International Journal of Current Advanced Research
ISSN: O: 2319-6475, ISSN: P: 2319-6505, Impact Factor: 6.614
Available Online at www.journalijcar.org
Volume 7; Issue 4(M); April 2018; Page No. 12216-12225
DOI: http://dx.doi.org/10.24327/ijcar.2018.12225.2141

Research Article

STEM ANATOMY OF SOME MEMBERS OF THE SUBFAMILY PAPILIONOIDEAE WITH REFERENCE TO THEIR IDENTIFICATION

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A R T I C L E I N F O

Article History:

Received 5th January, 2018
Received in revised form 20th February, 2018 Accepted 8th March, 2018
Published online 28th April, 2018

Key words:

Papilionoideae, stem anatomy, adavanced, primitive, characters.

A B S T R A C T

Quantitative and qualitative data are presented for ten species in the subfamily Papilionoideae in Ile-Ife, Nigeria with the view to document the stem characters of taxonomic value and that could be used in identifying the species within the subfamily. Species studied were *Desmodium tortuosum* (Sw.) DC., *Desmodium scorpiurus* (Sw.) Desv., *Desmodium adscendens* (Sw.) DC., *Cajanus cajan* (L.) Millsp., *Calopogonium mucunoides* Desv., *Centrosema molle* (Mart.) ex. Benth., *Mucuna pruriens* (Linn.) Walp., *Vigna unguiculata* (Linn.) Walp., *Crotalaria retusa* Linn. and *Gliricidia sepium* (Jacq.) Walp.

The stem anatomy of the species was studied by cutting the Transverse, Tangential Longitudinal as well as Radial Longitudinal Sections of the stem. with a Reichert microtome at a thickness of ten micrometre. Schultz's fluid was used for the maceration of wood.

Circular and undulating stem outline is diagnostic and unique for *Centrosema molle* while oval pith shape through the Transverse Section of the stem is also unique to *Centrosema molle* and *Vigna unguiculata*. Pith is central in all the other species studied except in *Mucuna pruriens* and *Vigna unguiculata*. *Cajanus cajan* is the only species with angular collenchyma cells in the cortical region. Axial parenchyma is generally paratracheal and range from scanty to banded confluent. Wood is generally diffuse porous. Vessel pore shape is generally oval to circular in shape except in *Desmodium adscendens*, *Mucuna pruriens, Vigna unguiculata* and *Centrosema molle* where they are largely circular to oval. Presence of tyloses in the vessels delimit *Glyricidia sepium* from all the other species studied.

Vessel length and diameter study suggest primitiveness of the vessels in *Vigna unguiculata* while they are more advanced in *Mucuna pruriens*. Among the three species of *Desmodium* studied, *Desmodium scorpiurus* seems to be more advanced and *Desmodium tortuosum* least advanced in stem characters. Homogeneous wood rays in the Tangential Longitudinal Section of the stem suggest that *Cajanus cajan* and *Gliricidia sepium* are more advanced in terms of wood rays than the other species. The dendrogram of both qualitative and quantitative data distinctly separates *Crotalaria retusa* from other species. Type of stem, presence of central hollow at the pith and vessel width are the major characters unique to *Crotalaria retusa*. *Desmodium tortuosum* is delimited in the second major cluster largely because of its non-storied fibres and heterogeneous wood rays which are primitive characters. The study has also revealed a close relationship in stem anatomy between *Desmodium adscendens* and *Calopogonium mucunoides*; between *Desmodium scorpiurus* and *Centrosema molle*; and between *Cajanus cajan* and *Gliricidia sepium*.

INTRODUCTION

The homogeneous subfamily Papilionoideae is the largest and most widespread group of legume generally distributed throughout the world (Hutchinson and Dalziel, 1958; Gurcharan, 2004; Duane and Paul, 2012). It consists of about 475 genera and nearly 14,000 species grouped in 14 tribes (APG, 2012; Duane and Paul, 2012) and about 335 Nigerian species (Hutchinson and Dalziel, 1958). The members of the subfamily are mainly herbs or herbaceous climbers; sometimes they are erect or climbing shrubs or trees, or lianas.

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The climbers have climbing stem but often they climb with leaf tendrils (Watson and Dallwitz, 1992; Gurcharan, 2004; ILDIS, 2005). The subfamily contains most of the important leguminous crop species (Gurcharan, 2004; Klitgard and Lewis, 2010) and finds a wide range of usefulness (ILDIS, 2005). The subdivision of the Papilionoideaeinto taxa of lower rank had created controversies and discrepancies for many decades while more recent phylogenetic studies provided no decisive answer to these problems (Wojciechowski *et al.*, 2004; Champagne *et al.*, 2007; El- Gazzar *et al.*, 2013).

The internal parts of plants and their structures are useful in the taxonomic delimitation of plants, since they are less affected by environmental changes and therefore are highly conservative in taxonomic variations (Stace, 1980). Anatomical features are of particular value to scientists who need to identify small scraps of plant material (Ahmad *et al.*,

2010). Studies at tribal or generic level show that anatomical features are informative and contribute to support different clades (Teresa and Salvador, 2002). Bisen and Sheldrake (1981) investigated the stem anatomy of pigeon pea (*Cajanus cajan* (L.) Papilionoideae). They gave reports that the primary vascular tissue of the stem was organized in strands connecting nodes. Also, they reported that xylem vessels werein an aliform confluent pattern, solitary or radial or more rarely, tangential multiples and that secretory duct occurred throughout the plant body.

Agbagwa *et al.* (2007) used variations such as number of layers of tissues in collenchyma, pericyclic parenchyma, sclerenchyma and pith cells in the genus Abrus Adanson species to demonstrate the importance of anatomical features in taxonomy. They reported the variability in number of tracheids and large solitary vessels occurring in radial chains between the species of *Abrus* Adanson (Papilionoideae) studied. They also gave reports on *A. canescens* that it had a distinct anatomical feature of stem, possessing epidermal hairs and degeneration of the pith cells, leaving a prominent central. The different arrangements of genera within the Papilionoideae are so great that a study based on a wider range of the plants' characters seemed urgently needed (El-Gazzar *etal.*, 2013). Although the stem anatomy of the Leguminosae is relatively well-known (Gasson, 1994; Lavin *et al*., 2001; Gasson *et al.*, 2004) the majority of the species of Papilionoideae remain to be studied. It has been suggested that wood anatomical characters are useful in studying relationships at tribal and generic levels in Leguminosae (Gasson, 2001). So little has been elucidated on the stem anatomy of the species of Papilionoideae, most especially in Nigeria. The aim of this study is therefore to document the stem anatomical characters that are of taxonomic value and which could be used in identifying the species within the subfamily.

MATERIALS AND METHODS

For the purpose of this work, ten species in the subfamily Papilionoideae were studied. The species studied include; *Desmodium tortuosum* (Sw.) DC., *Desmodium scorpiurus* (Sw.) Desv., *Desmodium adscendens* (Sw.) DC.,*Cajanus cajan* (L.) Millsp., *Calopogonium mucunoides*Desv., *Centrosema molle* (Mart.) ex. Benth., *Mucuna pruriens* (Linn.) Walp., *Vigna unguiculata* (Linn.) Walp., *Crotalaria retusa* Linn. and *Gliricidia sepium* (Jacq.) Walp. All plant species were collected at different locations in Ile-Ife, Osun State, Nigeria.

The stem anatomy of the species was studied by cutting the transverse, tangential longitudinal as well as radial longitudinal sections of the stem. All sections were made with the aid of a Reichert sliding microtome at a thickness of 10 micrometre and were preserved in 50% ethanol. The sections were stained for 5 minutes in 1% aqueous Safranin O, rinsed in three changes of water and counter stained for 3-5 minutes in 1% solution of Alcian Blue. The counter stained sections were rinsed thoroughly in water and dehydrated through series of ethyl alcohol: 50%, 70%, 90%, and 100% alcohol.

For the purpose of maceration and staining of wood samples' macerates, wood samples from each species were sliced into small pieces using pen knife and macerated using Schultz's fluid obtained by mixing equal volume of 10% chromic acid [dissolved 1g Potassium Nitrate (KNO_3) in 50ml concentrated Nitric Acid (HNO_3)] and 10% Nitric Acid. The maceration was carried out in beakers kept in the oven at 90^{o}C for 8 hours. The macerated wood samples were washed in five changes of water and stained using the same procedure as employed for the sections.

Each section and macerates were mounted on a glass slide in dilute glycerol as mountant after staining. Qualitatively, observations and descriptions of characters of the epidermis, cortex, pith, wood, axial parenchyma, vessels, rays, and fibres were studied and documented. Quantitative data taken were number of epidermal layers, number of periderm layers, number of parenchyma layers, collenchyma layers, and number of rows of ray cells, fibre length, vessel length and width.

Photomicrographs of the characters studied were taken using ACCU-SCOPE microscope. All microscopic measurements were made using an ocular micrometer (inserted into th\e eye piece of the microscope) and were multiplied by the ocular constant with respect to the power under which the measurements were taken to obtain the final values in micrometre. Data generated from this work were subjected to one - way Analysis of Variance using Duncan Multiple Range Test to show significant differences. Simple Descriptive Statistics from SPSS Analysis were also used to calculate minimum, maximum, means and standard error of mean. Cluster Analysis was also carried out on the data using Palaeontological Statistics (PAST).

RESULTS

Desmodium tortuosum (Sw.) Dc. (Plate 1, A-M; Tables 1-3).
Transverse Section: Woody and circular, periderm is present, 4 – 6 layers; parenchyma is 3 – 4 layers. Broken bands of pericyclic fibre and phloem fibres are present, medullary rays are present. Wood is diffuse. Axial parenchyma is paratracheal and vascicentric, medullary rays are present, vessels are in multiple of 2 – 5 occasionally in cluster, oval occasionally circular in shape. Pith is circular and central, crystals are present at the pith.

Rays are heterogeneous and largely uniseriate occasionally biseriate. Fibres are non-storied.

Radial Longitudinal Section: Rays are homocellular, consist upright cells only.

Macerates: Vessels: have simple pits, some have tail, 130.00 µm – 250.00 µm long and 20.00 µm – 120.00 µm wide, perforation plate simple, transverse and oblique end walls. Fibre: non septate, consists fibre tracheids, 70.00 µm – 820.00 µm long.

Desmodium scorpiurus (Sw.) Desv. (Plate 2, A-Q; Tables 1-3).

Transverse Section: Woody herbaceous and circular, cuticle is present, epidermis is 1 layered, parenchyma is 5 – 7 layers, and continuous band of pericyclic fibre is present. Wood is diffuse. Axial parenchyma is paratracheal and vascicentric; vessels are solitary occasionally in multiple of 2, oval occasionally circular in shape. Pith circular and central, crystals are present at the pith and all parenchyma cells at the cortex.

Tangential Longitudinal Section: Rays are heterogeneous and multiseriate ranging from 4 – 7 rows of cells. Fibres are storied.

Table 1 Summary of Qualitative Stem Anatomy of the species of Papilionoideae studied

Species	Stem Type	Stem Outline	Axial Parenchyma	Vessel Pore shape	Vessel Pore type	Vessel Pits
Desmodium tortuosum	Woody	Circular	Paratracheal-Vascicentric	Oval-circular	Multiple/ cluster	Simple
Desmodium scorpiurus	Woody herbaceous	Circular	Paratracheal-Vascicentric	Oval-circular	Solitary/ multiple	Simple
Desmodium adscendens	Woody herbaceous	Circular	Paratracheal-scanty	Circular-oval	Solitary/ multiple/ cluster	Simple/ helical
Mucuna pruriens	Woody	Circular	Paratracheal-Vascicentric	Circular-oval	Solitary/ multiple	Simple
Calopogonium mucunoides	Woody	Circular	Paratracheal-Aliform/ aliform confluent	Oval-circular	Solitary-multiple	Simple
Cajanus cajan	Woody	Circular	Paratracheal-Vascicentric	Oval-circular	Multiple-solitary	Simple
Crotalaria retusa	Woody	Circular	Paratracheal-Scanty	Oval-circular	Multiple/ cluster/ solitary	Simple/ helical
Vigna unguiculata	Woody herbaceous	Circular	Paratracheal-Vascicentric	Circular-oval	Solitary-multiple	Simple/ helical
Centrosema molle	Woody	Circular/undulating	Paratracheal-Vascicentric	Circular-oval	Solitary-multiple	Simple/helical
Gliricidia sepium	Woody	Circular	Paratracheal-banded confluent	Oval	Solitary-multiple	Simple

Species	Crystal	Tylose	Pith Position	Pith Shape	Fibre	Ray Homogeneity	Ray Homocellularity	Ray Cells Orientation
Desmodium tortuosum	+	-	Central	Circular/solid	Non-storied	heterogeneous	homocellular	Upright
Desmodium scorpiurus	+	-	Central	Circular/solid	Storied	heterogeneous	heterocellular	U/P/S
Desmodium adscendens	+	-	Central	Circular/solid	Storied	heterogeneous	homocellular	Upright
Mucuna pruriens	+	-	Not central	Circular/solid	Storied	heterogeneous	heterocellular	U/P/S
Calopogonium mucunoides	-	-	Central	Circular/solid	Storied	heterogeneous	heterocellular	U/P/S
Cajanus cajan	+	-	Central	Circular/solid	Storied	homogeneous	heterocellular	Procumbent/square
Crotalaria retusa	+	-	Central	Circular/hollow	Non-storied	heterogeneous	homocellular	Upright
Vigna unguiculata	-	-	Not central	Oval	Storied	heterogeneous	homocellular	Upright
Centrosema molle	+	-	Central	Elongated	Storied	heterogeneous	homocellular	Upright
Gliricidia sepium	-	+	Central	Circular	Storied	homogeneous	heterocellular	Procumbent/Upright

+ =Present, - = Absent, U/P/S = Upright/Procumbent/Square

Radial Longitudinal Section: Rays are heterocellular, consist upright, occasionally procumbent and square cells.

Macerates: Vessels; have simple pits, some have tail, 60.00 µm – 230.00 µm long and 30.00 µm – 200.00 µm wide, perforation plate simple, transverse and oblique end walls, fibre; non septate, consists libriform fibres and fibre tracheids, 856.50 µm – 1710.00 µm long.

***Desmodium adscendens (*Sw.) Dc. (Plate 3, A-O; Tables 1-3).**

Transverse Section: Woody herbaceous and circular, epidermis is 1 layered, periderm is 4 – 6 layers, parenchyma is 4 – 6 layers, continuous band of pericyclic fibre is present, medullary rays are present. Wood is diffuse. Axial parenchyma is paratracheal and scanty, vessels are solitary occasionally in multiple of 2 – 4 and cluster of 2, circular occasionally oval in shape. Pith is circular and central, crystals are present at the pith.

Tangential Longitudinal Section: Rays are heterogeneous and multiseriate ranging from 5 rows to 22 rows (compound rays) with many uniseriate and occasionally biseriate rays. Fibres are storied.

Radial Longitudinal Section: Rays are homocellular, consisting of upright cells only.

Macerates: Vessels: have simple and helical pits, some have tail, 70.00 µm – 350.00 µm long and 20.00 µm – 90.00 µm wide, perforation plate simple, transverse and oblique end walls. Fibre; non septate, consists libriform fibres and fibre tracheids, 330.00 µm – 1630.00 µm long.

Mucuna pruriens (Linn.)Walp.(Plate 4, A-N; Tables 1-3)

Transverse Section: Woody and circular, epidermis is 1 layered, periderm is present, 4 – 6 layers, parenchyma is 3 – 5 layers, crystals are present in the cortical parenchyma, continuous band of pericyclic fibre is present, phloem fibres also present. Wood is diffuse. Axial parenchyma is paratracheal and vascicentric; vessels are solitary occasionally in multiple of 2 – 5, circular occasionally oval in shape. Pith is circular and not central.

Tangential Longitudinal Section: Rays are heterogeneous and multiseriate, ranging from 3 – 6 rows, occasionally uniseriate. Fibres are storied.

Table 2 Minimum, Mean, Standard Error of Mean and Maximum Values of Stem Anatomical Characters

Species	Vessel length (µm)	Vessel width (µm)	Fibre length (µm)	Number of Epidermal Layer	Number of Periderm Layer	Number of Parenchyma Layer	Number of Collenchyma Layer	Number of Row of Ray Cells
	Min(Mean±S.E.M)Max	Min(Mean±S.E.M)Max	Min(Mean±S.E.M)Max	Min(Mean±S.E.M)Max	Min(Mean±S.E.M)Max	Min(Mean±S.E.M)Max	Min(Mean±S.E.M)Max	Min (Mean±S.E.M) Max
Desmodium tortuosum	130.00 (199.00 ± 9.06) 250.00	20.00 (77.50 ± 6.48) 120.00	70.00 (590.00 ± 45.76) 820.00	0.00 (0.00 ± 0.00) 0.00	4.00 (4.90 ± 0.20) 6.00	3.00 (3.90 ± 0.16) 5.00	0.00 (0.00 ± 0.00) 0.00	1.00 (1.30 ± 0.10) 2.00
Desmodium scorpiurus	60.00 (150.50 ± 9.75) 230.00	30.00 (104.00 ± 10.47) 200.00	500.00 (856.50 ± 79.43) 1710.00	1.00 (1.00 ± 0.00) 1.00	0.00 (0.00 ± 0.00) 0.00	5.00 (5.95 ± 0.20) 7.00	0.00 (0.00 ± 0.00) 0.00	4.00 (6.05 ± 0.21) 7.00
Desmodium adscendens	70.00 (207.00 ± 15.83) 350.00	20.00 (49.50 ± 4.89) 90.00	330.00 (799.50 ± 90.89) 1630.00	1.00 (1.00 ± 0.00) 1.00	4.00 (5.25 ± 0.20) 6.00	4.00 (4.90 ± 0.20) 6.00	0.00 (0.00 ± 0.00) 0.00	1.00 (10.7 ± 2.15) 22.00
Mucuna pruriens	110.00 (219.50 ± 20.31) 430.00	40.00 (181.00 ± 26.10) 400.00	650.00 (1055.00 ± 62.78)1520.00	1.00 (1.00 ± 0.00) 1.00	4.00 (5.20 ± 0.21) 6.00	3.00 (4.05 ± 0.20) 5.00	0.00 (0.00 ± 0.00) 0.00	1.00 (3.55 ± 0.39) 6.00
Calopogonium mucunoides	90.00 (289.00 ± 41.29) 540.00	30.00 (76.50 ± 9.44) 150.00	800.00 (1186.00 ± 101.74) 2960.00	0.00 (0.00 ± 0.00) 0.00	2.00 (4.00 ± 0.25) 5.00	3.00 (4.70 ± 0.23) 6.00	0.00 (0.00 ± 0.00) 0.00	1.00 (5.85 ± 0.68)10.00
Cajanus cajan	70.00 (148.00 ± 11.76) 220.00	50.00 (95.50 ± 13.19) 210.00	250.00 (617.00 ± 47.08) 1100.00	1.00 (1.00 ± 0.00) 1.00	0.00 (0.00 ± 0.00) 0.00	4.00 (4.45 ± 0.11) 5.00	2.00 (5.25 ± 0.46) 7.00	1.00 (1.50 ± 0.20) 4.00
Crotalaria retusa	130.00 (270.50 ± 18.98) 380.00	30.00 (71.00 ± 5.18) 100.00	310.00 (531.50 ± 26.30) 680.00	1.00 (1.00 ± 0.00) 1.00	0.00 (0.00 ± 0.00) 0.00	4.00 (5.05 ± 0.15) 6.00	0.00 (0.00 ± 0.00) 0.00	1.00 (4.95 ± 1.69) 25.00
Vigna unguiculata	50.00 (352.50. ± 48.13) 810.00	20.00 (112.5 ± 19.69) 310.00	1710.00 (1938.00 ± 40.38) 2220.00	1.00 (1.00 ± 0.00) 1.00	0.00 (0.00 ± 0.00) 0.00	4.00 (4.35 ± 0.11) 5.00	0.00 (0.00 ± 0.00) 0.00	1.00 (5.55 ± 0.82)10.00
Centrosema molle	110.00 (166.00 ± 9.10) 230.00	30.00 (132.50 ± 17.71) 230.00	370.00 (925.50 ± 75.09) 1250.00	0.00 (0.00 ± 0.00) 0.00	4.00 (5.20 ± 0.20) 6.00	3.00 (5.55 ± 0.32) 7.00	0.00 (0.00 ± 0.00) 0.00	1.00 (11.20 ± 1.95) 20.00
Gliricidia sepium	160.00 (206.50 ± 5.77) 240.00	60.00 (119 .50 ± 10.50) 190.00	680.00 (832.50 ± 25.60) 1020.00	0.00 (0.00 ± 0.00) 0.00	4.00 (4.85 ± 0.19) 6.00	6.00 (6.85 ± 0.20) 8.00	0.00 (0.00 ± 0.00) 0.00	1.00 (1.50 ± 0.15)3.00

Min = Minimum value, Mean = Mean value, S.E.M = Standard Error of Mean, Max = Maximum value

Radial Longitudinal Section: Rays are heterocellular, consist upright, procumbent and square cells.

Macerates: Vessels: have simple pits, some have tail, 110.00 µm – 430.00 µm long and 40.00 µm – 400.00 µm wide, perforation plate simple, transverse to oblique end walls. Fibre: non septate, consists libriform fibres and fibre tracheids, 650.00 µm – 1520.00 µm long.

Calopogonium mucunoidesDesv.(Plate 5, A-L; Tables 1-3)

Transverse Section: Woody and circular, periderm is 2 – 5 layers, parenchyma is 3 – 6 layers, broken band of fibre pericyclic is present, phloem fibres are also present. Medullary rays are present, wood is diffuse. Axial parenchyma is aliform to aliform-confluent paratracheal. Vessels are solitary occasionally in multiple of 2 – 3, oval occasionally circular in shape. Pith is circular and central.

Tangential Longitudinal Section: Rays are heterogeneous and multiseriate, ranging from 3 – 10 rows, occasionally uniseriate. Fibres are storied.

Radial Longitudinal Section: Rays are heterocellular, consist upright, square and occasionally procumbent cells.

Macerates: Vessels: have simple pits, some have tail, 90.00 µm – 540.00 µm long and 30.00 µm – 150.00 µm wide, perforation plates simple, transverse to oblique end walls, Fibre: non septate, consists fibre tracheids, 800.00 µm – 2960.00 µm long.

Cajanus cajan (L.) Millsp. (Plate 6, A-N; Tables 1-3)

Transverse Section: Woody and circular, epidermis is 1 layered, eglandular trichomes present at the epidermis, collenchyma cells present at the angle, 2 – 7 layers. Parenchyma is 4 – 5 layers. Continuous band of fibre is present, phloem fibres are also present. Medullary rays are present. Wood is diffuse. Axial parenchyma is paratracheal and vascicentric, vessels are in multiple of 2 – 5 occasionally solitary; oval occasionally circular in shape. Pith is circular and central, crystals are present at the pith.

Tangential Longitudinal Section: Rays are homogeneous and largely uniseriate occasionally biseriate and multiseriate ranging from 3 – 4 rows. Fibres are storied.

Radial Longitudinal Section: Rays are heterocellular, consist procumbent and occasionally square cells.

Macerates: Vessels: have simple pits, some have tail, 70.00 µm – 220.00 µm long and 50.00 µm – 210.00 µm wide, perforation plates simple, transverse and oblique end walls. Fibre: non septate, consists fibre tracheids, 250.00 µm – 1100.00 µm long.

Crotalaria retusa Linn.(Plate 7, A-P; Tables 1-3).

Transverse Section: Woody and circular, epidermis is 1 layered, eglandular trichomes are present at the epidermis, parenchyma is 4 – 6 layers, broken bands of pericyclic fibre are present, phloem fibres are also present. Medullary rays are present. Wood is diffuse. Axial parenchyma is paratracheal and scanty. Vessels are either in multiple of 2 – 6 or in cluster of 2 – 3 occasionally solitary, oval occasionally circular in shape. Pith is circular, hollow and central, crystals are present at the pith.

Tangential Longitudinal Section: Rays are largely homogeneous to heterogeneous and multiseriate ranges from 3 – 25 rows (compound rays) occasionally uniseriate. Fibres are non-storied.

Radial Longitudinal Section: Rays are homocellular, consist of upright cells only.

Macerates: Vessels: have simple and helical pits, some have tail, 130.00 µm – 380.00 µm long and 30.00 µm – 100.00 µm wide, perforation plates simple, transverse and oblique end walls. Fibre; non septate, consists fibre tracheids, 310.00 µm – 680.00 µm long.

Vigna unguiculata **(Linn.) Walp.(Plate 8, A-P; Tables 1-3).**
Transverse Section: Woody herbaceous, circular, epidermis is 1 layered, parenchyma is 4 – 5 layers, broken bands of pericyclic fibre are present, medullary rays are present. Wood is diffuse. Axial parenchyma is paratracheal and vascicentric. Vessels are solitary, occasionally in multiple of 2, circular occasionally oval in shape. Pith is oval and not central.

Tangential Longitudinal Section: Rays are heterogeneous and multiseriate ranging from 3 – 10 rows occasionally uniseriate and biseriate. Fibres are storied.

Table 3 Species grouping from Duncan's Multiple Range Test based on Stem Anatomy (Means with the same superscript down the column are not significantly different. P < 0.0001).

Species	Vessel Length (µm)	Vessel Width (µm)	Fibre Length (µm)	Number of Epidermal Layer	Number of Periderm Layer	Number of Parenchyma Layer	Number of Collenchyma Layer	Number of Rows of Ray Cells
Desmodium tortuosum	199.00^{cd}	77.50^{cde}	590.00^{e}	0.00^{b}	4.90^{a}	3.90^{f}	0.00^{b}	1.30^{c}
Desmodium scorpiurus	150.50^{d}	104.00^{bcd}	856.50^{d}	1.00^{a}	0.00^{c}	5.95^{b}	0.00^{b}	6.05^{b}
Desmodium adscendens	207.00^{cd}	49.50^{e}	799.50^{d}	1.00^{a}	5.25^{a}	4.90^{de}	0.00^{b}	10.70^{a}
Mucuna pruriens	219.50^{cd}	181.00^{a}	1055.00^{bc}	1.00^{a}	5.20^{a}	4.05^{f}	0.00^{b}	3.55^{bc}
Calopogonium mucunoides	289.00^{ab}	76.50^{cde}	1186.00^{b}	0.00^{b}	4.00^{b}	4.70^{de}	0.00^{b}	5.85^{b}
Cajanus cajan	148.00^{d}	95.50^{bcd}	617.00^{e}	1.00^{a}	0.00^{c}	4.45^{ef}	5.25^{a}	1.50^{c}
Crotalaria retusa	270.50^{bc}	71.00^{de}	531.50^{e}	1.00^{a}	0.00^{c}	5.05^{cd}	0.00^{b}	4.95^{b}
Vigna unguiculata	352.50^{a}	112.50^{bcd}	1938.00^{a}	1.00^{a}	0.00^{c}	4.35^{ef}	0.00^{b}	5.55^{b}
Centrosema molle	166.00^{d}	132.50^{b}	925.50^{cd}	0.00^{b}	5.20^{a}	5.55^{bc}	0.00^{b}	11.20^{a}
Gliricidia sepium	206.50^{cd}	119.50^{bc}	832.50^{d}	0.00^{b}	4.85^{a}	6.85^{a}	0.00^{b}	1.53^{c}

Radial Longitudinal Section: Rays are homocellular, consist upright cells.

Macerates: Vessels: have simple and helical pits, some have tail, 50.00 µm – 810.00 µm long and 20.00 µm – 310.00 µm wide, perforation plates simple, transverse and oblique end walls, tracheids are also present. Fibre; non septate, consist libriform fibres and fibre tracheids1710.00 µm – 2220.00 µm long.

Centrosema molleMart. ex. Benth.(Plate 9, A-O; Tables 1-3).

Transverse Section: Woody, circular and undulating. Periderm is 4 – 6 layers, parenchyma is 3 – 7 layers continuous band of pericyclic fibre is present, medullary rays are present. Wood is diffuse. Axial parenchyma is paratracheal and vascicentric. Vessels are solitary, occasionally in multiple of 2, circular occasionally oval in shape. Pith is elongated and central, crystals are present in the parenchyma cells surrounding the pith.

Tangential Longitudinal Section: Rays are heterogeneous and multiseriate ranging from 3 – 20 rows (majorly compound) occasionally with uniseriate and biseriate types. Fibres are storied.

Radial Longitudinal Section: Rays are homocellular, consist upright cells.

Macerates: Vessels: have simple and helical pits, some have tail, 110.00 µm – 230.00 µm long and 30.00 µm – 230.00 µm wide perforation plates simple, transverse and oblique end walls. Fibre; non septate, consists libriform fibres and fibre tracheids, 370.00 µm – 1250.00 µm long.

Gliricidia sepium(Jacq.)Walp.(Plate 10, A-K; Tables 1-3)

Transverse Section: Woody and circular, periderm is 4 – 6 layers, parenchyma is 6 – 8 layers, broken band of pericyclic fibres is present. Medullary rays are present. Wood is diffuse. Axial parenchyma is paratracheal and banded confluent, vessels are solitary, occasionally multiple of 2 – 3, oval in shape, tyloses are present in the vessels. Pith is circular and central.

Tangential Longitudinal Section: Rays are uniseriate, biseriate and occasionally triseriate. Fibres are storied and homogeneous.

Radial Longitudinal Section: Rays are heterocellular, consist upright and procumbent cells.

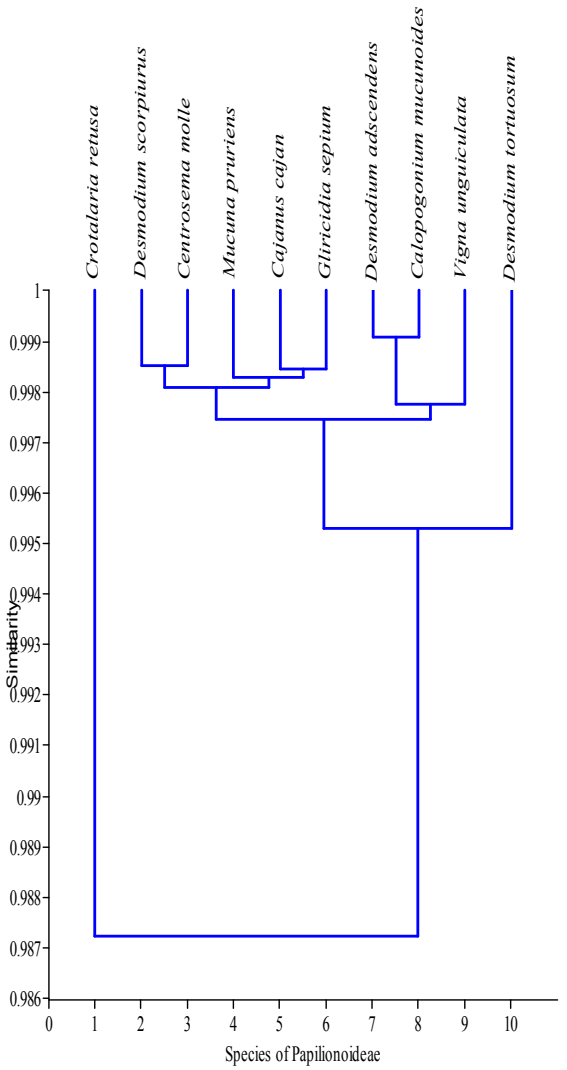


Figure 1: Dendrogram of the Papilionoideae species studied based on the quantitative and qualitative stem anatomical characters.

Macerates: Vessels: have simple pits, some have tail, 160.00 µm – 240.00 µm long and 60.00 µm – 190.00 µm wide perforation plate simple, transverse and oblique end walls. Fibre; non septate, consists fibre tracheids, 680.00 µm – 1020.00 µm long.

Plate 1: Stem Anatomy of Desmodium tortuosum (Sw.) Dc.
A to E –Transverse Section (T.S), D – Trichome at epidermis.
F – Tangential Longitudinal Section (T.L.S)
G – Pith
H & I, K, L, M – Macerates
J – Radial Longitudinal Section (R.L.S)

P – Periderm
Tr - Trichome
Pr – Parenchyma; VA – Vascicentric Paratracheal Axial Parenchyma-Sparse
PF – Phloem Fibre; BF – Broken band of fibre; F – Fibre
SV – Solitary Vessel; PM – Vessel Pore Multiple; V – Vessel
MR – Medullary Ray; UP – Upright Ray
UR – Uniseriate Rays; BR – Biseriate Rays
Cr – Crystal
T – Tail
Pt – Pith

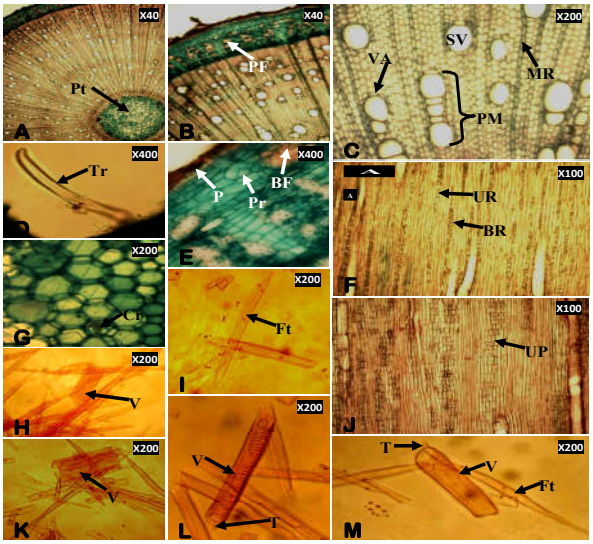


Plate 2 Stem Anatomy of *Desmodium scorpiurus* (Sw.) Desv

A to C –Transverse Section (T.S), C – Pith
D & G – Tangential Longitudinal Section (T.L.S)
E & H –Radial Longitudinal Section (R.L.S)
F – Transverse Section (T.S)
I to Q – Macerates
Ct – Cuticle
Ep – Epidermis
Pr – Parenchyma; VA – Vascicentric Paratracheal Axial Parenchyma
PF – Phloem Fibre; CF – Continuous band of fibre; F – Fibre; FT – Fibre tracheid
SV – Solitary Vessel; PM – Vessel Pore Multiple; V – Vessel element; Vs - Vessel
MR – Medullary Ray; UP – Upright Ray; Pb – Procumbent ray; Sq – Square cells
MsR – Multiseriate Rays
Cr – Crystal
T – Tail

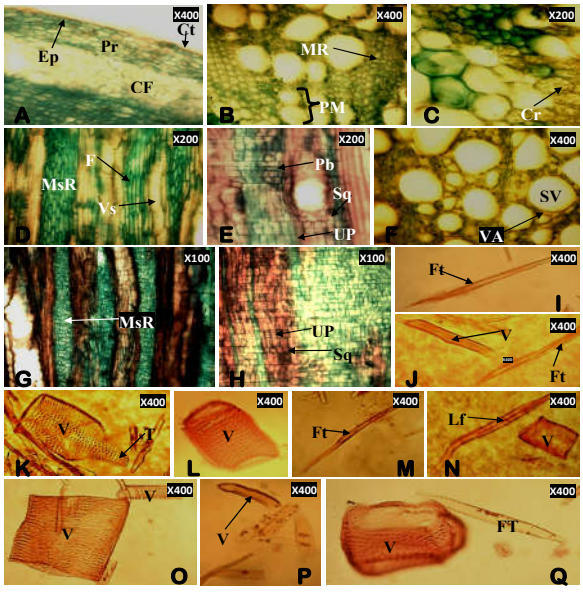


Plate 3 Stem Anatomy of *Desmodium adscendens* (Sw.) Dc
A to E – Transverse Section, D - Pith
F to H – Tangential Longitudinal Section
I & J – Radial Longitudinal Section
K to O – Macerates
EP – Epidermis
P – Periderm
Tr – Trichome
Pr – Parenchyma; SA – Scanty Paratracheal Axial Parenchyma
PF – Phloem fibre; CF – Continuous band of fibre; F – Fibre
SV – Solitary vessel;PM – Vessel Pore Multiple; PC – Vessel Pore Cluster
V – Vessel element; Vs - Vessel
MR – Medullary Ray; UP – Upright Ray; UR – Uniseriate ray; BR – Biseriate ray
MsR – Multiseriate ray; CR – Compound ray
Cr – Crystal

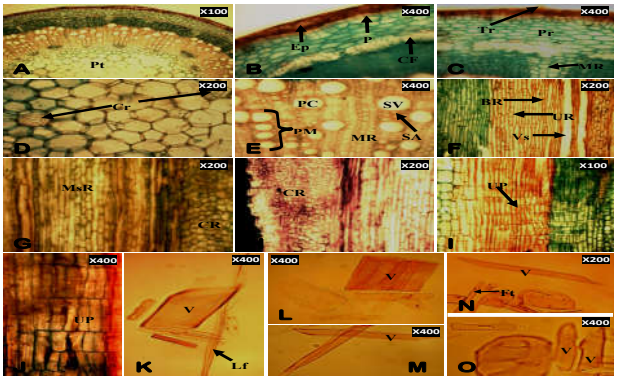


Plate 4 Stem Anatomy of *Mucuna pruriens* (Linn.) Walp

A to D –Transverse Section
E – Tangential Longitudinal Section
F & G – Radial Longitudinal Section
H to N – Macerates
Ep – Epidermis
P – Periderm
Tb – Trichome base
Pr – Parenchyma; VA – Vascicentric Paratracheal Axial Parenchyma
PF – Phloem fibre; CF – Continuous band of fibre; F – Fibre; FT – Fibre tracheid
SV – Solitary vessel;PM – Vessel Pore Multiple; V – Vessel element
MR – Medullary Ray; UP – Upright ray; Pb – Procumbent Ray; Sq – Square ray cell
BR – Biseriate ray; UR – Uniseriate ray; MsR – Multiseriate ray
T – Tail

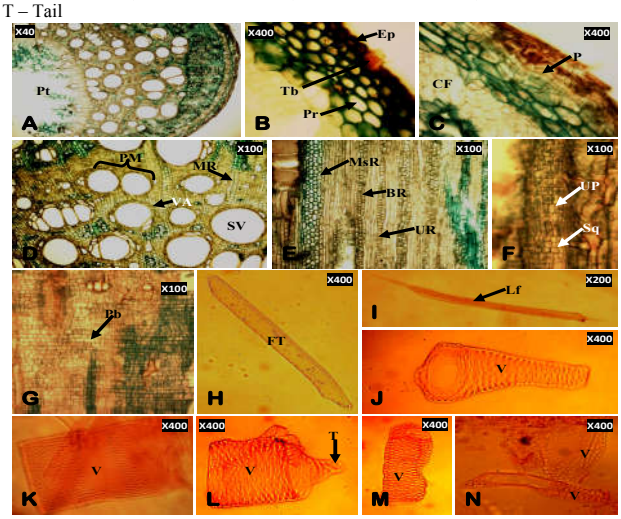


Plate 5 Stem Anatomy of*Calopogonium mucunoides* Desv

A to C –Transverse Section
D & E –Tangential Longitudinal Section
F & G – Radial Longitudinal Section
H to L– Macerates

P - Periderm
Pr – Parenchyma; AL – Aliform Paratracheal Axial Parenchyma
ALC – Aliform-Confluent Paratracheal Axial Parenchyma
Co – Collenchyma
PF – Phloem fibre; BF – Broken band of fibre; F – Fibre
SV – Solitary vessel;PM – Vessel Pore Multiple; V – Vessel element; Vs - Vessel
MR – Medullary Ray; Pb – Procumbent Ray; Sq – Square ray cell
UP – Upright ray; UR – Uniseriate ray; MsR – Multiseriate ray
RD – Resin deposit
T – Tail

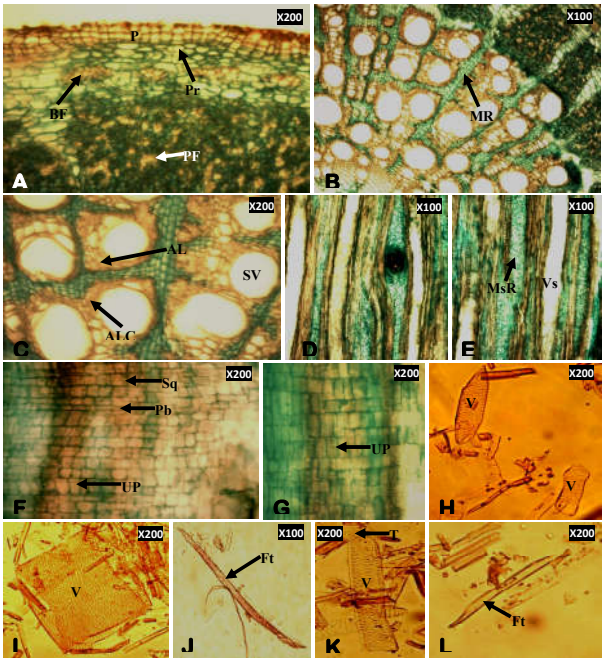


Plate 6 Stem Anatomy of *Cajanus cajan*(L.) Millsp

A to F –Transverse Section, D – Pith
G – Tangential Longitudinal Section
H – Radial Longitudinal Section
I to N – Macerates
Ep – Epidermis
P - Periderm
Pt – Pith
Tb – Trichome base; Tr – Trichome
Pr – Parenchyma; VA – Vascicentric Paratracheal Axial Parenchyma
Co – Collenchyma
PF – Phloem fibre; CF – Continuous band of fibre; F – Fibre; FT – Fibre tracheid
SV – Solitary vessel;PM – Vessel Pore Multiple; V – Vessel element; Vs - Vessel
MR – Medullary Ray; Pb – Procumbent Ray; Sq – Square ray cell
BR – Biseriate ray; UR – Uniseriate ray; MsR – Multiseriate ray
Cr – Crystal
T – Tail

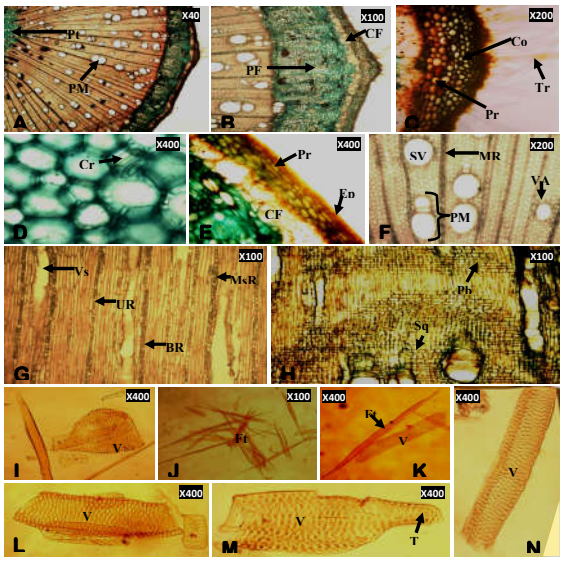


Plate 7 Stem Anatomy of *Crotalaria retusa* Linn.

A to G –Transverse Section, C – Trichome, G – Pith
H & I – Tangential Longitudinal Section
J – Radial Longitudinal Section
K to P – Macerates

Ep – Epidermis
Pt – Pith
Tb – Trichome base
Pr – Parenchyma; **SA** – Scanty Paratracheal Axial Parenchyma
BF – Broken band of fibre; **F** – Fibre; **FT** – Fibre tracheid
PM – Vessel Pore Multiple; **PC** - Vessel Pore Cluster
V – Vessel element
MR – Medullary Ray; **UP** – Upright Ray;
BR – Biseriate ray; **UR** – Uniseriate ray; **CR** – Compound ray
Cr – Crystal
T – Tail

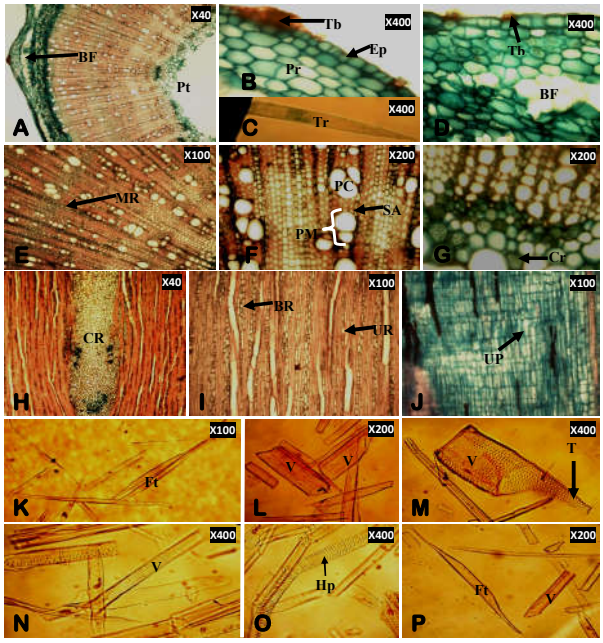


Plate 8 Stem Anatomy of *Vigna unguiculata* (Linn.) Walp

A to E – Transverse Section
F – Radial Longitudinal Section
G – Tangential Longitudinal Section
H to P – Macerates

Pt – Pith
Tb – Trichome base
Pr – Parenchyma; **VA**– Vascicentric Paratracheal Axial Parenchyma
PF – Phloem Fibre; **BF** – Broken band of fibre; **F** – Fibre; **FT** – Fibre tracheid
SV – Solitary Vessel; **PM** – Vessel Pore Multiple; **V** – Vessel element
MR – Medullary Ray; **UP** – Upright Ray;
MsR – Multiseriate Rays; **BR** – Biseriate ray; **UR** – Uniseriate ray
Cr – Crystal
T – Tail

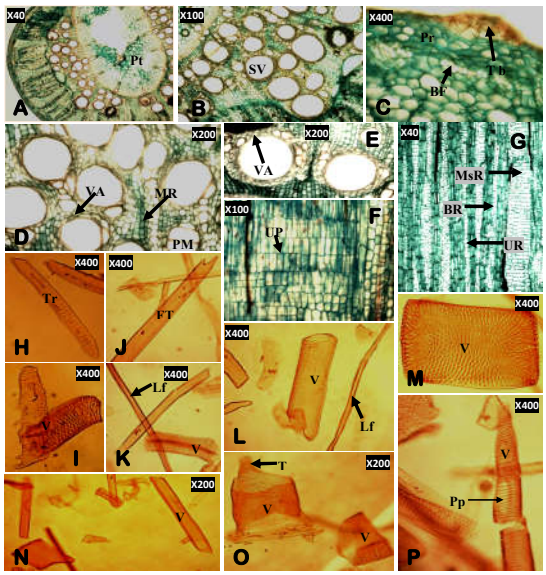


Plate 9 Stem Anatomyof *Centrosema molle* Mart. ex. Benth.

A to G –Transverse Section, E – Pith
H & I – Tangential Longitudinal Section
J – Radial Longitudinal Section
K to O – Macerates

P – Periderm
Pr – Parenchyma; **VA** – Vascicentric Paratracheal Axial Parenchyma
PF – Phloem Fibre; **CF** – Continuous band of fibre; **F** – Fibre; **FT** – Fibre tracheid
SV – Solitary Vessel; **PM** – Vessel Pore Multiple; **V** – Vessel element
MR – Medullary Ray; **UP** – Upright Ray; **CR** – Compound ray; **UR** – Uniseriate ray;
BR – Biseriate ray
Cr – Crystal
PT – Pith

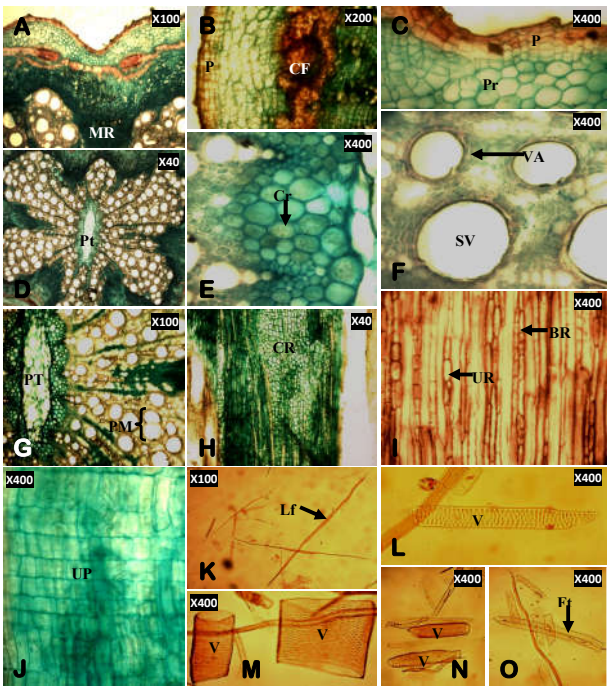
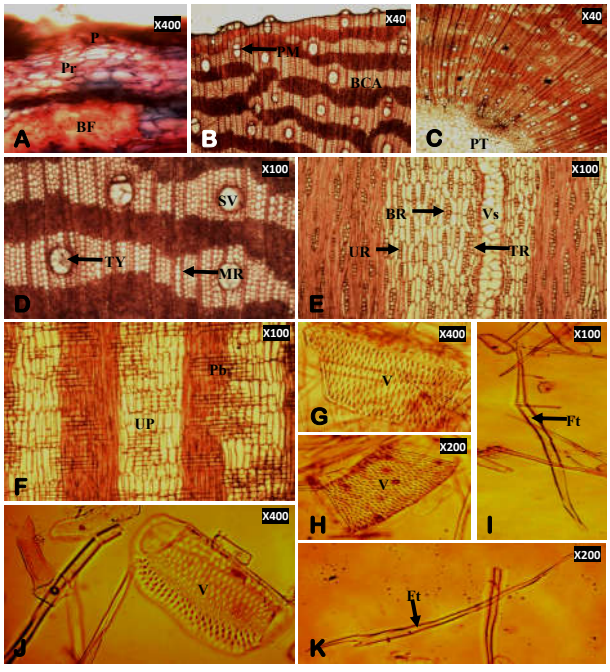


Plate 10: Stem Anatomy of *Gliricidia sepium*(Jacq.) Walp.
A to D –Transverse Section
E – Tangential Longitudinal Section
F – Radial Longitudinal Section
G to K – Macerates

P – Periderm
Pr – Parenchyma; **BCA** – Banded-Confluent Paratracheal Axial Parenchyma
PF – Phloem Fibre; **BF** – Broken band of fibre
SV – Solitary Vessel; **PM** – Vessel Pore Multiple; **V** – Vessel element; Vs – Vessel
MR – Medullary Ray; BR – Biseriate ray; UR – Uniseriate ray; TR – Triseriate ray
Pb – Procumbent ray;
TY – Tyloses
PT – Pith



DISCUSSION

Characters of the stem have been documented to be of taxonomic value (Aguoru and Okoli, 2012, Goremykina and Dinekina, 2016 and El-Chamery *et al.*, 2017). According to Dickison (2000), the secondary xylem has proved to be highly significant in elucidating higher level plant phylogenies. In no other vegetative tissues are the trends of structural evolution as clearly defined. Stepanova *et al.* (2013), reported that wood anatomical study of several members of all genera belonging to the tribe Podalyrieae has led to the insight that wood anatomical characters allow for the distinction between some genera, especially if unique combinations of characters are taken into account.

In this work, anatomy of stem of the members of this subfamily shows consistency in composition, distribution, as well as arrangements of the tissues. However, some of the species possess features which may be employed to delimit them. For instance, the stem of *Desmodiumtortuosum* is the only woody stem among the species of *Desmodium* studied. *Vigna unguiculata*, among the other species studied besides *Desmodium*, is also the only woody herbaceous specieswhile others are woody non-herbaceous. Similarly, stem outline is diagnostic for *Centrosema molle* in that it is circular and undulating whereas it is generally circular without undulations in all the other species studied. Also pith shape through the Transverse Section, in all the species is circular while it is oval in *Vigna unguiculata* and elongated in *Centrosema molle*. Positions of pith in *Mucuna pruriens* and *Vigna unguiculata* are not central, which delimits them from the other species.

Agbagwa *et al.* (2007), used variations in stem characters to demonstrate the importance of anatomical features in the taxonomy of *Abrus* species (Papilionoideae) they studied. They also gave reports on prominent central hollow present in the stem of *Abrus canescens*. In thisstudy, the pith of *Crotalaria retusa* is the only one that possesses a central hollow; other species possess piths that are solid with parenchyma cells filling them completely. Therefore, this character separates *Crotalaria retusa* from the other species studied; and also justifies its separation from the other species in the dendrogram. The cortical region of the stem in all the species is parenchymatous except in *Cajanus cajan* where collenchyma cells are present at the angles of the stem only. This clearly separates *Cajanus cajan* from the other species in this study. This was not reported in the findings of Shahanara*et al.* (2007), in their study on the anatomy of stem of Pigeon pea (*Cajanus cajan*).

Axial parenchyma is generally paratracheal in all the species studied. Metcalfe (1968) reported aliform confluent parenchyma to be more advanced, indicating that the species that possess aliform parenchyma are more recently evolved. In this study, axial parenchyma is of taxonomic value in delimiting the species. Generally, it is vascicentric, except in *Desmodium adscendens* and *Crotalaria retusa* where it is scanty; aliform in *Calopogonium mucunoides* and banded confluent in *Gliricidia sepium*. Axial parenchyma in the stem of *Cajanus cajan* were observed to be vascicentric, this does not agree with the report of Bisen and Sheldrake (1981) who reported that xylem vessels parenchyma in the stem of *Cajanus cajan* were in aliform confluent pattern.

Wood is generally diffuse porous in all species studied. Vessel pores are largely oval to circular (that is more oval than circular) in shape except in *Desmodium adscendens*, *Mucuna pruriens*; *Vigna unguiculata* and *Centrosema molle* where they are largely circular to oval (that is, more circular than oval). They are largely solitary but are occasionally in radial multiple in *Desmodium tortuosum*, *Cajanus cajan* and *Crotalaria retusa*. This is in agreement with the findings of Bisen and Sheldrake (1981) who reported that xylem vessels in the stem of *Cajanus cajan* were solitary, or radial multiple or more rarely tangential multiple. The presence or absence of tyloses in the vessels is an important taxonomic criterion (Singh and Jain, 2006). In this study, *Gliricidia sepium* is the only species where tyloses are present in the vessels, this can be used to delimit the taxon from other taxa. Tyloses prevent the leakage of water through damaged xylem and also prevent the invasion of pathogens through the vessels or tracheids.

Metcalfe and Chalk (1958) and Singh and Jain (2006), reported that vessel lengths show evolutionary advancement in plant species. Elongated or narrow vessels are regarded as a primitive feature or character while short and wide vessels are advanced characters. *Vigna unguiculata* is characterized by its significantly longest vessel than the other species studied and this suggests that it is the least specialized and more primitive. It was also observed that *Vigna unguiculata* has significantly longest fibre than the other species. These may justify its separation from the other species grouped together in the dendrogram. Among the three species in the genus *Desmodium*, *Desmodium tortuosum* is the only species that possesses non-storied fibres among others. This may also be the reason for its separation in the dendrogram. Among all other species studied, apart from the genus *Desmodium*, *Crotalaria retusa* is also the only species with non-storied fibre. This may also explain why it is distinctly separated from the other species studied. According to Singh and Jain (2006), the woods with storied structures are considered advanced over the non-storied woods. Consequently, it can be suggested that among the species of the genus *Desmodium* studied, *Desmodium tortuosum* is the most primitive, with *Crotalaria retusa* sharing in the primitiveness. Vessel length and diameter also delimits *Desmodium scorpiurus* from the other *Desmodium* species in that vessels are shortest in length and widest in diameter than in the other two species, which suggests that the taxon is more specialized or more advanced. Among the other species, besides those that belong to the Desmodium genus, *Crotalaria retusa* may also be more primitive because it has significantly narrowest vessel, while *Mucuna pruriens* may be more advanced because of its significantly widest vessel among all the species studied.

Intravascular pits are generally simple but helical type is also present in *Desmodium adscendens*, *Crotalaria retusa*, *Vigna unguiculata* and *Centrosema molle*. Perforation plates are generally simple, end walls transverse to oblique. Fibres are non-septate and are largely tracheid-like (i.e. fibre tracheids). Crystals are present in all species studied except in *Calopogonium mucunoides*, *Vigna unguiculata* and *Gliricidia sepium* where they are absent.

Gracielza and Regis (1997) in their study of *Jacaranda,* suggested a primitive position for species with exclusively heterogeneous wood rays and an advanced position for species with homogeneous wood rays. Van der Graaf and Baas (1974) also reported that specialization trend from heterogeneous to homogeneous is a reliable tool since it is unidirectional and irreversible. In the present study, wood rays in the Transverse Longitudinal Section of the stem, are generally heterogeneous but homogeneous in *Cajanuscajan* and *Gliricidia sepium*. This indicates that they are more advanced and more specialized than other species. In the Radial Longitudinal Section of the stem, rays are homocellular in *Desmodium tortuosum*, *Desmodium adscendens*, *Crotalaria retusa*, *Vigna unguiculata* and *Centrosema molle* but heterocellular in all other species.

The dendrogram from Cluster of both qualitative and quantitative data of stem/wood anatomical characters is presented in Figure 1. The first main cluster distinctly separates *Crotalaria retusa* from other species. The second main cluster are delineated into two sub clusters and distinctly separates *Desmodium tortuosum* (being the only species in the first sub cluster) from other eight species which are clustered together. The second sub cluster is further delineated into two groups, which separates *Desmodium adscendens*, *Calopogonium mucunoides* and *Vigna unguiculata* from other species but *Desmodium adscendens* and *Calopogonium mucunoides* are the most closely related species since they are at the highest similarity level. In the second group, *Desmodium scorpiurus* and *Centrosema molle* are more closely related and distinctly separated from the other three species which are clustered together. The last sub cluster separates *Mucuna pruriens* from *Cajanus cajan* and *Gliricidia sepium* which are more closely related.

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How to cite this article:

Adedeji, O and Owolabi, J. A (2018) 'Stem Anatomy of Some Members of the Subfamily Papilionoideae With reference to their Identification', *International Journal of Current Advanced Research,* 07(4), pp. 12216-12225.
DOI: http://dx.doi.org/10.24327/ijcar.2018.12225.2141

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