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NOTE

Relationships between tree size and reaction wood formation in 23 Japanese angiosperms

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Abstract The objective of this study is to clarify the relationships between tree sizes and the anatomical, chemical, and physical characteristics of reaction wood. Naturally inclined stems or branches in 23 Japanese angiosperms, including 3 tree sizes at adult trees, 7 arbor, 7 subarbor, and 9 shrub species, were used. The presence of a gelatinous (G-) layer, basic density, vessel frequency, and lignin content were examined on the upper and lower sides of each sample. Almost all the species showed a decrease in vessel frequency and lignin content on the upper side, which are typical characteristics of reaction wood in angiosperms. A G-layer was formed in four arbor, one subarbor, and two shrub species. The ratio of the upper and lower sides in vessel frequency and lignin content gradually decreased with an increase in tree size. Based on the results of the principal component and cluster analyses, shrub species were classified into the different groups: from arbor and subarbor groups. These results indicate that the magnitude of alteration in the characteristics due to reaction wood formation of arbor and subarbor might differ from that of shrub, although the characteristics changed by reaction wood formation might be the same in the three tree sizes at adult trees.

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Introduction

Woody plants develop special tissues, called reaction wood, to restore their inclined stems or branches to their original positions [1–10]. Reaction wood in angiosperms forms on the upper side of the inclined stems or branches, and it is known as tension wood. Tension wood is typically characterized by eccentric growth on the upper side, and lower lignin and higher cellulose contents of wood due to formation of a cellulosic layer (G-layer) in the innermost layer of wood fibers [1, 2, 5, 6, 8–10]. However, some angiosperms form reaction wood without forming a G-layer on the upper side of inclined stems or branches. This type of reaction wood has been found in *Magnolia, Liriodendron*, and others [1, 5, 7, 11–14]. In addition, a few angiosperms, such as *Buxus* spp., *Gardenia jasminoides*, and others, form reaction wood on the lower side of the stems with similar anatomical and chemical characteristics to compression wood in gymnosperms. Therefore, it is called compression-wood-like reaction wood [15–18].

Form and size of trees at adult trees have been considered to be related closely to diversity of reaction wood in angiosperms [1, 19, 20]. Onaka [1] summarized eccentric growth and anatomical characteristics of reaction woods in 347 angiosperms. He reported that many arbor trees in angiosperms showed eccentric growth on the upper side [1]. Fisher and Stevenson [19] examined the relationships between branch orientation of a tree and anatomical characteristics of reaction wood in angiosperms. They found that a tree with monopodial branching tended to form typical tension wood with a G-layer. However, information is still limited on the relationships between tree size and reaction wood formation in angiosperms.

The purpose of the current study is to clarify the relationships between tree size based on adult trees and reaction wood formation in 23 Japanese angiosperms collected from 3 different sites using principal component analysis (PCA) and cluster analysis.

Materials and methods

A total of 23 Japanese angiosperms from 16 families were used in the present study (Table 1). From 2009 to 2010, naturally inclined stems or branches were collected from natural forests in Funyu Experimental Forest, Utsunomiya University (36°46′ N, 139°49′ E, 12.6 °C in mean annual temperature and 1687 mm in mean annual precipitation), Nikko Experimental Forest, Utsunomiya University (36°48′ N, 139°28′ E, 7.1 °C in mean annual temperature and 2199 mm in mean annual precipitation), and forests in the southern part of Chiba (34°57′–34°58′ N, 139°50′–139°53′ E, 16.1 °C in mean annual temperature and 1856 mm in mean annual precipitation) in Japan. Before sampling, to confirm reaction wood formation, surface-released strain was measured for secondary xylem surface on the upper and lower sides of all samples by strain gauge method [11, 13, 20]. To increase the number of species, a sample of stem or branch was collected from a tree in each species. In the present study, tree size was defined as tree height at adult tree based on the references [21, 22], arbor (over 8 m in tree height), subarbor (3–8 m in tree height), and shrub (0.3–3 m in tree height). Seven arbor, seven subarbor, and nine shrub species were used in this study (Table 1). Most of species are diffuse porous wood, whereas four species [*Fraxinus lanuginosa* (Ar-5), *Kalopanax septemlobus* (Ar-7), *Toxicodendron trichocarpum* (Sh-5), and *Buckleya lanceolata* (Sh-6)] are ring-porous wood (Table 1).

One-cm-thick disks were obtained from inclined stems or branches. The disks were fixed with 3% glutaraldehyde in phosphate buffer (pH 7.0) for 1 day and then washed in running tap water for 1 day. After fixation, they were preserved in 50% ethanol solution until used for each experiment. To determine basic density, small wood blocks containing the current annual ring were collected from the

Table 1 Species used in the present study

Tree sizes	Codes	Families	Species	Sampling sites	IA (degree)
Arbor	Ar-1	Eupteleaceae	*Euptelea polyandra* Siebold et Zucc	II	42
	Ar-2	Sapindaceae	*Acer rufinerve* Siebold et Zucc	II	38
	Ar-3	Theaceae	*Stewartia pseudocamellia* Maxim	I	–
	Ar-4	Styracaceae	*Styrax obassia* Siebold et Zucc	II	30
	Ar-5	Oleaceae	*Fraxinus lanuginosa* Koidz. f. *lanuginosa*	II	42
	Ar-6	Aquifoliaceae	*Ilex macropoda* Miq	I	52
	Ar-7	Araliaceae	*Kalopanax septemlobus* (Thunb.) Koidz	II	34
Subarbor	Su-1	Hamamelidaceae	*Hamamelis japonica* Siebold et Zucc	II	52
	Su-2	Rosaceae	*Malus toringo* (Siebold) Siebold ex de Vriese	II	34
	Su-3	Rosaceae	*Pourthiaea villosa* (Thunb.) Decne. var. *villosa*	II	51
	Su-4	Rosaceae	*Sorbus commixta* Hedl. var. *rufoferruginea* C.K.Schneid	II	30
	Su-5	Styracaceae	*Pterostyrax hispida* Siebold et Zucc	II	57
	Su-6	Clethraceae	*Clethra barbinervis* Siebold et Zucc	II	41
	Su-7	Aquifoliaceae	*Ilex crenata* Thunb. var. *crenata*	I	34
Shrub	Sh-1	Stachyuraceae	*Stachyurus praecox* Siebold et Zucc	III	30
	Sh-2	Rosaceae	*Cerasus nipponica* (Matsum.) Ohle ex H.Ohba var. *kurilensis* (Miyabe) H.Ohba	II	-
	Sh-3	Betulaceae	*Corylus sieboldiana* Blume var. *sieboldiana*	II	34
	Sh-4	Celastraceae	*Euonymus oxyphyllus* Miq. var. *oxyphyllus*	II	41
	Sh-5	Anacardiaceae	*Toxicodendron trichocarpum* (Miq.) Kuntze	II	38
	Sh-6	Santalaceae	*Buckleya lanceolata* (Siebold et Zucc.) Miq	I	55
	Sh-7	Ericaceae	*Rhododendron wadanum* Makino	I	38
	Sh-8	Ericaceae	*Rhododendron kaempferi* Planch. var. *kaempferi*	II	63
	Sh-9	Ericaceae	*Elliottia paniculata* (Siebold et Zucc.) Hook.f	II	35

Ar, Su, and Sh in code correspond to arbor, subarbor, and shrub, respectively. IA, inclination angle of sample stems or branches; I, II, and III in sampling sites are Funyu Experimental Forest, Nikko Experimental Forest, and Forests in southern part of Chiba, Japan, respectively, –, no data upper and lower sides of the obtained disks. Basic density was calculated by dividing oven-dried weight by green volume. Small wood blocks [5 (L) ×5 (R)×5 (T) mm) containing the current or preceding annual rings were collected from the upper and lower sides of inclined stems or branches. Transverse sections of 15 µm in thickness were sliced with a sliding microtome (ROM-380, Yamatokohki). The sections were stained with 1% safranin in 50% ethanol to determine the vessel frequency. In the case of ring-porous wood, vessel frequency was determined in the outer-pore zone. To detect the G-layer, transverse sections were stained with a zinc chloride–iodine solution and mounted in a 50% glycerol solution. Lignin content was determined by the acetyl bromide method [23].

Upper-side-to-lower side (UP/LP) ratios of all measurements were calculated to clarify the relationship between tree sizes and the changes in anatomical, chemical, and physical characteristics of wood due to reaction wood formation. PCA was applied to UP/LP ratios of basic density, vessel frequency, and lignin content in all trees using R software [24]. In addition, principal component scores were calculated for these characteristics by PCA. After that, cluster analysis was conducted using Euclidean distance and the Ward's linkage method.

Results

Of the 23 species, four arbor, one subarbor, and two shrub species formed a G-layer on the upper side (Table 2; Fig. 1). UP/LP ratios in basic density in all samples ranged from 0.91 to 1.15 (Table 2). No tendency for changes in basic density by reaction wood formation was observed among the species and tree size at adult trees. Almost all the species showed a decrease in vessel frequency by reaction wood formation (Table 2). The UP/LP ratio in vessel frequency decreased with an increase in tree size at adult trees (shrub < subarbor < arbor). Lignin contents decreased

Table 2 Presence of G-layer, basic density, vessel frequency, and lignin content on the upper and lower sides of samples

Tree sizes	Codes	G-layer	Basic density (g/cm^3)			Vessel frequency (No./mm^2)			Lignin content (%)		
			UP	LP	UP/LP	UP	LP	UP/LP	UP	LP	UP/LP
Arbor	Ar-1	−	0.59	0.54	1.08	94	178	0.53	21.8	28.7	0.76
	Ar-2	+	0.42	0.42	1.00	58	139	0.42	22.4	30.2	0.74
	Ar-3	−	0.59	0.58	1.01	63	96	0.66	24.2	26.4	0.92
	Ar-4	+	0.58	0.55	1.07	37	71	0.52	20.2	31.3	0.64
	Ar-5	+	0.62	0.63	0.98	15	81	0.19	20.3	28.7	0.71
	Ar-6	−	0.51	0.45	1.13	124	138	0.90	24.1	27.2	0.89
	Ar-7	+	0.50	0.54	0.94	163	229	0.71	23.7	26.7	0.89
	Mean				1.03			0.56			0.79
Subarbor	Su-1	+	0.60	0.54	1.12	204	323	0.63	21.6	31.1	0.69
	Su-2	−	0.61	0.59	1.03	198	355	0.56	29.4	30.0	0.98
	Su-3	−	0.72	0.72	1.00	167	233	0.72	23.8	26.0	0.92
	Su-4	−	0.53	0.54	1.00	146	351	0.42	23.1	24.9	0.93
	Su-5	−	0.47	0.45	1.04	69	135	0.51	23.4	27.3	0.86
	Su-6	−	0.48	0.49	0.97	27	48	0.56	25.6	28.1	0.91
	Su-7	−	0.64	0.62	1.03	96	127	0.75	25.4	30.0	0.85
	Mean				1.03			0.59			0.88
Shrub	Sh-1	−	0.50	0.55	0.91	110	120	0.92	38.2	37.9	1.01
	Sh-2	+	0.56	0.59	0.95	279	255	1.09	20.5	26.3	0.78
	Sh-3	−	0.44	0.46	0.95	101	107	0.94	20.2	26.3	0.77
	Sh-4	−	0.62	0.65	0.96	366	398	0.92	33.8	30.4	1.11
	Sh-5	+	0.52	0.54	0.97	45	35	1.28	21.8	24.4	0.89
	Sh-6	−	0.61	0.53	1.15	228	266	0.86	26.3	31.9	0.82
	Sh-7	−	0.58	0.59	0.97	307	348	0.88	36.0	34.8	1.03
	Sh-8	−	0.49	0.50	0.99	316	518	0.61	36.6	37.7	0.97
	Sh-9	−	0.50	0.45	1.13	489	526	0.93	26.2	32.3	0.81
	Mean				1.00			0.94			0.91

Code refers to Table 1; Ar, arbor; Su, subarbor; Sh, shrub; LP, lower side; UP, upper side; "+" and "−" in G-layer indicate presence and absence of G-layer, respectively

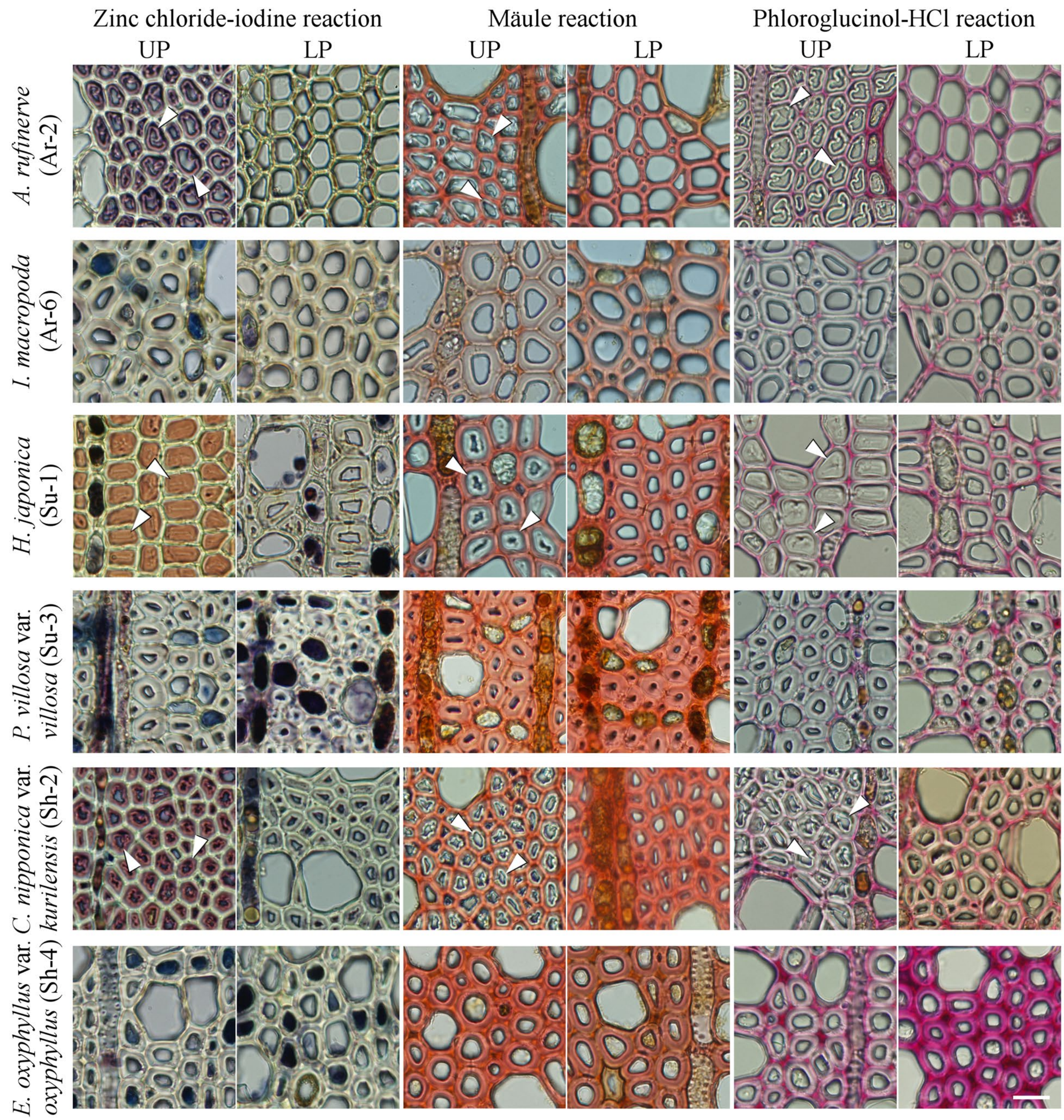

Fig. 1 Light microphotographs of transverse sections after zinc chloride–iodine reaction, Mäule reaction, and phloroglucinol–HCl reaction on the upper and lower sides of inclined stems or branches. *Arrowheads* indicate gelatinous (G)-layer. In the present study, G-layer was defined as the innermost layer of wood fibers stained with zinc chloride–iodine reagent, but not with Mäule and phloroglucinol–HCl reagents. *LP* lower side, *UP* upper side, *scale bar* 30 μm

on the upper side of almost all the samples (Table 2). Mean UP/LP ratios in arbor, subarbor, and shrub species were 0.79, 0.88, and 0.91, respectively.

The result of cluster analysis is shown in Fig. 2. Except for *Ilex macropoda* (Ar-6) and *Rhododendron kaempferi* var. *kaempferi* (Sh-8), shrubs form a distinct cluster from the other two tree sizes.

Discussion

In the present study, the G-layer formation, one of the characteristics of the typical tension wood, was observed on the upper side of seven species (30% of the total number of sample species; Table 2; Fig. 1). Concerning the tree size at adult trees, the number of species forming a G-layer in

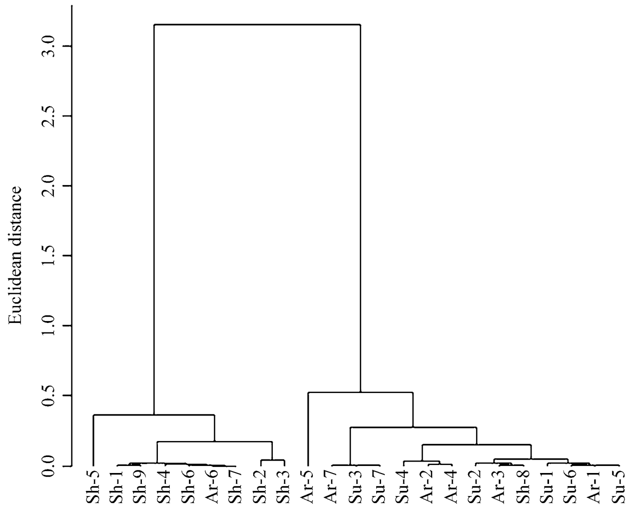

Fig. 2 Dendrogram based on principal component scores of basic density, vessel frequency, and lignin content. Code refers to Table 1. Shrubs form a distinct cluster from arbors and subarbors, except for *Ilex macropoda* (Ar-6) and *Cerasus nipponica* var. *kurilensis* (Sh-2)

arbors was much compared to that in subarbors and shrubs; percentages of formation of a G-layer were 57% (4/7 species) of arbors, 14% (1/7 species) of subarbors, and 22% (2/9 species) of shrubs, respectively. These results suggest that arbors mainly form typical tension wood with a G-layer. This tendency also was supported by the previous report [1].

In almost all the species, decreases in vessel frequency and lignin content were observed on the upper side (Table 2). These changes in anatomical and chemical characteristics due to reaction wood formation in angiosperms also have been reported by many researchers [1, 2, 4, 8–10, 12, 25, 26]. Ruelle et al. [25] reported that, in tropical angiosperms grown in French Guyana, vessel diameter and frequency were significantly decreased in 15 and 10 species, respectively. In Japanese shrub trees, eccentric growth and decreases in vessel frequency and lignin content were observed on the upper side by reaction wood formation [26]. Based on the obtained results in the present study and previous reports, eccentric growth, decreases in vessel diameter and/or frequency, and a decrease in lignin content on the upper side might be common characteristics of reaction wood in angiosperms, even though the tree size at adult trees differs (Table 2).

The UP/LP ratios of vessel frequency and lignin content tended to decrease with an increase in tree size at adult trees (Table 2). Therefore, it is considered that the magnitude of the alteration in reaction wood characteristics might differ among tree sizes at adult trees. Based on the obtained result of the cluster analysis, the magnitude of the alteration in vessel frequency and lignin content of arbor and subarbor species might differ from that of shrubs (Fig. 2), although the alteration in vessel frequency and lignin content due to reaction wood formation were the same. Studies on reaction wood formation in angiosperms mainly have focused on arbors [2–4, 8, 10, 12, 19, 20]. Arbor species tend to form wood fiber with a G-layer rich in cellulose in reaction wood. An increase in the cellulose-rich layer in the wood fibers might result in changes of the physical and mechanical properties of the wood—increases in basic density and longitudinal Young's modulus. For example, we found that, in tropical arbors, *Falcataria moluccana* and *Acacia auriculiformis*, an increase in cellulose content by reaction wood formation could cause an increase in basic density [8]. In addition, Yamamoto [3] reported that a development of a G-layer causes an increase in longitudinal Young's modulus. Thus, increases in basic density and longitudinal Young's modulus are considered to be due to an increase of the cellulose-rich layer and is one of the strategies to restore or support the tree shape in arbor species. In the present study, vessel frequency and lignin content commonly decreased in all three tree sizes at adult trees (Table 2). However, the occurrence percentage of G-layer formation was higher in arbor and subarbor species compared to shrubs (Table 2), suggesting that most arbor and subarbor species used in this study might have similar mechanisms to restore or support the tree shape. Thus, shrubs seem not always to form special layers, such as a G-layer, to restore or support a tree shape, although the lignin content decreases in reaction wood. On the other hand, our previous report [14] pointed out that degree of reaction wood formation could be affected by the difference in stem inclination angle. Because samples used in the present study were collected from fields, inclination angle of the sample trees was not always the same among different species. Further researches are still needed to clarify the relationship between tree size at adult trees and reaction wood formation in angiosperms with the same inclination angle.

Conclusion

This study aimed to clarify the relationships between tree sizes and the changes in anatomical, chemical, and physical characteristics due to reaction wood formation in naturally inclined stems or branches in 23 Japanese angiosperms. Almost all the species showed eccentric growth and decreases in vessel frequency and lignin content on the upper side. G-layer formation was found in seven species (four arbor, one subarbor, and two shrub species). However, UP/LP ratios in vessel frequency and lignin content decreased with an increase in tree size (shrub < subarbor < arbor). The results of PCA and cluster analysis showed that arbor and subarbor species were classified into the same group, whereas shrubs formed a separate group. Therefore, magnitude of alteration in characteristics due to reaction wood formation to support the stems or branches in arbor and subarbor species might differ from that in shrubs.

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