Wood Anatomy and Topochemistry of *Bombax ceiba* L. and *Bombax insigne* Wall.

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Wood anatomical characteristics, content of phenolic extractives, and topochemistry of two lesser known and underutilised hardwood species, Bombax ceiba and Bombax insigne were studied. Heartwood and sapwood material was obtained from logs originating from natural forests of Pyinmana District, Myanmar. The basic qualitative anatomical features agreed with descriptions reported for the species in other regions (e.g. India, Bangladesh, Southeast Asia). However, there were some light differences in the quantitative wood anatomical data among the regions due to the influence of environmental conditions. The amount of phenolic extractives obtained by gradual extraction with acetone-water was almost the same in heartwood and sapwood (about 1.2%) in B. insigne, while heartwood showed a higher amount (2.8%) than sapwood (2.5%) in B. ceiba. Topochemical distribution of lignin and phenolic deposits in heartwood tissues investigated by scanning UV microspectrophotometry (UMSP) revealed that B. insigne is more highly lignified than B. ceiba. For both species, a lower UV-absorbance by the fiber and ray cell wall as compared to that of the cell wall of vessels was observed. Also, phenolic compounds were mostly deposited in the lumina of parenchyma cells and vessels rather than in cell walls. The results further improve the knowledge on the wood anatomy and chemistry of the species and in this respect are useful in future research to broaden their utilisation potential.

Keywords: Macroscopic characteristics; Wood anatomical features; Phenolic extractives; UV microspectrophotometry; Lignin; Cell wall layers

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INTRODUCTION

Bombax is a genus of tropical and subtropical trees in the Malvaceae family (Mabberley 2008). *Bombax* species are native to Western Africa, the Indian subcontinent, Southeast Asia, as well as sub-tropical regions of East Asia and Northern Australia. They are among the largest trees in their regions, reaching 30 to 40 m in height and up to 3 m in trunk diameter (Kress *et al.* 2003; Seth 2004). *Bombax ceiba* L. is naturally distributed in Pakistan, India, Myanmar, Indochina, China, Taiwan, Thailand, Java, Borneo, the Philippines, Sulawesi, the Lesser Sunda Islands, the Moluccas, New Guinea, and Northern Australia (Sosef *et al.* 1998). The trees grow 58 to 78 cm in diameter and over 30 m in height and have a straight, cylindrical stem with buttresses at the base (Pearson

and Brown 1932). Due to its rapid growth and wide distribution, it is the most promising tree species in the afforestation and reforestation programme in the central dry zone of Myanmar to restore the environment and to satisfy the increasing timber demand (Chaturvedi and Pandey 2001; Tanvir *et al.* 2003). *Bombax insigne* Wall. naturally occurs in Laos, Myanmar, and Vietnam (Tang *et al.* 2007). It is also a large tree with a straight, cylindrical bole and is normally buttressed at the base. The tree can grow to a height of 24 to 36 m, up to 160 cm in diameter, with a clear bole of 12 to 18 m (Balan 1980). In the green forests of lower Myanmar, *B. insigne* trees attain a diameter of 58 to 68 cm and a branchless clear bole of about 24 m (Pearson and Brown 1932).

There is hardly any research work available for the properties and utilisation of *B. insigne*. In India, Sri Lanka, and Nepal, it is mainly used for building kattumarans (catamarans = a kind of boat) (Balan 1980; FAO 1984). On the other hand, several studies have been carried out on *B. ceiba* concerning chemical constituents of leaves and bark, gums, pollination, genetic diversity, growth and yield, formation of traumatic gums, and enhancement of wood durability (Babu and Shah 1987; Bhattacharya and Mandal 2000; Chaturvedi and Pandey 2001; Tanvir *et al.* 2003; Tarakanadha *et al.* 2006). Its timber is mainly used for sculpture production in Australia, for production of matches and plywood in India, and for matches, rubber boxes, boards for ceilings, and coffins in Myanmar (Chaturvedi and Pandey 2001; Griffiths *et al.* 2003).

Basic qualitative wood anatomical descriptions of the species are already available (*e.g.* Pearson and Brown 1932; Mohiuddin 1990). However, more detailed information on the wood anatomy and chemistry of the species is needed, which could be then used for understanding their influence on wood properties and for increasing the added value of timber. Thus, this study was focused on the description of quantitative wood anatomical characters as well as the chemical analyses of the extractive contents in the heartwood and sapwood of *B. ceiba* and *B. insigne*. Also, the topochemical distribution of lignin and phenolic deposits were investigated using cellular UV microspectrophotometry (UMSP).

EXPERIMENTAL

Material

Four 5-m logs, two of *B. ceiba* L. with base diameters 60 and 77 cm and two of *B. insigne* Wall. with base diameters 120 and 124 cm, were obtained from the Myanmar Timber Enterprise, Myanmar. The logs originated from the base of trees growing on alluvial flat soils at natural moist deciduous forests of Pyinmana District. The *B. ceiba* trees were 21 and 24 m height to the first branch, had 57 and 73 cm breast height diameter, and the respective data for the *B. insigne* trees were 27 to 31 m for the height to the first branch and 116 to 119 cm for the breast height diameter. The exact ages of the trees were not exactly known and could be only estimated to 30 and 40 years for *B. ceiba*, and 60 and 80 years for *B. insigne*.

The logs were converted into 50 mm thick boards, which were then kiln-dried at 50°C to around 12% moisture content. The boards for this study were taken further from ring 20 from the pith to avoid juvenile wood (heartwood boards) and at 5 cm from the bark (sapwood boards). Five heartwood and five sapwood boards per log were used for the experiments.

Light Microscopy

From each heartwood and sapwood board, three radial wood strips of approximately 15 mm were collected for anatomical investigations. Several transverse, radial, and tangential sections (15 to 25 μ m thick) were cut from each wood strip on a Reichert-Jung sliding microtome, double stained with safranine and astra blue, and mounted in a synthetic resin. Furthermore, material was taken randomly from the strips and macerated in a mixture of equal volumes of acetic acid and hydrogen peroxide at 60°C for 12 to 24 hours (Tsoumis 1991). The macerated material was mounted in glycerine for fiber length measurements. A digitized image analysis system (analySIS®, Olympus) mounted on an Olympus AX 70 microscope was used to record quantitative (histometric) anatomical data (comp. Table 1).

| Feature | Bombax ceiba | Bombax insigne | |
|--|------------------------------------|---------------------|--|
| | Heartwood + Sapwood | Heartwood + Sapwood | |
| Vessels | | | |
| Tangential vessel diameter | 122-336* (274±37)** | 175-467 (357±53) | |
| (µm) | | | |
| Vessel element length (µm) | 309-673 (506±79) | 351-559 (429±42) | |
| Vertical Diameter of | 4-7 (6±0.6) | 8-15 (12±1.6) | |
| intervessel pits (µm) | | | |
| Fibers | | | |
| Fiber length (µm) | 1231-2956 (1832±312) | 968-2875 (2215±500) | |
| Double cell wall thickness | 4-9 (6±0.9) | 5-9 (7±0.9) | |
| (µm) | | | |
| Axial parenchyma | | | |
| Number of cells per axial | 2-5 (3.3±0.8) | 3-5 (4±0.6) | |
| parenchyma strand | | | |
| Rays | | | |
| Width (number of cells) | 1-11 (5±2) | 1-7 (3±1) | |
| Height (µm) | 404-5027 (1957±1222) | 417-4270 (1560±973) | |
| * values outside parenthesis indicate the minimum and maximum values | | | |
| ** mean values ± standard dev | viations are given in the parenthe | esis | |

Table 1. Some Quantitative Wood Anatomical Features of *B. ceiba* and *B. insigne*

The IAWA standard list of anatomical features was used as a guideline for the description of wood structures (IAWA Committee 1989). The quantitative data were based on 50 measurements for each feature and species, respectively (Hapla and Saborowski 1987). The numerical values presented in the descriptions are expressed as minimum-maximum and mean in brackets.

Quantitative Determination of the Phenolic Extractives

For a quantitative chemical analysis, wood shavings from sapwood and heartwood specimens of *B. ceiba* and *B. insigne* were prepared. The samples were ground in a mill with rotating knife (Retsch) using a 3 mm screen. The gradual extraction was performed on 2 g dried wood powder using an accelerated solvent extraction (ASE 200, Dionex): solvent acetone-water (9:1): temperature 60°C, pressure 100 bar, heating time 5 min; static time 10 min; flush volume 100 %; purge time 120 s; static cycles: 1.

For the quantification of the total content of extractives, the extracts were concentrated in vacuo at 40°C purged with nitrogen and dried over phosphorus

pentoxyde. The dry extracts were weighted and their content in the samples was expressed as percentage dry mass of the original sample. It should be noted that the extraction with acetone-water is the most effective and established method for the quantitative determination of phenolic extractives in wooden tissue (Puls 1993; Koch *et al.* 2003; Koch *et al.* 2006; Mayer *et al.* 2006).

Subcellular UV Microspectrophotometry

The subcellular distribution of lignin and phenolic extractives were topochemically investigated using scanning UV microspectrophotometry according to Koch and Kleist (2001) and Koch and Grünwald (2004). Cellular UV microspectrophotometry is an established technique for characterising lignin *in situ* and for its semi-quantitative determination in the various layers of wood cell walls according to Lambert-Beer's law. It is based on the ultraviolet illumination of semi-thin transverse sections of woody tissue (*e.g.* Fergus *et al.* 1969; Fukazawa 1992; Koch and Kleist 2001; Takabe 2002, Koch and Grünwald 2004) and enables direct imaging of the lignin distribution within the individual cell wall layers.

Small blocks (approximately 1 x 1 x 5 mm) were prepared from the heartwood of the two species. After a usual dehydration in acetone, the blocks were impregnated with a series of Spurr's epoxy resin (Spurr 1969). Finally, they were immersed in pure resin and polymerized at 70°C for 24 hours. Ultrathin sections of 1 μ m in thickness were cut from the polymerized blocks with a diamond knife, transferred to non-reflective quartz slides, immersed in a drop of non-UV-absorbing glycerine, and covered with non-reflective quartz slides.

The analyses were carried out using a Zeiss UMSP 80 microspectrophotometer equipped with a scanning stage which enables the determination of image profiles at defined wavelengths with the scan software APAMOS® (Zeiss). The scan program digitizes rectangular fields on the tissue with a local geometrical resolution of 0.25 x 0.25 μ m and a photometrical scale resolution of 4096 grey scale level, which are converted to 14 basic colors to visualize the UV absorbance intensities.

Photometric point measurements were also performed on a spot size of $1 \ \mu m^2$ between 240 and 560 nm wavelengths using the program LAMWIN® (Zeiss). For quantitative studies, 10 to 15 spectra were taken for each individual cell wall layer and cell type as well as extractives deposited in the cell lumina, respectively.

RESULTS AND DISCUSSION

Anatomical Features

Bombax ceiba

The anatomical structure of *B. ceiba* wood is described in Table 1, and Fig. 1 shows representative microscopic images of the three anatomical directions. In detail, the individual structural parameters and cell types (vessels, axial, and ray parenchyma) can be described as follows:

Macroscopic characteristics: Wood diffuse-porous. Growth ring boundaries indistinct to fairly distinct in both heartwood and sapwood zones; when present, marked by denser fiber zones. The grain is straight.

Microscopic characteristics: Vessels mostly solitary or in radial multiples of 2 to 3, mostly oval in outline (Fig. 1a); thin-walled, tangential vessel diameter 122-336 (274)

 μ m, vessel element length 309-673 (506) μ m; perforation plates simple, intervessel pits alternate (Fig. 1f), 4-7 (<u>6</u>) μ m in diameter (vertical); tyloses common.



Fig. 1. Light micrographs of *Bombax ceiba*. **(a)** Heartwood, transverse section. Solitary oval vessels, multiseriare heterogeneous rays and a uniseriate ray (arrow); **(b)** Heartwood, radial section. Non-septate fibers; **(c)** Heartwood, tangential section. Apotracheal axial parenchyma and multiseriate rays.; **(d)** Sapwood, transverse section. A heterogeneous ray with abundant starch grains; **(e)** Sapwood, radial section. Heterogeneous rays and axial parenchyma arranged alternately with the fibers **(f)** Sapwood, tangential section. Alternate intervessel pits (arrow), axial parenchyma strands of 4-5 cells and a heterogeneous ray, 8 cells wide. Scale bars for **(a)**, **(c)**, **(e)** = 500 µm; for **(b)**, **(d)**, **(f)** = 200 µm

Fibers arranged alternately with narrowly banded or diffuse-in-aggregates located parenchyma strands (Fig. 1b-f), 1231-2956 (<u>1832</u>) μ m long, double wall thickness 6 μ m on average, with simple to minutely bordered pits, and non-septate (Fig. 1b). Fibers are storied.

Axial parenchyma very abundant but indistinct to naked eye, mostly apotracheal and diffuse-in-aggregates arranged alternately with the fibers, and vasicentric in 1- to 2-layered sheaths, in strands of 2-5 cells (Fig. 1c-f); simple starch grains very abundant. Axial parenchyma strands and vessel elements storied.

Rays 1-11 seriate, 404-5027 (<u>1957</u>) μ m height; heterogeneous (Fig. 1d-f), uniseriate rays mostly composed of procumbent cells, sheath cells and single row of square to upright marginal cells often present; large starch grains abundant (Fig. 1d). Low rays storied, high rays non-storied.

Bombax insigne

The wood anatomical structure of *B. insigne* is illustrated in Fig. 2 and Table 1 and characterized by the following features:

Macroscopic characteristics: Wood diffuse-porous. Growth ring boundaries fairly distinct marked by denser fiber zones. The grain is straight.

Microscopic characteristics: Vessels solitary and in radial multiples of mostly 2-3, mostly oval in outline (Fig. 2a); thin walled, tangential vessel diameter 175-467 (<u>357</u>) μ m, vessel element length 351-559 (<u>429</u>) μ m; perforation plates simple, intervessel pits alternate (Fig. 2f), moderately large in diameter (vertical), 8-15 (<u>12</u>) μ m; vessel-ray pits with much reduced borders; tyloses abundant.

Fibers arranged alternately with the parenchyma strand (Fig. 2b-f), narrower than parenchyma cells (Fig. 2b), length 968-2875 (2215) μ m, double wall thickness 7 μ m on average, with simple to minutely bordered pits, non-septate.

Axial parenchyma very abundant but indistinct to naked eye, mostly apotracheal in tangential rows (somewhat wavy) of 4-5 cells forming a fine reticulum with the rays (Fig. 2a), paratracheal inconspicuous 1-2 seriate and laterally flattened (Fig 2d); arranged alternately with the fibers, in strands of 3-5 cells (Fig. 2c, e-f); large starch grains very abundant (Fig 2b). Rays and/or axial elements irregularly storied.

Rays 1-7 seriate (Fig. 2d, f), 417-4270 (<u>1560</u>) μ m height; heterogeneous (Fig. 2b-f); large starch grains very abundant (Fig. 2b).

The anatomical features of *B. ceiba* and *B. insigne* observed in this study are similar to those reported in different geographical distributions, *e.g.* India (Pearson and Brown 1932), Bangladesh (Mohiuddin 1990), and throughout South East Asia (Sosef *et al.* 1998). However, some differences in the quantitative anatomical data exist among the different localities (Table 2).

B. ceiba of Myanmar origin (this study) can have wider and higher rays than of India and Bangladesh, while its vessels can be smaller in diameter. Vessels are generally shorter in India, and no differences should be expected in fiber length among the regions. Rays of *B. insigne* also seem to be the highest in Myamar but they are equal in width. Vessels can attain higher length and diameter in Bangladesh and Myanmar, respectively. *B. insigne* growing in India appears to have the shortest fibers. These differences should be related to different environmental conditions such as seasonal or geographical conditions or nutrient regimes (Panshin and de Zeeuw 1980).



Fig. 2. Light micrographs of *Bombax insigne*. (a) Heartwood, transverse section. Solitary vessels and characteristic lines of somewhat wavy axial parenchyma; (b) Heartwood, radial section. Heterogeneous rays and axial parenchyma with abundant starch grains; (c) Heartwood, tangential section.; Rays variable in size and axial parenchyma in strands arranged alternately with the fibers; (d) Sapwood, transverse section. Axial parenchyma mostly apotracheal, and paratracheal laterally flattened (arrow); (e) Sapwood, radial section. Fibers are narrower than the axial parenchyma strands; (f) Sapwood, tangential section. Vessel elements with alternate intervessel pits (arrow). Scale bars for (a), (c), (e) = 500 μ m; for (b), (d), (f) = 200 μ m

| Feature | This Research | Mohiuddin (1990) | Pearson & Brown (1932) | |
|---------------------------------|---------------|------------------|------------------------|--|
| Bombax ceiba | | | | |
| Vessel element length (µm) | 309-673 | 216-649 | 280-500 | |
| Tangential vessel diameter (µm) | 122-336 | 206-453 | 375-410 (largest cell) | |
| Fiber length (µm) | 1231-2956 | 860-3000 | 650-3000 | |
| Height of rays (µm) | 404-5027 | 186-3173 | >3900 (largest ray) | |
| Width of rays (number of cells) | 1-11 | 1-7 | 1-7 | |
| Bombax insigne | | | | |
| Vessel length (µm) | 351-559 | 442-1164 | 430-550 | |
| Tangential vessel diameter (µm) | 175-467 | 103-319 | 300-360 (largest cell) | |
| Fiber length (µm) | 968-2875 | 1080-3080 | 1300-2500 | |
| Height of rays (µm) | 417-4270 | 309-1885 | >3500 (largest ray) | |
| Width of rays (number of cells) | 1-7 | 1-6 | 1-7 | |

Table 2: Comparison of Quantitative Wood Anatomical Features among Different

 Localities

Wood anatomical data are indices of physical and mechanical properties, and are therefore significant for wood utilization (Bauch *et al.* 2006). As shown in Table 1, *B. ceiba* and *B. insigne* have very thin cell-walls, large vessel tangential diameters, and abundant parenchyma, and are thus expected to exhibit low density and strength properties (Sint and Hapla 2008). These features also point out the readiness of the species to pick up impregnating solutions for enhancing wood properties (Sint *et al.* 2011, 2012). Scientists and processors working with the timber of the species could further explore the anatomical data presented here in explaining the properties and behavior of wood.

Extractive Content

The amounts of phenolic extractives (extracted gradually with acetone-water) of the selected xylem wood zones of *B. insigne* and *B. ceiba* are given in Table 3. In *B. insigne*, heartwood and sapwood of the selected specimens contain almost the same amount (1.16% in heartwood and 1.20% in sapwood) of acetone extracts, whereas heartwood (2.80%) contains a slightly higher amount than sapwood (2.51%) in *B. ceiba*.

Table 3. Total Content of Acetone-Water (9:1) Extractives of Heartwood and Sapwood of *B. ceiba* and *B. insigne*

| | V | |
|--|--------------------------------------|--|
| Species | Total Content of Acetone-Water (9:1) | |
| | Extractives (%)* | |
| Bombax ceiba | | |
| Heartwood | 2.80 | |
| Sapwood | 2.51 | |
| Bombax insigne | | |
| Heartwood | 1.16 | |
| Sapwood | 1.20 | |
| * On a dry weight basis, mean values of three replicate measurements | | |
| | | |

Extractives are generally deposited in greater amounts in heartwood than in sapwood, especially in the tropical hardwoods and impart heartwood a darker color (*e.g.*

Hillis 1972, 1987; Yatagai and Takahashi 1980). However, some fast growing tropical species were reported to contain small extractive contents. For example, the ethanolbenzene extractive content of heartwood ranges from 1.4 to 2.6% in balsa (*Ochroma lagopus*), obeche (*Triplochiton scleroxylon*), and okoumé (*Aucoumea klaineana*) (Fengel and Wegener 2003).

The amount of extractives of *B. ceiba* and *B. insigne* is quite low compared to other commercial and durable timber species of the region such as *Tectona grandis*, *Eucalyptus*, *Prosopis*, *etc.* (Haupt *et al.* 2003; Thulasida and Bhat 2007; Chafe 1987; Carrillo *et al.* 2008), which reflects their low natural durability. Scheffer and Morrell (1998) classified both species as "non-resistant or perishable". Similarly, Pearson and Brown (1932) described the species as "easily perishable". Sint (2010) tested their resistance to Basidiomycetes and to soft rot fungi and soil-inhabiting microorganisms, found very high mass losses (66.3% and 73.9% due to attacks by *Trametes versicolor* in 16 weeks; 84.9% and 72.6% due to attacks by soft rot fungi and soil inhabiting microorganisms in 32 weeks) and classified them as "not durable".

Topochemical Distribution of Lignin and Phenolic Extractives

Bombax ceiba

The topochemical distribution of lignin and phenolic extractives within individual cell types (fibers, vessels, and parenchyma) and cell wall layers were analysed by using scanning UV microspectrophotometry. Figures 3a-d show representative UV scanning profiles of heartwood tissue of B. ceiba at a defined wavelength of 278 nm (absorbance maximum of hardwood lignin). Each scanning is depicted in a matched pair of two- and three- dimensional views (Fig. 3a and 3c; 3b and 3d). In the three-dimensional view, the higher UV-absorbing regions such as cell corner (CC), compound middle lamella (CML), and locally deposited extractives can be clearly distinguished from the lower absorbing S2-layers (Figs. 3c-d). The S2-layers of fibers are characterized by a relative uniform UV absorbance in the range of 0.23 AU to 0.29 AU (white arrow in 3a). CC and CML offer a higher deposition of lignin evaluated by UV absorbance values in the range of 0.35 AU to 0.48 AU (black arrow in 3a; white arrow in 3b). Deposits of phenolic extractives are not detectable in the cell walls and cell lumen of fibers (yellow arrow in 3a). In contrast, some local deposits of phenolic extractives are detectable in the cell lumina of axial and ray parenchyma, which are characterized by varying degrees of UV-absorbance from about 0.10 AU (lowly condensed phenolics) to 0.94 AU (highly condensed phenolics) (black arrow in 3b).

The evaluation of the point analysis spectra (measured at the same morphological regions of the wooden tissue) fully confirms the results obtained from the scanning profiles (Figs. 3e-f). The maximum absorbance at the wavelength of 278 nm amounts to 0.21 AU for the fiber S2 layer and 0.31 AU for the CML, respectively, while that of the ray S2 layer is 0.29 AU. The vessel S2 layer is most strongly lignified among the cell types and represented by a value of 0.46 AU (Fig. 3e). The extractives deposited in the axial and ray parenchyma cells are characterized by the highest UV absorbance values up to 1.20 AU (Fig. 3f). These results confirm early and recent findings of Fergus and Goring (1970a), Koch and Kleist (2001), Koch and Grünwald (2004), and Carrillo *et al.* (2008) who demonstrated the applicability of this technique for the topochemical detection of lignin and phenolic extractives within individual cell wall layers of several hardwoods.

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Fig. 3. UV microscopic scanning 2D profiles **(a-b)**, 3D profiles **(c-d)** and point measurements **(e-f)** of heartwood tissue of *Bombax ceiba*. The colour pixels represent different UV absorption values of the cell wall layers and phenolic extractives measured at 278 nm. **(a)** White arrow represents fiber S2 layer, black arrow compound middle lamella, yellow arrow cell lumen of truncated fiber. **(b)** Deposits in axial parenchyma lumen (black arrow) and high deposition of lignin in compound middle lamella (white arrow). Compound middle lamella **(c)** and extractives **(d)** stand out as high intensity bands with histograms representing the statistics of the areas. **(e)** UV absorbance spectra of S2 layers of vessel, fiber, ray, and parenchyma (paren). **(f)** UV absorbance spectra of compound middle lamella (CML), cell corner (Corner) extractives in ray cell lumen (ext ray) and lumen of truncated fiber. F = fiber, LP = axial parenchyma cell

Bombax insigne

The individual cell types and cell wall layers of *B. insigne* are characterized by significant higher absorbance values as compared to *B. ceiba* (Fig. 4). In detail, the UV absorbance values of the S2 of fibers amount to 0.42 AU (white arrow in Fig. 4a), while the compound middle lamella shows the UV-absorbance of 0.61 AU (black arrow in Fig. 4a) representing a higher lignification of the CML. The cell corners and cell lumen of truncated fibers reveal varying degrees of UV-absorbance from 0.55 AU to over 0.93 AU (yellow arrow in Fig. 4a; white arrow in Fig. 4c). Furthermore, the tissue of *B. insigne* shows higher concentration of locally deposited phenolic extractives in the cell lumen of ray and axial parenchyma with UV absorbance values varying from 0.55 AU to over 1.00 AU (white arrows in Figs. 4b and 4d).



Fig. 4. UV microscopic scanning 2D profiles **(a-b)**, 3D profiles **(c-d)**, and point measurements **(e-f)** of heartwood tissue of *Bombax insigne*. The colour pixels represent different UV absorption values of the cell wall layers and phenolic extractives measured at 278 nm. **(a)** White arrow is pointing to S2 layer of a fiber, black arrow compound middle lamella, yellow arrow cell lumen of truncated fiber. **(b)** Black arrow represents extractives in ray cells. Cell lumen of truncated fiber **(c)** and extractives in ray cells **(d)** pointed by white arrows stand out as high intensity bands with histograms representing the statistics of the areas. **(e)** UV absorbance spectra of S2 layer of vessel, fiber, ray and parenchyma cells. **(f)** UV absorbance spectra of compound middle lamella (CML), extractives in ray cell lumen (Ext ray), extractives in parenchyma cell lumen (Ext paren) and extractives in vessel cell lumen (Ext vessel). F = fiber, LP = axial parenchyma cell, R = ray parenchyma cell

The evaluation of the point measurement spectra reveals analogous results and verifies the higher lignification of *B. insigne* (Figs. 4e-f). The maximum absorbance of the S2 layers of different cell types (vessels, fibers, ray, and axial parenchyma cells) detected at the wavelength of 278 nm ranges from 0.50 AU in vessels to 0.16 AU in axial parenchyma cells (Fig. 4e). The absorbance of fiber S2 layer amounts to 0.46 AU, while that of ray S2 layer is 0.31 AU. The deposits of phenolic extractives in the lumen of different cells show the maximum absorbance of 0.94 AU in vessels to 1.28 AU in ray cells, detected at the wavelengths of 279 nm to 282 nm (Fig. 4f). The bathochromic shift to a wavelength of 282 nm and slight shoulder at a wavelength range of 300 nm can be explained by the presence of chromophoric groups, *e.g.*, conjugated double bonds. The

higher degree of conjugation stabilizes π - π * transitions resulting in absorbance bands shifted to higher wavelengths (Goldschmid 1971) which can be detected by UV microspectrophotometry.

Remarks

In both species, the vessel S2 layer (absorbance: 0.46 AU in *B. ceiba* and 0.49 AU in B. insigne) is found to absorb the UV-light more strongly than the fiber S2 layer (absorbance: 0.21 AU in B. ceiba and 0.46 AU in B. insigne). Similarly, the cell wall of ray cells shows less absorbance than the cell wall of vessels. The lower absorbance by the fiber and ray cell wall can be attributed to the different chemical constitution of lignin in the individual cell types. Fergus and Goring (1970a, b) and Terashima et al. (1986) proved that the lignin located in vessel cell walls consists predominantly of the strongly absorbing guaiacyl type units, while the fiber cell wall lignin contains more syringyl units showing a lower UV absorbance at increasing OCH_3/C_9 ratio (Musha and Goring 1975). The cell corners and CML are generally represented by higher UV-absorbance as their lignin contains both guaiacylpropane and syringylpropane units in higher concentrations (Fergus and Goring 1970a). The UV-absorbance results obtained for each morphological region in both species are in agreement with those of other hardwoods such as beech, birch or merbau, with vessel S2 layers, CML, and cell corners absorbing more UV-light than fiber and ray S2 layers (Fergus and Goring 1970a; Musha and Goring 1975; Koch 2004; Koch et al. 2006).

In both species, the detectable (traceable) phenolic compounds are mostly deposited in cell lumina of ray and axial parenchyma and vessels rather than in cell walls. Only extractives deposited in the cell walls are responsible for increased dimensional stability and durability of heartwood (Hillis 1972).

CONCLUSIONS

The present study provides wood anatomical characteristics, topochemical information on the distribution of lignin and phenolic extractives within individual morphological regions, and the extractive content of two light Bombax species of Myanmar origin, B. ceiba and B. insigne. Based on anatomical descriptions, both wood species are quite similar in macroscopic and microscopic structure. Very thin cell-walls, large vessel tangential diameters, and large parenchyma cells are responsible for low density and strength of both species. The phenolic extractives are mostly deposited in the cell lumina rather in cell walls and their content, although higher in *B. ceiba*, is low when compared to other commercial species of the region. These findings explain the low durability of the species. The individual cell walls of *B. insigne* are more highly lignified than B. ceiba. In both species, the lower UV absorbance by the fiber and ray S2 layer can be attributed to the chemical composition of syringyl residues, while the higher absorbance of the vessel cell walls to the guaiacyl residues of lignin. The basic results are helpful for further research on the improvement of the physical and mechanical properties, and durability with modification techniques of the species to widen their utilization prospects.

REFERENCES CITED

- Babu, A. M., and Shah, J. J. (1987). "Unusual tissue complexes formed in association with traumatic gum cavities in the stem of *Bombax ceiba* L," Ann Bot-London 59, 293-299.
- Balan, R. (1980). Investment Reduction and Increase in Service Life of Kattumaram Logs. Report BOBP/WP/1, Bay of Bengal Programme, Development of Small-Scale Fisheries, FAO, Madras, India.
- Bauch, J., Koch, G., Puls, J., Schwarz, T. and Voiß, S. (2006). "Wood characteristics of *Podocarpus oleifolius* var. *macrostachyus* (Parl.) Buchholz and Gray native to Costa Rica: Their significance for wood utilization," *Wood Sci. Technol.* 40, 26-38.
- Bhattacharya, A., and Mandal, S. (2000). "Pollination biology in *Bombax ceiba* Linn.," *Current Sci. India* 79(12), 1706-1712.
- Carrillo, A., Mayer, I., Koch, G., and Hapla, F. (2008). "Wood anatomical characteristics and chemical composition of *Prosopis laevigata* grown in the Northwest Mexico," *IAWA J.* 29(1), 25-34.
- Chafe, S. C. (1987). "Collapse, volumetric shrinkage, specific gravity and extractives in Eucalypts and other species. Part 2. The influence of wood extractives," *Wood Sci. Technol.* 21, 27-41.
- Chaturvedi, O. P., and Pandey, N. (2001). "Genetic divergence in *Bombax ceiba* L. germplasms," *Silvae Genet*. 50, 3-4.
- FAO (1984). Report of Investigations to Improve the Kattumarans of India's East Coast. Bay of Bengal Programme, Development of Small-Scale Fisheries, FAO, Madras, India.
- Fengel, D., and Wegener, G. (2003). *Wood: Chemistry, Ultrastructure and Reactions*, Kessel Verlag, Germany.
- Fergus, B. J., and Goring, D. A. I. (1970a). "The location of guaiacyl and syringyl lignins in birch xylem tissue," *Holzforschung* 24, 113-117.
- Fergus, B. J., and Goring, D. A. I. (1970b). "The distribution of lignin in birch wood as determined by utraviolent microscopy," *Holzforschung* 24, 118-124.
- Fergus, B. J., Procter, A. R., Scott, J. A. N., and Goring, D. A. I. (1969). "The distribution of lignin in sprucewood as determined by ultraviolet microscopy," Wood Sci. Technol. 3, 117-138.
- Fukuzawa, K. (1992). "Ultraviolet microscopy," In: *Methods in Lignin Chemistry*, S.Y. Lin, S. Y., and Dence, C. W. (eds.), Springer-Verlag, Berlin, pp. 110-131.
- Goldschmid, O. (1971). "Ultraviolet spectra," In: *Lignins: Occurrence, Formation, Structure and Reactions*, Sarkanen and Ludwig CH (eds.), Wiley Interscience, New York, pp. 241-266.
- Griffiths, A. D., Philips, A., and Godjuwa, C. (2003). "Harvest of *Bombax ceiba* for the aboriginal arts industry, Central Arnhem Land, Australia," *Biol. Conserv.* 113, 295-305.
- Hapla, F., and Saborowski, J. (1987). "Stichprobenplanung für holzanatomische Untersuchungen," *Holz Roh- Werk*. 45, 141-144.
- Haupt, M., Leithoff, H., Meier, D., Puls, J., Richter, H. G., and Faix, O, (2003).
 "Heartwood extractives and natural durability of plantation-grown teakwood (*Tectona grandis* L.) A case study," *Holz Roh- Werk.* 61, 473-474.
- Hillis, W. E. (1972). "Formation and properties of some wood extractives. Review article," *Phytochemistry* 2, 1207-1218.

Hillis, W.E. (1987). Heartwood and Tree Exudates, Springer-Verlag. Berlin.

- IAWA Committee (1989). "List of microscopic features for hardwood identification," *IAWA Bull*. 10, 219-332.
- Koch, G. (2004). "Topochemical characterization of lignins and phenolic extractives in wood cell walls," *Lenzinger Berichte* 83, 6-12.
- Koch, G., and Grünwald, C. (2004). "Application of UV microspectrophotometry for the topochemical detection of lignin and phenolic extractives in wood fibre cell walls," In: *Wood Fibre Cell Walls: Methods to Study their Formation, Structure and Properties,* Schmitt *et al.* (eds.), Swedish University of Agricultural Sciences, Uppsala, pp. 119-130.
- Koch, G., and Kleist, G. (2001). "Application of scanning UV microspectrophotometry to localise lignins and phenolic extractives in plant cell walls," *Holzforschung* 55, 563-567.
- Koch, G., Puls, J., and Bauch, J. (2003). "Topochemical characterisation of phenolic extractives in discoloured beechwood (*Fagus sylvatica* L.)," *Holzforschung* 57, 339-345.
- Koch, G., Richter, H. G., and Schmitt, U. (2006). "Topochemical investigation on phenolic deposits in the vessels of afzelia (*Afzelia* spp.) and merbau (*Intsia* spp.) heartwood," *Holzforschung* 60, 583-588.
- Kress, W. J., DeFilipps, R. A., Ellen, F., and Kyi, Y. Y. (2003). *Checklist of the Trees, Shrubs, Herbs, and Climbers of Myanmar*, Dep. Syst. Biol.-Botany, Nat. Mus. Nat. Hist., Washington, DC.
- Mabberley, D. J. (2008). *Mabberley's Plant-Book: A Portable Dictionary of Plants*, 3rd Edn., Cambridge University Press, Cambridge, UK.
- Mayer, I., Koch, G., and Puls, J. (2006). "Topochemical investigations of wood extractives and their influence on colour changes in American black cherry (*Prunus serotina* Borkh.)," *Holzforschung* 60, 589-594.
- Mohiuddin, M. (1990). Wood Anatomy of Six Low Density Hardwoods (Alstonia scholaris, Anthocephalus chinensis, Bombax ceiba, Bombax insigne, Excoecaria agallocha and Trewa nudiflora) of Bangladesh, Bulletin 9, Wood Anatomy Series. Bangladesh For. Res. Inst., Chittagong.
- Musha, Y., and Goring, D. A. I. (1975). "Distribution of syringyl and guaiacyl moieties in hardwoods as indicated by ultraviolent microscopy," *Wood Sci. Technol.* 9, 45-58.
- Pearson, S. R., and Brown, H. P. (1932). Commercial Timbers of India: Their Distribution, Supplies, Anatomical Structure, Physical and Mechanical Properties and Uses, Vol I. Government of India, Central Publication Branch, Calcutta, India.
- Puls, J. (1993) "Substrate analysis of forest and agricultural wastes," In: *Bioconversion of Forest and Agricultural Plant Residues*, Saddler, J. N. (ed.)., CAP International, Wallingford, U.K., pp. 13-32.
- Scheffer, T. C., and Morrell, J. J. (1998). *Natural Durability of Wood: A Worldwide Checklist of Species*. Forest Research Laboratory, Oregon State University, Research Contribution 22.
- Seth, M. K. (2004). "Trees and their economic importance," Bot. Rev. 69(4), 321-376.
- Sint, K. M. (2010). "Promoting utilization potential of *Bombax ceiba* Linn and *Bombax insigne* Wall through enhancement of wood quality and technological properties by modification with melamine resin," Dissertation, Sierke Verlag, Goettingen.
- Sint, K. M., and Hapla, F. (2008). "Utilization potential of Myanmar lesser-used timber species," *Forstarchiv* 80, 129-131.

- Sint, K. M., Militz, H., Hapla, F., and Adamopoulos, S. (2011). "Treatability and penetration indices of four Myanmar lesser-used timber species," *Wood Res-Slovakia* 56(1), 13-22.
- Sint, K. M., Adamopoulos, S., Koch, G., Hapla, F., and Militz, H. (2012). "Impregnation of *Bombax ceiba* and *Bombax insigne* wood with a methylol melamine compound," *Wood Sci. Technol.*, DOI 10.1007/s00226-012-0482-y
- Sosef, M. S. M., Hong, L. T., and Prawirohatmodjo, S. (1998). Plant Resources of South-East Asia. No 5(3). Timber Trees: Lesser-Known Timbers, Backhuys Publishers, Leiden.
- Spurr, A. R. (1969). "A low-viscosity epoxy resin embedding medium for electron microscopy," *J. Ultrastruct. Res.* 26(1-2), 31-43.
- Takabe, K. (2002). "Cell walls of woody plants: autoradiography and ultraviolet microscopy," In: *Wood Formation in Trees*, Chaffey N, (ed.), Taylor & Francis, London, pp. 159-177.
- Tang, Y., Gilbert, M.G., and Dorr, L. J. (2007). "Bombacaceae," In: Flora of China, Wu et al. (eds.), Vol. 12, pp. 299-301.
- Tanvir, M. A., Khan, R. A., Siddiqui, M. T., and Khaliq Ch, A. (2003). "Growth behaviour and price variations of farm grown *Bombax ceiba* (Simal) in Punjab," *Int. J. Agric. & Biol.* 5(2), 154-156.
- Tarakanadha, B., Rao, K. S., Narayanappa, P., and Morrell, J. J. (2006). "Marine performance of *Bombax ceiba* treated with inorganic preservatives," *J. Trop. For. Sci.* 18(1), 55-58.
- Terashima, N., Fukushima, K., and Takabe, K. (1986). "Heterogeneity in formation of lignin," *Holzforschung* 40, 101-105.
- Thulasida, P. K., and Bhat, K. M. (2007). "Chemical extractive compounds determining the brown-rot decay resistance of teakwood," *Holz Roh- Werk*. 65, 121-124.
- Tsoumis, G. (1991). *Science and Technology of Wood: Structure, Properties, Utilization.* Van Nostrand Reinhold, New York.
- Yatagai, M., and Takahashi, T. (1980). "Tropical wood extractives: Effects on durability, paint curing time and pulp sheet resin spotting," *Wood Sci.* 12(3), 176-182.

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