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STEM ANATOMICAL DESCRIPTORS OF FOUR *SESBANIA* SCOP. SPECIES AND THEIR SYSTEMATIC IMPLICATION

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Abstract

Stem anatomical features of four *Sesbania* Scop. species *viz. S. bispinosa* (Jacq.) W. Wight, *S. cannabina* (Retz.) Poir., *S. sesban* (L.) Merr., and *S. rostrata* Bremek. & Oberm., were examined to add some insights for identification of these species using quantitative anatomical descriptors. *Sesbania* stem is composed of epidermis, cortex, vascular tissues – phloem, cambium zone and xylem, and pith, which exhibit significant variations among the species in terms of their area and thickness. *Sesbania sesban* showed the largest area and widest epidermal cells. The close relationship between *S. bispinosa* and *S. rostrata* was found in the stem anatomical descriptors. Moreover, *S. rostrata* and *S. cannabina* were closer to some extent according to some anatomical descriptors; also rationalizing the external morphological similarities of these species. A dichotomous key of the studied *Sesbania* species was made. Dendrograms based on Agglomerative Hierarchical Cluster analysis of stem anatomical descriptors also confirmed close relationships identified in previous phylogenetic analyses.

Introduction

The genus *Sesbania* Scop. includes about 70 species of which 27 species produce nodules (De Faria *et al*., 1989; Farruggia *et al*., 2018). *Sesbania* can fix atmospheric nitrogen through Legume-*Rhizobium* symbiosis and form nitrogen-fixing nodules on its roots, surprisingly *S. rostrata* forms nodules both on root and stem (Allen and Allen, 1981). The *Sesbania* species are broadly used and cultivated in agroforestry for soil improvement, as green manures and other products (Evans, 1990). The economic importance of *Sesbania*, especially in Africa and Asia, comprises shade plants, windbreaks, cover crops, ornamentals, fish poisons (source of isoflavones), fibre sources, construction materials, food and medicinal uses for both humans and livestock (Gillett, 1963; Powell *et al*., 1976; Laladhas *et al*., 2010; Mythilli and Ravindhran, 2012), and bioremediation of lead, zinc and copper from industrial discharged sites and contaminated soils as well (Qadir *et al*., 2002; Sahi *et al*., 2002; Yang *et al*., 2003; Branzini *et al*., 2012). In Bangladesh, *Sesbania* is represented by 5 species *viz. S. bispinosa* (Jacq.) W. Wight, *S. cannabina* (Retz.) Pers., *S. grandiflora* (L.) Pers., *S. javanica* Miq. and *S. sesban* (L.) Merr. (Ahmed *et al*., 2009); among these, three species *viz. S. sesban, S. bispinosa* and *S. cannabina*, and an exotic species, *S. rostrata* Bremek. & Oberm., are widely cultivated as green manure crops. The first three species are commonly known as *dhaincha* and the last one is African *dhaincha* (Sarwar *et al*., 2015). However, the identification of three native *dhaincha* species, especially *S. bispinosa* and *S. cannabina* is very difficult and confusing to separate in the field based on morphological features.

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Several plant micro-morphological features including stem anatomical structure provide evidence connecting to the interrelationships of higher taxonomic groups, such as families, sections and tribes and serve to generate true resemblances of genera having indecisive taxonomic status (Metcalfe and Chalk, 1950; Aziagba and Okeke, 2017); although many anatomical characters are influenced by environmental factors (Metcalfe and Chalk, 1950). Anatomical features of the stem, leaf and other plant parts are used for identification and inferring taxonomic relationships among the taxa in many plant families including Leguminosae (Barykina and Kramina, 2006; Aziagba and Okeke, 2017; Nwachukwu *et al*., 2017). Several studies have been made on the utility of different morphological descriptors for the better identification of *Sesbania* species (Prodhan *et al*., 1998; Prodhan and Sarkar, 2002; Sarkar and Prodhan, 2001; Sarwar *et al*., 2015; Chanda *et al*., 2018, 2019a,b, 2020a,b, 2021). The present study was undertaken to investigate quantitative stem anatomical descriptors to add new insight for the identification of closely related *Sesbania* species.

Materials and Methods

Healthy mature seeds of four *Sesbania* species were collected from the Laboratory of Plant Systematics, Department of Crop Botany, Bangladesh Agricultural University. Seeds were previously collected from the field during field surveys, multiplied, identified and maintained/stored in the Laboratory. The plants were raised in the Field Laboratory of the Department of Crop Botany in 2017 following Chanda *et al*. (2020a). Stem samples, of 60 days old, of four *Sesbania* species were collected from the experimental field and preserved in vials containing FAA solution for further anatomical studies (Ruzin, 1999).

For anatomical exploration, both freehand sectioning and paraffin methods of micro-techniques were followed (Prodhan and Sarkar, 2002). The paraffin sections were made habitually on the outcome of hand sections. After proper dehydration with ethyl alcohol and clearance with xylene, the sections were stained with safranin and fast green and mounted in Canada balsam (Ruzin, 1999; Sarkar and Prodhan, 2001; Prodhan and Sarkar, 2002). Three cross-sections from at least three different individual plants of each species were measured for each sample to assess the constancy of anatomical features. The length/width and size were measured under × 4, 10 and 40 magnifications using an optical microscope with Carl Zeiss Primo Star camera Model Axiocam ERc5s. Ten replicated measurements focusing lengths/width and size (area) were done on each slide and thirty (3x10) measurements for individual descriptors (Fig. 1). For individual cell lengths/width, measurements were done at three positions (central and two sides from the centre) and the average value was used as a single replicate. The diameter was measured through the longest axis of the xylem vessel.

The collected data were analyzed following the ANOVA using the statistical computer package program MSTAT-C. The mean differences of different parameters among the species were adjudged with Duncan's New Multiple Range Test (DMRT) (Gomez and Gomez, 1984). The agglomerative hierarchical cluster (AHC) analysis was performed on the dissimilarity of accession characteristics and the dendrogram was generated using the XLStat software program (https://www.xlstat.com/en/).

Results and Discussion

Sesbania species stem comprises four distinct layer/region(s) – epidermis, cortex, vascular zone, and pith, which are common phenomena of dicot stem ultrastructure (Fig. 2a–d). However, significant variations were observed in different stem anatomical components (Table 1).

Table 1. Dimensions of anatomical descriptors in stem of four *Sesbania* species.

Species	Thick-ness of cuticle (µm)	Epidermal cell			Hypodermal cell		General cortex cell		Endodermal cell	
		Size (μm^2)	Width (µm)	Wall thick-ness (µm)	Width (µm)	Size (μm^2)	Width (µm)	Wall thickness (µm)	Size (μm^2)	Width (µm)
S. bispinosa	6.02 c	857.9 ab	23.6 b	3.50 b	113.6 c	740.2 bc	32.3 b	2.05 c	1060.2 b	24.3 bc
S. cannabina	7.56 a	667.8 b	22.3 b	1.52 c	163.5 ab	814.1 b	62.2 a	2.94 a	1220.8 a	32.7 a
S. rostrata	7.19 ab	693.2 b	23.4 b	4.27 ab	177.4 a	627.1 c	31.03 b	2.25 bc	1029.4 b	29.2 ab
S. sesban	6.44 bc	999.8 a	28.3 a	4.82 a	144.2 b	968.7 a	33.08 b	2.64 ab	1061.1 b	21.7 c
Level of Significance	*	*	*	**	**	**	***	*	*	**
$LSD_{0.05}$	1.04	223.0	3.09	1.24	23.6	146.9	3.94	0.53	132.5	5.36

Right side of the table.

Vascular cylinder width (µm)	Bundle cap width (µm)	Phloem width (µm)	Sieve tube		Cambial layer thickness (µm)	Xylem thickness (µm)	Xylem Vessel diameter (µm)		Size of pith cell (μm^2)	
			Size (μm^2)	Wall thickness (µm)			Meta-	Proto-	Peripheral	Central
187.48 a	30.18	32.41 a	872.3 a	3.20 ab	27.8 bc	118.28 a	93.0 a	43.3 a	158.7 a	387.6 a
173.99 b	30.78	28.17 b	640.9 c	4.16 a	22.4 c	92.98 b	39.7 d	26.6 b	181.4 a	312.0 b
190.99 a	30.23	26.40 b	720.7 b	4.15 a	32.2 ab	121.65 a	74.4 b	27.5 b	164.5 a	295.1 b
154.19 c	28.44	28.13 b	414.6 d	2.98 b	35.9 a	85.17 c	62.2 c	40.1 a	115.9 b	295.8 b
***	NS	***	***	*	**	***	***	*	*	***
5.23	2.32	2.06	39.5	0.97	5.90	3.9	7.17	12.2	36.9	30.8

In a column figure (s) with the same letter do not differ significantly at 5% level by LSD Test; * = Significant at 5% level, ** = Significant at 1% level,

*** = Significant at 0.1% level; LSD = Least Significant Difference.

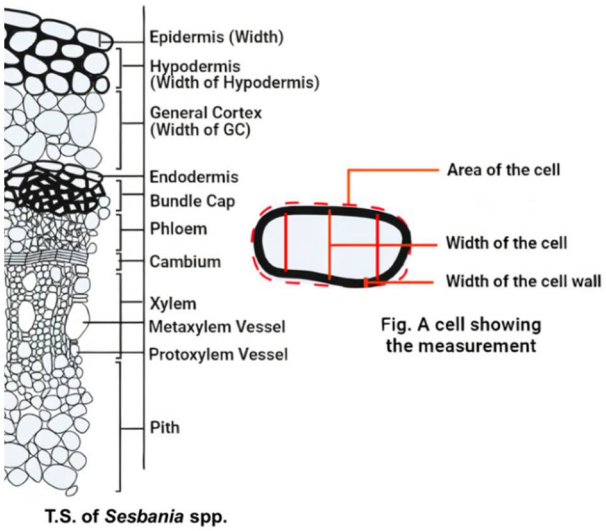

Fig.1. Schematic diagram of transverse section of a *Sesbania* sp.

Epidermis

The transverse section of the stem of all *Sesbania* species showed a single-layered epidermis covered with a thick cuticle. The epidermal cells are more or less square or slightly rectangular in shape (Fig. 2c). The result of previous studies, the single layer of epidermis with slightly rectangular cells, of *Sesbania* spp. were in an agreement with the present study (Prodhan and Sarkar, 2002; Sarkar and Prodhan, 2002). Statistically, the largest epidermal cell area was observed in *S. sesban* (999.8 μm^2) followed by *S. bispinosa* (857.9 μm^2) and the lowest in *S. cannabina* (667.8 μm^2) followed by *S. rostrata* (693.2 μm^2) (Table 1). Furthermore, the width of epidermal parenchyma cells was preeminent in *S. sesban* (28.3 µm) whereas lowest in *S. cannabina* (22.3 µm). In the case of an epidermal cell wall, the maximum value was recorded in *S. sesban* and it was statistically similar to *S. rostrata*. However, statistically significant results were found in *S. rostrata*, *S. bispinosa* and *S. cannabina* in thickness of epidermal cell wall. The thinnest epidermal cell wall was observed in *S. cannabina* (1.53 µm). On the contrary, the thickest cuticle was observed in *S. cannabina* and it was statistically insignificant to *S. rostrata* and significant to *S. bispinosa* (Table 1). From Table 1, it is evident that *S. bispinosa* and *S. rostrata* exhibited similar results followed by *S. cannabina* and *S. sesban* in terms of their epidermal characteristics which supported the taxonomic evidence and cladistics relationship of different *Sesbania* species (Farruggia *et al*., 2018). In addition, *S. rostrata* and *S. cannabina* were also closer according to their epidermal attributes that also justifies the observations reported by Chanda *et al.* (2020b) for the identification of *Sesbania* species based on external morphological descriptors.

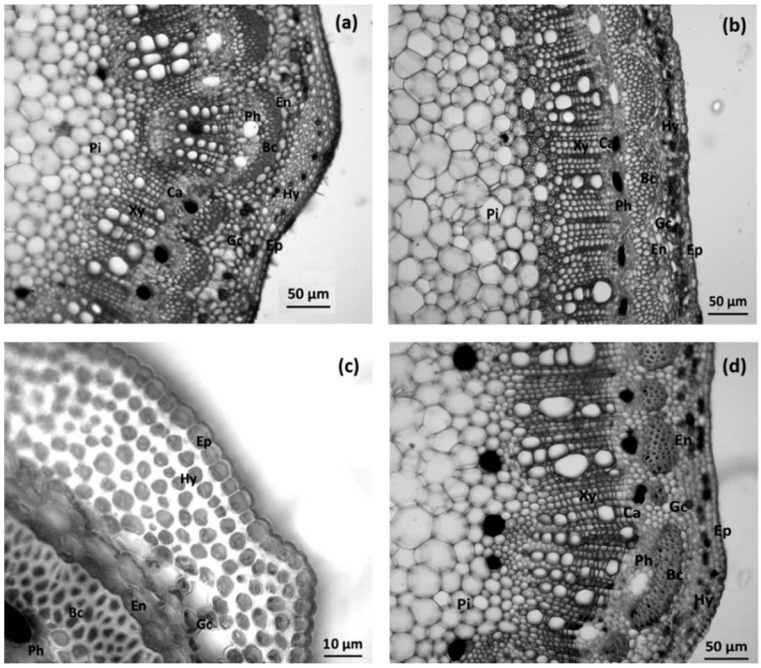

Fig. 2. Transverse section (TS) of *Sesbania* stem. a. *S. cannabina*, b. *S. sesban*, c. *S. rostrata*, d. *S. bispinosa*. Ep: Epidermis; Hy: Hypodermis; Gc: General cortex; En: Endodermis; Bc: Bundle cap; Ph: Phloem; Ca: Cambium; Xy: Xylem and Pi: Pith.

Cortex

Just beneath the epidermis, the position belongs to the cortex. It is a few to several cells in thickness. The cortex comprises (i) the hypodermis, (ii) the general cortex and (iii) the endodermis, which is located next to the vascular bundle cap (Fig. 2c). The number of cortical layers varies according to the age, size and level of secondary growth of the organ concerned (Prodhan and Sarkar, 2002). The collenchymatous hypodermal layers varied from two to six among the species. The cortex, especially the endodermis, is disorganized and disintegrated due to the stress of secondary growth (Fig. 2). Results revealed that the thickest hypodermal layer was found in *S. rostrata* (177.4 µm) followed by *S. cannabina* (163.5 µm), *S. sesban* (144.2 µm) and *S. bispinosa* (113.6 µm) (Table 1). There were significant variations of individual hypodermal cell areas among the *Sesbania* species. The largest hypodermal cell was observed in *S. sesban* and the smallest in *S. rostrata* (Table 1). The thickness of the general cortex was highest in *S. cannabina* whereas the other three species exhibited statistically similar and lowest thickness. In addition, the thickness of the cell wall of ground tissue exhibited highest in *S. cannabina* and lowest in *S. bispinosa*. Lots of tanniniferous cells are found in the middle zoned cortex (Fig. 2, Sarkar and Prodhan, 2001). Secretory cells are common in the cortex of many plants. The endodermis is a wavy layer of one cell in thickness. It lies at the innermost boundary of the cortex (Fig. 2). From Table 1, *S. cannabina* exhibited the largest endodermal cell (1220.8 μm^2) which was statistically different from other species. The width of the endodermal parenchyma cells was also statistically significant and the highest width was found in *S. cannabina* (32.7 µm) whereas the lowest was in *S. sesban* (21.7 µm). Results from the cortical area brought to light that statistical similarities were found among *S. bispinosa* and *S. rostrata* in most of the cases. With some exceptions, *S. rostrata* and *S. cannabina* were statically identical according to their hypodermal thickness and width of the endodermis. These anatomical findings justified the phylogenetic relationships among the *Sesbania* species and the external morphological similarities among the species (Farruggia *et al*., 2018; Chanda *et al*., 2020b). Chanda *et al*. (2020b) reported close similarities in stem base diameter of *S. rostrata* and *S. bispinosa* which confirmed the present results of the anatomical measurements.

Vascular bundle

The vascular bundles, consisting of bundle cap, phloem, cambium and xylem, are arranged in a ring as seen in the transverse section of *Sesbania* species (Fig. 2). The vascular bundles are of two types – large and small, and they were positioned alternately. However, one or two small vascular bundles in between two large bundles were also observed in *Sesbania* (Sarkar and Prodhan, 2001). The large vascular bundle contains 4-5 strands of xylem (Fig. 2). The number of xylem strands in the small vascular bundle is one or two. The xylem strand consists of proto- and meta-xylem vessels. Protoxylem vessel remains towards the centre while metaxylem vessel towards the periphery. The vessels are arranged radially. The vessels are round or oval with prominent secondary thickening (Fig. 2). The widest vascular cylinder was observed in *S. rostrata* (190.99 µm) and it was statistically identical in *S. bisponosa* (187.48 µm) however, the lowest value (154.19 µm) was observed in *S. sesban.* Phloem thickness was maximum in *S. bispinosa* (32.41 µm) while the other three species exhibited identical results. The thickness of the sieve tube wall was highest in both *S. cannabina* and *S. rostrata* and it was statistically insignificant to *S. bispinosa*, however, it was statistically identical with *S. sesban* (Table 1). The primary phloem consists of several sieve elements and a lot of parenchymatous cells. The first phloem appears in the external parts and xylem in the internal part of a pro-cambial filament. New phloem elements appear closer to the middle of the stem and the xylem differentiates oppositely. The vascular cambium arising in the two positions are called fascicular and inter-fascicular cambium (Prodhan

and Sarkar, 2002; Sarkar and Prodhan, 2001). Prodhan and Sarkar (2002) stated that a large vascular bundle consists of a lot number of sieve tubes and parenchymatous cells while in the small bundle are parenchymatous tissue with or without functional sieve element. Just beneath the endodermis, there was a discontinuous bundle cap (Fig. 2). The thickness of the bundle cap was statistically significant and the highest bundle cap thickness was found in *S. cannabina* which was statistically identical to *S. rostrata* and *S. bispinosa* whereas the lowest thickness was found in *S. sesban* (Table 1). At maturity, the cambial zone is composed of 1-3 layers of cells (Fig. 2). Cambial thickness was the maximum in *S. sesban* and it was statistically similar to *S. rostrata*, however, *S. cannabina* exhibited minimum cambial thickness. Prodhan and Sarkar (2002) reported that at an early stage, the cambium becomes active and gives rise to secondary phloem and secondary xylem. In the active stage, the cambial zone consists of 4-5 layers of cells consisting of cambial initiates and their derivatives.

Xylem thickness was the highest in *S. rostrata* (121.65 µm) which was statistically identical to *S. bispinosa* (118.28 µm) and the lowest (85.17 µm) in *S. sesban* (Table 1). The thickness of the metaxylem vessel showed statistically significant results among the four species. The highest value was found in *S. bispinosa* (93.0 µm) followed by *S. rostrata* (74.4 µm), *S. sesban* (62.2 µm) and *S. cannabina* (39.7 µm). The protoxylem vessel thickness was highest in *S. bispinosa* which was statistically similar to *S. sesban* and lowest in *S. cannabina*. Prodhan and Sarkar (2002) reported that the increase of secondary xylem was a result of secondary growth. They further stated that the secondary phloem lies abaxial to the cambial zone. The secondary phloem consists of sieve elements, phloem parenchyma and phloem fibre. Among the elements of secondary phloem, axial parenchyma has been found to occupy the major area. The phylogenetic (cladistics) relationship of different *Sesbania* species manifested that *S. bispinosa* and *S. rostrata* are closer species (Farruggia *et al*., 2018) which are in an agreement considering most of the anatomical vascular features of these taxa (Table 1). Chanda *et al.* (2021) explained the morphological and physiological characteristics of different *Sesbania* species and gave information about the close similarities of *S. rostrata* and *S. bispinosa* according to their stem base diameter. These findings also supported the close anatomical relationship between the *S. bispinosa* and *S. rostrata*. From the same study, the highest biomass yield was obtained from *S. bispinosa* (Chanda *et al*., 2020a) which also justifies the highest vascular cylinder, bundle cap, phloem, xylem, proto- and meta-xylem vessel thicknesses of *S. bispinosa*. The larger vascular components helped to transfer water and minerals from soil to leaves, and photosynthates from source to sink, which might be the inherent cause of higher biomass yield in *S. bispinosa*.

Pith

Pith occupies the central portion of the stem. The area of pith peripheral cell was statistically significant in *S. cannabina*, *S. bispinosa* and *S. rostrata*, however, insignificant in *S. sesban* (Table 1). The highest significant area of the pith central cell was found in *S. bispinosa* (387.6 μm^2) and other *Sesbania* species showed identical results. Prodhan and Sarkar (2002) reported that the pith is the central core of the stem and is composed of thin-walled parenchymatous cells.

A dichotomous key of four *Sesbania* species based on stem anatomical descriptors –

1a. Epidermal parenchyma cell 850-1010 μm^2, cuticle thickness 6.0-6.5 µm, hypodermal collenchyma width 110-145 µm, endodermal parenchyma width 20-25 µm, protoxylem vessel diameter 40-45 µm – 2

1b. Epidermal parenchyma cell 650-700 μm^2, cuticle thickness 7.0-7.7 µm, hypodermal collenchyma width 160-180 µm, endodermal parenchyma width 26-35 µm, protoxylem vessel diameter 25-30 µm – 3

2a. Epidermal parenchyma cell width 27-29 µm, epidermal parenchyma cell wall thickness 4.5-5.0 µm, hypodermal collenchyma cell 960-970 µm^2, vascular cylinder width 150-160 µm, sieve tube cell 410-420 µm^2, xylem thickness 80-90 µm – *S. sesban*

2b. Epidermal parenchyma cell width 23-24 µm, epidermal parenchyma cell wall thickness 3.3-3.7 µm, hypodermal collenchyma cell 735-745 µm^2, vascular cylinder width 180-190 µm, sieve tube cell 865-875 µm^2, xylem thickness 110-125 µm – *S. bispinosa*

3a. Epidermal parenchyma cell wall thickness 1.45-1.60 µm, hypodermal collenchyma cell 810-820 µm^2, cortical parenchyma cell width 60-65 µm, endodermal parenchyma cell 1200-1250 µm^2, vascular cylinder width 170-180 µm, sieve tube cell 630-660 µm^2, xylem thickness 90-100 µm – *S. cannabina*

3b. Epidermal parenchyma cell wall thickness 4.2-4.4 µm, hypodermal collenchyma cell 620-630 µm^2, cortical parenchyma cell width 30-35 µm, endodermal parenchyma cell 1000-1050 µm^2, vascular cylinder width 185-197 µm, sieve tube cell 710-730 µm^2, xylem thickness 118-125 µm – *S. rostrata*

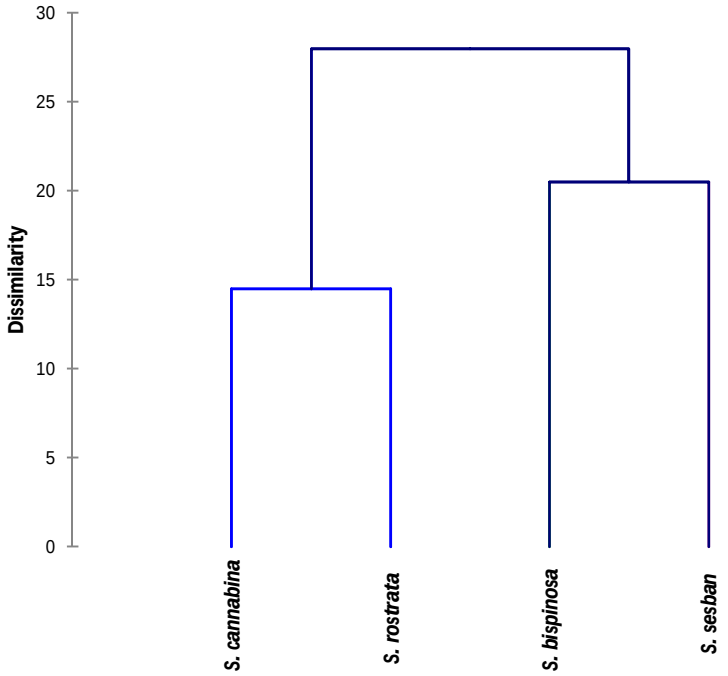

Fig.3. Agglomerative Hierarchical Cluster analysis dendrogram based on quantitative stem anatomical descriptors.

Multivariate analysis of quantitative stem anatomical descriptors

Agglomerative clustering is the most common type of hierarchical clustering used to group objects in clusters based on their similarity, resulting in a tree-based representation of the objects, named dendrogram. The dendrogram, based on stem anatomical descriptors, identified two distinct clades indicating the close relationships between *S. bispinosa* and *S. sesban* vs. *S. cannabina* and *S. rostrata* (Fig. 3). The close relationships were also well represented in both morphological (Chanda *et al*., 2020b) and molecular data (Farruggia *et al.*, 2018). Farruggia *et al.* (2018) also

concluded that *S. rostrata* might be a probable ancestor of *S. bispinosa* and *S. sesban*, although the closest similarity in morphological descriptors between *S. bispinosa* and *S. cannabina* in the field. *Sesbania sesban* possessed a relatively lower amount of lignified xylem, phloem fibres and xylem fibres which made it more digestible as animal forage (Guines *et al*., 2003).

From the results, it is evident that *S. bispinosa* and *S. rostrata* exhibited more or less similar results in terms of stem anatomical descriptors – epidermal, cortical and vascular bundle, which is also an indication of the close phylogenetic relationship of these two taxa. In addition, *S. rostrata* and *S. cannabina* were closer to some extent according to some anatomical descriptors that also justifies the external morphological similarities of these species. It might be concluded that quantitative stem anatomical descriptors could be useful to identify *Sesbania* species especially phenologically and floral morphologically similar, *S. bispinosa* and *S. cannabina*. Anatomical variations observed in *Sesbania* species need further studies, mainly from eco-physiological points of view, for a better understanding of plant adaptations to diverse ecosystems.

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