MATERIAL AND METHODS

The material for this investigations were collected between 1983 to 1985 from the following locations
1. F.R.I. arboretum Dehra Dun.
2. Gauhati district of Assam.
5. Hills of Mussoorie, Dehra Dun district.

The species were accurately identified by the Botanist of Systematic Botany Branch of F.R.I. and the herbarium material is housed in the herbarium of F.R.I. Dehra Dun. For the collections of samples in general, the following procedures were adopted. The culm and leaf samples from at least five living culm were collected from the 8th internode. In addition some authentic material from the herbarium was used for additional investigation and confirmation of the results.

To determine the structural variability on the culm surface, within one internode, samples of 1 cm length were cut out and divided into two to adjust in the stub size of cambridge Stereoscan - S-600 Scanning Electron Microscope. The observations were repeated in 5 culm samples of each species. In order to see variation from bottom to top of the culm, in a few species, one complete culm leaving two internodes alternately were observed. After gaining some basic information and the extent of variations on culm surface from these samples 8th internode was found to contain average and consistent appendages or encrustations. Accordingly five pieces from the five different culms at 8th internode were taken. The leaf samples studied were collected from different branches of varying ages, which include young to mature leaves.

The culm and leaf samples of each species were fixed in 4% glutaraldehyde, prepared in phosphate buffer of 6.8 pH. The samples were then dried by critical point drying method using liquid Co2. Since the epidermal structures on the culm and leaf surface of bamboos are quite sturdy, drying by critical point drying method is not necessary. Barring few initial observations in the beginning of this study, all samples were processed by dehydration in graded series of alcohol (10-100%) followed by 1:1 ratio of absolute alcohol: Amyl acetate. The samples were ultimately kept in pure Amyl acetate and were dried by pressing in case of leaves between two microscope slides, the culm samples were kept in covered syracuse and allowed to dry at room temperature. This method of drying did
not show any difference than that of samples dried by critical point drying apparatus. The herbarium material can also be used effectively without any treatment.

For SEM observations, the samples were mounted on the stubs using colloidal silver for culm and double coated scotch tape for leaves. The samples were coated with Gold or Gold/Palladium in Edwards 306 Vacuum Coater using filament and sputter coating accessories and in Polaron sputter coater having a cooling stage. The results obtained from the samples coated by Polaron sputter coater having cooling stage were much better. The observations were made under Cambridge Stereoscan S-600 Scanning Electron Microscope at Wood Anatomy Branch of F.R.I. The leaf samples were observed on both adaxial and abaxial surface.

Diagnostic SEM features

Stomata - Each bamboo leaf stoma opening is surrounded by a pair of guard cell having narrow middle and enlarged ends and a subsidiary cell adjacent to each guard cell thus giving a typical dumb-bell shape, characteristic of monocot stomata. The subsidiary cells have a distinct shape, thus can be distinguished easily from a regular epidermal cell. The stomata can be classified according to the shape of these subsidiary cells as seen in surface view. Four types of subsidiary cells are recognised as by Metcalf (1960).

(a) Parallelsided
(b) Low-domed
(c) High-domed
(d) Triangular

The subsidiary cells of bamboo stomata have special features. They can be categorised as simple and papillate. The papillate type are further classified on the basis of position and shape of the papillae (1) globose (2) elliptical (3) papillae not overarching (4) papillae overarching. In some bamboos the number of overarching papillae are definite irrespective of locality, age, and altitude e.g. Melocanna baccifera, Bambusa khasiana. Occasionally, the stomata are obscured by overarching
papillae from adjacent epidermal cells or are sunken in grooves so that the shape of the subsidiary cells can not be determined.

Frequency of stomata vary in species to species and within the same species from different locality and age of the leaf. However it is indicated according to broad catagories absent infrequent, common, abundant. The location of stomata is generally intercostal in bamboos, but in some bamboos costal stomata are also observed, especially on the mid or parallel veins. Generally stomata occur in one or more horizontal bands in the intercostal (between veins) zones in single or several adjacent rows.

**Long cell** - There are two types of epidermal cells in bamboos as in other grasses, long and short cells. Major portion of intercostal zone consists of long cells, which are generally longer horizontally than vertically. The shape of long cells are as described by Palmer and Tucker 1981.

**Square** - Cell about as long horizontally as it is wide vertically.

**Rectangular** - Cell about two to three times as long as wide.

**Long and narrow** - Cell more than three times as long as wide, width uniform.

**Long and narrow** - Cell more than three times as long as wide, width non uniform. The outline of the long cell walls as they abut cells in an adjacent horizontal row is categorised as non-sinusoidal, slightly sinusoidal or markedly sinusoidal, interlocking flat. The presence or absence of papillae on long cell is noted. If papillae are present, the number, distribution (i.e. row or rows, paired, irregular), shape (globular or elongate) and size (uniform or variable) are described.

**Interstomatal Cell** - The long cell that occur in the same horizontal row as the stomata and that separate individual stomata are termed interstomatal cells or interstomatal (i.e. cells between stomata), the catagories of these cells are the same as that of long cells. The manner in which the end walls fit against the stomata is described as straight to convex, slightly concave or U-shaped or markedly concave or V-shaped.
The outline of the interstomatal cell walls as they abut cells in an adjacent horizontal row is categorized as same as that of long cells. The presence or absence of papillae on interstomatal cell is noted. If papillae are present their number distribution, shape and size are described.

**Papillae** - Protrusions from the outer walls of epidermal cells are termed papillae. Papillae occur primarily on long and interstomatal cells in bamboos they are also observed on cork cell, subsidiary cell and at the raised base of macrohairs. The papillae in bamboo are thick, and heavily cutinised or silicified and remain rigid. Since papillae protrude from cell surface they are well suited for examination with SEM and the taxonomic importance of papillae may be greater than realised with light microscopy (Palmer and Tucker 1981). This fact is confirmed in this study. The papillae described are as by palmer and Tucker. On the papillae possessing cells, notably long cells number of papillae per cell, single or in pairs, in a row in several rows, uniform or variable in size, large, small or warty, single, double or forked are noted.

**Short Cell** - Epidermal cells that contain silica bodies (Silica cell) or that are suberised (Cork cell) are referred to as short cell. Generally they are similar in their length and width, but dimensions are variable. In bamboos they are generally observed on costal (over the vein) and rarely intercostal (between veins) and are usually single, sometimes paired (cork cell and a silica cell). Cork cells are not easily detectable with SEM, many times they are overlooked, hence they are described together as silica cells, (Cork cell may or may not present).

**Silica Bodies** - A silica cell usually contain a silica body, which may or may not assume the same shape as the cell. Differences in the shape of silica body within the cell are of great importance in bamboo identification. Assuming the shape of silica body is the same as that of silica cell, seen on the surface with SEM, following types have been recognised which are basically those of Metcalf (1960) and Palmer and Tucker (1981).
a) Tall and narrow  
b) Crenate vertical  
c) Figure-eight  
d) Saddle-shaped  
e) Elliptical  
f) Round  
g) Crescent-shaped  
h) Cross-shaped  
i) Acutely angled  
j) Square  
k) Elongate-smooth  
l) Nodular  
m) Dumb-bell-shaped  
n) Rounded or straight or concave or middle  
o) Middle wide-narrow  
p) Middle long or middle short.

**Prickles** - Prickles are stout, sharply pointed epidermal appendages with swollen bases. Metcalf (1960) described these as prickle hairs. These appendages occur in both costal and intercostal. The absence or presence along with their type help in identifying bamboos at species level. When present, their frequency of occurrence is not that reliable as their presence. However it is noted as infrequent, common or abundant.

In bamboo two main types are recognised which are similar to Palmer and Tucker's categories.

Prickles - large with a pointed apex and a swollen base  
Hooks - small with abrupt pointed apex and a prominent swollen base.

Besides these two common type of prickles, some bamboos are characterised by prickles - long, straight with abrupt blunt apex and prickles - short having swollen base, with blunt apex, described as papillate hooks prickles. In few bamboos bicellular prickles are also observed. (*Bambusa nana*).

**Microhairs** - Microhairs are small, two-celled, rarely unicellular epidermal appendages comprised of a basal cell and an apical cell. The cells of the microhairs are thin and fragile, specially the apical cell. In bamboo leaf surface the microhairs are found usually on intercostal zone, but in some bamboos they are observed on costal zone too. The microhairs are very useful for the identification of bamboo genera and their species. The diagnostic character of microhairs used are the same that recognised by Palmer and Tucker (1981).
a) One celled - not observed in this study.
b) Two celled - basal and apical cells equal in length - short (cells one to
times and long as wide).
c) Two celled - basal and apical cells equal in length - medium (cells three
to four times as long as wide).
d) Two celled - basal and apical cell equal in length - long (cells five or
more times as long as wide).
e) Two celled - basal cell longer than apical cell.
f) Two celled - basal cell shorter than apical cell.

**Nature of apex of apical cell** -

a) Rounded
b) Tapered.

**Macrohairs** - Long unicellular hairs, which can be seen by unaided eye or with a
very low magnification are termed as Macrohairs usually occur in costal region,
have raised or flat base, sometimes surrounded by a ring of globose papillae.

The presence or absence of macrohairs at costal or intercostal zone deter-
mine the bamboo genera. However it is observed that these hairs are most fragile
amongst all epidermal appendages in bamboos. They very often fall during process-
ing or with the age. This fact is of much importance in case of culms. It is
observed that most of the young bamboo culm have macrohairs, but are not seen
during observations. These hairs contribute bulk of silicious deposition on the culm
surface; observed as encrustation, formed by mixture of wax with silica present
in these hairs. When present, the frequency is divided into three categories: infrequent, common, and abundant.
Format for species description

The format for species description of epidermal appendages in bamboos followed is the same as that of Palmer and Tucker (1981).

Stomata - Frequency; subsidiary cell shape; distribution; special features.
Interstomatal cell - shape, and walls; outline of walls. Papillae: Number/cell, distribution on each cell, shape, size.
Long cell - shape; outline of walls. Papillae: Number/cell, distribution on each cell, shape, size.
Prickles or Hooks - Frequency; location and type.
Silica bodies - location and shape.
Microhairs - frequency; number of cells; shape; shape of apex.
Macrohairs - frequency; location; nature of base.
Special features - Description of unique features not covered in other categories.