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Anatomical characteristics of reaction wood in naturally inclined *Gardenia* sp. tree grown in South Kalimantan, Indonesia
インドネシア・南カリマンタンに生育するクチナシ属種あて材の組織学的特徴

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ABSTRACT

Anatomical characteristics (vessel and wood fiber morphologies, and secondary wall layer structure), lignin distribution, and lignin content were investigated for a *Gardenia* sp. naturally inclined tree grown at South Kalimantan, Indonesia. Four coppiced stems (ca. 40 cm in length; sample A, B, C, and D) were sampled from the *Gardenia* sp. tree. Sample A had almost straight stem and was regarded as normal wood. The other samples (B, C, and D) were naturally inclined about 40 to 50 degree from vertical. Based on the preliminary experiments for identifying the reaction wood formation position by using sample A (normal wood) and inclined samples, secondary xylem in lower side of inclined stems was strongly stained with phloroglucinol-HCl reagent compared to normal wood. Thus, characteristics of reaction wood in *Gardenia* sp. were clarified by comparing those between upper side (opposite wood) and lower side (reaction wood) in inclined stem samples (sample B, C, and D). Compared to opposite wood, reaction wood had significantly shorter wood fiber and larger lignin contents. In reaction wood fibers, S_3 layer was not observed, while the secondary walls were strongly stained with lignin color reactions. Based on the results and comparison with previous reports, it is concluded that type of reaction wood and reaction wood characteristics of tropical *Gardenia* sp. used in this study were the same as those of other *Gardenia* species growing in temperate regions which form "compression-wood-like-reaction wood".
Keywords: *Gardenia* sp., "compression-wood-like-reaction wood", microfibril angle, lignin distribution

要　旨

本研究では，熱帯に生育するクチナシ属種（*Gardenia* sp.）の組織学的特徴（道管形態，木部繊維形態および木部繊維二次壁壁層構造）とリグニン分布およびリグニン量を調査するため，インドネシア・南カリマンタンで自然に傾斜して生育していたクチナシ属種一個体から長さ約 40 cm の萌芽幹を 4 本採取した．このうち 1 本はほぼ鉛直方向に生育していたため，正常材（サンプル A）とし，鉛直方向に対して 40 ～ 50 度に傾斜して生育していた残りの 3 本（サンプル B，C および D）をあて材を含むサンプルとした．正常材とあて材を含むサンプルを用いた予備試験の結果，傾斜下側の木部がフロログルシン－塩酸反応により強く染色されることを確認した．この結果から，あて材が傾斜の下側に形成されていると考え，サンプル B，C および D の傾斜の上側（オポジット材部）と下側（あて材部）の組織学的特徴およびリグニン分布を調査し，比較することにより，本種のあて材の特徴を明らかにした．オポジット材とあて材を比較した結果，道管形態，木部繊維の直径および壁厚，ミクロフィブリル傾角の変化に有意差はなかった．しかしながら，あて材部において，木部繊維長の減少およびリグニン量の増加が認められた．一方，あて材部の木部繊維には S_3 層の形成は認められず，リグニン呈色反応後の木部繊維壁には強い染色性が認められた．得られた結果から，本研究で調査した熱帯で生育する *Gardenia* sp. が形成するあて材の特徴は，温帯で生育する *Gardenia* 種でこれまでに報告された "compression-wood-like-reaction wood" で認められるものと一致することが明らかになった．
キーワード：*Gardenia* sp.，"compression-wood-like-reaction wood"，ミクロフィブリル傾角，リグニン分布

1. Introduction

When woody angiosperms are exposed their stems or branches to inclination stimulus, they form specific tissue, termed as reaction wood, to maintain stems' or branches' original position (Onaka 1949; Yoshizawa et al. 1993; Evert 2006; Aiso et al. 2013; Groover 2016). Based on the position forming reaction wood and anatomical characteristics, at least three types of reaction wood are found in angiosperms: (1) tension wood, (2) tension-wood-like-reaction wood, and (3) compression-wood-like-reaction wood (Onaka 1949; Yoshizawa et al. 1993; Evert 2006; Aiso et al. 2013; Groover 2016, Table 1). Of these three types, formation of (3) compression-wood-like reaction wood has been reported only a few species, such as *Buxus* spp., *Gardenia jasminoides*, and *Sarcandra glabra* (Onaka 1949; Yoshizawa et al. 1993; Baillères et al. 1997; Aiso et al. 2013; Hiraide et al. 2016).

Even though habitat is different, characteristics of reaction wood are usually the same in a genus. For example, reaction wood characteristics of seedlings of *Acacia mangium* grown in Indonesia (Nugroho et al. 2013) resemble those of seedlings of *A. auriculiformis* grown at greenhouse in Japan (Aiso et al. 2016b). Similar results were obtained in *Buxus* species: compression-wood-like reaction wood characteristics were found in *Buxus microphylla* var. *insularis* and *B. microphylla* var. *japonica* grown in Japan (Onaka 1949; Yoshizawa et al. 1993; Hiraide et al. 2016), and *B. sempervirens* grown in France (Baillères et al. 1997). Thus, it is considered that reaction wood characteristics are almost the same within a genus. However, further researches are needed for other genus.

Genus of *Gardenia* is known as evergreen shrubs or tree species of family Rubiaceae (Yonekura and Kajita 2003-). According to Low (2013), about 200 species of old genus are found in the world. It is most commonly found in Africa, Asia, and the Pacific islands which are the warm and tropical regions (Low 2013). In *Gardenia jasmioides*, which is one of the *Gardenia* species growing in temperate regions, we reported that it forms reaction wood with "compression-wood-like" type (Aiso et al. 2013). However, there is no information on reaction wood characteristics of other *Gardenia* species growing tropical region.

In this study, we collected naturally inclined stems of a *Gardenia* sp. tree grown in a tropical region, South Kalimantan, Indonesia. Using these samples, anatomical characteristics and lignin distribution of cell wall were examined. Based on the results, type of reaction wood of tropical *Gardenia* species was discussed.

2. Materials and methods

Four coppiced stems (ca. 40 cm in length) were sampled from a tree of *Gardenia* sp. grown at Banjarbaru, South Kalimantan, Indonesia (Figure 1). In this study, sampled stems were referred as sample A, B, C, and D. Of these samples, inclination angle of stem of sample A was almost zero from vertical. Thus, sample A was regarded as normal wood. Stem inclination angle of remaining three samples ranged from 40 to 50 degree from vertical (Table 2). All collected stems were cut into 1-cm-thick disks for the following experiments.

In this study, sample A was used to identify where reaction wood was formed. As preliminary experiments, transverse sections (15 μm in thickness) were prepared from all samples using a sliding microtome (REM-710, Yamatokohki). Then, the sections were treated with phloroglucinol-HCl reagent (Yoshizawa et al. 1993). As a result, the secondary xylem at certain position on the lower side of samples B, C, and D showed different staining characteristics compared to those in sample A (Figure 2). Thus, the position with different staining characteristics was regarded as reaction wood. All anatomical observations were carried out at the reaction wood positions (lower side of inclined stems) as well as opposite wood positions (upper side of inclined stems) in this study. Detail experimental scheme was shown in Figure 3.

Based on the results of preliminary experiments, 15-μm-thick transverse sections were prepared from reaction and opposite wood in disks of three inclined stem samples. Permanent slides were prepared from some sections stained with 1% safranin. Using transverse sectional images of

Table 1 Major reaction wood characteristics of angiosperms

Characteristics	Type of reaction wood		
	(1) Tension wood	(2) Tension-wood-like-reaction wood	(3) Compression-wood-like-reaction wood
Position forming reaction wood	Upper side	Upper side	Lower side
Eccentric growth	Upper side	Upper side	Lower side
Fiber morphologies	Forming gelatinous (G-) layer Microfibril angle (MFA) of G-layer shows almost 0 degree from vertical. Three types of secondary wall structure · S_1+G · S_1+S_2+G · S_1+S_2+S_3+G	No G-layer formation Two types of secondary wall structure · S_1+S_2 · S_1+S_2+S_3 MFA of S_2-layer shows almost 0 degree from vertical.	No G-layer formation No S_3-layer formation (S_1+S_2) MFA of S_2-layer shows large value compared to normal wood fiber.
Vessel morphologies	Tend to decrease diameter and number	Tend to decrease diameter and number	No certain tendency
Cellulose content	Increase	Increase	Decrease
Lignin content	Decrease	Decrease	Increase

permanent slide, anatomical characteristics of vessel and wood fiber (diameter of vessel and wood fiber, vessel frequency, and wall thickness of wood fiber) were measured by the methods described in our previous studies (Aiso et al. 2016a, b). Diameter and wall thickness were measured for 30 vessels and wood fibers, respectively. Vessel frequency were measured for four transverse sectional images in each sample. All images were taken with a light microscope (BX51, Olympus) equipped with a digital camera (E-P3, Olympus).

To measure microfibril angle (MFA) of S_2 layer in wood fiber, radial sections (ca. 20 μm in thickness) were prepared. By the iodine method described by Senft and Bendtsen (1985), MFA was measured for 30 wood fibers in each position.

Small wood sticks were also collected from the disks and macerated with Schulze's solution (6 g potassium chlorate in 100 mL 35% nitric acid). Length of 60 wood fibers and vessel elements were measured according to the method described by Aiso et al. (2013).

To observe lignin distribution, two lignin color reactions, phloroglucinol-HCl color reaction and Mäule color reaction, were carried out for transverse sections (15 μm in thickness) according to Yoshizawa et al. (1993). In addition, lignin contents were determined by acetyl bromide method (Iiyama and Wallis 1988) using transverse sections as samples.

Small wood blocks were made from the disks to observe secondary wall structure in wood fibers. Blocks were dehydrated with ethanol series and acetone, then embedded in epoxy resin containing Quetol 812, dodecenyl succinic anhydride (DDSA), methyl-5-norbornene-2,3-dicarboxylic anhydride (MNA), and 2, 4, 6-tris(dimethylaminomethyl) phenol (DMP-30, Oken). Volume ratio of reagent was Quetol 812 : DDSA : MNA : DMP-30 = 100 : 25 : 75 : 1. One-μm thick transverse sections were cut using a rotary ultramicrotome (LKB2128, Bromma). Secondary wall structure of wood

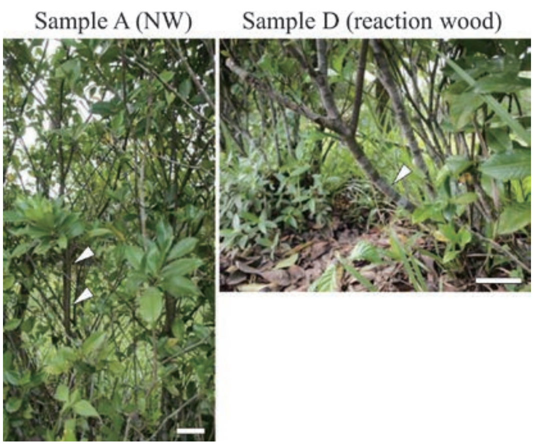

Figure 1 Photographs of coppiced stems from a tree of *Gardenia* sp. used in this study

Note: Arrowheads indicate sampled stems (samples A and D); scale bar = 10 cm. After cut stems from ground side, disks with 1 cm interval were obtained from almost 50% position in length in each sample.

Table 2 Inclination angle and diameter of stems in Gardenia sp. investigated in this study

Sample	IA (degree)	Diameter (cm)
A (NW)	1	1.6
B	45	1.5
C	50	1.1
D	41	1.9

IA, inclination angle; NW, normal wood; Stem diameter was measured at the position about 25 to 30 cm above the ground.

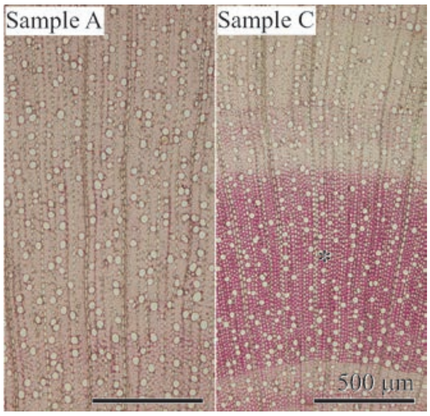

Figure 2 Photomicrographs of transverse sections of normal wood (sample A) and reaction wood (sample C) in a *Gardenia* sp. tree after phloroglucinol-HCl color reaction

Note: Asterisk indicates reaction wood which showed strong pinkish color compared to normal wood after staining.

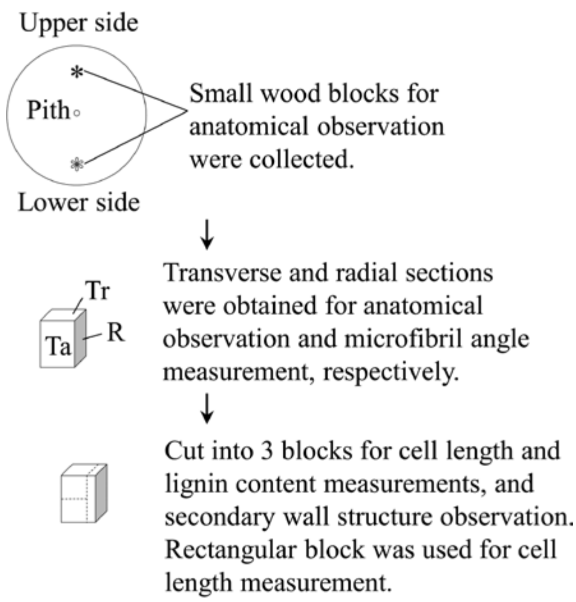

Figure 3 Diagram of experimental scheme in this study

Note: After preliminary experiment, small wood blocks were obtained from both upper side (black asterisk) and lower side (white asterisk) of disks of inclined samples. Then, for anatomical observation including lignin color reactions and microfibril angle measurement, transverse and radial sections were obtained. After that, blocks were cut into three blocks including one rectangular block. Rectangular block was used for cell length measurement. Remaining two blocks were used for secondary wall structure observation (embedding in epoxy resin) and lignin content measurement, respectively. Tr, transverse section; R, radial section; Ta, tangential section.

fibers was observed by a polarized light microscopy (BX51, Olympus).

To detect the significant difference between opposite and reaction wood in each characteristic, paired t-test was performed at 5% level using R (version 3.6.3, R Core Team 2020).

3. Results

When mean values of three inclined stems in each characteristic were calculated, significant differences between opposite and reaction wood were found only for wood fiber length and lignin content ($p < 0.05$, Table 3). Other characteristics, such as vessel morphologies, and diameter, wall thickness, and MFA of wood fibers, did not show significant differences between two positions ($p > 0.05$, Table 3).

Table 3 Means and standard errors of measured characteristics in reaction and opposite wood of inclined samples

Position	Opposite wood	Reaction wood	*p*-value
Vessel frequency (No./mm^2)	149.0 ± 7.0	166.6 ± 23.0	0.457
Vessel diameter (μm)	32.4 ± 2.0	32.8 ± 1.8	0.460
Vessel element length (mm)	0.54 ± 0.04	0.56 ± 0.01	0.650
Wood fiber diameter (μm)	17.8 ± 0.4	17.7 ± 0.4	0.828
Wood fiber wall thickness (μm)	4.1 ± 0.2	4.1 ± 0.3	0.903
Wood fiber length (mm)	1.08 ± 0.04	0.97 ± 0.06	0.040
MFA of wood fiber (°)	18.8 ± 0.8	24.0 ± 1.7	0.068
Lignin content (%)	24.9 ± 0.1	25.8 ± 0.1	0.046

Number of samples = 3; The *p*-values were calculated by paired *t*-test.

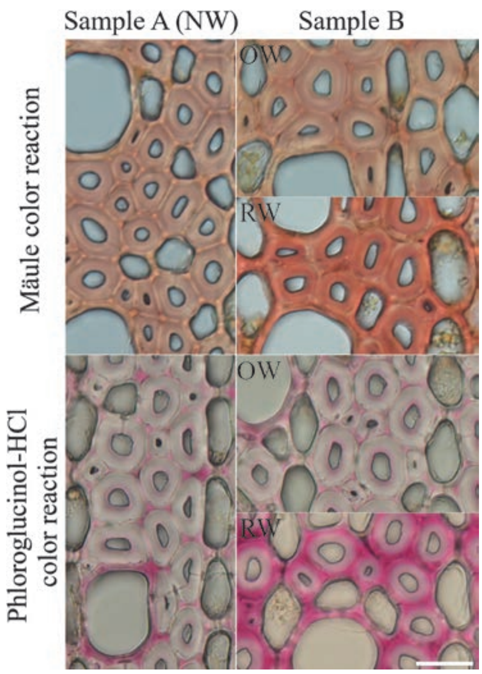

Figure 4 Photomicrographs of transverse sections after lignin color reactions in samples A and B of a *Gardenia* sp. tree

Note: NW, normal wood; OW, opposite wood; RW, reaction wood; scale bar = 20 μm. No distinct changes in staining were observed between sample A and opposite wood of sample B. In reaction wood of sample B, wood fiber walls were strongly stained by both reactions, and intercellular layer showed pinkish color after phloroglucinol-HCl color reaction.

Compared between sample A and opposite wood of sample B, there were no apparent staining differences after both Mäule and phloroglucinol-HCl color reactions (Figure 4). However, after Mäule color reaction, walls in wood fiber and vessel were strongly stained in reaction wood of sample B, whereas wood fiber wall and intercellular layer in reaction wood were strongly stained by phloroglucinol-HCl color reaction. Other inclined samples also showed the same tendencies as sample B.

In sample A, distinct S_3 layer was observed for walls of wood fibers and vessels (Figure 5). On the other hand, although wood fiber in reaction wood sample D showed no S_3 layer formation, it was observed only in vessels (Figure 5).

4. Discussion

"Compression-wood-like" type of reaction wood is formed in certain angiosperms which form vessel element in secondary xylem, such as *Buxus* spp. (Yoshizawa et al. 1993; Baillères et al. 1997; Hiraide et al. 2016), and *Gardenia jasminoides* (Onaka 1949; Aiso et al. 2013). Among these genera, the following changes in wood fibers are known as common characteristics for "compression-wood like" type: decrease in length, increase in MFA, no S_3 layer formation, and increase in lignin concentration (Table 1, Yoshizawa et al. 1993; Baillères et al. 1997; Aiso et al. 2013; Hiraide et al. 2016). In this study, significant decrease in wood fiber length and lignin content in reaction wood was found compared with opposite wood (Table 3). Although there was no significant difference, MFA of wood fibers tended to increase in reaction wood samples (24.0°) compared to that in opposite wood (18.8°, $p = 0.068$, Table 3). By using microscopy, no S_3 layer formation was observed in wood fiber of reaction wood (Figure 5). Corresponding to the changes in lignin content, walls of wood fiber in reaction wood were strongly stained by both Mäule and phloroglucinol-HCl color reactions (Figure 4), suggesting that lignin concentration

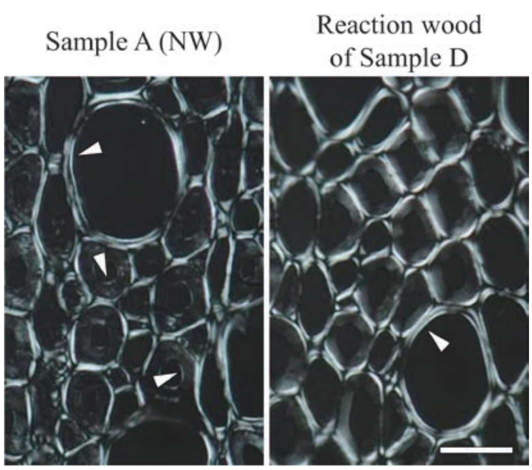

Figure 5 Polarizing photomicrographs of transverse sections without staining in samples A and D of a *Gardenia* sp. tree

Note: Arrowheads indicate the S_3 layer; scale bar = 20 μm. S_3 layer was clearly observed for both sample A and reaction wood in sample D, but it was observed for wood fiber only in sample A.

increase by reaction wood formation. Based on the results, it is considered that *Gardenia* sp. tree growing at tropical region forms "compression-wood-like" reaction wood on the lower side of inclined stem.

In the previous study (Aiso et al. 2013), we reported that reaction wood in *G. jasminoides* showed strong staining in wood fiber walls especially for after phloroglucinol-HCl color reaction. However, there were no significant changes in lignin content between normal and reaction wood. Because Mäule and phloroglucinol-HCl color reactions indicate the presence of syringyl and guaiacyl units in lignin, respectively (Lin and Dence 1992), we concluded that alteration of nature in lignin with inclination stimulus of *G. jasminoides* corresponds to increase in lignin concentration especially for guaiacyl unit in lignin. As shown in Table 3 and Figure 4, *Gardenia* sp. showed different tendency compared with *G. jasminoides* in the change of chemical characteristics in relation to lignin: although reaction wood in *G. jasminoides* showed only apparent color change after phloroglucinol-HCl reaction, both lignin content and lignin color reactions under microscopic observation in *Gardenia* sp. tested here significantly differed between opposite and reaction wood. It is concluded that type and characteristics of reaction wood in tropical Gardenia sp. used in this study are almost same as those of temperate *G. jasminoides*.

5. Conclusion

In the present study, reaction wood characteristics of tropical *Gardenia* sp. were investigated. Vessel and wood fiber morphologies, and lignin content were determined. In addition, walls of wood fiber structure and lignin distribution were observed by microscopy. No significant tendencies in changes in vessel morphologies, and thickness, diameter, and MFA of wood fiber were observed in reaction wood of inclined samples. However, significant decrease in wood fiber length, increase in lignin content, and no S_3 layer formation in wood fiber were found in reaction wood. Wood fibers in reaction wood samples were strongly stained by Mäule color reaction. By phloroglucinol-HCl color reaction, both wood fiber walls and intercellular layer were strongly stained in reaction wood. Based on the results, it is considered that decrease in length, and increase in lignin concentration in wood fiber walls by reaction wood formation are common characteristics in *Gardenia* species, even though the growth conditions are quite different between tropics and temperate regions.

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