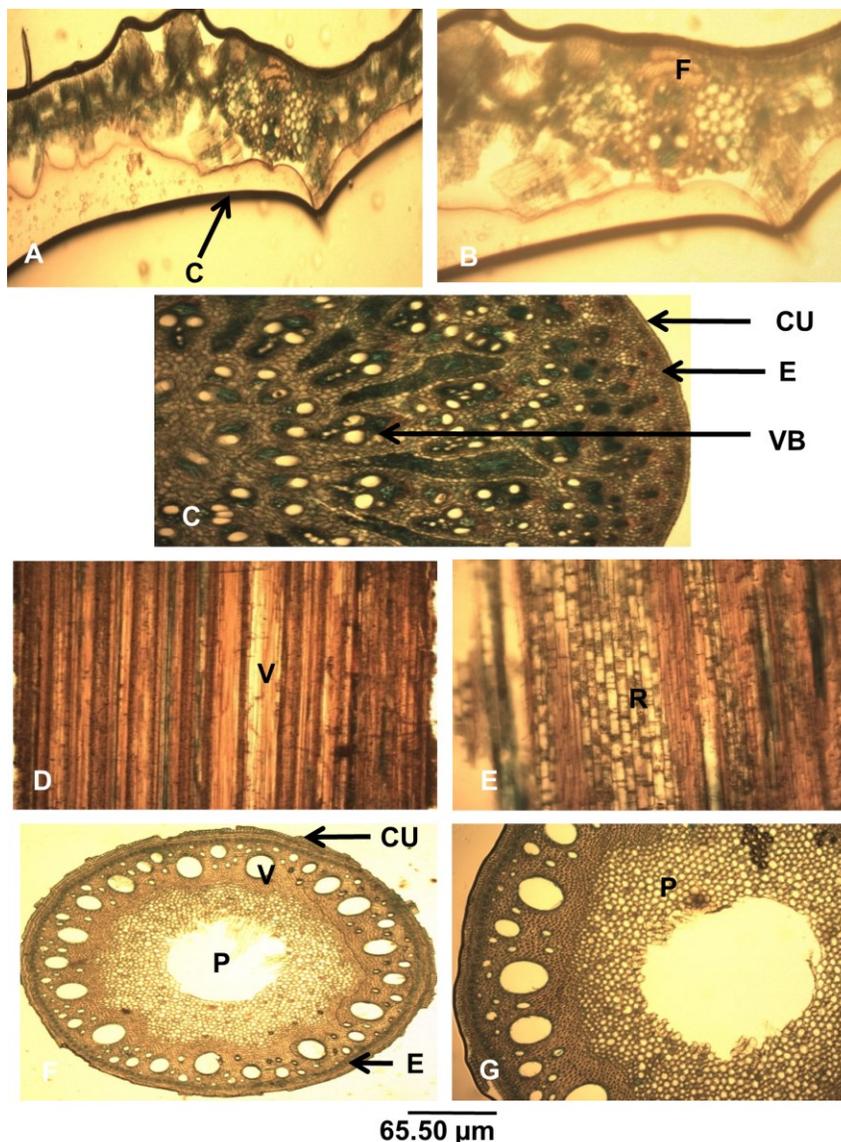


Fig. 3 Photomicrographs of *Bambusa vulgaris* accession BV3. A and B = transverse section of the leaf; C = transverse section of the stem; E = tangential longitudinal section of the stem; F = radial longitudinal section of the stem; G and H = transverse section of the root. BC = bulliform cell, VV = vascular vessel, E = epidermis, P = phloem, PX = protoxylem, F = fiber, CU = cuticle, VB = vascular Bundle, C = cortex, R = ray, V = vessel.

Seen attached to the vascular bundle. Few starch grains or granules were found littered within the parenchyma cells of the pith (Fig. 3C).

Tangential longitudinal section of the stem

The tangential longitudinal section of the stem was rayless (Fig. 3D).

Radial longitudinal section of the stem

Rays were clustered, heterocellular and irregular in

arrangement (majorly upright bar-shaped cells and occasionally squared cells) (Fig. 3E).

Transverse section of the root

The epidermis and cortex were not distinguishable and were laden with biseriate cells of sclerenchyma tissues called brachysclereid. The xylem cells were arranged in a ring pattern across the entire transverse section of the root. Paratrachea type of parenchyma cells was encountered. Sheaths of fiber elements surround the parenchyma cells. Starch grains were

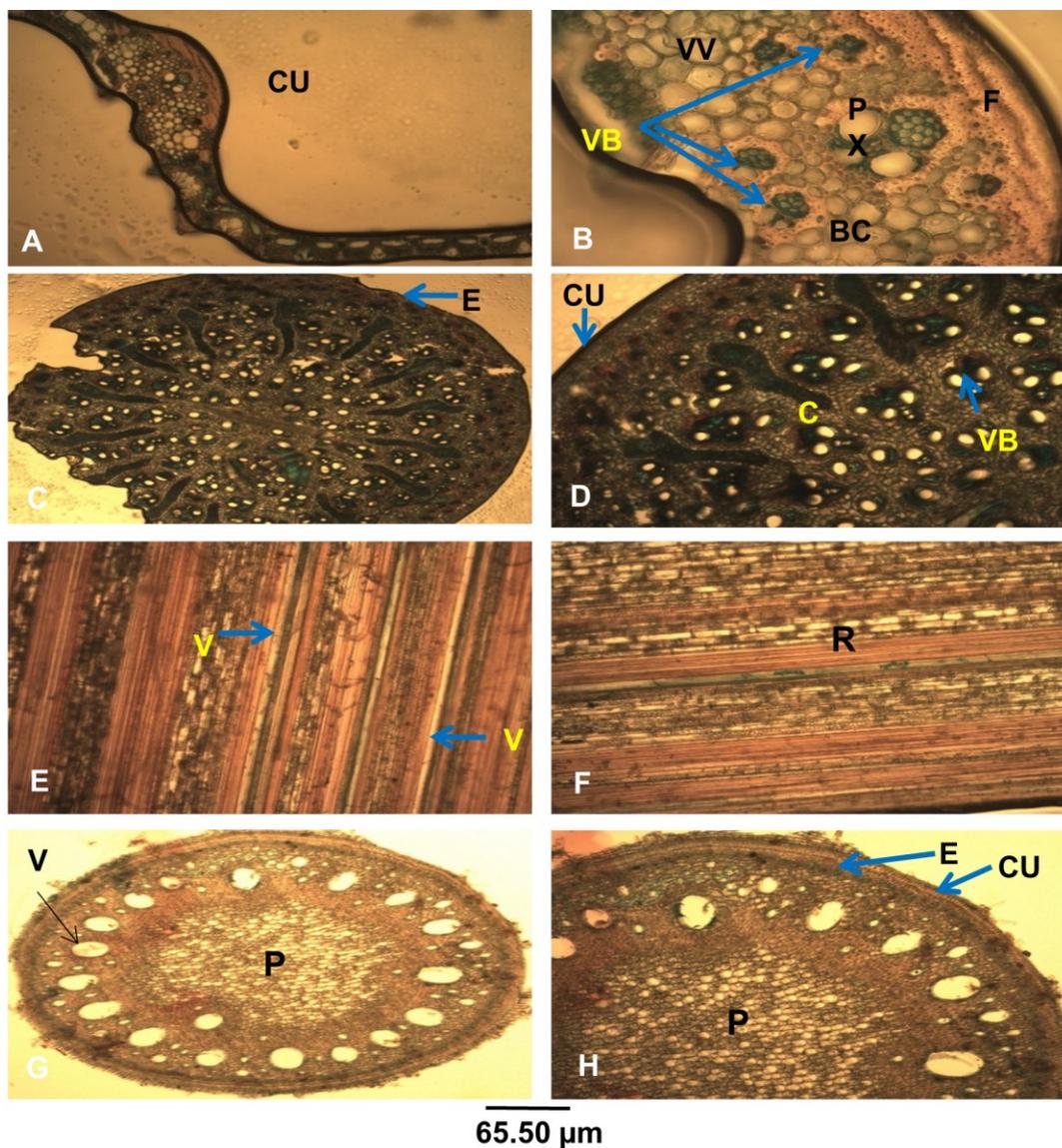


Fig. 4 Photomicrographs of *Bambusa vulgaris* accession BV4. A and B = transverse section of the leaf; C and D = transverse section of the stem; E = tangential longitudinal section of the stem; F = radial longitudinal section of the stem; G and H = transverse section of the root. BC = bulliform cell, VV = vascular vessel, E = epidermis, P = phloem, PX = protoxylem, F = fiber, CU = cuticle, VB = vascular Bundle, C = cortex, R = ray, V = vessel.

found within the parenchyma cells that occupied the pith (Fig. 3F and G).

Anatomical study of BV4

Transverse section of leaf

The uniseriate epidermis on both surfaces was covered by thick layers of cuticle. The cells of the epidermis were majorly rectangular, occasionally squared or oval in shape with straight to undulating periclinal walls. There was the presence of starch grains and abundant fiber in the vascular region, and

in the cortical region (Fig. 4A and B).

Transverse section of stem

The cells of the cuticle were uniseriate. The epidermal layer comprised two to four layers of collenchyma cells that were of varying sizes and shapes. The pith was occupied with many vascular bundles of varying sizes and shapes. The vessel elements were surrounded by bundles of parenchyma cells that are paratracheal in nature. The phloem cells were situated in between the xylem cells at an angle to the metaxylem. Bundles of fiber elements were seen

Table 2 Qualitative stem and root anatomical characters of four accessions of *Bambusa vulgaris*.

Plant section	Accessions characters	Accessions of <i>Bambusa vulgaris</i>			
		BV 1	BV 2	BV 3	BV 4
General morphology	Culms	Glossy, bright-green, erect below, arching above			
	Branching	Clustered severally, occurring from mid-culm to top			
	Leaves	Narrow and tender	Narrow and tender	Narrow and tender	Narrow and tender
TS of leaf	Cuticle	Thick	Thick	Thick	Thick
	Epidermis	Uniseriate	Uniseriate	Uniseriate	Uniseriate
	Periclinal wall	Straight/ undulating/ slightly oblique			
	Fiber	Present and abundant	Present and abundant	Present but few	Present and abundant
TS of stem	Epidermis	Uniseriate	Uniseriate	Uniseriate	Uniseriate
	Parenchyma	Paratracheal	Paratracheal	Paratracheal	Paratracheal
	Sheath of fiber around parenchyma	Present, numerous	Present, numerous	Present, numerous	Present, numerous
TLS of stem	Starch grains	Present, few	Present, few	Present, few	Present, few
RLS of stem	Rayless	Rayless	Rayless	Rayless	Rayless
TS of root	Ray cells	Heterocellular	Heterocellular	Heterocellular	Heterocellular
	Epidermis and cortex	Not distinguishable	Not distinguishable	Not distinguishable	Not distinguishable
	Cells of the epidermis	Biseriate, brachysclereid	Biseriate, brachysclereid	Biseriate, brachysclereid	Biseriate, brachysclereid
	Type of parenchyma	Paratracheal	Paratracheal	Paratracheal	Paratracheal
	Starch grains	Present	Present	Present	Present

TS = transverse section; TLS = Tangential longitudinal section; RLS = radial longitudinal section

attached to the vascular bundle. Few starch grains or granules were found littered within the parenchyma cells of the pith (Fig. 4C and D).

Tangential longitudinal section of the stem

The tangential longitudinal section of the stem was rayless (Fig. 4E).

Radial longitudinal section of the stem

Rays were clustered, heterocellular and irregular in arrangement (majorly upright bar-shaped cells and occasionally squared cells) (Figure 4F).

Transverse section of the root

The epidermis and cortex were not distinguishable and were laden with biseriate cells of sclerenchyma tissues called brachysclereid. The xylem cells were arranged in a ring pattern across the entire transverse section of the root. Paratracheal type of parenchyma

cells was encountered. Sheaths of fiber elements surrounded the parenchyma cells. Starch grains were found within the parenchyma cells that occupy the pith (Figure 4G & H).

Discussion

The use of anatomical features in plants to delimit members of a family, genus, or between species is well documented in the literature [7-15]. Anatomical characters are particularly appropriate to elucidate the relationship between forms, functions and adaptation at a macro-evolutionary scale. In this study, close similarities in the anatomical character of the investigated species were encountered. Micro-anatomical data from the four accessions of *B. vulgaris* considered in this study were subjected to one-way analysis of variance (ANOVA). Investigations into the micro-anatomy of vegetative organs of plants have been observed to play vital roles

Table 3 Quantitative stem and root anatomical characters of four accessions of *Bambusa vulgaris* (BV).

Accessions	Stem anatomy		Root anatomy	
	Mean vessel length (μm)	Mean vessel width (μm)	Mean vessel length (μm)	Mean vessel width (μm)
BV 1	49.70 \pm 1.37 ^a	37.00 \pm 1.12 ^a	124.10 \pm 3.64 ^a	86.30 \pm 3.18 ^a
BV 2	97.12 \pm 2.74 ^c	66.60 \pm 1.90 ^c	153.20 \pm 7.90 ^b	101.60 \pm 6.09 ^b
BV 3	73.00 \pm 2.26 ^b	42.80 \pm 1.08 ^b	130.40 \pm 7.73 ^a	88.55 \pm 5.49 ^a
BV 4	68.80 \pm 2.00 ^b	42.60 \pm 1.07 ^b	135.60 \pm 2.40 ^a	95.32 \pm 1.89 ^a

Values are expressed as mean \pm Standard Error (n = 25)

Values in each column with different superscripts are significantly different ($p < 0.05$)

in establishing inter-relationship at both infra- and supra-specific levels [16]. In this study, qualitative stem and root anatomical characters of accessions of *B. vulgaris* showed similarities between the accessions studied using some morphological and anatomical characters, this corroborated the fact that the accessions are same species but from different locations (Table 2). The quantitative results showed that there were significant differences between BV3, BV4 and others in terms of their stem and root anatomy (Table 3). BV1, BV2 and BV3 shared similar values for vessel lengths and widths, and hence the accessions could be classified as one taxon based on their stem and root micro-anatomy. Meanwhile, BV2 is delineated from other accessions by its highly significant vessel lengths and width (Table 3). Similar results have previously been reported by Akinsulire et al. [17], who separated closely related species of the family Combretaceae using quantitative information from stem anatomy, as well as [15] for the genus *Diospyros* L. Meanwhile, information from the qualitative leaf, stem and root anatomical investigation was increasingly uninformative for this comparative analysis as all four accessions exhibited highly similar qualitative micro-anatomical characters (Table 2). Nevertheless, the qualitative leaf, stem and root micro-anatomical characteristics such as thick cuticle, uniseriate epidermis and straight / undulating / slightly oblique periclinal epidermal walls and the presence of abundant fiber in BV1, BV2 and BV4 but scanty in BV3 (Fig. 1-4) in the leaves; uniseriate stem cuticle, paratracheal stem parenchyma, presence of fiber sheath around parenchyma cells in the stem, presence of starch grains in the stem as well heterocellular cells in the tangential longitudinal sections can all serve great species' identification purpose for *B. vulgaris*. Several scientists have previously used qualitative wood anatomical characteristics in the identification of plant groups [15, 18]. In this study, though the fact remains that the four accessions represent a single species and share a monophyletic origin, but the fact that BV2 might probably have been influenced by

ecological environmental factors cannot be ignored. The findings, based on combined stem and root micro-anatomical characters revealed that the differences between the accessions are connected to the differences in their quantitative stem and root anatomical characters. Similar results were reported by other researchers in the delimitation of *Terminalia* species [19].

Conclusions

This study has provided information on the stem and root micro-anatomical characters of four accessions of *B. vulgaris* thereby enhancing its taxonomy. It is concluded that quantitative stem and root anatomical characters are important in delineating species accessions and should be employed in separating closely related species as well as different accessions of the same species. It is, however, recommended that an ecological investigation should be conducted on the species to further enhance their taxonomic position and responses of their accessions to various environments.

Conflict of interest

The authors claim no conflicts of interest.

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