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The Assessment of Phylogenetic Relationships in Fabaceae Family with Reference to Anatomical Characterization of Some Genera

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Abstract: *Phylogeny is the study of relationships among different groups of organisms and their evolutionary development. Phylogeny attempts to trace the evolutionary history of all life on the planet. It is based on the phylogenetic hypothesis that all living organisms share a common ancestry. In this study relationships are determined by anatomical similarities. Anatomy is tightly correlated, as cell and tissue structure has changed with respect to the evolution as novel functional mechanisms. So, it can provide valuable characteristics in phylogenetic analyses, but these are less frequently acquired today than in the past. Therefore, anatomical features used directly to generate a Phylogeny tree or cladistics or a cladogram. Finally, it is concluded that Anatomical characters of vegetative parts of flowering plants have been successfully employed to solve taxonomic problems and for the elucidation of phylogenetic relationships.*

Keywords: Anatomy, Cladogram, Fabaceae, Phylogeny, Tissue

I. INTRODUCTION

All life on Earth is part of a single phylogenetic tree, indicating common ancestry.

Phylogeny is the study of relationships among different groups of organisms and their evolutionary development. Phylogeny attempts to trace the evolutionary history of all life on the planet. It is based on the phylogenetic hypothesis that all living organisms share a common ancestry. The relationships among organisms are depicted in what is known as a phylogenetic tree. Relationships are determined by shared characteristics, as indicated through the comparison of genetic, morphological and anatomical similarities (Brooks *et al.,*1991).

A phylogeny is represented in a diagram known as a phylogenetic tree. The branches of the tree represent ancestral and/or descendant lineages. Relatedness among taxa in a phylogenetic tree is determined by descent from a recent common ancestor. Phylogeny and taxonomy are two systems for classifying organisms in systematic biology. While the goal of phylogeny is to reconstruct the evolutionary tree of life, taxonomy uses a hierarchical format to classify, name, and identify organisms (Gregory, T. R. 2008).

Plant anatomy is the study of the tissue and cell structure of plant organs. The term anatomy, as applied to plants, generally deals with structures that are observed under a high-powered light microscope or electron microscope. Anatomy is tightly correlated, as cell and tissue structure has changed with respect to the evolution of novel functional mechanisms (Cui, Y. *et al.,* (2022)

Anatomical characters of vegetative and floral parts of flowering plants have been successfully employed to solve taxonomic problems and for the elucidation of phylogenetic relationships. It was (Bureau, E. 1864) who for the first time used anatomical characters in plant classification for the delimitation of taxa of various levels, within the family Bignoniaceae.

After the discovery of Electron microscopy, it has brought revolution in all biological fields, and so also in the field of taxonomy. Plant micromorphological studies with the help of SEM and TEM aided by ultra microtome techniques, have proved a powerful tool in studying various anatomical aspects of taxonomic significance. However, till date only a few ultra structural characters have been exploited and applied in plant classification

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Therefore, it can provide valuable characteristics in phylogenetic analyses, but these are less frequently acquired today than in the past. Therefore, anatomical features used directly to generate a cladogram. (Michael G. Simpson, in Plant Systematics (Third Edition), 2019).

The Fabaceae family is chosen for this analysis because it is one of the largest families of flowering plants with 750 genera and approximately 18,000 species (Wojciechowski *et al.,* 2004), and can serve as a model for such an approach. Fabaceae is the third largest family of flowering plants (Angiosperms),which further subdivided into three widely accepted sub-families: the Caesalpiniodeae, the Mimosoideae and the Papilioniodeae. The high-quality protein source from the legumes can be substituted to dietary animal protein (Anderson et al. 1999). Wide range of secondary metabolites from leguminous plants have been utilized as nutraceuticals and various by-products have also been developed for industrial application including biofuels (Lewis *et al.* 2005). Moreover, legumes were playing an important role in sustainable agriculture: managing soil fertility via symbiotic nitrogen fixation and used to grown as a rotation crop with cereal and vegetable (Arianoutsou and Thanos 1996; Velazquez *et al.* 2010). It is widely distributed on every continent and in a wide variety of habitats, and many species are agriculturally and economically important.

Now, it has been accepted facts that Anatomy could be used successfully for phylogenetic studies in specific plant group. With this view, the present work was planned to explore new possibilities to reconsider phylogenetics of some wild members of Fabaceae from Amravati Division.

II. MATERIALS AND METHODS

Collection of Plants from Study Area

It was collected from different natural habitats from Washim and Akola districts of Vidarbha region (MS) India. The collected plant species were identified taxonomically by using flora of Vidarbha (Naik, 1998) and flora of Maharashtra (Karthikeyan and Singh, 2001).

In present work following plants were undertaken.

List of Plants

1. Acacia catechu (L.f.)Willd.
2. Canavalia gladiata (Sw.)DC.
3. Crotalaria junicea L.

Protocol for Anatomy

Suitable sections of plant material were first washed in clean water and then stained with 1% aqueous safranine. Keep sections in safranine for 5 minutes. A section was then placed on clean slide and few drops of aceto-butanol (1:3) were added to it and allowed to act for few seconds. Due to this a moisture is repelled instantaneously, a counter stain (light green) was added on the slide itself and section again washed with aceto-butanol (1:3) in order to remove extra stain. At this stage the material is dehydrated and can even be washed with absolute alcohol, the material then transferred to Xylol:Butyl Alcohol (1:1) grade for fraction of minute and finally transferred it into pure Xylol. The section was now ready for mounting (Wadoodkhan, Marathwada University Journal, 1980).

Anatomical Study of the Selected Plants

The selected plants after correct taxonomic identifications were proceed for anatomical characterization. Fresh material of each species was used for anatomical study. The transverse sections of stem were studied. The fresh stem materials were sectioned using fine razor and proceed for double staining of the sections. After that, the most suitable and stained sections were photographed using Carl Zeiss Binocular Microscope. The anatomical study of each section was done as per the standard method (Metcalfe and Chalk, 1979 and 1985; Fahn, 1990 and Rudall, 1994). Apart from the internal structure as seen in transverse sections of the material, trichome study was also done as trichomes are unique feature of some genus in fabaceae family.

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Observations

PLANT No. – 1

Acacia catechu (L.f.) Willd.

Morphological Characterization-

Habit: Small trees, 5-7 m tall; bark dark-brown; young parts with dense spreading hairs, often purplish.

Leaves: Leaves 10-15 cm long; rachis white-villous with glands between many of the pairs of pinnae and a large gland near the middle of the petiole; stipular spines hooked; pinnae 20-30 pairs, 3-5 cm long, subsessile. Leaflets 30-50 pairs, linear, 4-6 x 1 mm, sessile, subacute, often ciliate.

Flowers: Flowers sessile, in 1-4-nate axillary spikes 5-10 cm long; rachis white pubescent. Calyx campanulate, 1-1.5 mm long, hairy outside; teeth deltoid, ciliate. Corolla 4-5 mm long, dull white; lobes oblong, obtuse. Stamens much exserted.

Fruit: Pods stalked, 6-10 x 1.5 cm, flat, thin, brown, tapering at both ends, glabrous. Seeds 3-10, compressed, brown, polished.

Flowering and Fruiting Time: June to October.

Anatomical Characterization of Stem

In transverse section, stem shoes circular in outline. The present section shows secondary growth.

In transverse section stem shows following parts-

1. Epidermis – it is single layered, thick walled and parenchymatous in nature. The cells of epidermis are somewhat circulars in shape.
2. Hypodermis – it is collenchymatous in nature, it is 2-3 celled thick.
3. Cortex – Hypodermis is followed by cortical region, it consists of two layers i.e. outer cortex and inner cortex. Outer cortex is parenchymatous in nature while inner cortex is sclerenchymatous in nature. Both outer cortex and inner cortex is separated by Layer of Stone cells.
4. Endodermis and Pericycle is not seen.
5. Vascular tissue – secondary vascular tissue is observed, , the secondary tissues are also consisting presence of parenchymatous medullary rays, medullary rays transversing the secondary phloem and xylem tissues.
6. Xylem – large xylem cylinder is seen, it consists of large metaxylem vessels, xylem tracheids and xylem parenchyma.
7. Phloem – secondary phloem cylinder is observed above the xylem cylinder.
8. Pith- it small and pentangular in shape, it consists of parenchyma cells without intercellular cells.

PLANT No. –2

Canavalia gladiata (Sw.)DC.

Morphological Characterization-

Habit: Stout, glabrous, twining, annual herbs.

Leaves: Leaves 3-foliolate; petioles 10-15 cm long, striate, glabrous; stipule triangular, deciduous. Leaflets membranous, ovate-elliptic, 6-15 x 4-10 cm glabrous.

Flowers: Its flowering shoot is about 30 cm long and found at the leaf axils. Its purple flowers are sweetly-scented and notched at the tips.

Fruit: Its fruits are dry, dehiscent pods (legumes), linear-oblong, 6–15 by 1.5–3 cm, and contain many dark brown seeds.

Flowering and Fruiting Time: July to December.

Anatomical Characterizationof Stem

In transverse section, stem shoes circular in outline.The present section shows secondary growth.

In transverse section stem shows following parts-

1. Epidermis – it is single layered, thin walled and parenchymatous in nature. The cells of epidermis are somewhatcirculars to elliptical in shape.
2. Hypodermis – it is collenchymatous in nature, collenchyma is present in 3 - 4 layers.

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3. Cortex – Hypodermis is followed by cortical region, it consists of parenchymatous cells. The cortical region has 6-7 layers of compactly arranged parenchyamatous cells.
4. Endodermis – it is prominently observed in the present transverse section, it is single layered, and consists of elongated and thick-walled cells.
5. Pericycle – it is present in sclerenchymatous patches. These patches are interrupted by presence of parenchyma cells. The sclerenchymatous cells are compactly arranged.
6. Vascular tissue – in the present section secondary vascular tissues are observed, the secondary tissues are also consisting presence of parenchymatous medullary rays running from sclerenchymatous tissues to pith region. These medullary rays are 1-3 celled in thickness.
7. Xylem – secondary xylem cylinder consisting of xylem vessels and xylem tracheids and parenchyma cells. The xylem in primary vascular bundles are also seen prominently. The secondary phloem cylinder is present above the secondary xylen cylinder. The interesting characteristics of secondary phloem is, at some places the amount of secondary phloem produced by the vascular cambium is more. It results into presence of soft phloem tissue more in quantity supporting its twinning habitat.
8. Pith –it is the largest region present in the centre of stem. And it consists of parenchymatous cells.

PLANT No. – 3

Crotalaria junicea L.

Morphological Characterization

Habit: Erect, simple or branched, appressed hairy, annual herbs, 1-2 m tall; stems conspicuously striated.

Leaves: Leaves short-petioled, lanceolate-oblong to linear, 4-15x0.7-4 cm, acute at base, obtuse, mucronate; stipules linear, 1-2 mm long.

Flowers: Flowers 3-15 in lax racemes 3-20 cm long; pedicels 8-10 mm long, pubescent; bracts minute, linear-subulate; bracteoles linear. Calyx 2-2.5 cm long, clothed outside
with dense brown hairs; teeth long, linear. Corolla golden yellow, slightly exserted.

Fruit: Pods cylindric, 2-3x0.4-0.6 cm, brown-silky, 10-15 seeded. Seeds reniform, ash-grey, polished.

Flowering and Fruiting Time: August to November.

Anatomical Characterization of Stem

1. Stem is pentagular in outline, having five prominent angular outgrowths.
2. Presence of uniseriate trichomes.
3. Present transverse section showing secondary growth.
4. T.S. of stem is devided into following layers.

- Epidermis – Single layered, consisting thick walled cells, and some what circular in shape cells.
- Hypodermis – In angular growth region, it is Collenchymatous in nature. While in rest of the regions hypodermis is consisting Palisade tissue in 2-3 layers.
- Cortex – Hypodermal region is followed by cortex. Cortex consists of 3-4 layers of Parenchymatous cells.
- The inresting feature is presence of external vascular bundles in cortical regions. Which are conjoint, collateral and open and may be showing formation of secondary Vascular tissues.
- Vascular tissue – In the present material secodary vascular tissue are prominent seen. The secondary xylem on inner side of cambium and secondary Phloem on the outer side. The cylinder of secondary Xylem is seen interuppted by the presence of uniseriate medullary rays. At some places secondary xylem is consisting Metaxylem, Protoxylem, Tracheids and Xylem Parenchyma.
- Pith – pith is large situated in the centre. It consists of compactly arranged large parenchyma cells.

III. DISCUSSION

In the present study it is observed that the anatomical characters differ as per habit of the genus.

In *Acacia catechu* (L.f.) Willd. the Transverse section of Stem shows well developed secondary xylem cylinder and secondary phloem cylinder having medullary rays, While the pith region is small. In case of *Canavalia gladiata(Sw.) DC.* the habit is twining herb. Anatomically stem shows secondary growth by the formation of secondary cylinders of

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xylem and phloem. The pith is large. The quantity of phloem is more supporting twining habit of plant. The erect herbaceous plant *Crotalaria junicea L.*in anatomy of stem secondary growth is seen, The outline is pentangular, with uniseriate hairs. The large parenchymatous pith is present in the centre. The presence of additional external vascular bundles are interesting features in *Crotalaria juniceaL.*

IV. CONCLUSION

Anatomical characters of vegetative parts of flowering plants have been successfully employed to solve taxonomic problems and for the elucidation of phylogenetic relationships.

In the present study, three different genera having tree, herb and climber stem are studied anatomically, these characters are conserved as important evidence for evolution.

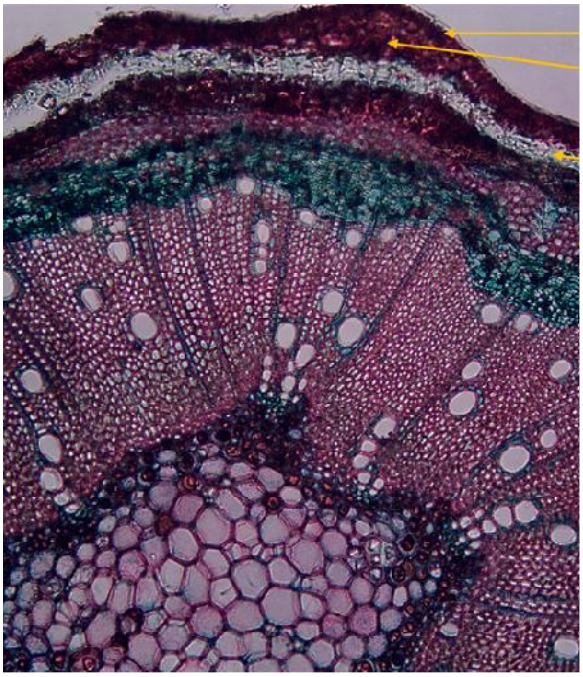
Some characteristics in all genera are found similar viz. single layered epidermis, presence of secondary vascular tissue and uniseriate medullary rays. But presence of less pith region in *Acacia catechu*as compare to other genera.

The sclerenchyma is present in cortical region, which is considered as primitive characters supporting tree habit. While formation of more secondary phloem is considered as advanced character in support of twining habit in *Canavalia gladiata.*

In the present study it is seen that the secondary xylem is more in Acacia catechu indicating it's woody nature and tree habit while in case of *Crotalaria junicea* the Scleroids are seen in pericycle region and supporting herbaceous nature. In *Canavalia gladiata* anatomical study showing anomalous secondary growth to explain the twining habit.

Finally, it is concluded that, The Anatomical characters has proved to be useful in tracing the evolutionary trends and for creation of interrelationship among the taxa. Woody *Acacia catechu* might be considered as primitive, while *Crotalaria junicea* and *Canavalia gladiata* are herbaceous but evolved.

PHOTO PLATE NO. – 1



T.S. stem-*Acacia catechu* (L.f.)Willd.-

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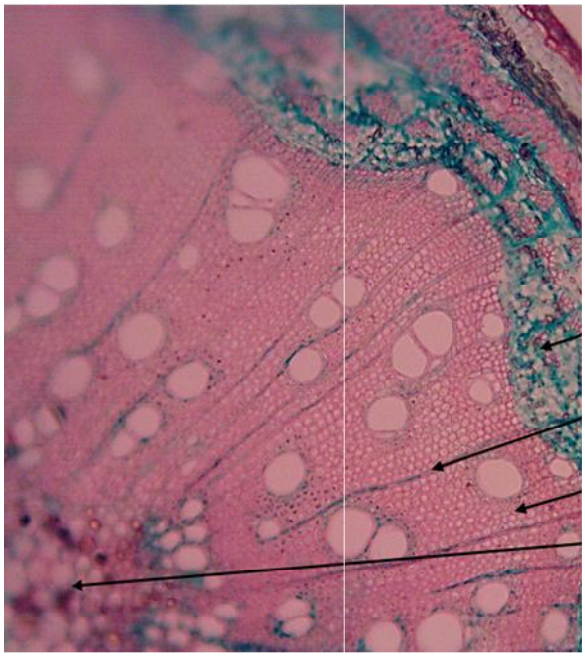
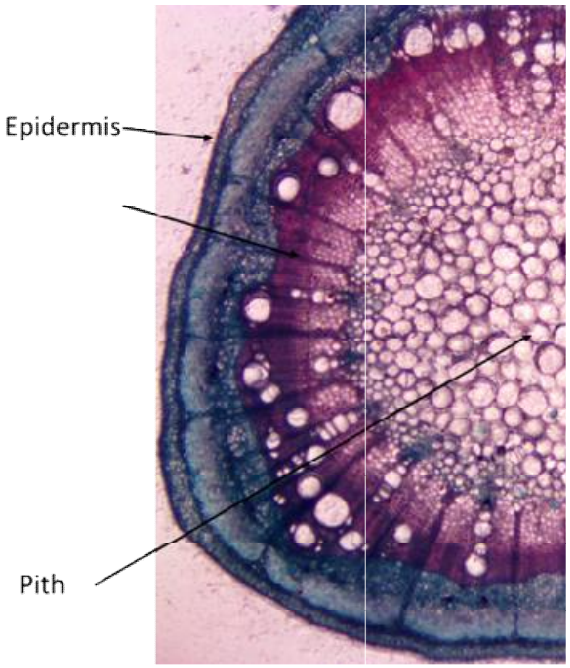


T.S. stem-*Acacia catechu* (L.f.)Willd.-

PHOTO PLATE NO. – 2



T.S. stem-*Canavalia gladiata (Sw.)DC.*

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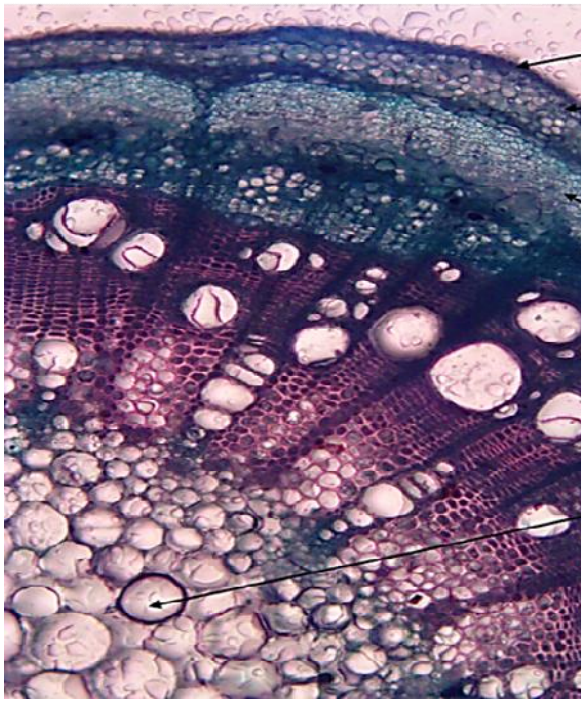
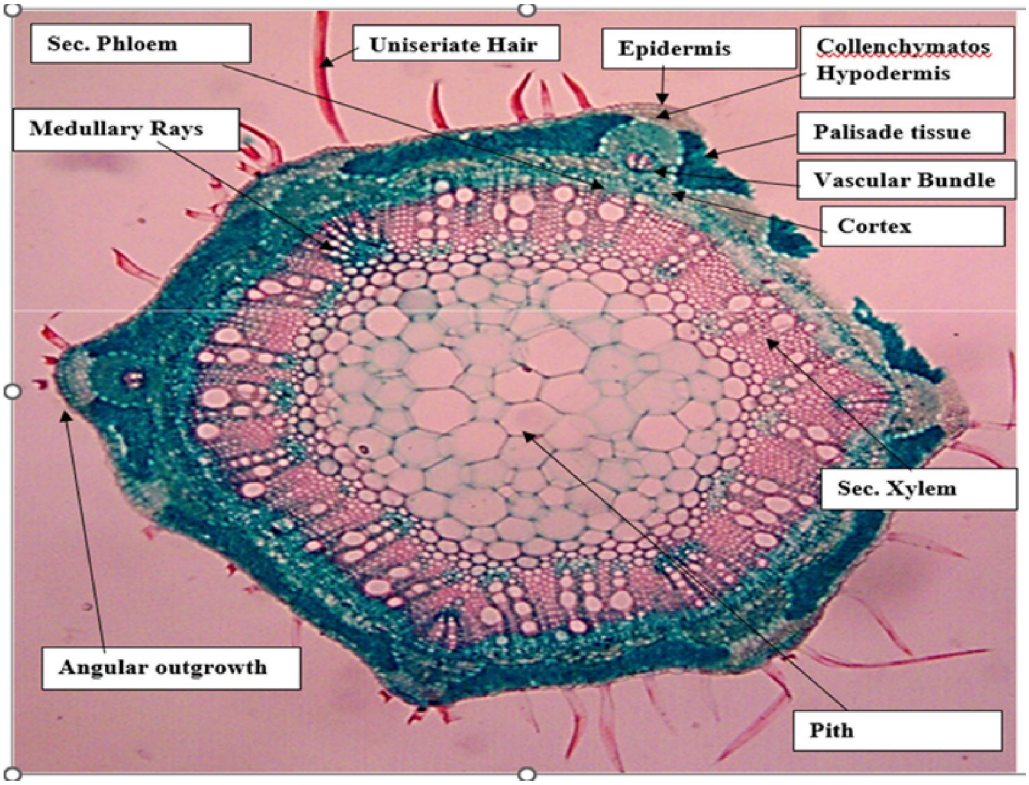


T.S. stem-*Canavalia gladiata (Sw.)DC.*

PHOTO PLATE NO. – 3



T.S. stem-*Crotalaria junicea L.*

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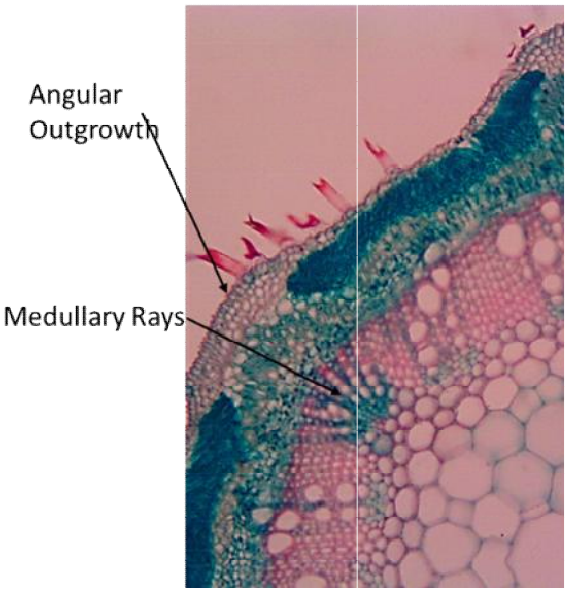

T.S. stem-*Crotalaria junicea L.*

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