Taxonomic and Anatomical Study of Pongamia pinnata
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**INTRODUCTION**

As a result of successful infection of host plant by the pathogen, a number of physiological changes occur in the plant. The physiological changes occurring in the diseased plant have their counterparts in the anatomical and morphological changes – *morbid anatomy*. Anatomical and morphological changes are brought about by affecting individual cells and growth of entire plant or of an organ, vegetative as well as floral. Thus, changes in physiology of plants due to infection result into several anatomical and morphological changes which are expressed externally in the form of visible symptoms. Symptoms are characteristic of a disease by which it can be recognized in the field\(^{(4)}\).

*Agrobacterium* species produces galls on the leaves of the tree *Pongamia pinnata*. The disease gains its name from the large tumour like swellings (galls). Apart from reducing the marketability of nursery stock, which other types of damages are caused by this disease is an important aspect of study. For this, differentiating study of anatomy of
healthy plant parts against the anatomical study of galls on leaf was carried out. The following investigations, therefore, deal with these differentiating observations.

Also the taxonomic study of *Pongamia pinnata* was carried out using standard literature to determine the exact position of this plant in Plant Kingdom.

**MATERIAL AND METHODS**

**Taxonomic study:**

Taxonomic position of *Pongamia pinnata* in plant kingdom was determined using Bentham and Hooker Classification system\(^{(70,71,72)}\).

**Anatomical study:**

**Sample collection:**

A small piece of root was cut in the primary state of growth from a young *Pongamia pinnata* plant. Similarly the sample for anatomical study of stem of pongam tree was collected in primary state of growth. A mature healthy leaf as well as a mature leaf with galls were selected for studying leaf anatomy of *Pongamia pinnata*. Thin transverse sections of these samples were selected for staining.
**Slide preparation:**

Permanent slides of transverse sections of collected samples were prepared using standard method as follows \(^{73,74}\) :-

The sections were placed in aqueous safranine and then washed with water until these became colourless. Afterwards dehydration of sections with 30% alcohol, 50% alcohol, 70% alcohol and 90% alcohol was followed sequentially. The sections were stained with light green for a few seconds and then destained with 90% alcohol for 5 minutes. Lastly sections were kept in absolute alcohol for 5 minutes and then cleared with xylol. Mounting was done in DPX (or Canada Balsam) and slides were allowed to dry for 4 days.

The slides were observed under low power objective of microscope and studied with the help of standard literature\(^{75,76,77,78}\).

To study the fibres observed in the centre of the galls, two tests were performed. First, freshly cut sections of gall were stained with cotton blue stain and observed under low power of microscope.
Second, the section was taken on slide with a little water, treated with iodine for some time and then a drop of concentrated sulphuric acid was added on to the section. On addition of water, change in colour is observed under microscope (10X)\(^{(73,74)}\).

**RESULTS**

**Taxonomic study of *Pongamia pinnata***:

According to Bentham and Hooker Classification System, the plant *Pongamia pinnata* belongs to Division Spermatophyta as it is a seed bearing plant. As the ovules in pongam tree are present within the ovary leading to presence of seed within the fruit and also its flowers are complex so it is included in subdivision Angiospermae.

It belongs to class Dicotyledonae because embryo of the seed of pongam tree possesses two cotyledons. Flowers are pentamerous, reticulate venation in leaves and tap-root system is present. The subclass is Polypetalae as the petals of the flower are free and series is Calyciflorae as the flowers of *Pongamia pinnata* are perigynous.

The plant is included in order Rosales due to monocarpellary, marginal placentation and family is
Leguminosae due to presence of legume fruit. Root nodules are observed in pongam tree in association with *Rhizobium* species.

Flowers are zygomorphic and floral formula is $\frac{2}{5}$ $K(5)$ $C_{1-2+2}$, $A_{110+1}$, $G_{1}$. Also corolla is papilionaceous, therefore *Pongamia pinnata* is placed in subfamily Papilionaceae.

Genus is *Pongamia* and species name is *pinnata* due to the presence of pinnately compound leaves.

The position of *Pongamia pinnata* in Plant kingdom can be represented as

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Anatomical study of *Pongamia pinnata*:

**Stem Anatomy:**

Internal structure of stem shows three distinct regions – the epidermis, the cortex and the stele (photograph No. 1 & 2).

*Epidermis* consists of a single layer of cells and is the outermost layer of the stem. It contains stomata. The outer cell walls are greatly thickened and heavily cutinized. The cells are compactly arranged and do not possess intercellular spaces. In transverse section, the cells appear almost rectangular. It serves mainly for restricting the rate of transpiration and for protecting the underlying tissue.

The region that lies next to the epidermis is the *cortex*. The innermost layer of the cortex is *endodermis*. The part of the cortex situated between the epidermis and the endodermis is generally divided into two regions, an outer zone of collenchyma and an inner zone of parenchyma cells.

The parenchyma cells are regular in shape, have comparatively thin walls (than collenchyma cells), and are not greatly elongated. When they are exposed to the light they develop chloroplasts and are known as chlorenchyma cells.
Photo No. 1: Transverse section through stem of *Pongamia pinnata*.

Photo No. 2: Transverse section through stem of *Pongamia pinnata*. 
The innermost layer of the cortex is the endodermis consisting of barrel-shaped, elongated, compact cells, having no intercellular spaces among them. The cells contain starch grains and thus the endodermis may be termed as starch sheath. It consists of a single layer of cells which surrounds the stele.

The part of the stem inside the cortex is known as the stele. It consists of three general regions – the pericycle, the vascular – bundle region and the pith.

The region between the vascular bundles and the endodermis is known as the pericycle. The vascular bundles are arranged in the general form of a broken ring. Each vascular bundle consists of three parts – that nearest the centre of the stem contained thick walled cells and is known as xylem. The peripheral portion of the bundle is composed of thin-walled cells called phloem. The xylem and phloem are separated by a cambium layer, which is composed of meristematic cells.

The xylem is composed of three different types of cells-tracheary cells, that include trachieds and vessels, wood fibres and wood parenchyma.
The primary phloem of the dicotyledonous stems consists of 3 types of cells – sieve tubes, companion cells and phloem parenchyma. The arrangement of xylem and phloem is collateral. Presence of cambium makes the bundles open.

The vascular bundles are separated from each other by radial rows of parenchyma cells known as pith rays. The pith ray cells are elongated in a radial direction.

The centre of the stem is composed of thin-walled parenchyma cells and is known as the pith. The cells have distinct intercellular spaces (75,76,77,78).

**The root anatomy:**

The tap root of *Pongamia pinnata* is thick and long. Lateral roots are numerous and well developed. A cross section through root in the primary state of growth shows the following tissues from outside within (photograph no. 3,4).

*Epidermis* is the outermost layer of the primary root. It is made up of elongated, thin walled parenchymatous cells that are compactly arranged. The root epidermis is typically uniseriate. Many of the epidermal cells extend out in the form of tubular unicellular root hairs. They are absent in the nearest proximity of the apical meristem, and they die off in the older root part. The root epidermis is devoid of
stomata. The main functions of the epidermis are protection and absorption of water and solutes.

Next to the epidermis on inner side lies the cortex. The cortex is massive and consists of thin-walled polygonal parenchyma cells having sufficiently developed intercellular spaces among them. The parenchyma cells of the cortex contain abundant starch grains in them. The cortex of roots is devoid of chlorophyll.

*Endodermis* is the innermost distinct layer of cells differentiated from innermost layer of cortex. The endodermis is uniseriate. The cells of endodermis are living and characterized by the presence of Casparian strips or bands in their radial and transverse walls. Guttenberg (1943) says that the suberin like materials are found in the strips that extend completely around the cells. This band is a part of the primary wall.

The layer next to endodermis is *pericycle*. It constitutes the outer boundary of the primary vascular cylinder.

The phloem of the root occurs in the form of strands distributed near the periphery of the *vascular cylinder*, beneath the pericycle. The xylem forms discrete
strands, alternating with the phloem strands. The roots typically show an exarch xylem i.e. the protoxylem is located near the periphery of the vascular cylinder, the metaxylem farther inward. The phloem is also centripetally differentiated i.e. the protophloem occurring closer to the periphery than the metaphloem.

The protoxylem consists of annular and spiral vessels whereas metaxylem of reticulate and pitted vessels. The phloem consists of sieve tubes, companion cells and phloem parenchyma. The parenchymatous conjunctive tissue occurs in between xylem and phloem strands (75,76,77,78).

**The leaf anatomy:**

In case of *Pongamia pinnata*, the alternate, compound imparipinnate leaves consist of 5 to 7 leaflets which are arranged in 2 to 3 pairs and a single terminal leaflet. Leaflets are 5-10 cm long, 4-6 cm wide and pointed at the tip.

In the transverse section of the healthy leaf of *Pongamia pinnata*, following tissues were observed (photograph no. 5 & 6).

The leaf consists of the dermal system, the vascular system and the ground tissue system. The *epidermis*
Photo No. 5: Transverse section of healthy leaf of *Pongamia pinnata* through midvein.
persists as the dermal system. Compact arrangement of epidermal cells and presence of cuticle and stomata are the main features of the leaf epidermis. The leaf is covered on both surfaces by epidermis. The outer surfaces of the epidermis are frequently covered with a cuticular layer formed of cutin. As the outer walls of the epidermis are thick, and cutinized, water does not pass through them rapidly and the transpiration from the surface of the epidermis is greatly reduced. Stomata are present only on the lower epidermis.

The main part of the ground tissue of a leaf blade is mesophyll characterized by an abundance of chloroplasts and a large intercellular space system. The mesophyll is differentiated into palisade parenchyma and spongy parenchyma. The cells lying below the upper epidermis are elongated and arranged in a compact layer. The layer is called palisade parenchyma. Below the palisade parenchyma, the cells are loosely arranged and have varying shapes. This region is called spongy parenchyma. These are thin-walled cells having big intercellular spaces among them. As the chloroplasts are more dense in the palisade tissue than the spongy tissue the upper surface of the leaf appears to be deeper green than the lower surface. Rod shaped crystals are
recorded in the palisade tissue of *Pongamia* leaf\(^{76}\). Secretary cavities of various types usually appearing as transparent dots are recorded in *Pongamia* leaf \(^ {76}\).

Many large and small *vascular bundles* are present. Vascular bundles are conjoint, collateral and closed. Vascular strands form an interconnected system in the median plane of the blade parallel with the surface of the leaf. The vascular bundles in the leaf are called veins and the pattern formed by these veins. In *Pongamia pinnata*, leaf shows reticulate venation having the largest vein along the median longitudinal axis of the leaf. This is the midvein. It is connected laterally with somewhat smaller lateral veins. Each of these veins is connected with still smaller veins, and from them, other smaller veins diverge. The ultimate branchings form meshes delimiting small areas, or areoles, of mesophyll. The tissue associated with the larger veins rises above the surface of the leaf and thus forms ribs on the dorsal side.

Each vascular bundle is surrounded by bundle sheath. Bundle sheath is parenchymatous and in case of large bundles it extends up to the epidermis with the help of thin-walled parenchymatous cells. The xylem is present
towards the upper epidermis and the phloem is situated towards the lower epidermis.

**The anatomy of galls on leaves:**

As compared to the above anatomical features of healthy leaf, the anatomy of the galls shows considerable difference.

Gall manifests itself initially as small swelling on the leaf surface and resembles the callus tissue. As galls become older, their shape becomes quite irregular and mostly they are connected to the leaf surface by only a narrow bit of tissue\(^ {18} \). Anatomical study of the transverse section through gall shows that though the tissues visible in healthy leaf are also observed here but these are completely disorganized (photograph no. 7). Young tumours consist largely of parenchyma, but later there is some differentiation, some groups of cells becoming transformed into strands of vessels and trachieds. But these vascular elements, which may be numerous and widely distributed do not become organized into a well-defined system and they have little or no connection with the vascular bundles of the host plant\(^ {79} \) (photograph no. 8).

In the centre of the gall, the fibres are observed (photograph no. 9 and 10). These fibres did not take cotton
Photo No. 9: Transverse section through gall showing fibres in the centre.

Photo No. 10: Transverse section through gall showing fibres in the centre.
blue stain thus discarding the presence of fungal hyphae. When treated with iodine and concentrated sulphuric acid, the fibres turned blue thus confirming the presence of cellulose in these fibres (photograph no. 11, 12).

**DISCUSSION:**

From the observations and results, it is clear that anomalies are produced in the anatomy of the diseased foliage of *Pongamia pinnata* as compared to the healthy leaf. The anatomical features of stem and root of such pongam plant with diseased foliage were observed to be normal i.e. similar as in healthy plant.

Such galls with disorganized tissue system, may interfere with water and nutrient transport and result in unthrifty plants, however in many cases the damage is mainly cosmetic\(^ {17} \). As the tumor is not protected by an entire epidermis, leaves the tissue susceptible to secondary pathogens, insects and saprophytes.

Thus, morphological and anatomical abnormalities produced in the foliage of *Pongamia pinnata* due to gall formation not only reduces its marketability and medicinal value but gives a shabby look to the plant, which otherwise is a good ornamental plant.
Photo No. 11: Fibres after adding iodine.

Photo No. 12: Fibres from galls turned blue on addition of iodine and concentrated H₂SO₄.