MATERIALS AND METHODS

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Crataeva nurvala Buch. Ham. (Capparaceae) and Salvadora persica L. (Salvadoraceae) were selected for this study mainly for the following reasons (i) during preliminary survey, the arrangement of their vascular system in the petiole was found to be convenient for the proposed investigation (ii) the plant materials were easily available in the locality and no work of the nature proposed to be investigated has hitherto been reported in these plants (iii) Crataeva nurvala is a deciduous tree in which leaf fall occurs after 4 or 5 months and Salvadora persica is considered an evergreen tree in which leaves remain for about one year and there is no simultaneous leaf fall.

The leaves, shoot apices and bark used for this study were collected from the trees growing in the university campus and the arboretum of the M.S.University of Baroda.

2.1 Elongation of petiole and dating of leaves

Young leaves were tagged on the current year's shoot just after they emerged and developed from their buds. This method was found to be suitable for dating the leaves and

facilitated the monthly collection. In Crataeva the petiole was equally divided as basal, middle and distal regions and marked with India ink and the length was measured to the nearest millimeter at a fixed time of the day till it ceased elongation. The data were collected from about 20 leaves.

As the petiole of Salvadora is very short compared to Crataeva no study was undertaken on petiole elongation.

2.2 Microtechnique

Details of preparing 8-10 μm paraffin sections of FAA or Craf III fixed materials were obtained from Berlyn and Miksche (1976). The sections were stained with safranin O - fast green FCF (Johansen,1940), with toluidine blue O (Berlyn and Miksche,1976) or with decolourized aniline blue (O'Brien and McCully,1981) for fluorescence microscopy. Aniline blue is generally considered as a specific indicator for callose, but this has been questioned (Smith and McCully,1978). However, I obtained comparable results using highly specific lacmoid technique on paraffin sections (Cheadle et al.,1953), for fluorescence microscopy. Starch was localized with I_2KI (Johansen,1940) and mercuric bromophenol blue (MBB) was used to localize phloem-specific proteins (Mazia et al.,1953). Phloroglucinol-HCl test was conducted for lignin (Johansen, 1940).

Photomicrographs were taken with a Zeiss photomicroscope or Carl Zeiss Fluoval epifluorescence microscope on ORWO NP 55 (125 ASA) black and white negative film. Konica or Kodak film (100 ASA) was used for colour photography. For aniline blue induced fluorescence the absorption maximum for the dye tissue complex was ca 430-510 nm (with exciter B 224 combined with barrier G 247). Polarized light micrographs were taken with a Leitz Vario Orthomat 2 polarization microscope.

For thin sections,materials were processed according to the method of Roland (1978). Sections of 1 um thickness were obtained from Spurr-resin embedded blocks with glass knives on a Porter Blum MT-2 or Reichert Ultracut E ultramicrotome. For light microscopy sections were stained with toluidine blue 0 (Berlyn and Miksche,1976). For electron microscopy ultrathin sections were collected on uncoated copper grids, stained with uranyl acetate and lead citrate and viewed in Philips CM 10 electron microscope.

Serial transverse, radial and longitudinal sections of the bark were obtained with a Leitz sliding microtome at 15-20 µm. Sections were stained with tannic acid-ferric chloride-resorcin blue (Cheadle et al.,1953).

The diagrammatic representation of the inter-node-node-petiole continuum was constructed from camera

lucida drawings of serial sections taken from the respective regions of the continuum. Measurements were made by using an ocular micrometer.

2.3 Clearing

Leaf clearings were prepared following the method of O'Brien & McCully (1981). Cleared lamina were stained with toluidine blue O and dried in the herbarium press. Prints were made directly from the cleared leaves which served as negatives.

Pieces of young nodes with the petiole were cleared in boiling lactic acid for 1 hr and kept in the same solution for 3 days at room temperature. Cleared specimens were washed in water and stained with dilute aqueous safranin O. Photographs were taken with a Wild-Leitz Stereozoom microscope.

2.4 Localization of metabolic activity of the phloem in the petiole

The physiological activity of the phloem elements which is assumed to be reflected by their ability to reduce the metabolites, was investigated by the use of thiazolyl blue-MTT (Van der Schoot,1989). Hand sections of fresh

petiole were continuously shaken in a thiazolyl blue solution (1 mg/ml 10 mol. m^{-3} phosphate buffer, pH 6.5) of 27°C for 2-3 hrs; washed for 5 minutes in tap water (27°C) and studied microscopically. Alternatively,a thiazolyl blue solution (27°C) was allowed to be sucked up for an hour by transpiration pull followed by 15 min chasing period with tap water (27°C). Then free-hand sections were obtained and they, after washing in water, were examined microscopically and photographed.

2.5 Determination of the vascular architecture in the internode/leaf-system

Two kinds of experimental system were used: (i) an internode/leaf-systems (IL-system) consisting of an internode-node-unit with the attached leaf and (ii) a petiole/leaflet-systems (PL1-system) consisting of a petiole with one, two or three leaflets. In both experimental systems materials were excised under water and placed with the cut end in tap water for 30 minutes prior to the experiment.

Dyes were supplied separately to the basal splits of the internode of an IL-system and a petiole of PL1-system and sucked up through the various vascular bundles. The dyes, fast green and safranin O were used in 0.1% aqueous

solution and toluidine blue O in 0.05% aqueous solution respectively.

2.6 Wound cambium and wound phloem in Crataeva

Young and mature petioles were wounded (during summer months and under natural light conditions) to regenerate wound cambium and wound phloem by a vertical and horizontal razor blade incision which partly severed the petiole vascular system. Wounded petioles were usually kept for 8-10 days to obtain wound cambium and would phloem, which were later processed for microscopy. Transverse and longitudinal sections were taken from the wound region. Sections of wound phloem were observed under UV light for aniline blue induced fluorescence of the regenerated wound sieve elements.