Unit - III

MATERIALS & METHODS
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This research work was carried out at the Department of Botany, Aligarh Muslim University, Aligarh, India.

III [i] Selection of plants

Adult and normal trees of comparable age and vigour were selected for the study of each species. Nine trees each of C. limon and C. sinensis were picked up in random from the plantation at the Botanical garden located in University fort, Aligarh Muslim University, Aligarh. Nine trees each of C. reticulata var. kinnow and C. paradisi were similarly selected at random from the plantation at Government Jawahar Park, District Aligarh.

III [ii] Collection of samples

Blocks of 1-2 sq. inch size containing sapwood along with attached bark were dug out in the middle of the trunks from the southern side of the tree in morning hours. The samples were collected at regular intervals for a period of three consecutive calendar years (2005, 2006 & 2007). Three samples were collected from three trees on each month with a gap of ten days. The next sets of samples were collected from the same set of trees at least after three months. Care was taken to collect the samples at least 10 cm away from the wounded spot whenever the tree was used the next time.

The samples for ontogenetic study were collected only once during the period of study, i.e. in April 2005 for C. limon, June 2005 for C. sinensis, September 2005 for C. paradisi and December 2005 for C. reticulata var. kinnow. Six samples of each species
were collected by the aforesaid method at different height levels from individual tree
on the same day of the month already stated.

III [iii] Fixing & Preservation

All samples were fixed on spot in F.A.A. (Berlyn & Miksche 1976) and then
transferred to 70% ethanol after 72 hrs. for preservation.

III [iv] Sectioning

Suitably trimmed small pieces of these samples were sectioned in transverse,
tangential and radial longitudinal planes at a thickness of 6-12 μm on a Reichert
sliding microtome (Austria). Double stained sections in different combinations are
mounted in Canada balsam after dehydration in ethanol series and clearing in xylol
(Sass 1958).

III [v] Stains used

The following stains were used alone and in combination depending on the
purpose of study:

1. For bark and wood
   a. Heidenhains Haematoxylin - Safranin (Johansen 1940).
   b. Heidenhains Haematoxylin – Bismark brown (Johansen 1940).
   c. Tannic acid – Ferric chloride – Lacmoid (Cheadle et al. 1953).

2. For cambium
   a. Heidenhains Haematoxylin.
   b. Tannic acid – Ferric chloride (Foster 1934).
III [vi] Maceration

For the study of individual cells maceration was done following the method of Ghouse et al. 1974. Safranin was used for phloem and xylem fibres and vessel elements while lacmoid for sieve-tube elements.

III [vii] Quantitative estimation of the tissue

The relative proportions of different types of elements were determined on the basis of the area occupied by the respective elements following the method described by Ghouse & Iqbal (1975).

III [viii] Biometrical analysis

Measurement of cambial initials and their derivatives (except for length of vessel elements, sieve-tubes and fibers) were carried from transverse and tangential longitudinal sections with the help of an ocular micrometer scale under the specific magnification of Quasmo IS:3686 compound microscope. The dimensions of the fibers, vessel elements and sieve-tubes were taken after macerating the bark and wood separately following the method of Ghouse et al. 1974. An average of 400 measurements, macerated or sectioned, was taken on random basis. The mean and range of cell dimensions were determined after pooling the readings obtained from different samples.

Rays of varying heights were measured and categorized as short (1 to 10 cells), medium (11 to 20 cells) and tall (21 to above cells), whereas widthwise they were categorized as uniseriate (one cell wide), biseriate (2 cells wide) and multiseriate (3 or more cells wide).
Double stained sections were viewed under an Olympus 20CHI trinocular optical source compound microscope while macerated materials were viewed under a Metzer 555 monocular optical source compound microscope. Original light photomicrographs were recorded digitally using an Olympus 5 mega pixels camera.

III [ix] Biostatistical analysis

The data was subjected to statistical analysis and the range, mean, standard error, standard deviation, coefficient of variation and least significant difference were calculated. This was performed with the SPSS 15.0 and Minitab 11.12 statistical computer softwares.

1. Range

The first value represents the lowest limit and the second value represents the highest limit of observations in different sets of data.

2. Mean (X)

The arithmetic mean is the sum of the number of values divided by the total number of observations.

3. Standard deviation (SD)

It determines the statistical significance of values obtained and is a measure of variability or of dispersion which is the positive square root of mean of the square of deviation of the individual observations from their arithmetic mean.

4. Standard error (SE)

It is the computational error measuring the variability or fluctuations among sample means.
5. Coefficient of variation (CV\%)

It measures the relative magnitude of variation present in observation relative to magnitude of their arithmetic mean. It is the ratio of standard deviation to arithmetic mean expressed as percentage and in a unitless number.

6. Least significant difference (LSD)

If the difference between the averages of two treatments exceeds the estimated LSD at 5\% or 1\% level the difference between those treatments is said to be significant at 5\% or 1\% level.