6.1 Introduction

Anatomy or the internal form and structure of plant organs, is another classical source of data used in plant taxonomy. Anatomical data are often extremely useful in solving problems of relationships because they can often suggest with greater confidence the homologies of morphological character states, and they can help in the interpretation of evolutionary directionality (=Polarity). It is some time held that comparative anatomy has outlived its usefulness and that it is a sterile discipline, bereft of new advances. Nothing could be further from the truth. A glance at the papers in recent symposia volumes (e.g., Robson, Cutler and Gregory 1970; Baas 1982b; White and Dickison, 1984) plus the stimulating ecological hypotheses of Carlquist (1975) and the architectural forms and function analysis of Tomlinson (1978; Honda, Tomlinson and Fisher, 1981, 1982; Fisher, 1984) reveal many new challenges; in fact, interest is now focused clearly on the function and adaptive value of anatomical features, even in fossil plants (e.g., Taylor, 1981), which will help to reveal more clearly the homologies of structure for purpose of classification and the reconstruction of phylogeny.

6.1.2 Types of Anatomical Data

Anatomical data can be viewed as consisting of two types: endomorphic (as contrasted with exomorphic, or morphological, data) and ultrastructural. The former are the observable, largely with the light microscope and latter by the use of the transmission electron microscope (TEM). As one approaches comparative cellular structure with TEM the transition with cytology is reached.

6.1.3 Vegetative Anatomical Characters

In contrast to vegetative morphological features, vegetative anatomical characters have been used with more regularity than floral ones. This is probably due to the view point that if additional data are believed desirable to solve a
Taxonomic problem, then looking inside the leaves, stems and roots could potentially yield different information than that from reproductive organs. Data from floral and fruit anatomy usually correlate well with observed reproductive morphological features, and hence serve to refine the relationship already documented instead of offering totally new insights.

6.2 REVIEW OF LITERATURE

6.2.1 Leaves

Leaves provide many anatomical characters as shown by the following examples in the Bromeliaceae (Robinson, 1969); Leguminosae (Lackey, 1978); Palmae (Glassman, 1972); and Myrtales (Keating, 1984). Within leaves, data can be taken from the petiole, blade or cotyledons. An example of the former comes from Schofield (1968) in the Guttiferae, and the latter by Philipson (1970) in Rhododendron of Ericaceae.

Most leaf features of taxonomic significance derive from the blade. The epidermis (and hypodermis when present) provide many useful characters as shown by Stace (1966); Baranova (1972); and Cutler, Carton and Harris (1980). These are ultra structural studies of epidermal cell features, such as the general cell variations in the Irrami cae (Brown and Johnson, 1962); Phytoglyphs in Eucalyptus species (Carr, Milkoritis, and Carr 1971). The mesophyll offers some useful features, including also the presence of crystals (Heintzelman and Howard 1948, Icacinaceae, Malthew and Shah 1984, Verbanaceae). The structure of the bundles can also vary (Brittan 1970; D’Arcy and Keating 1979), especially in Graminae with C₄ Photosynthesis (Kranz Anatomy) in which bundle/sheath cells have chloroplorst centrifugally localized and without grana (Johnson and Brown 1973; Brown 1977). Patterns of venation are also useful as has been shown on numerous occasions (Dickison, 1969). Sclereids in leaves are taxonomically valuable too, as indicated by Rao, Bhattacharya and Das 1978; Rhizophoraceae/Tucker (1977; Magnoliaceae). Leaf anatomical data as with most all other types of data, have also been treated phenetically for a more quantitative view of relationships (e.g., Lubke and Philipps, 1973).
6.2.2 Stems

Tissues and cells of stems also provide many helpful lines of taxonomic evidence. The stems of both herbaceous and woody plants form starch grains that are of some limited taxonomic utility (Czaja, 1978). In plants that have laticifers (or latex-containing duct, as in the Euphorbiaceae), the anatomy of these structures, their starch grains, as well as the chemistry of the latex itself is taxonomically important (Mahlberg, 1975; Biesboer and Mahlberg, 1981; Mahlberg and Pleszczynska, 1983; Mahlberg, Raugh, and Schnepf, 1983). At the ultrastructural level, it has been shown that members of the closely related Cruciferae and Capparidaceae (Capparales) have dilated cisternae of the endoplasmic reticulum (ER) in phloem parenchyma cells filled with protein granules, filaments, or tubules (Hoefert, 1975). These features are also known from root cap and epidermal cells and in leaves (Iversen, 1970a; Behnke, 1977a, c) as well as in the inner integuments of ovules (Ponzi, Pizzolongo and Caputo, 1978).

Many significant taxonomic features have been derived from stem tissue. Leaf gaps and nodal anatomy are two of these useful features, and they can be helpful in both herbaceous and wood plants (Dickison, 1969, 1975; Howard, 1970; Keating, 1970. Generally transverse (Cross-), radial, and tangential sections are made of the secondary xylem and the arrangement of tissues are compared and contrasted for taxonomic utility. Detailed microscopic study of the individual cell types may also prove significant (Findlay and Levy, 1970) for good SEM photos of details of wood structure.

6.3 Materials and Methods

6.3.1 Histochemical Studies

Histochemical localization (Krishnamurthy, 1999)

Histochemical studies play an important role in understanding the structure and content of plant cells at subcellular level. It gives adequate as well as significant information to the researchers about the plant structure. It also imparts a detailed account of the chemical components and their localization in a cell or tissue. Hence the histochemical analysis gives a dynamic approach to know the chemical
properties of an intact cell or tissues (both quantitatively and qualitatively). The following histochemical tests are involved in the present study.

6.3.2 Test for polyphenols

(Toluidine blue O method) Feder and Wolf, 1965; McCully, 1966).

Procedure: The fresh plant specimens (in vitro and in vivo) leaf and stem of Pueraria phaseoloides were sectioned, washed and dipped in water. Then the sections were stained for 1-5 minutes and washed in running tap water for 1 minute [0.05% stain in benzoate buffer (Benzoic acid 0.25 g, sodium benzoate 0.29 g in 200 ml water) at pH 4.4] until most of the stain was washed out. Then the stained sections were mounted.

Result: The presence of Polyphenol indicates turquoise green or blue green.

6.3.3 Test for lignin (Toluidine blue 'O' method)

Procedure: Fresh leaf and stem materials (in vitro & in vivo) of Pueraria phaseoloides were sectioned and kept in water and they were stained for 1-5 minutes and washed in slow running tap water for one minute [0.05% stain in benzoate buffer (benzoic acid 0.25 g, sodium benzoate 0.29 g) in 200 ml water at pH 4.4] until most of the stain was removed. The stained sections were mounted for observation.

Result: The appearance of blue green confirms the presence of lignin.

6.3.4 Macroscopic studies

Morphological characters of different parts of in vivo and in vitro plants of Pueria phaseoloides were studied using fresh plants with the help of a binocular dissection microscope.

6.3.5 Anatomical Studies

The required samples of the different plant parts such as leaf and stem of in vivo and in vitro plants were fixed in FAA (Formalin 5 ml + Acetic acetic acid 5 ml + 70% Ethyl alcohol 90 ml). After 24 hours of fixing, the specimens were
dehydrated with gracial series of TBA (Testing Butyl Alcohol). The sections were stained with TBO (as per the method of O’ Brien et al., 1964). Since TB is a polychromatic stain, the staining results were distinct. The dye rendered pink colour to the cellulose of cell walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies, etc. Certain sections were also stained with saffranin.

Microscopic description of tissues were supplemented with photomicrographs wherever necessary. Photography were taken using Nikon E-400 (Japan) Fluorescent microscope. For normal observations bright field was used. Descriptive terms of the anatomical features were as given in the standard anatomical book (Easu, 1964).

6.4 Observation

6.4.1 Anatomy of in vivo Plant stem

Plants grown in the nursery of Research Centre were used in this study. A transverse section of the stem with primary growth have pith in the centre, with vascular bundles forming a distinct ring visible when the stem is viewed in cross section. The stem is covered with an epidermis, with a layer of water proof cuticle. The epidermis also possesses stomata for gas exchange. A cortex of parenchyma cells lies between the epidermis and vascular bundles (Plate 36 a-f). The epidermal layer is followed by 2-3 layered collenchyma cells. After collenchyma 2-3 layered chlorenchyma cells are seen beneath, and then the lignified sclerenchymatous cells (Plate 36:4). Well developed phloem cells lie beneath the sclerenchymatous cortical layers (Plate 36:5). Between the xylem and phloem there is cambium (Plate 36:6). Between the vascular bundles there are multilayered medullary rays (Plate 36:6) with lignified walls and is blue in colour. It forms a continuous layer beneath the cortical layers. There is a distinct central pith with thin walled parenchymatous cells.

6.4.2 Anatomy of in vitro plant stem

When the in vivo stem structure is compared with in vitro stem much similarity is noticed in both. (Plate 36 a-f) The content of lignin and polyphenols
were more in \textit{in vitro} plant sections than in \textit{in vivo}. This may be due to the heavy accumulation of nutrients during \textit{in vitro} cultures.

\subsection*{6.4.3 Anatomy of \textit{in vivo} and \textit{in vitro} plant leaf}

The transverse section of the leaf of \textit{Pueraria phaseoloides} consists of the following tissues. An epidermis that covers the upper and lower surfaces. An inner chlorenchymatous mesophyll and the vascular tissues. The polyphenol is prominently seen in leaf (Plate 38 g, 5, 6). The epidermis is single layered (plate 38g.1) and includes several differentiated cell types, such as epidermal cells, guard cells, subsidiary cells, and epidermal hairs (trichomes) in both \textit{in vivo} and \textit{in vitro} leaves. The stomata are more numerous over the abaxial (lower epidermis) than the adaxial (upper epidermis).

\subsection*{6.4.4 Mesophyll}

Between the upper and lower epidermis both \textit{in vivo} and \textit{in vitro} leaf sections there is a parenchymatous or chlorenchymatous tissue called the mesophyll (plate 38 c and d). An upper palisade layer of tightly packed, vertically elongated cells, one to two cells thick, directly beneath the adaxial epidermis is present. Its cells contain more chloroplasts than the spongy layer. These long cylindrical cells are regularly arranged in one to three rows. Beneath the palisade layer is the spongy layer. The cells of the spongy layer below the palisade are more rounded and tightly packed. There are large intercellular air spaces. These cells contain fewer chloroplasts than those of the palisade layer. The pores or stomata of the epidermis open into substomatal chambers, connecting to air spaces, between the spongy layer cells. Veins are located in the spongy layer of the mesophyll. They are the vascular tissues of the leaf. Xylem and phloem are present (Plate 38 g, h). Xylem lies over the phloem. Both are embedded \textit{in vitro} in a dense parenchymatous tissue, called pith. \textit{In vitro} leaf seems to contain greater amount of lignin and polyphenols (Plate 38 4, g, 6 respectively).

\subsection*{6.5 Discussion}

Histochemical test for polyphenols and lignin clearly proves the presence of polyphenol and lignin in the leaf and stem of \textit{Pueraria phaseoloides} and in large
amount. The *in vitro* leaf and stem include more lignin and polyphenol, than those *in vivo* leaves and stem. This may be due to the nutrients absorbed by explants. All the vascular bundles are of similar type and pith is very large both *in vivo* and *in vitro* (Plate 38. f, 3) sections.

Histochemical localization of stem and leaves both *in vitro* and *in vivo* showed positive results for the presence of polyphenols and lignins in the cells, of various tissues. Transverse sections of stem & leaves stained with Toluidine blue -O exhibited greater positivity for the presence of polyphenols, and lignins. Earlier phytochemical studies (Gonzalez et al., 1996; Hussein et al., 1999, Alvarenga et al., 1999) have shown the presence of a number of phenolic compounds which could be responsible for the high incidence of antibacterial activity.

The presence of biologically active compounds such as phenols, polyphenols, tannins, alkaloids flavanoids and terpenoids in various plants are known to possess antibacterial activity (Panizzi et al., 2002).

Similarly the extracts of *Momordica dioica* and *Bridelia micrantha* demonstrated inhibitory activities against different bacterial species including *Staphylococcus aureus*, *Salmonella typhi*, *Bacillus cereus*, *Kebsiella pneumoniae* and *Pseudomonas aeruginosa*. In the present study *Pueraria phaseoloides* also possesses the active compounds viz. polyphenols and lignins (Plate 38 f, g).

Based on previous literature about 20% of the reported plants showed inhibitory activities against pathogenic microorganisms due to the presence of the above mentioned bioactive compounds (Panizzi et al., 2002). This study has also confirmed the role of those compounds for antimicrobial activity.

**Histochemical analysis**

- T.S of stem and leaf stained with Toluidine blue ‘O’ showed great positivity to polyphenol in the xylem region.

- T.S of stem and leaf stained with Toluidine blue ‘O’ showed polyphenols in the sclerechyma region.
PLATE: 36. Cross section of Pueraria phaseoloides stem

a)
1. epidermis with polyphenols
2. collenchyma with polyphenols
3. chlorenchyma
4. sclerenchyma
5. Phloem
6. cambium
7. xylem vessel

Scale of magnification

a-d × 100

e,f × 200
PLATE: 37 Cross section of *Pueraria phaseoloides* stem

C

1. Sclerenchyma with lignified walls
2. Collenchyma with polyphenols

E-1. Primary xylem

*Scale of magnification*

d,f×200

a,b,c,e×100
PLATE: 38 Cross section of leaf of *Pueraria phaseoloides*

- **a,b**
  1. vascular bundles

- **c,d**
  1. palisade
  2. epidermis
  3. cuticle
  4. spongy tissue

- **e,f**
  1. metaxylem
  2. protoxylem
  3. pith
  4. Lignified wall of bundle sheath.

- **g,h**
  1. cuticle
  2. epidermis
  3. Sclerenchyma
  4. collenchyma
  5. palisade tissue
  6. Leaf veins

Scale of magnification

- a × 200
- b-h × 100