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The anatomical structure of the root, stem, and branch of *Gyrinops versteegii* trees from different growing sites

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Abstract. *Adimahavira A, Lukmandaru G, Pujiharti R, Prastiwi FW, Nugroho WD. 2023. The anatomical structure of the root, stem, and branch of* Gyrinops versteegii *trees from different growing sites. Biodiversitas 24: 4590-4598. Gyrinops versteegii* (Gilg) Domke is an agarwood (*Gaharu*)-producing species with a unique color and very expensive fragrance. Even so, studies on the effects of growing sites on the wood anatomical structure of several parts of *G. versteegii* have not been thoroughly investigated. Therefore, this research aims to investigate the anatomical structure of *G. versteegii* on the tree's root, stem, and branch at two different growing sites. Three five-year-old *G. versteegii* trees from Pacitan and Klaten Districts (Indonesia) were used for the experiment and were harvested from the root, stem, and branch. The wood samples were then put in a glutaraldehyde solution for 24 hours and later in an alcohol solution. The samples were sliced in microtome (15-20 µm) and observed in the transverse, tangential, and radial sections. The results showed that the anatomical characteristics, such as vessel diameter, ray height, ray frequency, interxylary phloem proportion, and frequency differed significantly between growing sites. Furthermore, fiber length, vessel diameter, ray height, ray frequency, and interxylary phloem proportion significantly differed among plant parts. In addition, the branch part has the highest interxylary phloem value, indicating the possibility of a high agarwood resin deposit when agarwood stimulation is applied.

Keywords: Branch, *Gyrinops versteegii,* Klaten, Pacitan, root, stem

INTRODUCTION

Agarwood is a high-value non-timber forest product traded in over 18 Southeast and Middle Eastern countries (Liu et al. 2017; Pasaribu et al. 2021; Abdulah et al. 2022). National Standardization of Indonesia (2011) defined agarwood as a forest product with various unique shapes and a high content of fragrant resin from the tree that grows naturally and has died due to an artificial or natural infection process. Agarwood is one of the most popular forest products due to its various uses, which include perfume, traditional medicine, and religious rituals (de Alwis et al. 2019; Kang 2021). High-quality agarwood can be worth approximately 30,000 USD per kg, making it one of the most expensive natural resources compared to other oleoresin (Jayachandran 2015). Agarwood-producing trees in tropical areas consisted of 3 families, namely Thymelaeaceae (*Aquilaria* sp., *Gyrinops* sp., and *Gonystylus* sp.), Fabaceae (*Dalbergia* sp.), and Euphorbiaceae (*Exoecaria* sp.) (Wahyudi 2013; Susmianto et al. 2014; Lukman et al. 2022).

Gyrinops versteegii (Gilg) Domke is one of the agarwood-producing trees that naturally grows in Lombok, Sumbawa, Flores, Sumba, and Timor Island, Indonesia (Yelnititis 2014) and is classified in Appendix II of Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES 2023). The different growing sites of *G. versteegii* might affect their anatomical structure and quality, primarily when the tree is cultivated at different sites. Rocha et al. (2019) reported that there is a possibility that the wood characteristics of the tree are different between the natural and outside its natural habitat. The growing site greatly influences wood characteristics by interacting with the tree's genetic potency (Talbert and Jett 1981; Zobel and van Buijtenen 1989; You et al. 2021). Furthermore, Chaabouni (2014) stated that different growing sites could affect the wood characteristics because of the different environmental conditions such as temperature, light intensity, and elevated CO_2 levels. In the previous study, Hietz et al. (2022) reported that different growing sites affect the vessel area, vessel fraction, and wood density of *Quercus robur* L. from Austria (Weyerburg, Wels, and Weistrach). This is also supported by Arnič et al. (2021), where vessel area, vessel density, and tree ring width of *Fagus sylvatica* L. significantly differ among the trees from three selected sites in Slovenia.

Commonly, agarwood is only harvested from the tree's stems, leading to ineffective utilization. In order to increase the productivity and utilization of agarwood-producing trees, it is necessary to harvest all of its parts, not only the stem but also the root and branch (Liu et al. 2013). The anatomical characteristic in agarwood-producing trees is vital to support resin production. Furthermore, Zobel and van Buijtenen (1989) stated that the tree's anatomical characteristics differed. A previous study was conducted by Andianto (2010) on the anatomical structural differences in the stem and root of agarwood species *Aquilaria microcarpa* Baill. and *Aquilaria malaccensis* Lam. showed differences in vessel average diameter and length, ray's average composition and height, cell wall thickness, and fiber length. Furthermore, Longui et al. (2018) reported differences in vessel diameter and frequency, fiber length, fiber wall thickness, ray height and frequency, and density on the root, stem, and branch wood of 10-year-old *Inga laurina* (Sw.) Willd. The difference or variation in the wood anatomical structure on a plant is vital to ease water flow, which minimizes cavitation from root to crown (Gerolamo and Angyalossy 2017).

Moreover, the anatomical structure differences in various parts must be studied to understand the wood characteristics, function, and physiological interaction between the trees (Fortunel et al. 2014; Marcati et al. 2014; Longui et al. 2017). Therefore, this study aims to investigate the anatomical structure of *G. versteegii* agarwood species on the root, stem, and branch parts from two different sites on Java Island, namely Pacitan and Klaten Districts. The information from this research is expected to increase the knowledge of *G. versteegii* cultivation and agarwood production.

MATERIALS AND METHODS

Study area

The *G. versteegii* trees in this research were selected from two forests in Pacitan District, East Java and Klaten District, Central Java, Indonesia. The study site in Pacitan District, Kebonagung Sub-district (8° 12'43.0" S and 111° 09'00.1" E, 16 m asl) with temperature range of 33°, humidity ranges from 24.4 to 26%, average rainfall from 2013 to 2017 of 2,790.5 mm (Badan Pusat Statistik Kabupaten Pacitan 2014-2018), and latosol soil type (Wulandari 2014). Meanwhile, the other study site was in Klaten District at South Klaten Sub-district (7° 44'21.8" S and 110° 35'10.4" E, 144 masl) with a temperature range from 23.8 to 28.1°, humidity ranges from 76.2 to 86.2%, average rainfall from 2013 to 2017 of 1,860.1 mm (Badan Pusat Statistik Provinsi Jawa Tengah 2013-2017; Badan Pusat Statistik Kabupaten Klaten 2013), and grey regosol soil type (Badan Pusat Statistik Kabupaten Klaten 2013). The samples were obtained from the designated areas and then observed at the Faculty of Forestry, Universitas Gadjah Mada, Yogyakarta Province, Indonesia.

Plant materials and sample preparation

This study used 6 five-year-old selected *G. versteegii* trees, with an average diameter of 17.5 cm (Pacitan District) and 9.67 cm (Klaten District), as shown in Figure 1. Wood sample blocks (2 x 2 x 2 cm) were harvested from the root, stem, and branch with chisel and hammer. One sample in the selected part (18 sample blocks) was taken from *G. versteegii* trees. Furthermore, Figure 2 shows that on the root part, wood sample blocks were taken about 10 cm from the root base. On the stem part, wood blocks were sampled at Diameter at Breast Height (DBH), while on the branch part, wood blocks were sampled at the branch (close to the ground) with > 2cm diameter. Wood sample blocks were then fixed in 4% glutaraldehyde in 0.1 M phosphate buffer (pH 7.3) for 24 hours and then in a 30% alcohol solution for preservation.

Wood sample blocks (2 x 2 x 2 cm) were divided into microtome (1 x 1 x 1 cm) and maceration (1 x 1 x 2 mm). The samples were sliced with a microtome (Yamatokohki, Saitama, Japan) with 15-20 µm thickness in the transverse, tangential, and radial sections. These slices were stained with 0.1% safranin solution (WAKO Pure Chemical Industries), cleared with xylol solution, and mounted on a glass slide with resin (Entellan New, Merck, Darmstadt, Germany). Furthermore, wood maceration was conducted by boiling the wood sample in Franklin solution (CH_3COOH 100% and H_20_2 50% (1:10)) until it was disintegrated into fibers.

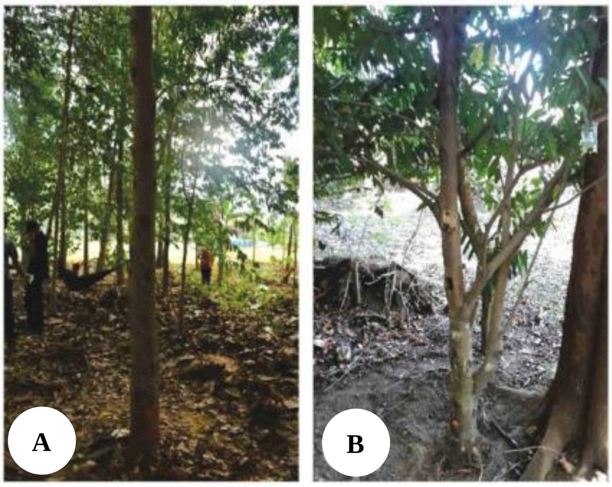

Figure 1. A. The *G. versteegii* tree in the community forest in Pacitan District (Indonesia), and B. in Klaten District (Indonesia)

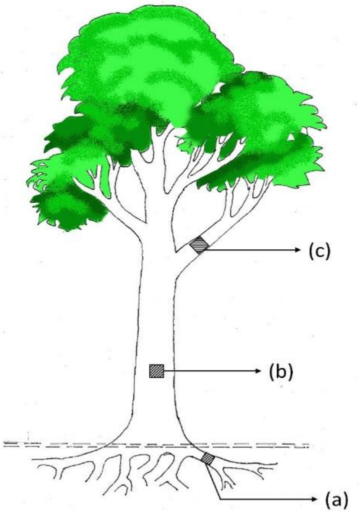

Figure 2. The illustration of *G. versteegii* tree and wood sample-blocks sampling scheme: A. Root sample, taken about 10 cm from the root base; B. Stem sample, taken at Diameter at Breast Height (DBH); C. Branch sample, taken at the branch (close to the ground) with > 2cm diameter

Sample observation

The samples were observed through an Olympus BX51 series light microscope and captured with a digital camera (DP70 Olympus Corporation) connected to the microscope with a mounting connector 0.5x. The parameter was measured using Image-Pro Plus ver. 4 software according to the IAWA list of microscopic features for hardwood identification (Wheeler et al. 1989). The anatomical characteristics such as wood fiber (fiber length, fiber wall thickness, and fiber diameter), vessel (diameter and frequency), ray parenchyma (height and frequency), and interxylary phloem (proportion and frequency) were observed and measured in this study. The measurement of wood fiber length used 50 randomly selected fibers in each sample, while fiber wall thickness, fiber diameter, vessel diameter, and ray height used 30 random cells in each sample. Vessel, ray, and interxylary phloem frequency were determined by counting their presence in 1 mm^2 in five selected areas in each sample. Interxylary phloem proportion was measured by tracing the area of it in 1 mm^2 in three selected areas. The amount of repetition on measurement in this study was based on the minimum amount determined by the IAWA list of microscopic features for hardwood identification (Wheeler et al. 1989).

The results were statistically analyzed using SPSS software (SPSS Statistics ver. 17, IBM Corp., USA) at 95% and 99% confidence. The effect of the wood part (root, stem, branch) and growing site (Pacitan and Klaten) on the anatomical characteristics were analyzed by two-way Analysis of Variance (ANOVA) and then Tukey's Post Hoc Test at p<0.05 and p<0.01.

RESULTS AND DISCUSSION

Wood fibers

The fiber length of *G. versteegii* in Pacitan was shorter than Klaten, with an average length of 591.3 ± 12.8 µm and 633.6 ± 15.4 µm, respectively (Table 1). The fiber length both in Klaten and Pacitan were classified as short fiber (<900 µm) (Wheeler et al. 1989). In Pacitan, the highest fiber length was found in the branch part (633.6 ± 24.3 µm), while the lowest was found in the root (554.9 ± 12.9 µm). The fiber length of *G. versteegii* in two different growing sites and tree parts is shown in Figure 3. Furthermore, in Klaten, fiber length in the stem part has the highest value (634.2 ± 29.6 µm), while the lowest was found in the branch (546.4 ± 2.1 µm). The fiber length of *G. versteegii* in Klaten showed a similar result to Dunham et al. (2007), Yaman (2014), and Kiaei and Moya (2015), which stated that the highest fiber length value was in the stem part, while the lowest was in the branch. Compared to the other study, the fiber length of *G. versteegii* in Pacitan and Klaten was shorter than the same species from Gorontalo with a value of 758.45 µm (Asdar 2006) and Lombok with a value of 738 µm (Mandang and Wiyono 2002).

Moreover, based on the statistical analysis, the interaction between the growing site (Pacitan and Klaten) was not significantly different (Table 1), which means that the different growing sites did not affect the fiber length of *G. versteegii*. On the other hand, the tree part factor has influenced the 1% test level with a significance value of 0.003. This showed that the interaction between the three factors significantly influenced the fiber length of *G. versteegii*. This was supported by Zobel and Buijtenen (1989), where the fiber length varies greatly within and among trees, resulting from genetic control or the different environments.

The differences in the fiber wall thickness of tree parts in two different growing sites are presented in Figure 4. The fiber wall thickness of *G. versteegii* in Pacitan was thicker than Klaten's, which is 2.7 ± 1.1 µm and 2.1 ± 0.5 µm respectively (Table 1). In Pacitan, the highest fiber wall thickness was in the root part (3.2 ± 1.5 µm), while in Klaten, the highest value was in the stem part (3.4 ± 1.2 µm). Compared to *G. versteegii* trees in Pacitan, the trees in Klaten have more branches that protrude from the stem closer to the ground. This shows that the thick fiber wall thickness in the stem of *G. versteegii* in Klaten was caused by the high need for mechanical power of the trees with many branches and broad tree crowns. The tree needed high mechanical power to withstand the strong wind and intense rain (Longui et al. 2018).

Table 1. Fiber length, fiber wall thickness, and fiber diameter of *Gyrinops versteegii*'s root, stem, and branch in Pacitan and Klaten

	Fiber length (µm)	**Fiber wall thickness (µm)**	**Fiber diameter (µm)**
Pacitan			
Root	554.9 ± 12.9	3.2 ± 1.5	26.5 ± 2.0
Stem	585.4 ± 1.2	1.7 ± 0.2	26.2 ± 3.3
Branch	633.6 ± 24.3	3.2 ± 1.1	24.5 ± 2.3
Average	591.3 ± 12.8	2.7 ± 1.1	25.8 ± 2.3
Klaten			
Root	579.2 ± 4.0	1.5 ± 0.1	23.7 ± 1.3
Stem	634.2 ± 29.6	3.4 ± 1.2	23.7 ± 2.1
Branch	546.4 ± 2.1	1.5 ± 0.1	23.6 ± 3.1
Average	633.6 ± 15.4	2.1 ± 0.5	23.7 ± 2.2
Sig. value (P)			
Growing sites	0.563 (ns)	0.200 (ns)	0.083 (ns)
Parts of plant	0.003 (**)	0.895 (ns)	0.701 (ns)

Note: **: Significantly different at *P*<0.01; ns: Not significantly different

Besides that, the fiber wall thickness in Pacitan and Klaten showed similarity with *G. versteegii* in Nusa Tenggara Timur (Andianto 2010), which has an average fiber wall thickness of 2.2 ±0.2 µm but showed a different value of *G. versteegii* in Gorontalo (Asdar 2006) that has a higher value (3.975 µm). The different fiber wall thicknesses of *G. versteegii* in different places showed that the growing site condition, especially soil moisture, affects the fiber wall thickness (Zobel and van Buijtenen 1989). Furthermore, from the statistical analysis, the interaction between the two factors has a non-significant value, which shows that the interaction does not influence the cell wall thickness of *G. versteegii*.

The fiber diameter of *G. versteegii* in Pacitan was bigger than Klaten's, with an average value of 25.8 ± 2.3 µm and 23.7 ± 2.2 µm (Table 1). The tree roots have the highest value of fiber diameter in both sites, which affects the efficiency of *G. versteegii* in transporting water (Longui et al. 2018). Furthermore, the fiber diameter in this study was similar to *G. versteegii* in Lombok, with an average fiber diameter of 27.6 ± 1.3 µm (Mandang and Wiyono 2002) and higher than the same species in Nusa Tenggara Timur with a value 35.87 µm (Dwianto 2019). Based on statistical analysis, none of the factors significantly influences the fiber diameter.

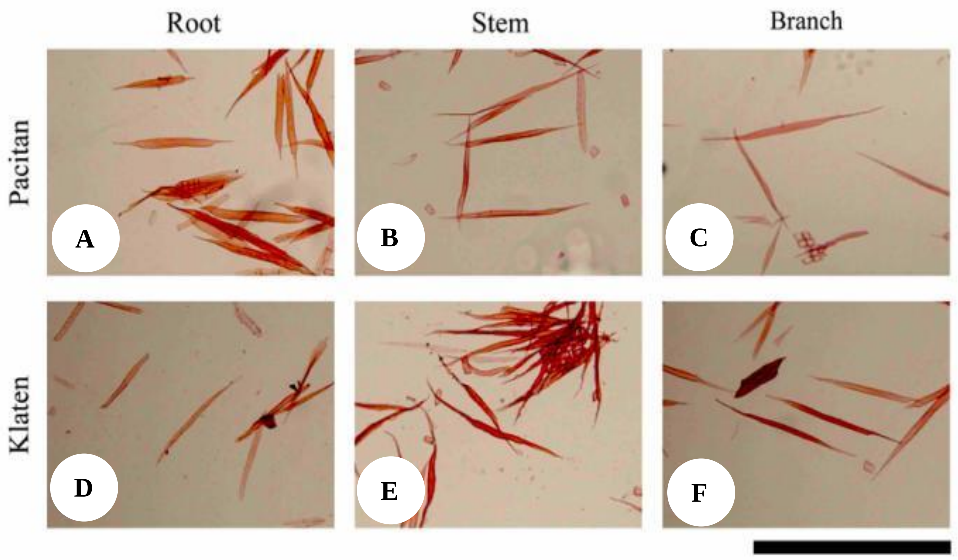

Figure 3. Photomicrograph of wood fibers of the root, stem, and branch part of *G. versteegii* growth in Pacitan, Indonesia (A, B, C) and Klaten, Indonesia (D, E, F). Scale bar: 1 mm

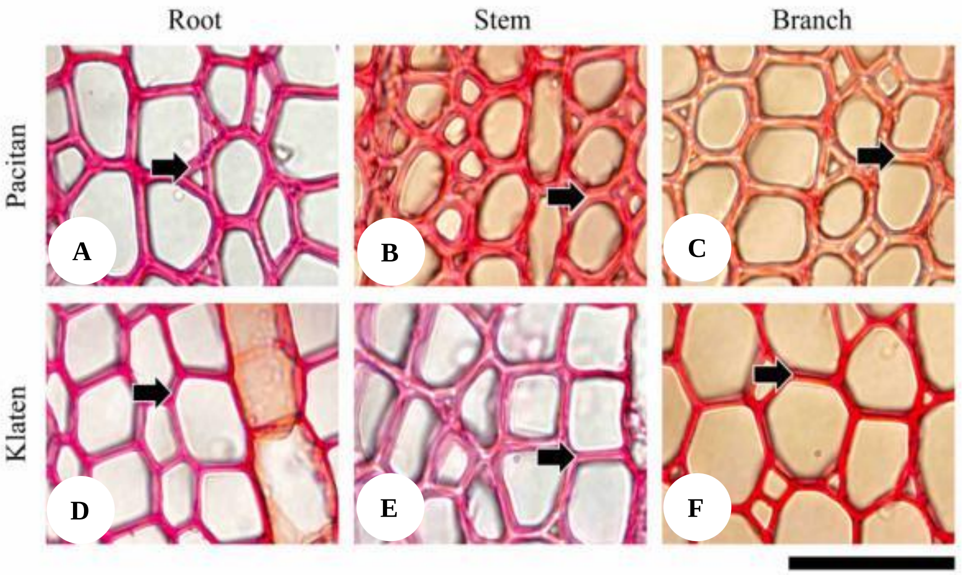

Figure 4. Photomicrograph of a transverse section of the root, stem, and branch part of *Gyrinops versteegii* growth in Pacitan, Indonesia (A, B, C) and Klaten, Indonesia (D, E, F). The arrows show the fiber's cell walls (Scale bar: 50 µm)

Vessels

The vessel diameter of *G. versteegii* in Pacitan was higher than in Klaten, with an average of 61.8 ± 6.9 µm and 46.4 ± 2 µm, as shown in Table 2 and Figure 5. In both growing sites, the highest value of vessel diameter was in the stem part, with 68.1 ± 4.2 µm (Pacitan) and 54.1 ± 3.6 µm (Klaten). The lowest values were located in the branch part with a vessel diameter of 53.3 ± 2.5 µm (Pacitan) and 41.5 ± 1.7 µm (Klaten). The vessel diameter of *G. versteegii* in Pacitan tends to decrease from the root to the branch. This result was supported by Schuldt et al. (2013) on the change of its distribution pattern in trees growing in tropical areas, where the vessel diameter tends to decrease acropetally. Wide vessel diameter was related to greater hydraulic conductivity, increasing water conduction efficiency (Gerolamo and Angyalossy 2017; Miranda et al. 2018). Furthermore, the different vessel diameter in Pacitan and Klaten (P=0.00) was related to the growing site conditions. February (1993) showed that plants growing in a wet environment have a wider vessel diameter than plants in dry areas. The difference in vessel diameter in tree parts of *G. versteegii* (P=0.00) might be inherited (Zobel and van Buijtenen 1989).

For the vessel frequency, the average value was higher in Klaten, which is 23.9 ± 9.9/mm^2, while in Pacitan, *G. versteegii* only has 18.2 ± 6.2/mm^2 vessels (Table 2). Within the tree, vessel frequency showed a different value in both sites; the highest value is in the branch part in Pacitan with 21.9 ± 9.4/mm^2 and in the root part in Klaten with 32.7 ± 19.6/mm^2, while the lowest value was in the root part in Pacitan with 15.9 ± 2.0/mm^2 and in the stem part in Klaten with 18.3 ± 2.4/mm^2. The higher value of vessel frequency in Klaten might be caused by the high availability of soil macro as well as a micronutrient and the organic matter content in the soil, as studied by Melo Junior et al. (2018). This phenomenon is supported by Lima et al. (2010) research, which showed that the increase in soil fertilizer dosage leads to a rise in the vessel frequency. Based on the statistical analysis, the growing site and the tree factors do not influence the vessel frequency of *G. versteegii*.

Table 2. Vessel diameter and vessel frequency of *Gyrinops versteegii*'s root, stem, and branch in Pacitan and Klaten (Indonesia)

		Vessel Diameter (µm)	Vessel Frequency (per mm^2)
Pacitan			
	Root	64.1 ± 13.4	15.9 ± 2.0
	Stem	68.1 ± 4.2	16.6 ± 7.2
	Branch	53.3 ± 2.5	21.9 ± 9.4
	Average	61.8 ± 6.9	18.2 ± 6.2
Klaten			
	Root	43.5 ± 2.1	32.7 ± 19.6
	Stem	54.1 ± 3.6	18.3 ± 2.4
	Branch	41.5 ± 1.7	20.7 ± 7.6
	Average	46.4 ± 2.5	23.9 ± 9.9
Sig. value (P)			
	Growing sites	0.0002 (**)	0.246 (ns)
	Parts of plant	0.009 (**)	0.502 (ns)

Note: **: Significantly different at P<0.01; ns: Not significantly different

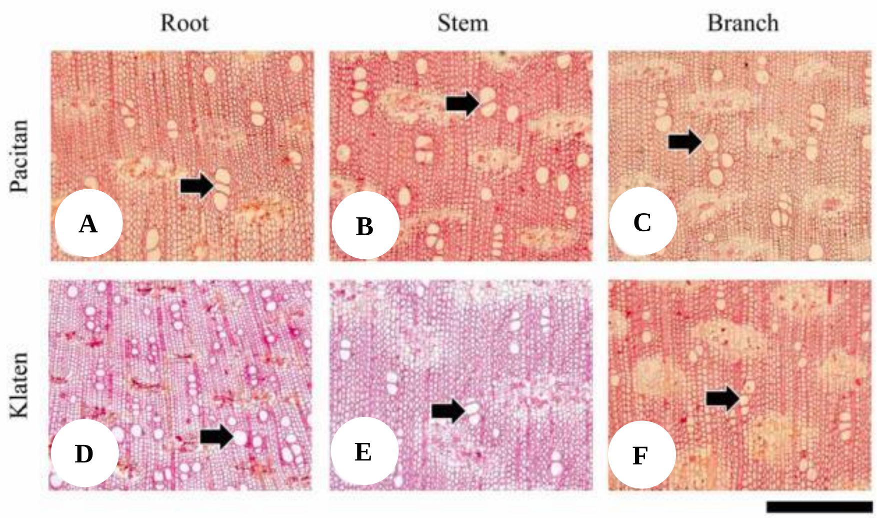

Figure 5. Photomicrograph of a transverse section of the root, stem, and branch part of *Gyrinops versteegii* growth in Pacitan, Indonesia (A, B, C) and Klaten, Indonesia (D, E, F). The arrows show the vessels of wood of *Gyrinops versteegii* (Scale bar: 500 µm)

Rays

Based on Table 3, the average ray height in Pacitan was higher with 255.1 ± 13.6 µm compared to Klaten with 219.6 ± 13.4 µm (*P*=0.00). Compared to the same species in Lombok (Mandang and Wiyono 2002) and Nusa Tenggara Timur (Andianto 2010), the ray height of *G. versteegii* in this study has a similar result. In the part of the tree, ray height showed a different result (*P*=0.01), where the highest value of ray height in Pacitan was in the branch part 283.7 ± 26.9 µm and in stem part in Klaten 228.0 ± 27.3 µm. The same result was reported by Longui et al. (2018), where the highest ray height in *I. laurina* was in the stem part. The differences in ray height in tree parts of *G. versteegii* in different growing sites are presented in Figure 6.

The average ray frequency of *G. versteegii* in Pacitan was higher than Klaten, with 9.5 ± 0.7/mm^2 and 8.4 ± 1.3/mm^2 (Table 3). Mohamed et al. (2013) and Faizal et al. (2022) stated that living ray parenchyma was the only cells synthesizing agarwood resin. Zhang et al. (2022) also reported that agarwood was mainly formed and accumulated in ray cells and interxylary phloem. Furthermore, the abundant ray in *G. versteegii* in Pacitan might cause the trees' bigger diameter. It has been known that a bigger tree diameter leads to higher ray frequency, which eases the distribution of water and nutrients in the tree in the radial direction (Slupianek et al. 2021). The high ray frequency has a role in the metabolic processes related to the storage and mobilization of carbohydrates (Pfautsch 2016). Based on Table 3, the ray frequency of *G.versteegii* in tree parts shows different values (*P*=0.01), where the highest value was in the stem part for both sites

Table 3. Ray height and ray frequency of *Gyrinops versteegii*'s root, stem, and branch in Pacitan and Klaten (Indonesia)

		Rays Height (µm)	Rays Frequency (per mm^2)
Pacitan			
	Root	222.1 ± 10.1	9.5 ± 0.7
	Stem	259.4 ± 3.9	10.6 ± 0.6
	Branch	283.7 ± 26.9	8.4 ± 0.1
	Average	255.1 ± 13.6	9.5 ± 0.5
Klaten			
	Root	215.0 ± 3.5	7.1 ± 1.4
	Stem	228.0 ± 27.3	9.7 ± 1.4
	Branch	215.9 ± 9.5	8.4 ± 1.2
	Average	219.6 ± 13.4	8.4 ± 1.3
Sig. Value (P)			
	Growing sites	0.001 (**)	0.040 (*)
	Parts of Plant	0.017 (*)	0.012 (*)

Note: *: Significantly different at P<0.05; **: Significantly different at P<0.01

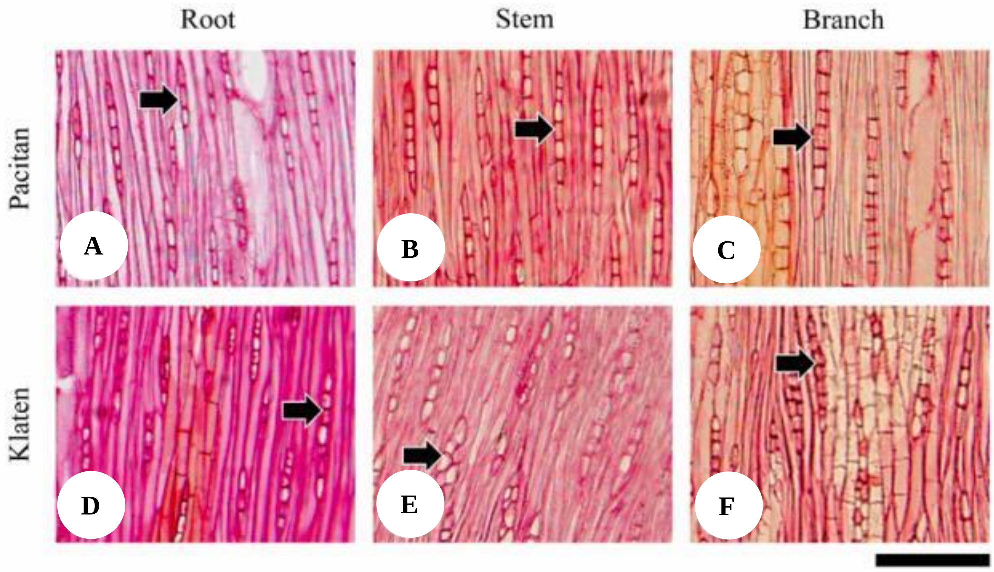

Figure 6. Photomicrograph of tangential section of the root, stem, and branch part of *Gyrinops versteegii* growth in Pacitan, Indonesia (A, B, C) and Klaten, Indonesia (D, E, F). The arrows show the ray cells of the wood of *G. versteegii* (Scale bar: 200 µm)

Interxylary phloem

In this study, *G. versteegii* in Klaten has a higher proportion of intexylary phloem than Pacitan (*P*=0.03), with values of 17.5 ± 2.2 % and 14.2 ± 3.2 %, respectively (Table 4.). The highest value of the proportion of intexylary phloem in the tree part from Pacitan was a branch part with 18.1 ± 3.7%, while the Klaten stem part showed the highest value with 21.7 ± 1.8 % (*P*=0.00). Liu et al. (2019) and Zhang et al. (2022) showed that interxylary phloem and ray parenchyma are the main parts of the formation of agarwood resin and its storage.

In addition, Liu et al. (2019) stated that the living ray parenchyma cells are distributed in the interxylary and xylem, which form a living cell network on the wood. Meanwhile, at the initial process of agarwood formation, mitochondria and plastids on the parenchyma cells become the place and source of energy in the resin. Based on those studies, it concluded that a higher proportion and frequency of interxylary phloem increases agarwood resin in the tree.

Table 4. shows that the frequency of interxylary phloem of *G. versteegii* in Pacitan has a higher frequency than Klaten with values of 9.49 ± 1.1 per mm^2 and 8.4 ± 1.3 per mm^2, respectively (*P*=0.03). This is presented in Figure 7. The interxylary phloem of *G. versteegii* in Pacitan is more frequent, although it is smaller than the interxylary phloem in Klaten. The environmental condition differences between Pacitan and Klaten might cause these differences. The highest value of interxylary phloem frequency from the tree parts is in the branches and stem parts for both sites. Zhang et al. (2022) stated that interxylary phloem is the main part of agarwood resin formation and deposition. Therefore, the development of interxylary phloem might be an indicator for the assessment of the potencies of agarwood production.

Table 4. Interxylary phloem proportion and frequency of *Gyrinops versteegii*'s root, stem, and branch in Pacitan and Klaten (Indonesia)

		Interxylary Phloem Proportion (%)	**Interxylary Phloem Frequency (per mm^2)**
Pacitan			
	Root	8.7 ± 1.6	9.5 ± 1.0
	Stem	15.6 ± 4.4	10.6 ± 1.8
	Branch	18.1 ± 3.7	8.4 ± 1.0
	Average	14.2 ± 3.2	9.49 ± 1.1
Klaten			
	Root	11.6 ± 1.7	7.1 ± 2.3
	Stem	21.7 ± 1.8	9.7 ± 1.4
	Branch	19.3 ± 3.0	8.4 ± 0.8
	Average	17.5 ± 2.2	8.4 ± 1.3
Sig. Value (P)			
	Growing sites	0.030 (*)	0.031 (*)
	Parts of Plant	0.0003 (**)	0.084 (ns)

Note: *: Significantly different at *P*<0.05; **: Significantly different at *P*<0.01; (ns): Not significantly different

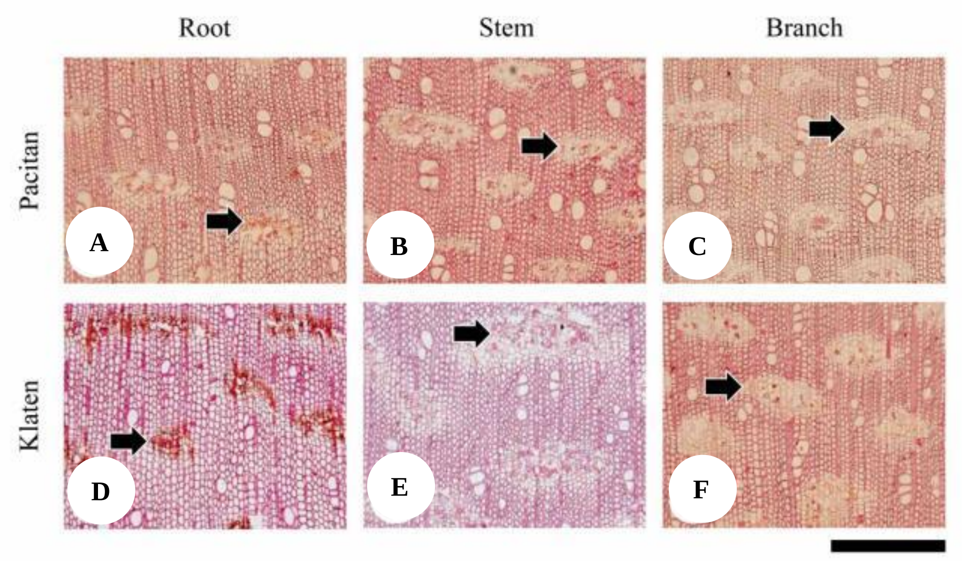

Figure 7. Photomicrograph of a transverse section of the root, stem, and branch part of *Gyrinops versteegii* growth in Pacitan, Indonesia (A, B, C) and Klaten, Indonesia (D, E, F). The arrows show the interxylary phloem of *G. versteegii* wood. (Scale bar:5 00 µm)

In conclusion, the results show that anatomical characteristics, such as vessel diameter, ray height, ray frequency, interxylary phloem proportion, and frequency, differed significantly between growing sites. The *G. versteegii* growing in Klaten District has higher interxylary phloem proportion and frequency than Pacitan District's *G. versteegii*. In contrast, the *G. versteegii* from the Pacitan District has a higher ray frequency and ray height compared to Klaten District. Interxylary phloem and ray parenchyma are the main parts of agarwood resin formation and deposition. The *G. versteegii* from each growing site has specific tissue that might play an essential role in agarwood formation. Furthermore, fiber length, vessel diameter, ray height, ray frequency, and interxylary phloem proportion significantly differ among plant parts. In addition, the branch and stem part has a higher proportion of interxylary phloem, ray parenchyma height and ray parenchyma frequency, which indicates the possibility of a high agarwood resin deposit when agarwood stimulation is applied.

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