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WOOD ANATOMICAL CHARACTERISTICS OF AGARWOOD-PRODUCING SPECIES (*AQUILARIA* SP.)

Widyanto Dwi Nugroho, Rini Pujiarti, Novena Puteri Tiyasa
Universitas Gadjah Mada
Yogyakarta, Indonesia

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ABSTRACT

Aquilaria sp. is one of the tree species that produce agarwood. Agarwood is a kind of wood that has high content of fragrant resin and high economic values. Sufficient information about wood anatomical characteristic of agarwood-producing species is essential in agarwood trading, especially for identification and verification of wood. Therefore, this research aims to observe wood anatomical features of *Aquilaria* sp. and to observe its cell morphology. Five trees and five seedlings of *Aquilaria* sp. were used for the experiment. The results showed that the wood of *Aquilaria* sp. can be determined according to IAWA List of Hardwood for Identification with anatomical features nos. 2, 5, 10, 13, 22, 24, 29, 30, 40, 48, 49, 52, 58, 62, 66, 68, 71, 79, 92, 97, 105/104, 112, 115 and 134. Furthermore, seedling and trees of *Aquilaria* sp. have similar anatomical features but they have some differences on the cells morphology.

KEYWORDS: Agarwood, anatomical characteristics, *Aquilaria* sp., wood identification.

INTRODUCTION

Agarwood is one of the commercial woods and has high economical value that is often used by the people as fragrances, cosmetics and pharmaceuticals. *Aquilaria* sp. is one of the woody species that produces agarwood. Agarwood is defined as a kind of wood from agarwood-producing species that has unique colour and shape with high content of aromatic resin as a result from natural or artificial infection on that wood (SNI 2011). Agarwood is a product that has a form of dense clumps of dark brown to black and smelled fragrant wood on the woody plant which has undergone physical and chemical changes triggered by biotic and abiotic factors in agarwood-producing species (Mohamed et al. 2014, Rahayu 2011, Siran 2011, Tan et al. 2019).

Agarwood-producing species belong to spermatophyte division, dicotyledoneae class and three families such as Thymeleaceae, Euphorbiaceae dan Leguminoceae. Agarwood is produced by several genera of plants, namely Aetoxylon, Aquilaria, Enkleia, Gonystylus, Gyrinops, Phaleria, Wikstroemia, Dalbergia and Exxocaria (Wahyudi 2013, Wyn and Anak 2010).

Indonesia has approximately 25 agarwood-producing species (Siran 2011). Ministry of Environment and Forestry of Republic Indonesia reported that agarwood-producing species in tropical zone are *Aquilaria*, Gyrinops, and Gonystylus genera. Aquilaria Genus has 15 member species and is naturally distributed in India, Pakistan, Myanmar, Laos PDR, Thailand, Cambodia, China, Malaysia, Philippines and Indonesia (Wahyudi 2013).

Historically, agarwood has been used by the royal elite and tribal communities in the form of incense for the religious ritual, body and room deodorizers, cosmetic ingredients and pharmaceuticals (Siran 2011). The use of agarwood started as early as 2000 years ago and the demand for agarwood continues to increase (Lee et al. 2016). The worldwide demand for agarwood with high economical values has continued to increase in recent years. In contrast, the supply of agarwood from natural forest decreases. The Convention on International Trade in Endangered Species of Wild Fauna and Flora has listed *Aquilaria malaccensis* in Appendix II and more recently all species of Aquilaria were included so that worldwide trade in agarwood is monitored and controlled (CITES 2004). In worldwide wood trading, the information about the wood species is very important. It is often confusing for the wood trader to determine the wood species. Wood anatomical characteristics have close relation to the wood properties, physiological function of woody tissues and typical features for wood identification. Therefore, sufficient information about anatomical characteristic of agarwood-producing species is very important in agarwood trading, especially for the identification and verification of wood.

Moreover, many researchers show great interest on agarwood-producing species such as *Aquilaria* sp. propagation, cultivation and artificial induction of agarwood. However, it is often difficult to find *Aquilaria* sp. trees for the experiments. Agarwood-producing species in tree stage is quite difficult to find, and also expensive, therefore it is necessary to find the plant model instead the tree stage. Agarwood-producing species in seedling stage is easier to find and manage. But, we are facing some questions; is seedling possible for plant model on wood anatomical observation and is there any difference on wood anatomical characteristics compared to trees?

According to the situation explained above, this research aims to observe anatomical features of wood of *Aquilaria* sp. according to International Association of Wood Anatomist (IAWA) List of Microscopic Features for Hardwood Identification (IAWA Committee 1989) and to observe cell morphology of wood of *Aquilaria* sp. in seedling and tree stages.

MATERIALS AND METHOD

Plant materials

Five trees and five seedlings of *Aquilaria* sp. were used for the experiment. The seedlings were approximately 70 cm tall and were planted in straight position in 20 cm diameter pots, filled with regosol soil, in a greenhouse at the nursery of the Faculty of Forestry, Universitas Gadjah Mada, Yogyakarta, Indonesia. *Aquilaria* sp. trees were approximately 6 years old that were planted in community forest. Wood samples from *Aquilaria* sp. trees were obtained using increment borer and wood samples from seedlings were obtained using knife.

Observation and measurement of characteristics of wood anatomy

The transverse, tangential and radial sections of 15-20 μm thickness were sectioned on sliding microtome (NS-31; Yamato Koki, Saitama, Japan). The transverse, tangential and radial sections of the wood from seedlings and trees were stained with 1% safranin solution, dehydrated in a graded ethanol series, mounted on glass slides, fixed with resin (Entellan New;

Merck, Darmstadt, Germany) and covered with cover slips (Nugroho et al. 2012). Images were recorded from each slide under the light microscope with the digital camera. Digital images of transverse, tangential and radial sectional area were taken in order to measure cell morphology such as diameter of vessels, frequency of vessels, length of vessels, length of fibers, diameter of fibers, thickness of fiber wall, diameter of fibers lumen, ray height, frequency of ray, frequency of Included phloem and proportion of Included phloem. Furthermore, the digital images were observed to determine the anatomical features of wood of seedlings and trees of *Aquilaria* sp. according to IAWA List of Microscopic Features for Hardwood Identification. The morphology of cells was measured with image-analysis software (Image Pro Plus ver. 4.5; Media Cybernetics, Rockville, USA).

Measurements of wood fiber length

A small piece, approximately 2 x 2 x 10 mm, were removed from each wood samples from seedlings and trees. The pieces of wood were macerated with Franklin solution, which is a mixture of equal volumes of glacial acetic acid (100%) and hydrogen peroxide (30%) and heated in boiled water. Images of wood fibers were captured under a light microscope with a digital camera (Nugroho et al. 2012). Lengths of wood fibers were measured with image-analysis software (Image Pro Plus ver. 4.5; Media Cybernetics, Rockville, USA).

Analysis of data

The anatomical characteristics of wood of *Aquilaria* seedlings and trees were described according to IAWA List of Microscopic Features for Hardwood Identification (IAWA Committee 1989). Furthermore, analysis of variance was conducted to determine the significant differences of data on the cell morphology of wood. Statistical analyses were performed using PRISM5 for Mac OS X (GraphPad Software Inc, California, USA). The data of each measured parameters were analyzed using one-way analysis of variance (ANOVA) followed by Tukey`s post hoc test with the level of significance differences at $P < 0.05$.

RESULTS AND DISCUSSION

Vessels

Figs. 1A and 1B showed the cross sections of seedlings and trees of *Aquilaria* sp. wood. The vessels of seedlings and trees of *Aquilaria* sp. are almost similar.

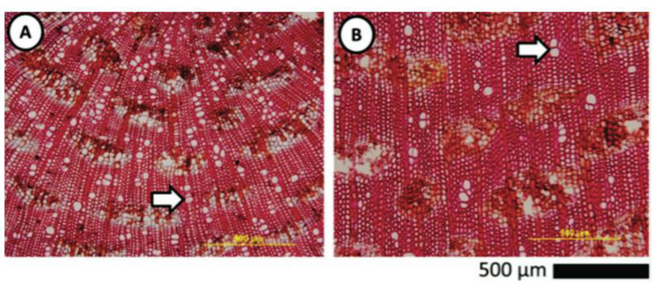

Fig. 1: Cross sections of seedlings (A) and trees (B) of Aquilaria sp. wood. Arrows indicate the type of vessel grouping (radial pattern).

The vessels are arranged in diffuse porous (feature no. 5), grouping in radial pattern (feature no. 10), simple perforation plates (feature no.13), intervessel pits alternate (feature no. 22), intervessel size minute ≤ 4µm (feature no. 24), pits vestured (feature no. 29), vessel ray pitting similar to intervessel pit (feature no. 30), no helical thickening. *Aquilaria* sp. trees have deposit in the vessels (feature no. 58) while the seedlings have no deposit.

Figs. 2A and 2B showed the arrangement and size of intervessel pits. Growth rings are indistinct in both seedlings and trees of *Aquilaria* sp (feature no. 2).

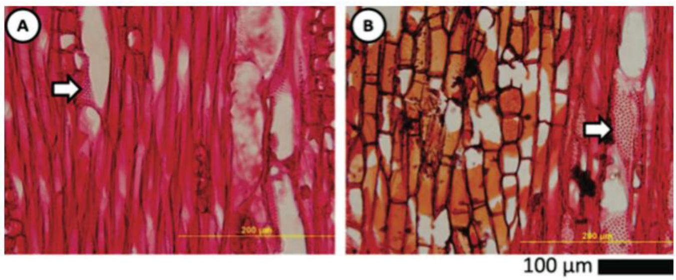

Fig. 2: Radial sections of seedlings (A) and trees (B) of Aquilaria sp. wood. Arrows indicate the type of intervessel pitting (alternate).

Detailed anatomical features are shown in Tab. 1. The anatomical features of vessels of seedlings and trees of *Aquilaria* sp. are in accordance to previous report (Ogata et al. 2008). There are no differences in the anatomical features of vessel between seedling and tree stage because the seedlings have already formed secondary xylem. The axial parenchyma, fibers, vessel element and ray cell are the products from the activity of vascular cambium (Chaffey 2002). Even though the seedlings were still 1 year old, but the seedlings have already formed secondary xylem that can be proven by the presence of various types of woody cells and the presence of vascular cambium.

Tab. 1: Anatomical features of wood of seedlings and trees of Aquilaria sp. according to IAWA List of microscopic features for hardwood identification.

No	Anatomical features	Seedlings	Trees	(IAWA features nos.)
1	**Growth rings**	Indistinct	Indistinct	2
2	**Vessels**			
	a. Porosity	Diffuse porous	Diffuse porous	5
	b. Vessel arrangement	-	-	-
	c. Vessel groupings	Radial pattern	Radial pattern	10
	d. Solitary vessel outline	-	-	-
	e. Perforation plates	Simple	Simple	13
	f. Intervessel pits: arrangement and size	Alternate, Minute ≤ 4µm	Alternate, Minute ≤ 4µm	22, 24
	g. Vestured pits	Vestured	Vestured	29
	h. Vessel ray pitting	Similar to intervessel pit	Similar to intervessel pit	30
	i. Helical thickening	No	No	-

	j. Tangential diameter of vessel lumina	33.68 ± 3.25	48.91 ± 13.20	40
	k. Vessels per square millimetre	42 ± 6.69	23 ± 4.87	48, 49
	l. Mean vessel element length	259.62± 59.05	237.89 ± 43.79	52
	m. Tyloses and deposits in vessels	No	Deposit	58
	n. Wood vesselless	No	No	-
3	**Tracheids and fibres**			
	a. Ground tissue fibres	Distinct bordered pits	Distinct bordered pits	62
	b. Septate fibres and parenchyma-like fibre bands	Non septate fibers present	Non septate fibers present	66
	c. Fibre wall thickness	Very thin (1.24±0.17µm)	Very thin (1.30±0.12µm)	68
	d. Mean fibre lengths (µm)	608.54 ± 37.36	550.90 ± 79.30	71
4	**Axial parenchyma**			
	a. Apotracheal axial parenchyma	-	-	-
	b. Paratracheal axial parenchyma	Scanty paratracheal	Scanty paratracheal	78
	c. Banded parenchyma	-	-	-
	d. Axial parenchyma cell type / strand length	Four (3-4) cells per parenchyma strand	Four (3-4) cells per parenchyma strand	92
5	**Rays**			
	a. Ray width	1-3 cells	1-3 cells	97
	b. Aggregate rays	-	-	-
	c. Ray height	354.14±57.23 µm	242.59 ± 79.52 µm	-
	d. Rays of two distinct sizes	-	-	-
	e. Rays: cellular composition	Upright/ square	Procumbent/ square	105/104
	f. Sheath cells	-	-	-
	g. Tile cells	-	-	-
	h. Perforated ray cells	Yes	Yes	112
	i. Disjunctive ray parenchyma cell walls	-	-	-
	j. Rays per millimeter	11 ± 2.55	15 ± 4.44	115
	k. Wood rayless	No	No	-
6	**Storied structure**	-	-	-
7	**Secretory elements and cambial variants**			
	a. Oil and mucilage cells	-	-	-
	b. Intercellular canals	-	-	-
	c. Tubes/ tubules	-	-	-
	d. Cambial variants	Included Phloem-Diffuse	Included Phloem-Diffuse	134

8	**Mineral inclusion**			
	a. Prismatic crystals	-	-	-
	b. Druses	-	-	-
	c. Other crystal types	-	-	-
	d. Other diagnostic crystal features	-	-	-
	e. Silica	-	-	-
9.	**Non-anatomical features information**			
	a. Heartwood colour	-	-	-
	b. Sapwood colour	White-yellowish	White-yellowish	-
	c. Odor	-	-	-
	d. Wood weight	-	-	-
10	**Determined species**			
	Family	Thymelaeaceae		
	Genus	*Aquilaria*		
	Species	*Aquilaria* sp.		
	Local name	Gaharu		

The cell morphology of vessels such as diameter of vessel and frequency of vessel are significantly different between seedling and trees (P=0.0037 and P=0.001). Diameter of vessels and vessel frequency of seedlings are 33.68 ± 3.25 μm and 42 ± 6.69/mm^2 while trees are 48.91 ± 13.20 μm and 23 ± 4.87/mm^2, respectively. However, the length of vessels was not significantly different between seedlings and trees of *Aquilaria* sp. The lengths of vessel are 0.26 ± 0.06 mm in seedlings and 0.24 ± 0.04 in trees. The differences in diameter of vessel and vessel frequency might be related to the cambial age of seedlings and trees of *Aquilaria* sp. The morphological aspects of vessels are in accordance to previous reports (Ogata et al. 2008, Andianto 2010).

Fibers

Figs. 3A and 3B showed that wood from seedlings and trees have very thin fibers cell walls. The anatomical features of fibers between seedling and tree stage of *Aquilaria* sp. are very similar.

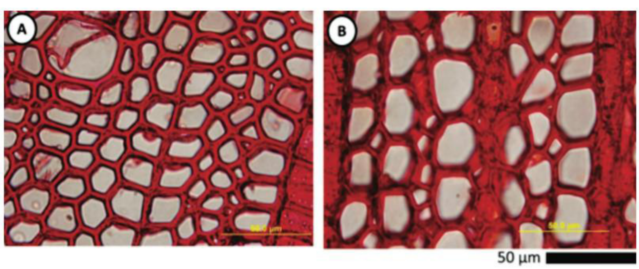

Fig. 3: Cross sections of seedlings (A) and trees (B) of Aquilaria sp. wood. All samples showed very thin of cell wall.

The fibers can be characterized as fibers with distinct bordered pith (feature no. 62), no septate fibers present (feature no. 66), very thin cell walls (feature no. 68) and fiber length of wood from seedlings and trees were ≤ 900 μm (feature no. 71). Detailed anatomical features of fibers are shown in Tab. 1. The measurement revealed that length of fibers, diameter of fibers, thickness of fiber wall and diameter of fiber lumen in seedlings are 0.61 ± 0.04 mm, 14.91 ± 1.19 µm, 1.24 ± 0.17 µm and 12.43 ± 1.17 µm while in the trees stages are approximately 0.55 ± 0.08 mm, 21.80 ± 2.55 µm, 1.30 ± 0.12 µm and 19.20 ± 2.40 µm. Diameter of fibers and diameter of lumen fibers between seedlings and trees stage are significantly different (P=0.001 and P=0.000). The anatomical features of fiber of seedlings and trees of *Aquilaria* sp. are in accordance to previous reports (Ogata et al. 2008, Andianto 2010). The differences in diameter of fibers and diameter of lumen fibers might be related to the cambial age of seedlings and trees of *Aquilaria* sp or other factors such as soil condition, climate, and other environmental factors.

Axial parenchyma

We found that the axial parenchyma of seedlings and trees have similar pattern. The type of paratracheal axial parenchyma is scanty paratracheal (feature no. 78) with 3-4 cells per parenchyma strand (feature no. 92). No modification concerning axial parenchyma in the maturation of trees. The axial parenchyma is also similar to the previous observation (Ogata et al. 2008, Andianto 2010).

Ray parenchyma

Figs. 4A and 4B showed the radial sections of seedlings and trees of *Aquilaria* sp. wood. Ray parenchyma cells of seedlings and trees of *Aquilaria* sp are almost similar in their anatomical features.

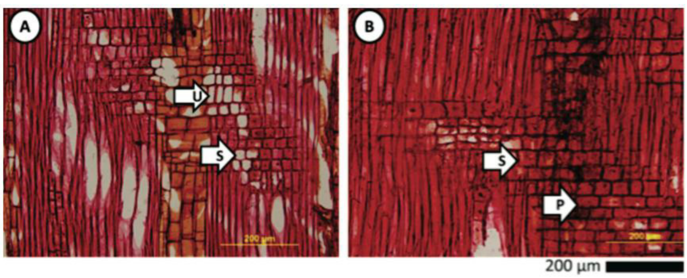

Fig. 4: Radial sections of seedlings (A) and trees (B) of Aquilaria sp. wood. Arrows indicate the type of cellular composition of rays. U: upright cells; S: square cells and P: procumbent cells.

In all of samples ray width is arranged by 1-3 cells (feature no. 97), ray high < 1 mm, perforated ray cells were found (feature no. 112), ray per millimeter is 4-12/mm (feature no. 115). The anatomical differences were found in the cellular composition of rays. Seedlings have upright and square type of ray cells while trees have procumbent and square types of ray cells (Figs. 4A, B). In term of cells morphological aspect, ray high is significantly different between seedlings and trees (P=0.034). The ray high of seedlings is 354.14 ± 57.23 µm while in the trees is 242.59 ± 79.52 µm. Detailed anatomical features and cell morphology of ray parenchyma are shown in Tab. 1 and Tab. 2.

Tab. 2: Cells morphology wood of seedlings and trees of Aquilaria sp.

Cells morphology	P	Type of samples	
		Seedlings	Trees
Vessels			
1. Diameter of Vessels (μm)	0.037*	33.68 ± 3.25	48.91 ± 13.20
2. Frequency of Vessels (number/mm²)	0.001**	42 ± 6.69	23 ± 4.87
3. Length of Vessels (mm)	0.541ns	0.26 ± 0.06	0.24 ± 0.04
Fibers			
1. Length of Fibers (mm)	0.180ns	0.61 ± 0.04	0.55 ± 0.08
2. Diameter of Fibers (μm)	0.001**	14.91 ± 1.19	21.80 ± 2.55
3. Thickness of Fiber Wall (μm)	0.533ns	1.24 ± 0.17	1.30 ± 0.12
4. Diameter of Fibers Lumen (μm)	0.000**	12.43 ± 1.17	19.20 ± 2.40
Ray parenchyma			
1. Ray Hight (μm)	0.034*	354.14 ± 57.23	242.59 ± 79.52
2. Frequency of Ray (number/mm²)	0.104ns	11 ± 2.55	15 ± 4.44
Included phloem			
1. Frequency of *Included Phloem* (number of *Included Phloem* /mm²)	0.001**	9 ± 1.66	3 ± 1.01
2. Proportion of Included Phloem	0.716ns	28.08 ± 8.18	29.76 ± 5.63

P - Level of significance * - Significantly different at P<5%

** - Significantly different at P<1% ns - No significantly different.

The result suggested that there was no modification on anatomical features of ray in the maturation process of trees. The modification was only on the term of cells size that is caused by the cambial activity and cambial age. From the previous report (Andianto 2010), the cellular composition of ray in seedlings is in accordance to *Aquilaria malacensis* while the cellular composition of ray in the tree indicated that the features in accordance to *Aquilaria microcarpa*.

Other anatomical and non-anatomical features

In term of other anatomical features, we found that there are no storied structure, oil or mucilage cells, intercellular canal, tubes and mineral inclusion. However, we found that the seedlings and trees have such kind of cambial variant namely included phloem. The included phloem is arranged in diffuse distribution (feature no. 134). Fig. 5 showed cross and tangential sections of seedlings (Fig. 5A,C) and trees (Fig. 5B,D) of *Aquilaria* sp. wood.

Arrows in the figures indicate the presences of included phloem. *Aquilaria* sp. has unique cambial variant such as included phloem (Ogata et al. 2008, Andianto 2010). Included phloem is arranged by sieve tube, companion cells and parenchyma cells or other cells that were formed in secondary xylem (Carlquist 2013). The changes of anatomical and morphological characteristics on the xylem and cambium such as cell division, cell differentiation, cell orientation, cell elongation and cell wall thickening can be influenced by mechanical, biological or climatic factors (Schwingruber 2007).

Tab. 2 showed that frequency of included phloem significantly different between seedlings and trees (P=0.001) while no significant differences between seedlings and trees in term of proportion of included phloem. Furthermore, the color of fresh cut woods is white-yellowish. The colour of wood is the typical color of wood of *Aquilaria* sp. that is in non infected condition. In infected woods, the woods became darker in their color (Sitepu et al. 2011).

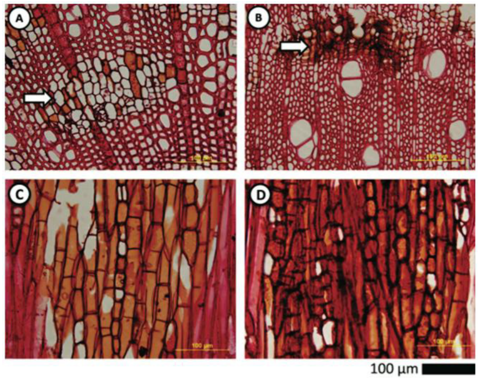

Fig. 5: Cross and tangential sections of seedlings (A, C) and trees (B, D) of Aquilaria sp. wood. Arrows indicate the presence of Included Phloem.

In summary, the plant level such as seedling and trees of *Aquilaria* sp. have very similar anatomical features but they have some differences on the cells morphology because of the age of vascular cambium and activity of vascular cambium. Mechanical, biological and climatic factor might be the first to affect the morphological aspects rather than their anatomical features.

CONCLUSIONS

The results showed that the wood of *Aquilaria* sp. can be determined according to IAWA List of Hardwood for Identification with anatomical features nos. 2, 5, 10, 13, 22, 24, 29, 30, 40, 48, 49, 52, 58, 62, 66, 68, 71, 79, 92, 97, 105/104, 112, 115 and 134. Furthermore, seedling and trees of *Aquilaria* sp. have similar anatomical features but they have some differences on the cells morphology. The differences are in their vessel diameter, vessel frequency, fibers diameter, fibers lumen diameter, ray height and the frequency of included phloem. Therefore, seedling is possible for plant model on the study wood anatomical characteristics in *Aquilaria* sp.

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Widyanto Dwi Nugroho*, Rini Pujiarti, Novena Puteri Tiyasa
Universitas Gadjah Mada
Faculty of Forestry
Department of Forest Product Technology
Jl. Agro No. 1 Bulaksumur
Yogyakarta, 55281
Indonesia
*Corresponding author: wdnugroho@ugm.ac.id