Study of radial variation in anatomical characteristics

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Study of radial variation in anatomical characteristics of three native fast-growing tree species of a secondary forest in South Kalimantan for evaluation as pulpwood

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SUMMARY

The anatomical characteristics of terap (*Artocarpus elasticus*), medang (*Neolitsea latifolia*) and balik angin (*Alphitonia excelsa*) were studied to identify new pulpwood resources among lesser-known species native to Indonesia. The mean values of fibre and vessel element lengths were 1.55 and 0.42 mm in terap, 1.21 and 0.56 mm in medang and 1.14 and 0.52 mm in balik angin. The mean proportions of vessel, fibre, ray parenchyma, axial parenchyma and cell wall were 10.5%, 48.5%, 18.3%, 22.7% and 45.4% in terap, 21.6%, 49.7%, 13.8%, 14.9% and 50.5% in medang and 15.6%, 67.7%, 10.3%, 6.4% and 46.4% in balik angin. The anatomical characteristics of the three species were similar to those of fast-growing tree species used as pulpwood. Fibre diameter and cell wall percentage were significantly correlated with basic density in all species, indicating that diameter of wood fibre and cell wall percentages affect basic density values of all three woods.

KEYWORDS

Cell morphology, fast-growing tree species, terap (*Artocarpus elasticus*), medang (*Neolitsea latifolia*) and balik angin (*Alphitonia excelsa*)

INTRODUCTION

Fast-growing tree species have become promising wood sources to lessen the gap between wood demand and supply in the pulp and paper sector *(1-4)*, however, woods from many of these species are used for lumber production. For this reason, other fast-growing tree species suitable for pulp and paper production should be identified among less-used tree species. Terap (*Artocarpus elasticus* Reinw. ex Blume), medang (*Neolitsea latifolia* (Blume) S. Moore) and balik angin (*Alphitonia excelsa* (Fenzel) Reissek ex Benth) are native fast-growing tree species growing abundantly in secondary forests in South Kalimantan, Indonesia *(4)*. In a previous study, we found significant differences in wood properties among sample trees in each species*(4)*.

To determine the suitability of wood as a raw material for pulp and paper, it is important to know its anatomical characteristics. Characteristics such as length, diameter, and wall thickness of cells and percentage of cell types, are important factors in pulp and paper production *(5-8)*. Wood fibre length affects the strength properties of paper, particularly tearing strength *(9, 10)*. The wall thickness of the fibre is associated with high tearing strength and surface characteristics in paper *(11)*. Vessel percentage and vessel frequency (vessels/mm^2) have high negative correlations with pulp yield and paper strength properties. Thus, increasing vessel percentage will increase sheet density of paper and decrease paper strength measured as tear strength and stretch *(5)*. In addition, vessels also pick up ink during printing, causing print defects *(12)*.

Studies of anatomical characteristics in fast-growing tree species have been reported by several researchers *(2, 3, 8, 13-15)*. However, information on anatomical characteristics of the three lesser-known species, terap, medang and balik angin is still limited. Study of the properties of these species is required before use of their woods as raw material for pulp and paper production.

The aim of this study was to obtain basic information regarding anatomical characteristics associated with pulp and paper quality of these three tree species from South Kalimantan, Indonesia. Based on the results, we discuss the possibility of tree breeding programs for pulpwood production.

MATERIALS AND METHODS

Materials

Pith-to-bark strips (20 mm in tangential direction) were obtained from discs 2 cm in thickness collected 1 m above the ground from trees of the three species. The trees were located in a naturally regenerating secondary forest in South Kalimantan, Indonesia *(4)*. The mean diameter and tree height were 19.4 cm and 20.8 m, 19.0 cm and 20.8 m and 18.8 cm and 18.8 m for terap, medang and balik angin, respectively *(4)*. The strips were divided into two parts 10 mm in width along the radial direction. One part was used for determining cell length, and the other for determining cell morphology.

Cell length

To determine the length of fibre and vessel elements, small sticks were cut from the strips at 1 cm intervals from pith to bark with a razor blade. The sticks were macerated with Schulz's solution. Fifty fibres and 30 vessel elements in each sample were measured with a digital calliper (Mitutoyo, CD-15CX) under a microprojector (Nikon, V-12B). Mean values were calculated for fibre length and vessel element length.

Cell morphology

Wood blocks collected at 1 cm intervals from the pith were softened with 25% glycerine at 90 °C for 6 to 50 hours. Transverse sections (15 to 30 µm) were prepared from the softened blocks and stained with safranin. The sections were then dehydrated and mounted in Bioleit. Transverse sectional images were captured using a digital camera (Olympus, EP3) attached to a microscope (Olympus, BX 51), transferred to a personal computer and analysed with ImageJ software (National Institute of Health).

The following vessel and fibre morphologies were measured: radial and tangential diameters, radial and tangential lumen diameters, cell cross-section area, lumen cross-section area, cell perimeter and lumen perimeter. Diameters of fibres and vessels were calculated as averages of radial and tangential diameters. Fibre and vessel wall thickness was calculated as follows:

$$\text{Wall thickness } (\mu\text{m}) = \frac{2 \times (\text{cell area} - \text{lumen area})}{(\text{Cell perimeter} + \text{lumen perimeter})}$$

All cell morphologies of 50 fibres and 30 vessels were measured at 1 cm intervals from pith. The mean value of the anatomical characteristics in a tree was calculated by averaging the data obtained at 1 cm intervals from the pith.

Proportion of cell types

The proportion of cell types was measured by the point-counting method *(14)*. Five transverse images were obtained at each radial position using the microscope and digital camera described above. Gridlines were drawn on the images at 100 µm intervals. A total of 40 grid points per image were drawn, resulting in 200 grid points in a radial position. Cells were categorised into the following types: vessel wall/lumen, fibre wall/lumen, ray parenchyma wall/lumen, and axial parenchyma wall/lumen. The proportion of cells in each type was calculated as a ratio of the counted number of each type to the total number of grids in five transversal images (five images by 200 grids = 1000 grids).

Basic density

To clarify the relationships between basic density (BD) and anatomical characteristics, small blocks (1(R) by 2(T) by 1(L) cm) were collected to determine BD. BD was calculated as the ratio of oven-dry weight to green volume determined by the water displacement method *(16)*.

RESULTS

Average value of anatomical characteristics and BD of terap, medang and balik angin and result of ANOVA test are shown in Table 1. The results of fibre length of these three species are compared to other fast-growing tree species commonly used in the pulp and paper industry: *Acacia mangium*, *A.* hybrid, *Falcataria moluccana*, *Eucalyptus globulus*, and *E. camaldulensis* (Table 1a).

Table 1. Average value of anatomical characteristics and basic density in three species and result of ANOVA tests										
Property	Terap (*n*=5)			Medang (*n*=5)			Balik angin (*n*=5)			Significance among species
	Mean	SD	Sig.	Mean	SD	Sig.	Mean	SD	Sig.	
VEL [mm]	0.42	0.04	**	0.56	0.05	**	0.52	0.02	**	**
VWT [µm]	3.2	0.0	ns	2.8	0.1	**	2.9	0.3	**	ns
VD [µm]	167	6	ns	104	10	**	141	9	**	**
FL [mm]	1.55	0.12	ns	1.21	0.09	**	1.14	0.03	ns	**
FWT [µm]	1.6	0.1	**	2.6	0.1	ns	1.0	0.1	ns	**
FD [µm]	24.5	2.8	**	17.7	1.6	**	17.3	1.0	**	**
Vessel [%]	10.5	1.5	ns	21.6	3.8	**	15.6	2.0	ns	**
WF [%]	48.5	4.5	**	49.7	3.5	ns	67.7	4.3	**	**
RP [%]	18.3	2.8	ns	13.8	1.9	*	10.3	1.7	ns	**
AP [%]	22.7	1.9	**	14.9	2.5	ns	6.4	1.3	**	**
Cell wall [%]	45.4	4.6	**	50.5	2.5	**	46.4	2.8	*	ns
Basic density [g/cm^3]	0.37	0.06	*	0.57	0.04	**	0.39	0.03	ns	**

Note: *n*, number of trees

VEL, vessel element length
VWT, vessel wall thickness
VD, vessel diameter
FL, fibre length
FWT, fibre wall thickness
FD, fibre diameter
WF, wood fibre
RP, ray parenchyma
AP, axial parenchyma
SD, standard deviation
Sig., significance among trees;
**, significant at 1% level; ns, not significant.

Table 1a.The mean values of fibre length of terap, medang, balik angin and other some fast-growing tree species				
Species	Fibre length[mm]			
	Min.	Mean	Max.	SD
Terap	1.39	1.55	1.72	0.12
Medang	1.08	1.21	1.33	0.09
Balik angin	1.12	1.14	1.19	0.03
Acacia mangium (8)	0.61	0.94	0.95	0.05
Acacia hybrid (17)	-	1.01	-	0.12
Falcataria moluccana (13)	0.69	1.07	1.27	0.15
Eucalyptus globulus (10)	0.56	0.71	0.82	0.07
Eucalyptus camaldulensis (18)	0.78	0.91	1.03	0.08

Figure 1 shows the radial variation of wood fibre and vessel element length in terap, medang and balik angin at 1.0 m above the ground. The fibre length variation in these three species show similar patterns from pith to bark. Terap wood fibre length is longer than two other tree species. Radial variations of cell morphology, including vessel and fibre wall thickness, and vessel and fibre diameter are shown in Fig. 2. These three species show similar patterns in radial variation of cell morphology. Radial variation of the proportion of cell type is shown in Table 2. Mean proportions of cell types (vessel, wood fibre, ray parenchyma and axial parenchyma) were calculated at three positions: near the pith (1 to 3 cm from pith), the middle part (4 to 6 cm from pith) and near the bark (7 to 9 cm from pith).

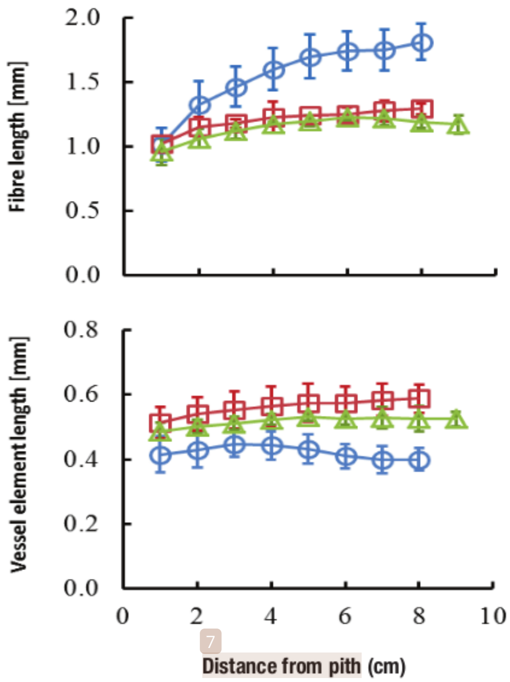

Fig. 1. Radial variation of wood fibre and vessel element length in terap, medang and balik angin at 1.0 m above the ground. Circles, squares and triangles indicate terap, medang and balik angin, respectively.

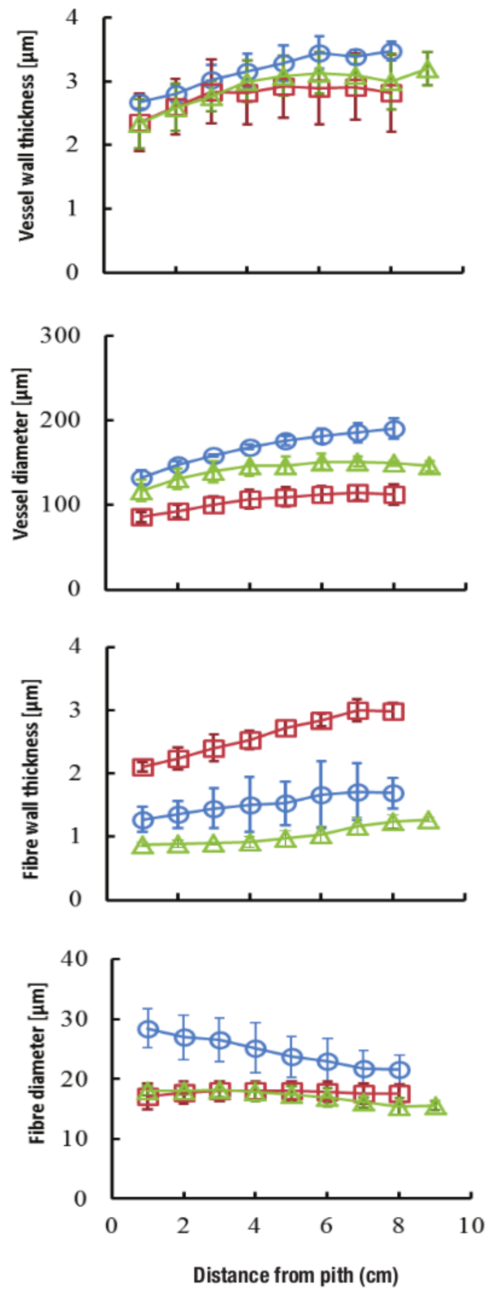

Fig. 2. Radial variation of cell morphology from terap, medang and balik angin at 1.0 m above the ground. Circles, squares and triangles indicate terap, medang and balik angin, respectively.

Table 2. Percentage of all types in each radial position									
Property	Terap (*n*=5)			Medang (*n*=5)			Balikangin (*n*=5)		
	Near the pith	Middle	Near the bark	Near the pith	Middle	Near the bark	Near the pith	Middle	Near the bark
Vessel [%]	9.2	10.8	11.6	19.0	20.2	25.7	15.0	14.2	17.6
Fibre [%]	52.6	48.3	44.6	52.4	50.2	46.4	68.3	71.6	63.2
Ray [%]	15.7	17.9	21.3	15.0	13.1	13.3	10.5	9.0	11.4
AP [%]	22.5	23.0	22.5	13.6	16.6	14.6	6.2	5.2	7.8
Cell wall [%]	42.9	47.4	45.7	49.5	50.4	51.4	43.8	46.7	48.8

Note: AP, axial parenchyma; *n*, number of trees

Table 3. Correlation coefficients between basic density and anatomical characteristics for terap, medang and balik angin							
Property		Terap (*n*=40)		Medang (*n*=41)		Balikangin (*n*=40)	
		r	Sig.	*r*	Sig.	*r*	Sig.
Vessel	VEL [mm]	– 0.59	**	– 0.24	ns	0.12	ns
	WT [µm]	0.79	**	– 0.49	**	0.29	ns
	D [µm]	0.68	**	– 0.20	ns	0.45	**
Wood fibre	L [mm]	0.83	**	0.15	ns	0.42	**
	WT [µm]	0.79	**	0.27	ns	0.68	**
	D [µm]	– 0.93	**	– 0.77	**	0.64	**
Percentage of cell type	Vessel	0.36	*	– 0.14	ns	0.29	ns
	WF	– 0.64	**	– 0.06	ns	– 0.39	*
	RP	0.42	**	0.40	ns	0.19	ns
	AP	0.28	ns	0.14	ns	0.31	ns
Cell wall percentage		0.59	**	0.63	**	0.36	*

Note: Sig., significant difference among trees; **, significant at 1% level; *, significant at 5% level; ns, no significance.

Relationships between basic density and anatomical characteristics

Table 3 shows the correlation coefficients between BD and anatomical characteristics of these three species, terap, medang, and balik angin woods. Almost all of the characteristics except axial parenchyma percentage have high significant correlations in terap wood, whereas those characteristics in two other species showed varied results. These results indicate that anatomical characteristics influence BD value in these three species.

DISCUSSION

Fibre length

The mean fibre lengths were 1.55, 1.21 and 1.14 mm and the mean vessel element length were 0.42, 0.56 and 0.52 mm in terap, medang and balik angin, respectively (Table 1). There were significant differences among the three species in length of fibre and vessel elements. Ogata *et al.(19)* reported that fibre length in *Artocarpus* spp. ranged from 1.2 to 2.6 mm and that those in species of Lauraceae, to which medang belongs, were around 1.3 mm. Fibre lengths of terap and medang in the present study were similar to those found in a previous study *(19)*.The fibre length of terap was clearly longer than that measured for other fast-growing trees *(8, 10, 13, 17, 18)*, whereas those in medang and balik angin were similar to those in *F. moluccana* and *A.* hybrid *(13, 17)* (Table 1a). Fibre length is positively correlated with tearing strength of paper *(11)*. Higher tearing strength is recognised as a desirable paper quality *(11)*; therefore, terap wood may provide raw material for paper with higher tearing strength than paper from the other two species tested.

Figure 1 shows the radial variations of fibre and vessel element length. Fibre length in all species increased from pith to bark.

Fibre length in terap increased up to 5 cm from the pith and then became stable toward the bark. In medang and balik angin, fibre length increased up to 4 cm from the pith and then showed almost constant values. In contrast, vessel element length in these three species was almost constant from pith to bark. In 10- and 13-year-old *A. mangium* trees, fibre length has been reported to be shortest near the pith and to gradually increase toward the bark, whereas vessel element length was almost constant from pith to bark *(20)*. Increase of fibre length from pith to bark has been reported in several other fast-growing tree species, *Acacia* spp, *F. moluccana* and *Eucalyptus* spp *(8, 13, 15, 17, 18, 21)*. The trend of constant vessel element length from pith to bark has also been reported in *Acacia* spp *(17)*.

Fibre wall thickness

The mean fibre wall thicknesses in terap, medang and balik angin were 1.6, 2.6 and 1.0 µm, respectively (Table 1). In comparison, the mean fibre wall thicknesses in 7-year-old *A.* hybrid, *A. mangium* and *A. auriculiformis* were 2.5, 2.6 and 2.8 µm, respectively *(2)*. In 13-year-old *F. moluccana* grown in Indonesia, the fibre wall thickness was 1.0 µm *(14)*. The mean fibre wall thicknesses in 14-year-old *E. camaldulensis* and *E. globulus* were 1.7 and 2.0 µm, respectively *(5)*. The fibre wall thicknesses in nine 50-month-old *Eucalyptus* species were 3.0 ± 0.5 to 5.0 ± 0.7 µm *(15)*. Hence these results show that fibre wall thickness of terap was similar to that of *E. camaldulensis (5)*, and that of medang was similar to those of *A. mangium* and *A. auriculiformis (2)*. Fibre wall thickness is associated with high tearing strength and surface characteristics in paper *(8, 11)*. Based on the fibre wall thickness value, medang may be a good raw material for producing paper with higher tearing strength compared with terap and balik angin. Fibre wall thickness in medang gradually increased from pith to bark and those in terap and balik angin slightly increased from pith to bark (Fig. 2). An increasing pattern of fibre wall thickness from pith to bark has been reported for other fast-growing tree species *(8, 14, 15, 21, 22)*.

Fibre diameter

The mean fibre diameters were 24.5, 17.7 and 17.3 µm in terap, medang and balik angin, respectively (Table 1). Ogata et al.*(19)* have reported that the fibre diameter in *Artocarpus* spp. was 25.0 to 45.0 µm. The fibre diameter of terap in the present study was similar to that obtained in the previous study *(19)*. Terap also showed a fibre diameter similar to that of *A.mangium* grown in Indonesia (23.8 to 25.2 µm) *(8)*, whereas medang and balik angin wood showed a fibre diameter similar to those of three *Acacia* species grown in Thailand (15.2 to 18.6 µm) *(1)*. The fibre diameter of terap gradually decreased from pith to bark (Fig.2). Balik angin showed an almost constant fibre diameter from pith up to 6 cm, and then it decreased toward the bark. In contrast, the diameter in medang was almost constant from pith to bark (Fig. 2). In the radial direction, a decreasing trend in fibre diameter from pith to bark has been reported for *F. moluccana*, *A. mangium*, and *A. auriculiformis (8, 14, 22)*. Veenin *et al.(18)* have reported that radial variation in five clones of *E. camaldulensis* was nearly constant from pith to bark. The pattern of radial variation of fibre diameter in terap was similar to those of *F. moluccana*, *A. mangium*, and *A. auriculiformis (8, 14, 22)*. Those of medang and balik angin were almost similar to those of *E. camaldulensis (18)*.

Vessel wall thickness

The mean vessel wall thicknesses in terap, medang, and balik angin were 3.2, 2.8 and 2.9 µm, respectively (Table 1). The reported mean values of vessel wall thickness in *E. camaldulensis* and *E. globulus* were 3.6 and 2.9 µm, respectively *(5)*. The mean values of vessel wall thickness in terap, medang and balik angin were similar to those of *E. camaldulensis* and *E. globulus (5)*. In addition, Ona *et al.(5)* reported that, in *E. camaldulensis*, vessel wall thickness was positively correlated with sheet density and burst factor of paper. Thus, terap may provide raw material for paper with higher sheet density and burst factor. Radial variation of vessel wall thickness was almost the same for terap, medang and balik angin (Fig. 2). Radial variation of vessel wall thickness gradually increased up to 6 cm from the pith and then became almost constant to the bark. Ohshima *et al. (23)* reported that *E. camaldulensis* and *E. globulus* had high wall thickness in the outer parts of the trunk. The trend of increasing wall thickness was observed from pith to bark in the radial direction. The radial variation in vessel wall thickness in these three species was similar to those in *E. camaldulensis* and *E. globulus (23)*.

Vessel diameter

The mean values of vessel diameter in terap, medang and balik angin were 167, 104 and 141 µm, respectively (Table 1). Ogata *et al. (19)* reported that the maximum tangential diameter of a solitary vessel ranged from 180 to 410 µm in *Artocarpus* spp. and from 70 to 300 µm in Lauraceae species. The mean vessel diameters reported for 14-year-old *E. camaldulensis* and *E. globulus* were 120 and 157 µm, respectively *(5)*. In other species commonly used for pulpwood, reported mean vessel diameters were 115 to 179 µm for *E. tereticorni*, 139 to 291 µm for *F. moluccana* and 132 to 142 µm for *A. mangium*, respectively *(8, 14, 21)*. The mean values of vessel diameters in terap, medang and balik angin were similar to those found in fast-growing pulpwood species *(5, 8, 14, 19, 21)*. Significant differences in vessel diameter were observed among the three species. Although the values differed among species, the radial variation of vessel diameter gradually increased from pith to bark in all species (Fig. 2). Similar patterns of vessel diameter in the radial direction have been reported for other fast-growing tree species *(8, 14, 18, 21- 23)*.

Proportion of cell types

The mean proportion of vessel, fibre, ray parenchyma, axial parenchyma and cell wall are shown in Table 1. Pirralho *et al.(15)* reported that the mean vessel, fibre, ray parenchyma and axial parenchyma proportions were 31.0%, 44.0%, 17.0% and 8.0%, respectively for *E. camaldulensis* and 25.0%, 33.0%, 19.0% and 21.0%, respectively, for *E. globulus*. The reported mean proportion of vessel, fibre, ray parenchyma, axial parenchyma and cell wall were 13.0%, 63.0%, 10.0%, 17.0% and 44.0%, respectively, in *A. auriculiformis* grown in Bangladesh *(22)*. The mean proportion of wood fibre in balik angin was similar to that in *A. auriculiformis (22)*, whereas the values in these three species were higher than those in *E. camaldulensis* and *E. globulus (15)*.

Vessel proportion increased from pith to bark in terap and medang, whereas in balik angin it decreased from near the pith to the middle area and then increased toward the bark. Fibre proportion showed a contrasting trend to that of vessel proportion. No consistent trend was found in ray and axial parenchyma proportion in any species. Cell wall percentage increased from pith to bark in medang and balik angin. These results were similar to those of previous studies *(14, 22)*.

Relationships between basic density and anatomical characteristics

Panshin and de Zeeuw *(24)* have pointed out that wood density variation is correlated mainly with cell diameter and thickness. In the present study, as shown in Table 3, fibre diameter showed a highly negative correlation with BD in terap (r = −0.93), medang (r = −0.77) and balik angin (r = −0.64), respectively. Significant positive correlations were found between BD and fibre wall thickness in terap (r = 0.79) and balik angin (r = 0.68). Fibre diameter showed a strong negative correlation (r = −0.71) with air-dried density, whereas fibre wall thickness showed a positive correlation (r = 0.67) in *A. auriculiformis (22)*. A similar result was reported by Ishiguri *et al. (14)* in *F. moluccana,* in which a significant negative correlation (r = −0.64) was found between the cell diameter of wood fibre and BD, whereas a high and significant positive correlation (r = 0.87) between cell wall thickness of wood fibre and BD.

In the correlation between BD and fibre percentage, significant negative correlations were found in terap (r= −0.64) and balik angin (r =−0.39) but not in medang. Cell wall percentage showed significant positive correlations with BD in all species. Ishiguri *et al. (14)* reported a negative correlation (r = −0.72) between BD and wood fibre proportion, and high positive correlation (r = 0.72) between BD and cell wall percentage in *F. moluccana*. In addition, Chowdhury *et al. (22)* reported a significant negative correlation (r = −0.35) between fibre proportion and air-dried density. In contrast, a significant positive correlation (r = 0.36) was reported between cell wall proportion and air-dried density in *A. auriculiformis*. The results of the present study were in accordance with those of the previous studies *(14, 22)*. From the results obtained in this study, BD values of terap, medang and balik angin are affected by the fibre diameter and cell wall thickness.

Among-tree variations

An ANOVA test was applied to test the differences in cell morphology, proportions of cell types and BD among trees and among species in terap, medang and balik angin (Table 1). Significant differences in vessel element length and fibre diameter were observed among trees in all species, whereas other properties showed varying results in all trees. Significant differences among species were found in vessel element length, vessel diameter, fibre length, fibre wall thickness, fibre diameter and proportion of cell types (vessel, wood fibre, ray parenchyma and axial parenchyma). Significant differences among the trees in BD was found in terap and medang wood, whereas high significant differences among species was found in this research. From these results, we conclude that tree-breeding program could be conducted to improve the quality of cell morphology, proportion of cell types and BD in terap, medang and balik angin.

CONCLUSION

The mean fibre and vessel element length were 1.55 and 0.42 mm in terap, 1.21 and 0.56 mm in medang and 1.14 and 0.52 mm in balik angin, respectively. Radial variation of fibre length increased from pith to bark, whereas vessel element length was almost constant from pith to bark in all three species. The mean proportions of vessel, fibre, ray parenchyma, axial parenchyma and cell wall were 10.5%, 48.5%, 18.3%, 22.7% and 45.4% in terap, 21.6%, 49.7%, 13.8%, 14.9% and 50.5% in medang, and 15.6%, 67.7%, 10.3%, 6.4% and 46.4% in balik angin. These measured anatomical characteristics in three species were similar to those in other commercial fast-growing tree species used for pulp wood production. Significant among-tree variation in cell length, cell morphology and proportion of cell types was recognised, suggesting the possibility of tree selection for breeding. BD showed negative correlation with fibre diameter, and significant positive correlation with cell wall proportion in the three species. Based on the results obtained, we conclude that BD values of terap, medang and balik angin are affected by the diameter of wood fibre and proportion of cell wall.

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