# Diversity in Foliar Micro-Morphology and Isoperoxidases of some *Bambusa* species of Tripura

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**ABSTRACT**

The present study is focused on the foliar micromorphometric and isoperoxidase of some *Bambusa* species found in Tripura. The species included *B. balcooa* Roxb., *B. bamboos* (L.) Voss., *B. cacharensis* Majumder, *B. pallida* Munro and *B. Vulgaris* Schrad ex Wendl. Unequal distribution of stomatal frequency between lower and upper epidermis is recorded in all the species. High incidence of Stomatal Index (SI) observed in lower epidermis of *B. cacharensis*, with lowest SI value (0.50) on the upper epidermis. Epidermal micromorphology also revealed species specific variation among the taxa with characteristic leaf micro-appendages. Micro-hairs are not found in upper epidermis of *B. balcooa* and *B. bamboos*. Biochemical investigation of leaf isoperoxidase showed distinct variation in their zymogram pattern with characteristic species bands. Present foliar micromorphpmetric and isoperoxidase analysis could be effective and appropriate measures to reveal species diversity and genetic variability among the species of *Bambusa.*

**Key Words:** *Bambusa*, Foliar epidermis, Isoperoxidase, Micromorphometric characters.

## INTRODUCTION

Eastern Himalayas represent a natural Bamboo diversity hub with a total of 63 species under 15 genera (Biswas, 1998). Germplasm resource characterization of Bamboo through conventional taxonomic work is very difficult and mainly based on vegetative characters. Since most Bamboo flower at long intervals vegetative features at micromorphological level play key role in characterization and identification of the taxa. Within species variation has been reported in several species of Bamboo (Kondas, 1982; Soderstrom and Ellis, 1983**;** Kochhar *et al.*, 1990). Moreover difficulties in identification and confusion in nomenclature of Bamboo is also attributed due to existence of mere polyploids of the same species and wide geographical distribution (Bedell, 1998). The foliar epidermis is one of the basic taxonomic characters from biosystemic point of view and taxonomic studies of a number of plant families. Leaf anatomy, in particular foliar epidermal characters are less studied in Bamboo. Recently leaf epidermal features and anatomy of bamboo species like *Dendrocalamus* (Richa and Sharma, 2001) and *Cylicodiscus gabunensis* (Kadiri *et al.,* 2005) have been reported.

The biochemical approach of isozyme studies have been utilized in the field of plant systematic, leading to a better understanding of the phylogenesis of plant kingdom (Chu *et al.,* 1972; Kiang and Wu, 1979). In view of the above context an effort has been made to reveal diversity in foliar epidermal characters of *Bambusa* species and their variability in isoperoxidase pattern.

## MATERIALS AND METHODS

Fresh leaf materials from identified natural populations of each species were collected and kept in a wet petridish. Only young leaflets were used to prepare uniform epidermal peelings in all the species. Epidermal peeling was taken by scrapping the leaf with razor blade on a microscopic slide. The peeling thus obtained were washed in water and suitably stained in aqueous safranin solution and mounted in glycerine. For each species three peels of both surfaces (upper and lower) were taken and 15 microscopic fields of both surfaces were randomly selected and studied. The quantitative data of different epidermal characters were documented and recorded in tabular form (Table 1-3). The Stomatal Index (SI) was calculated using the formula of Salisbury (1927).

$$SI = \frac{S}{S+E} \times 100$$

'S' denotes the number of stomata per microscopic field and 'E' the number of epidermal cells in the same unit area. Stomatal and epidermal cell frequency was calculated in $mm^2$ and size of the stomata, epidermal cell, hair, prickle were measured by using ocular micrometer.

**Biochemical studies**

1gm of fresh rolled leaf sample of Bamboo was taken and crushed in 0.1M Tris HCL Buffer (pH 6.8) and centrifuged at 10000rpm for 15 min. at 4°C. The supernatant was collected and used for quantitative estimation of protein. Quantitative estimation of total soluble protein was done by modification method of Lowery et al (1951) using BSA as a standard. The protein assay was based on precipitation steps.

For isoenzyme studies gel electrophoresis (Laemmli, 1970) was performed into 10×8 cm mini slab apparatus of 1mm thickness in a vertical gel electrophoresis unit. A 8% resolving gel of 7cm height was first polymerized and upon it a 5% stacking gel of 3cm was cast. Protein samples of 100 µl in extraction buffer containing 12.5% glycerol were loaded on to each lane along with indicator Bromophenol blue. The gel was subjected to electrophoresis for 2½ hours at $4^oC$. After the electrophoresis gels strip was immersed in respective buffer for 30 mins. followed by treatment with peroxidase staining solution (Hislop and Stahmann 1971) to obtain the isozyme pattern.

**RESULTS AND DISCUSSION**

Individual foliar size and number of veins recorded in five different species of *Bambusa* clearly reveled variation with a maximum of 4.30±0.20cm wide in *B. pallida* to minimum of 1.18±0.07cm in *B. bamboos*. Average vein number also varied from 8.40±0.16 to 21.60±1.50. Epidermal cells of the *Bambusa* species also revealed similar nature of elongated cells along with short cells closed to the stomatal complex. The outer boundary of the cell was notched and wavy and degree of undulation also varied from species to species. The nature of the stomata is simple and the guard cells were dumbbell shaped. In general, foliar epidermis is characterized by having coastal and inter-coastal regions. Distribution of stomata was restricted to the inter-coastal region and found in both surfaces. Stomatal index value is always higher in the lower surface with maximum 40.50 in *B. cacharensis*. However, wide range of variability in stomatal index among the species of *Bambusa* (table-1) was recorded. Low stomatal frequency (0.50) in upper surface of *B. cacharensis* is very significant compared to rest of the taxa investigated in the present study.

Epidermal cells of different species of *Bambusa* were also characterized by the presence of different types of appendages in either on the upper surface, or lower surface or in both surfaces. The size and shape of the epidermal appendages like microhairs and prickles was varied from species to species. Micro-hairs distribution is completely lacking in upper surface of *B. balcooa* and *B. bamboos*. Micro-morphometric measurements of leaf epidermal micro-hairs varied from 60.09±10.66 µm in *B. bamboos* to 42.96±9.39 µm in *B. cacharensis*(table-2). However, size variability in length and breath within the species is also very significant. Similarly, distribution of prickles is absent in upper surface of *B. balcooa* and *B. bamboos*. The size of micro-prickle of upper epidermis is much larger compared to lower except *B. cacharensis* (table-3). The significance of foliar micro-morphometric characters in differentiation of many plant taxa at species level have also been reported (Saikia *et al*, 2000; Sinha *et al*, 2005; Yang *et al*, 2006). Therefore, the present investigation on diversity of micro-morphometric epidermal characters of *Bambusa* leaves clearly showed its utility in demarcation and distinction among the related species.

Biochemical studies of the total soluble protein of young rolled leaf revealed almost similar contents among the taxa. Electrophoretic studies of leaf isoperoxidase among the members of *Bambusa* clearly indicated species specific variability in terms of Rm values as well as close relationship among the taxon (table-4; fig.1). Therefore, the present investigation aimed to study foliar micro-morphometric characters revealed significant diversity in epidermal characters of Stomatal Index, distribution of leaf appendages and their size variability. Variability in term of leaf isoperoxidase pattern was also noticed indicating the genetic diversity among the species of *Bambusa*.

**Table 1: Foliar morphometric features of five *Bambusa* species**

| Species | Width of the leaf (cm)Min. (Mean±SD) Max. | No. of Veins Min. (Mean±SD) Max. | Upper Epidermal cell frequency/ $mm^2$ | Lower Epidermal cell frequency/ $mm^2$ | Upper Stomatal Index | Lower Stomatal Index |
|---|---|---|---|---|---|---|
| *B.balcooa* | 3.90 (4.50±0.44) 5.10 | 18 (19.80±1.60) 22.00 | 666 | 895 | 12.30 | 28.80 |
| *B.bamboos* | 1.10 (1.18± 0.07) 1.30 | 8.00 (8.40±0.16) 10.00 | 737 | 997 | 13.30 | 37.00 |
| *B.cacharensis* | 2.10 (2.24± 0.10) 2.40 | 10.00 (10.80±0.98) 12.00 | 675 | 932 | 0.50 | 40.50 |
| *B.pallida* | 4.00 4.30±0.20) 4.60 | 20.00 (21.60±1.50) 24.00 | 496 | 1045 | 7.80 | 39.80 |
| *B. vulgaris* | 3.10 (3.48±0.27) 3.90 | 12.00 (14.80±1.60) 16.00 | 565 | 820 | 12.90 | 33.90 |

**Table 2: Micro-morphometric measurement of hairs in five *Bambusa* species**

| Species | | Size of the hair (μm) | | |
|---|---|---|---|---|
| | Upper Epidermis | | Lower Epidermis | |
| | Length Min.(Mean±SD) Max. | Breadth Min.(Mean±SD) Max | Length Min.(Mean±SD) Max | Breadth Min.(Mean±SD) Max |
| *B. balcooa* | - | - | 34.39 (50.08±10.81) 85.07 | 7.24 (8.21±0.90) 9.05 |
| *B.bamboos* | - | - | 43.44 (60.09±10.66) 70.59 | 7.24 (8.09±1.85) 12.67 |
| *B.cacharensis* | 28.96 (36.92±6.77) 50.68 | 7.24 (7.48±0.85) 9.05 | 28.96 (42.96±9.39) 54.30 | 7.24 (7.68±0.73) 9.05 |
| *B. pallida* | 36.20 (45.97±4.57) 50.68 | 7.24 (8.81±0.62) 9.05 | 27.15 (46.58±9.38) 65.16 | 9.05 (9.29±0.62) 10.86 |
| *B. vulgaris* | 43.44 (63.11±10.53) 81.45 | 7.24 (8.75±0.80) 9.05 | 28.96 (44.65±15.56) 81.45 | 7.24 (8.09±0.90) 9.05 |

**Table-3: Micro-morphometric measurement of prickle in five *Bambusa* species**

| Species | Size of the prickle (µm) | | | |
|---|---|---|---|---|
| | Upper Epidermis | | Lower Epidermis | |
| | Length Min.(Mean±SD) Max. | Breadth Min.(Mean±SD) Max | Length Min.(Mean±SD) Max | Breadth Min.(Mean±SD) Max |
| *Bambusa balcooa* | - | - | 21.72(29.08±4.51) 36.20 | 14.48(16.53±1.65) 18.10 |
| *Bambusa bamboos* | - | - | 21.72(26.55±4.27) 36.20 | 12.67(15.33±1.60) 18.10 |
| *Bambusa cacharensis* | 16.29(19.67±2.37) 25.24 | 10.86(13.64±2.07) 16.29 | 16.29(19.67±2.37) 25.34 | 10.86(15.20±1.96) 18.10 |
| *Bambusa pallida* | 28.96(89.06±30.03) 123.08 | 21.72(46.94±12.79) 68.78 | 23.53(28.60±2.66) 34.39 | 14.48(16.17±1.23) 18.10 |
| *Bambusa vulgaris* | 27.15(37.65±7.31) 52.49 | 18.10(21.60±2.33) 25.34 | 23.53(31.74±4.81) 36.20 | 16.29(16.27±19.91) 23.53 |

**Table-4: Isoperoxidase profile (Rm value) in five species of *Bambusa***

| | Name of the species | | | | |
|---|---|---|---|---|---|
| | *Bambusa bamboos* | *Bambusa balcooa* | *Bambusa cacharensis* | *Bambusa pallida* | *Bambusa vulgaris* |
| Rm values | - | 0.02 | 0.02 | 0.02 | 0.02 |
| | 0.03 | 0.03 | - | 0.03 | 0.03 |
| | - | 0.04 | - | - | - |
| | 0.05 | 0.05 | 0.05 | - | 0.05 |
| | 0.09 | 0.09 | - | 0.09 | 0.09 |
| | - | - | 0.10 | - | - |
| | - | - | - | 0.12 | - |
| | - | 0.14 | - | - | - |
| | 0.19 | - | - | - | - |
| | - | - | - | - | 0.21 |
| | - | 0.27 | - | - | - |
| | - | 0.29 | - | - | - |
| | 0.31 | - | - | - | 0.31 |
| | - | - | - | 0.33 | - |
| | - | - | 0.36 | - | - |
| | 0.38 | 0.38 | 0.38 | 0.38 | 0.38 |
| | - | - | - | - | - |
| | - | - | - | - | - |
| | 0.41 | 0.41 | - | 0.41 | 0.41 |
| | 0.42 | - | | - | - |
| | - | 0.44 | 0.44 | 0.44 | - |
| | - | 0.46 | | 0.46 | 0.46 |
| Total No. of Bands | 8 | 12 | 6 | 9 | 9 |
| Protein content mg/g fresh weight Mean±SD | 10.95±0.46 | 12.18±0.49 | 11.98±0.83 | 12.60±0.37 | 13.22±0.68 |


**Figure: 1A-E.** Zymogram pattern of leaf Isoperoxidases of *Bambusa* Species,
**A.** *B. cacharensis* Majumder,
**B.** *B. balcooa.* Roxb.
**C.** *B. vulgaris* Schrad ex Wendl,
**D.** *B. pallida* Munro
**E.** *B. Bamboos* (L.) Voss.

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**LITERATRUE CITED**

**Bedell PE, 1998.** Taxonomy of Bamboo. *APC Pub.,* India.

**Biswas, Sas.,1988.** Studies on Bamboo distribution in North Eastern region of India. *Ind. For*. **114** (9): 514-531.

**Chu YE, TS Chou YS Li, CY Shih and SC Woo, 1972.** Identification of Bammboo clones, *Dendrocalamus latiflorus* in Taiwan. Bot.Bull. *Academia Sinica***. 13**: 11-18.

**Hislop EC and Stahmann MA, 1971.** Peroxidase and ethylene production by barley leaves infected with Erysiphe graminis f.sp.hardei. *Plant Pathology*. **1**:297-312.

**Kadiri AB, Olowokudejo JD, Ogundipe OT, 2005.** Some aspects of foliar epidermal morphology of *Cylicodiscus gabunensis* (Taub.) Harms (Mimosaceae). *J.Sci.res.Dev*. **10**:33-38.

**Kiang YT and LWu, 1979.** Genetic studies of esterase on the Taiwan wild rice population . *Bot. Bull. Academia since***. 20**: 103-116.

**Kondas S, 1982.** Biology of two Indian Bamboo, their culm potential and problems of cultivation .*Ind. Forester*. **108**: 179-83.

**Kochhar S, Mal B and Chaudhury RG, 1990.** Population aspect of the phenologigal behaviour of Bamboo germplasm In: Proc. Intl. Bamboo workshop (I.V. Ramanujd Rao, R Gnanaharam and C.B. Sastry, eds). Bamboo current research KFRI, India and IDRC Canada.:51-58.

**Laemmli UK, 1970.** Cleavage of structural Proteins during the assembly of the head of bacteriophage $T_4$. *Nature*.**227**:680-685.

**Lowry OH, Rosenbrough NJ, Farr AL. and Randall RJ, 1951.** Protein measurement with folin phenol reagent. *J. Bio cheme*. **193**:265-275.

**Richa and Sharma, ML, 2001.** Leaf epidermal studies in some Indian species of Bamboos-ii.Genus *Dendrocalamus* Nees .*J. Tree Sci* . **20**(1& 2): 64-71.

**Salisbury, EJ, 1927.** On the causes and ecological significance of stomatal frequency with special reference to woodland flora. *Philosophical Transactional of the Royal Society of Landon*, Series B, **216**:1-65.

**Saikia N, Nath SC and Dutta S, 2000.** Foliar micromorphgological characters of few taxa of the genus Aquilaria Lamk. Growing in North-East India. *Ad. Plant Sci.* **13**(11):551-558.

**Sinha RK, Roy K and Sinha S, 2005.** Foliar morphometric analysis in two species of *Tinospora*-an ethnobotanically important taxon of Tripura. *Ad. Plant Sci.* **18**(11):629-633.

**Soderstrom TR and Ellis RP, 1987.** The position of Bamboo genera and allies in system of grass classification. In: Grass Systematics and Evolution (T.R. Soderstrom, Ed).*Smithsonian Institution Press*, Washington,DC: 225-238.

**Yang H-Q,Wang H and Li D-Z, 2006.** Micromorphological study on leaf epidermis of *Schizostachyum* and its allies (Poaceae: Bambusoidae). *Acta Botanica Yunnanica*. **28**:261-267.