

Comparison of Anatomical Features in the Three *Syzygium* Species

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This study was conducted to provide foundational anatomical information of three infrequently used wood species growing in tropical areas. Three species of the genus *Syzygium*, namely the clove tree, kupa, and spicate eugenia, were selected. The representative anatomical features of these species were classified using the International Association of Wood Anatomists (IAWA) anatomical feature list. The representative anatomical features of the clove tree included the distribution of small vessels with tangential diameters of approximately 60 µm in cross-surface, a dense spacing of vessels, the axial parenchyma in narrow bands or lines up to three cells wide, and the body ray cells procumbent with over four rows of upright and/or square marginal cells. The kupa showed axial parenchyma confluent and the body ray cells were procumbent with over four rows of upright and/or square marginal cells. In the spicate eugenia, the axial parenchyma was diffused in aggregate with exclusively uniseriate rays and the body ray cells were procumbent with one row of upright and/or square marginal cells. These three species were easily identified by optical microscopy via the anatomical features of the woods.

Keywords: Anatomical features; Indonesian wood; *Syzygium aromaticum*; *Syzygium polycephalum*; *Syzygium zeylanicum*; Clove tree; Kupa; Spicate Eugenia

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INTRODUCTION

Korea relies on imports for 84.8% of its total wood demand and is therefore faced with difficulties in securing high-quality wood for industrial purposes (Korea Forest Service 2020). To solve this problem, Korea has diversified the importation of its wood from Southeast Asia to include New Zealand, China, and South America.

According to the Korea Forest Service (2019), 36 Korean companies are involved in overseas plantations in 14 countries including Australia, New Zealand, and Indonesia to secure carbon emission rights, wood resources, and bioenergy resources. Despite the increase in the importance of overseas plantations to secure high-quality wood resources, there has only been a focus on a few plantation tree species (FAO 2001; Sanchez 2019), such as pine (*Pinus* spp.), eucalyptus (*Eucalyptus* spp.), the rubber tree (*Hevea* spp.), and acacia (*Acacia* spp.). For more efficient overseas plantations, high value-added species should be investigated and reliable fundamental information must be provided to consumers and industries.

Research regarding the efficient use of imported wood resources (Jo *et al.* 1976, 1977; Choi and Yun 1982; Kang *et al.* 1982) has been undertaken in various fields with the increase in imported wood since the 1970s; however, studies have decreased owing to log export prohibition by timber exporting countries and increasing timber prices. Previous studies (Ogata *et al.* 2001; Alma *et al.* 2007; Kwon 2008; Park and Jung 2009; Park and Oh 2011; Korea Green Promotion Agency 2012; Hamdan *et al.* 2016; Hamad *et al.* 2019) have been undertaken on wood quality because of the recognition of such research for securing wood resources by overseas plantations. However, most of the imported woods produced from natural forests have large diameters and are tall, whereas recently imported wood from southeast Asia is produced from plantation forests or community forests that have small diameters. Therefore, there could be a difference in wood quality between the woods produced from natural forests and those from plantation forests. Previous studies (Kim *et al.* 2012; Jang *et al.* 2014; Kim *et al.* 2014) on the characteristics of plantation wood have provided identification and utilization indicators of infrequently used species grown in the tropical zone. However, information regarding recently planted species and promising species is still inadequate as an indicator for effective utilization.

The genus *Syzygium* contains over 1,000 species and has been widely grown in African and Asian tropical countries as a fruit source and for medicinal purposes. The major region of endemism is southeast Asia, including the Philippines, New Guinea, Indonesia, and Peninsular Malaysia. *Syzygium* species are small- to medium-sized trees. However, sometimes large trees have been reported up to 50 m tall with a bole up to 2 m in diameter (Lemmens *et al.* 1995). Despite the potential for the utilization of this species, their availability as a wood resource is unclear due to the lack of information on their wood quality. Therefore, in the present study, three representative *Syzygium* species from Java, Indonesia, *i.e.*, the clove tree (*Syzygium aromaticum*), kupa (*S. polycephalum*), and spicate eugenia (*S. zeylanicum*), were selected to investigate their anatomical characteristics to evaluate their potential as a wood resources and to provide fundamental information.

EXPERIMENTAL

Materials

Three tree species (clove tree, *i.e.*, *Syzygium aromaticum*; kupa, *i.e.*, *Syzygium polycephalum*; and spicate eugenia, *i.e.*, *Syzygium zeylanicum*) were collected from the research forest of Bogor Agricultural University in Bogor, West Java, Indonesia. The wood discs were obtained at breast height and wood blocks for microscopy were collected from near the pith and near the bark. Detailed information of sample trees and geographical features is shown in Table 1.

Microscopy

Based on the typical method (Park *et al.* 1993), slices of 15 to 20 μm in thickness from the transverse, radial, and tangential surfaces were prepared from each species using a sliding microtome (MSL-H model; Nippon Optical Works, Nagano, Japan). These surfaces were treated with 1% safranin and 1% light-green solution for staining lignin and cellulose, respectively, followed by dehydration with graded ethanol series (50%, 70%, 90%, 95%, and 99%) for 5 min per each stage, clearing with xylene for 20 minutes and mounting on a slide glass with Canada balsam. Photomicrographs were taken with a digital camera (INFINITY I-IM; Teledyne Lumenera, Ottawa, Canada) mounted on a microscope

(Eclipse E600; Nikon, Tokyo, Japan) and were used to measure the anatomical features. C-Mount connector (TV-Lens C-0.45x; Nikon, Tokyo, Japan) and Tube adaptor (Modelless; Nikon, Tokyo, Japan) were applied for adjustment between digital camera and microscope with 2.5x magnification. The objective lens of 10x and 20x (Plan fluor, Nikon, Tokyo, Japan) was applied for the observation of transverse and longitudinal surfaces, respectively. The qualitative and quantitative anatomical characteristics were analyzed using image analysis software (i-Solution lite; IMT Solutions, Version 9.1, British Columbia, Canada).

Table 1. Information on the Sample Trees and the Site

Species	D.B.H. (cm)	Climate of Habitat	Location
Clove tree (<i>Syzygium aromaticum</i>)	21.5	Tropical Rainforest Region, Average Relative Humidity 70%, Mean Precipitation 4,086 mm, Average Temperature 25.2 °C	Bogor, West Java, Indonesia (-6.554°S, 106.723°E)
Kupa (<i>Syzygium polycephalum</i>)	12.0		
Spicate eugenia (<i>Syzygium zeylanicum</i>)	12.0		

Note: D.B.H. : Diameter at Breast Height

Analysis of Qualitative and Quantitative Anatomical Characteristics

The qualitative anatomical features such as porosity, vessel arrangement, tyloses in vessels, axial parenchyma pattern, ray composition, prismatic crystals, and ray width were examined from cross, radial, and tangential sections.

The quantitative anatomical characteristics on vessel and ray properties were also investigated in each surface. The tangential diameter of the vessels from the cross-section and the height of the rays from the tangential surface were measured using 50 elements per species to improve the reliability of the results. The number of vessels per square millimeter from each cross-surface was measured six times. The number of rays per millimeter from the tangential surface was measured 30 times, and the average of these was calculated.

Classification of Anatomical Features

The anatomical characteristics observed in the present study were classified based on the International Association of Wood Anatomists (IAWA) hardwood feature list (Wheeler *et al.* 1989).

Statistical Analysis

An independent sample t-test was performed at a 95% confidence interval to determine the significance between near-the-pith and near-the-bark vessels and rays. A one-way analysis of variance (ANOVA) test was performed to verify the relationship among the species. Duncan's test was applied as a *post hoc* verification of the ANOVA test with a 5% significance level. Statistical analyses were computed using SPSS 24.0 for Windows (IBM Corp., Armonk, NY, USA).

RESULTS AND DISCUSSION

Qualitative Anatomical Characteristics

Figure 1 shows the optical micrographs of the cross-surfaces of the sample species. All species were identified as diffuse-porous wood. Vessels in radial multiples consisting of two to three vessels existed frequently, and vessel clusters were rarely seen in the clove tree (Fig. 1a). In the kupa (Fig. 1b), solitary pores coexisted with vessels in radial multiples consisting of two to three vessels, whereas spicate eugenia (Fig. 1c) was mostly composed of a solitary pore. Tyloses were observed in the vessels of all sample species. Regarding the arrangement of the axial parenchyma, the clove tree had axial parenchyma in narrow bands or lines up to three cells wide, the kupa had axial parenchyma confluent, and the spicate eugenia showed axial parenchyma that was diffuse in aggregates.

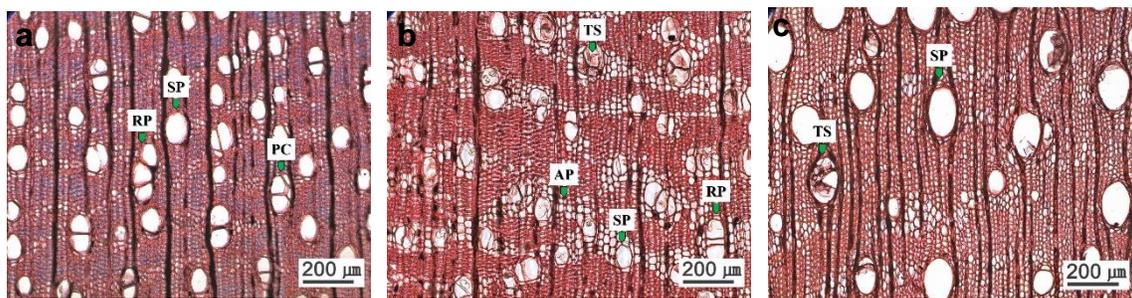


Fig. 1. Optical micrographs of the cross-surfaces of three *Syzygium* species: (a) clove tree (*S. aromaticum*); (b) kupa (*S. polyccephalum*); (c) spicate eugenia (*S. zeylanicum*); (SP) solitary pore; (RP) radial pore multiple; (PC) pore cluster; (AP) axial parenchyma; and (TS) tyloses

According to Lemmens *et al.* (1995), the genus *Syzygium* showed diffuse-porous vessels with radial multiples of 2 to 3 (up to 8) pores, tyloses were sparse to abundant in the vessels, and paratracheal and aliform parenchyma. In addition, Ogata *et al.* (2008) showed that the anatomical characteristics of *Syzygium* spp. included solitary vessels and radial multiples of 2 to 8 pores (mostly 2 to 4 pores to 2 to 6 pores), well-developed axial parenchyma with the paratracheal type as irregularly confluent, and tyloses were sometimes present. Based on the information from PlantUse (2020), *Syzygium* spp. have vessels that are diffuse with solitary and radial multiples of 2 to 3 (up to 8) pores and occur less frequently in clusters. Tyloses were sparse to abundant in the vessel lumina. The axial parenchyma was sparse to abundant and was entirely or predominantly classified into paratracheal as aliform, locally confluent, or completely confluent axial parenchyma. Other researchers (Richter and Dallwitz 2000) have stated that the genus *Syzygium* showed growth ring boundaries that were rarely distinct or indistinct or absent, diffuse-porous with tyloses, vessels that were arranged in a none-specific pattern and in multiples and commonly in short (2 to 3 vessels) radial rows, and axial parenchyma of the paratracheal type such as vasicentric, aliform, confluent, unilateral, or aliform parenchyma. Previous research studied the anatomical features of white jabon (*Anthocephalus cadamba*) and red jabon (*Anthocephalus macrophyllus*) to provide anatomical classification indicators within a genus (Kim *et al.* 2013). In the *Jabon* species, there were significant differences in the tangential diameters of fiber and vessel, the vessel number per square millimeter, and the frequency of the radial pore multiple.

In this study, it is found that the vessel arrangement of the three *Syzygium* species in cross-surface showed different patterns. Clove tree had solitary and radial multiple pores with pore clusters. In comparison, pore cluster was not found in kupa and spicate eugenia. In particular, exclusively solitary pore was observed in spicate eugenia. Axial parenchyma also showed different arrangements among the three species. Axial parenchyma was well-developed in kupa as a confluent arrangement on the cross-surface. Clove tree showed narrow banded axial parenchyma. In spicate eugenia, it was apotracheal type with diffuse-in-aggregate arrangement.

Optical micrographs of the radial surface of the sample species are presented in Fig. 2. The ray type of the clove tree (Fig. 2a) and kupa (Fig. 2b) was procumbent body ray cells with over four rows of upright and/or square marginal cells. Spicate eugenia (Fig. 2c) frequently had body ray cells that were procumbent with one row of upright and/or square marginal cells. In the clove tree and kupa, prismatic crystals in the ray and axial parenchyma cells were frequently observed; however, in the spicate eugenia, they were only found sometimes. Silica bodies were not found in the three species.

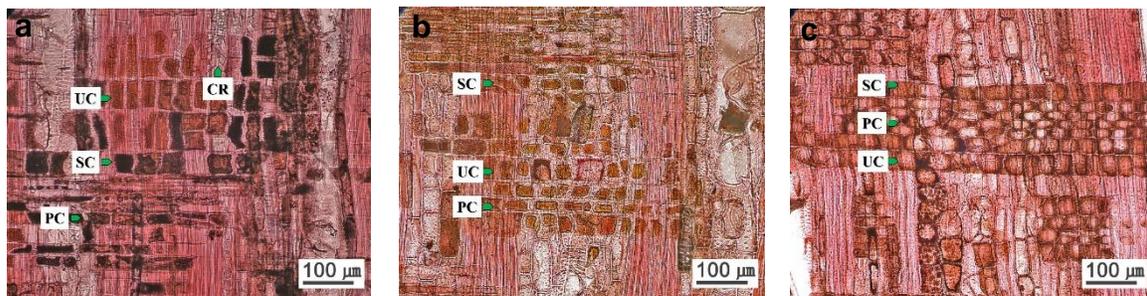


Fig. 2. Optical micrographs of the radial surfaces of three *Syzygium* species: (a) clove tree (*Syzygium aromaticum*); (b) kupa (*Syzygium polycephalum*); (c) spicate eugenia (*Syzygium zeylanicum*); (SC) square cell; (UC) upright cell; (PC) procumbent cell; and (CR) prismatic crystals

The ray type of the genus *Syzygium* could be classified into heterogeneous ray types II or III as a mixed structure with procumbent cells and square marginal cells using the Kribs method (Lemmens *et al.* 1995). Additionally, the authors reported that some species were also homogeneous with procumbent ray cells, prismatic crystals, and silica bodies in the parenchyma cells. Genus *Syzygium* additionally was reported to have prismatic crystals without silica grains in the axial parenchyma (Ogata *et al.* 2008). PlantUse (2020) stated that the rays were composed of mixed procumbent cells and were upright to the square cells; however, in some species they were wholly or predominantly composed of procumbent cells (heterogeneous II to III and homogeneous using the Kribs classification), and the uniseriate rays consisted wholly of upright cells. Prismatic crystals were comparatively rare in the apotracheal parenchyma; when present, they were solitary or in clusters, and in some species, they were found in chambered cells. Richter and Dallwitz (2000) stated that the rays of the genus *Syzygium* were generally composed of heterocellular rays with square and upright cells restricted to the marginal rows, with mostly one marginal row of upright or square cells, or 2 to 4 marginal rows of upright or square cells, or with more than four marginal rows of upright or square cells. Additionally, they reported the presence of prismatic crystals in the axial parenchyma and the absence of silica bodies.

Optical micrographs of the tangential surface of the sample species are shown in Fig. 3. The ray was mostly uniseriate and biseriate in the clove tree (Fig. 3a) and multiseriate in the kupa (Fig. 3b), whereas it was mostly uniseriate in the spicate eugenia (Fig. 3c). The clove tree and kupa showed abundant prismatic crystals in the axial parenchyma cells.

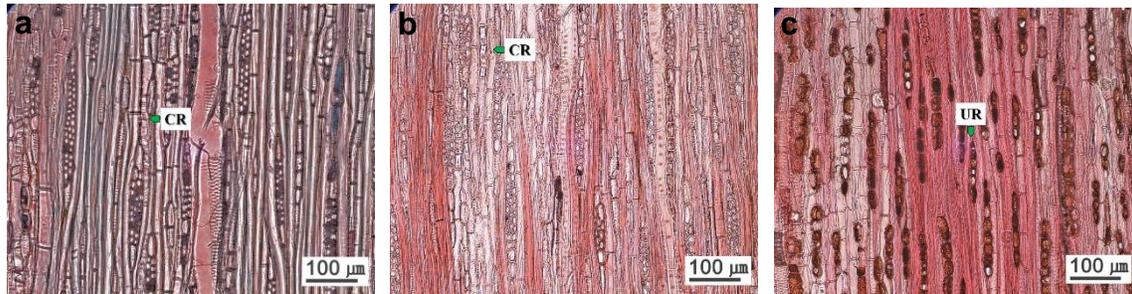


Fig. 3. Optical micrographs of the tangential surfaces of three *Syzygium* species: (a) clove tree (*S. aromaticum*); (b) kupa (*S. polycephalum*); (c) spicate eugenia (*S. zeylanicum*); (CR) prismatic crystals; and (UR) uniseriate ray

The main anatomical indicators of the tangential surface are shown from the ray properties of ray height, ray width, and ray number per millimeter (Wheeler *et al.* 1989). For example, cork oak (*Quercus suber*) has multiseriate rays composed of 0.45 to 0.53 mm widths, and the genus *Cinnamomum*, an Indonesian tropical species, was found to have uniseriate to multiseriate (up to 5) ray cells (Leal *et al.* 2006; Andianto *et al.* 2015). In a study of *Eucalyptus grandis*, the wood was found to have uniseriate and biseriate rays, ray heights of 1 to 16 cells in uniseriate and 15 to 26 cells in biseriate, and a ray number per millimeter of 9 to 11 ray cells (Jiang *et al.* 2007). The genus *Syzygium* was found to have a range from uniseriate to 10-seriates in a previous study by Lemmens *et al.* (1995), whereas Ogata *et al.* (2008) found it to have 1 to 3 to 1 to 5 seriates. Additionally, uniseriate rays were sparse to as numerous as multiseriate rays, and ray numbers per millimeter were 8 to 18 and 3 to 5 seriates (Richter and Dallwitz 2000; PlantUse 2020).

The present study found that the ray widths were in the range of uniseriate and biseriate in the clove tree, uniseriate to multiseriates (3 to 4) in the kupa, and mostly uniseriate in the spicate eugenia. Therefore, the ray characteristics in the tangential surface of the genus *Syzygium* were considerably different depending on the species.

Quantitative Anatomical Characteristics

The vessel properties of the three *Syzygium* species are shown in Table 2. The average tangential diameter of the vessels showed some differences among the clove tree, kupa, and spicate eugenia trees, being 60 µm, 81 µm, and 109 µm, respectively. The mean number of vessels per square millimeter was similar between kupa and spicate eugenia. However, the clove tree had approximately two-fold higher vessel number per square millimeter than the other two species. Comparing the vessel properties near the bark and near the pith of the clove tree and kupa, the tangential diameter of the vessels near the bark was wider than that near the pith. In contrast, in spicate eugenia, both parts showed similar tangential diameters in the vessel. There were significant differences in the tangential diameter among the species at the 5% significance level. The average vessel number near the bark was less than that near the pith; however, there was no significant difference in vessel numbers between kupa and spicate eugenia. There was a significant difference in

vessel number near the pith and near the bark in the clove tree. The clove tree had a significantly higher vessel number than that of the other species; however, there were no significant differences between kupa and spicate eugenia.

Table 2. Vessel Properties of the Three *Syzygium* Species

Sample		Tangential Diameter of Vessel (μm)	Vessels Per mm^2 (Number)
Clove (<i>S. aromaticum</i>)	NP	52.8 ± 5.2	47.0 ± 8.9
	NB	68.2 ± 5.8	34.3 ± 2.7
	Mean	$60.5^a \pm 9.5$	$40.7^a \pm 9.1$
	p-value	**	**
Kupa (<i>S. polycephalum</i>)	NP	70.4 ± 11.0	18.3 ± 3.9
	NB	92.5 ± 14.6	15.5 ± 2.4
	Mean	$81.5^b \pm 16.9$	$16.9^b \pm 3.4$
	p-value	**	ns
Spicate eugenia (<i>S. zeylanicum</i>)	NP	108.0 ± 10.6	16.2 ± 1.6
	NB	109.8 ± 11.6	14.5 ± 2.6
	Mean	$108.9^c \pm 11.1$	$15.3^b \pm 2.2$
	p-value	ns	ns

* $p < 0.05$
** $p < 0.01$
Notes: NP: near the pith, NB: near the bark
The p-value indicates the reliability between NP and NB in the three species.
The superscript letters beside the mean values in columns denote significant outcomes at the 5% significance level using Duncan's test.

Table 3. Ray Properties of the Three *Syzygium* Species

Sample		Rays Per mm^2 (Number)	Ray Height (μm)
Clove (<i>S. aromaticum</i>)	NP	11.3 ± 2.5	310 ± 93
	NB	11.6 ± 2.1	343 ± 79
	Mean	$11.4^a \pm 2.3$	$326^a \pm 88$
	p-value	ns	ns
Kupa (<i>S. polycephalum</i>)	NP	11.1 ± 2.6	151 ± 53
	NB	10.4 ± 1.8	250 ± 65
	Mean	$10.8^a \pm 2.2$	$201^b \pm 77$
	p-value	ns	**
Spicate eugenia (<i>S. zeylanicum</i>)	NP	11.0 ± 1.6	171 ± 46
	NB	15.0 ± 2.3	177 ± 42
	Mean	$13.0^b \pm 2.8$	$182^b \pm 55$
	p-value	**	*

* $P < 0.05$
** $P < 0.01$
Notes: NP: near-the-pith, NB: near-the-bark
The P-value indicates the reliability between NP and NB in the three species.
The superscript letters beside the mean values in columns denote significant outcomes at the 5% significance level using Duncan's test.

According to Lemmens *et al.* (1995), the genus *Syzygium* showed a range of 70 to 200 μm in tangential diameter of vessels and 5 to 20 (up to 38) in the number of vessels per square millimeter. The genus *Syzygium* had a tangential vessel diameter of 100 to 200 μm and number of 6 to 20 vessels per square millimeter (Ogata *et al.* 2008). Tangential vessel diameters of 120 to 170 μm with 11 to 13 vessels per square millimeter in the genus *Syzygium* have also been reported (Richter and Dallwitz 2000). Comparing the vessel properties with previous studies, the clove tree showed a significantly narrower diameter and denser spacing of the vessels than that of the other two species.

Table 4. Classification of Anatomical Features Based on the IAWA Feature List

Surface	Parameter	Clove (<i>S. aromaticum</i>)	Kupa (<i>S. polycephalum</i>)	Spicate eugenia (<i>S. zeylanicum</i>)
Cross-surface	Growth ring	Growth ring boundaries indistinct or absent (Feature 2)		
	Porosity	Diffuse-porous wood (Feature 5)		
	Vessel grouping	Solitary pore (Feature 9) Vessels in radial multiples (Feature 10) Vessel cluster (Feature 11)	Solitary pore (Feature 9) Vessels in radial multiples (Feature 10)	Solitary pore (Feature 9)
	Tangential diameter of vessel lumina	60 \pm 9 μm	81 \pm 17 μm	109 \pm 11 μm
		50 to 100 μm (Feature 41)		100 to 200 μm (Feature 42)
	Vessels per mm^2	40.7 \pm 9.1	16.9 \pm 3.4	15.3 \pm 2.2
		40 to 100/ mm^2 (Feature 49)	5 to 20/ mm^2 (Feature 47)	
	Tyloses	Tyloses common (Feature 56)		
Axial parenchyma	Axial parenchyma in narrow bands or lines up to three cells wide (Feature 86)	Axial parenchyma confluent (Feature 83)	Diffuse-in-aggregate (Feature 77)	
Radial surface	Ray cellular composition	Body ray cells procumbent with over four rows of upright and/or square marginal cells (Feature 108)		Body ray cells procumbent with one row of upright and/or square marginal cells (Feature 106)
	Prismatic crystals	Prismatic crystals in chambered axial parenchyma cells (Feature 142)		
Tangential surface	Ray width	Uni- or biseriates	Multiseriates	Exclusively uniseriate
		Ray width 1 to 3 cells (Feature 97)		Exclusively uniseriate (Feature 96)
	Rays per mm	11.4 \pm 2.3	10.8 \pm 2.2	13.0 \pm 2.8
		4 to 12 per mm (Feature 115)		\geq 12 per mm (Feature 116)
Ray height	326 \pm 88 μm (-)	201 \pm 77 μm (-)	182 \pm 55 μm (-)	

Table 3 shows the number of rays per millimeter along the tangential direction in the cross-surface and the ray height in the tangential surface. There were no significant differences between the clove tree and kupa in the number of rays per millimeter, with these being 11.4 and 10.8, respectively. However, spicate eugenia was significantly different from that of the other two species. Comparing the number of rays near the pith and near the bark, the average ray number in spicate eugenia was higher near the bark than that near the pith. However, there was no significant difference between the clove tree and kupa.

The ray height of the three sample species ranged from 150 to 340 μm . The ray heights of the three species were $326 \pm 88 \mu\text{m}$ in the clove tree, $201 \pm 77 \mu\text{m}$ in the kupa, and $182 \pm 55 \mu\text{m}$ in the spicate eugenia. There were no significant differences in ray height between the kupa and spicate eugenia; however, the clove tree was significantly different in ray height to the other species. Ray heights near the bark in the three species were higher than those near the pith. Specifically, there was a significant difference in the ray height between near-the-bark and near-the-pith in the kupa tree.

The ray spacing of the genus *Syzygium* was in the range of 8 to 18 per mm and uniseriate rays consisting of wholly upright cells with 1 to 10 cells high and multiseriate rays (up to 10-seriate) up to 0.8 mm high (Lemmens *et al.* 1995; PlantUse 2020). Regarding the ray height of the genus *Syzygium*, Ogata *et al.* (2008) reported a maximum ray height of 600 to 2,500 μm . The height of the large rays was reported to be 500 to 1,000 μm (Richter and Dallwitz 2000).

Classification of Anatomical Features

The anatomical features of the three species based on the IAWA anatomical hardwood feature list are summarized in Table 4.

CONCLUSIONS

1. Obvious anatomical differences among the three species were identified through this study.
2. Some qualitative anatomical features such as diffuse-porous wood, tyloses in the vessels, and prismatic crystals in axial parenchyma were commonly observed in the three species. In contrast, inherent features of each species such as the type of vessel grouping and arrangement of axial parenchyma were distinctly observed as the anatomical differences among the three *Syzygium* species, and these differences could be utilized as an identification indicator.
3. In quantitative anatomical observation, tangential diameter of vessel lumina showed significant differences among the sample species. Clove tree had outstandingly higher vessels per square millimeter and ray height compared to the other species.

In conclusion, it is suggested that vessel grouping, tangential vessel diameter, and axial parenchyma arrangement can be used for identification key of the species. For further accurate identification of wood, quantitative anatomical characteristics should be analyzed with quantitative characteristics for *Syzygium* species.

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