MATERIALS AND METHODS

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All the plant parts, i.e., the leaves, shoot apices and bark of the two trees, viz. Gmelina arborea Roxb. (Verbenaceae) and Tabebuia rosea DC. (Bignoniaceae), used for this study were collected from the trees growing in the university campus and arboretum of the Maharaja Sayajirao University of Baroda.

2.1 Sequential elongation of the petiole

To study the petiole elongation in relation to time, about 20 young leaves with 1.5 cm long petioles in Gmelina arborea and 5 cm long petioles in Tabebuia rosea were tagged on the current year's shoot. By this method it was easy to date the leaves and it also helped in monthly collections. Each petiole was equally divided with India ink into the basal which includes the pulvinus region (PB), middle region (PM) and distal region (PT). The petioles in Tabebuia rosea have a dilated distal end which is also included in the distal region of the petiole. The length of each part was measured to the nearest millimeter every alternate day at a fixed time till it ceased elongation.

2.2 Microtechnique

2.2.1 Fixation and preparation of the block

Leaves and shoot apices collected for the anatomical study were fixed on the spot in FAA of Craf III (Berlyn and

Miksche, 1976), dehydrated in tertiary butyl alcohol and embedded in paraffin wax with ceresin, m.p., 58-60 °C (Johansen, 1940).

2.2.2 Microtomy and staining

Transverse and longitudinal sections were obtained at 8-12 um thickness on a rotary microtome. Ribbons were spread on glass slides using 1% formalin and Haupt's adhesive (Berlyn and Miksche, 1976) and deparaffinized in xylene and stained.

Transverse, radial and longitudinal sections of the bark were obtained with a Leitz sliding microtome at 15 - 20 µm thickness.

The details of stains and histochemical reagents and techniques used are summarized in Table I.

2.2.3 Photography

Photomicrographs were taken on a Zeiss photomicroscope or a Leitz dialux 22 fluorescent microscope with bright field attachment and using different coloured filters. Polarized light micrographs were taken with a Leitz Vario Orthomat 2 polarization microscope.

ORWO NP 55 (125 ASA) black and white negative film and Konica or Kodak colour film (100 ASA), were used for the photomicrography.

Table I. Details of stains, histochemical reagents and techniques used.

Purpose	Stains/ techniques	References
1. General staining	a. Toluidine blue O pH 4.6	Berlyn and Miksche, 1976
	b. Safranin O-Fast green FCF	Johansen, 1940
2. General staining for phloem and callose	Tannic acid-Ferric chloride Resorcin blue (lacmoid)	Cheadle et al., 1953
3. Identification of phloem specific proteins	a. Mercuric bromophenol blue	Mazia et al., 1953
	b. Coomassie brilliant blue	Krishnamurthy, 1988
4. Starch localization	Iodine-Potassium iodide	Johansen, 1940
5. Lignification	Phloroglucinol HCl solution	Johansen, 1940

2.3 Diagrammatic representation and measurements

Diagrammatic representations were constructed from camera lucida drawings taken from the respective regions of the petiole.

Two dimensional drawings to represent the internode-node-petiole vascular continuum were constructed with the help of serial camera lucida drawings of the transections of the shoot apex and by identifying vascular strands from successive sections associated with the leaf

trace. The two dimensional diagrams are presented as if the node and the petiole are split longitudinally and the vasculature viewed from the outside of the node. The vascular strands are shown considerably straightened in a simplified pattern and represented in these diagrams.

All measurements were taken by the aid of the camera lucida drawings and using an ocular micrometer.

2.4 Clearing

Leaf clearings were prepared following the method of O'Brien and McCully (1981). Cleared lamina were then washed thoroughly in running water and stained with toluidine blue O. They were dried and then pressed in a herbarium press. Prints were made directly from these clearings which served as negatives.

Pieces of mature node and petiole were cleared in boiling lactic acid for one hour and kept in the same solution for 2-3 days. The cleared pieces were then thoroughly washed in water and stained with dilute aqueous safranin O. Photographs were taken with a Wild-Leitz Stereozoom microscope.