Inheritance of the wood properties of the Japanese red pine (*Pinus densiflora* Siebold et Zucc.) from the open-pollinated families selected as resistance to the pine wood nematode

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Abstract

Pine wilt disease is one of the most serious tree diseases occurring worldwide. Clones of *Pinus densiflora* Siebold et Zucc with pine wood nematode resistance were selected. In addition to resistance, wood quality is also an important criterion in the breeding program of *P. densiflora* because of its use as construction lumber. However, little information is available on the wood qualities of the progenies of resistant clones. The repeatabilities of the wood properties were investigated for 11 open-pollinated families of *P. densiflora* selected for their pine wood nematode resistance. Oven-dry density, latewood tracheid length, the microfibril angle (MFA) of the S₂ layer in latewood tracheids, modulus of elasticity (MOE), and modulus of rupture (MOR) were measured in the third or fourth annual ring from the pith. No significant correlations were found between the wood properties and the stem diameter or tree height. However, significant correlations were found between oven-dry density and MOE or MOR, which suggests that oven-dry density is a good indicator for selecting wood with higher bending properties. Among the measured wood properties, oven-dry density had the highest repeatability ($R=0.47$), followed by MOR ($R=0.33$), tracheid length ($R=0.21$), and MFA ($R=0.14$). MOE had the lowest value ($R=0.01$). The 11 families examined were

classified into three groups according to their growth characteristics, wood properties, and resistance to pine wilt disease. On the basis of the results, we conclude that genetic improvement of wood properties especially for wood density and MOR is possible for the resistant *P. densiflora*.

Keywords: tree breeding for wood quality, wood density, microfibril angle, tracheid length, modulus of elasticity, modulus of rupture, pine wilt disease

Introduction

Pine wilt disease is a serious tree disease affecting the genus *Pinus* that is caused by the pine wood nematode (*Bursaphelenchus xylophilus* [Steiner and Buhner] Nickle) (Kiyohara and Tokushige, 1971; Futai and Furuno, 1979; Fujimoto et al., 1989; Toda and Kurinobu, 2002; Toda, 2004; Kuroda et al., 2007; Kurinobu, 2008; Zhao et al., 2008; Ribeiro et al., 2012; Vicente et al., 2012; Carrasquinho et al., 2018; Menéndez-Gutiérrez et al., 2018; Hirao et al., 2019; Iki et al., 2020; Matsunaga et al., 2020). In Japan, the disease mainly occurs in the forestry species *P. densiflora* Siebold et Zucc. and *P. thunbergii* Parl. (Fujimoto et

al., 1989; Toda and Kurinobu, 2002; Toda, 2004; Kuroda et al., 2007; Kurinobu, 2008; Zhao et al., 2008; Hirao et al., 2019). As the disease has been spreading in East Asia and Europe, it is now recognized as one of the most serious tree diseases in forests worldwide (Zhao et al., 2008; Ribeiro et al., 2012; Vicente et al., 2012; Carrasquino et al., 2018; Menéndez-Gutiérrez et al., 2018; Hirao et al., 2019).

Resistance or susceptibility to the pine wood nematode differs among the *Pinus* species (Futai and Furuno, 1979). The Japanese *Pinus* species such as *P. densiflora* and *P. thunbergia* are susceptible to the nematode, but many *Pinus* species native in North America, such as *P. taeda* L., are resistant. In addition, resistant clones have been developed even among the susceptible *Pinus* species. Thus, resistance breeding programs of *P. densiflora* and *P. thunbergii* against pine wilt disease have been conducted in Japan. Through these programs, many resistant clones of *P. densiflora* and *P. thunbergii* have been selected, and the progenies of these selected clones have been evaluated (Fujimoto et al., 1989; Toda and Kurinobu, 2002; Toda, 2004; Kuroda et al., 2007; Kurinobu, 2008; Zhao et al., 2008; Hirao et al., 2019; Iki et al., 2020; Matsunaga et al., 2020).

Pinus densiflora is one of the important coniferous species for establishing plantations in Japan. The wood is relatively strong compared with other Japanese softwoods; hence, it has been traditionally used as structural lumber in Japanese wooden architectural structures (Wood Technological Association of Japan, 1984). Thus, the wood quality, growth characteristics, and nematode resistance of the seedling are important for establishing *P. densiflora* plantations. However, little information is available on the wood quality of the progenies of resistant clones.

The aim of the present was to obtain basic knowledge for understanding the inheritance of wood properties of *P. densiflora* with pine wood nematode resistance. We investigated wood properties in the third or fourth annual ring from the pith in 11 open-pollinated families resistant to pine wood nematode. Herein, we discuss the relationships among the measured characteristics and the repeatabilities of the wood properties of the pine wood nematode-resistant *P. densiflora* progeny on the basis of our results.

Materials and Methods

Materials

The sample trees used in the study were 18-year-old Japanese red pine (*P. densiflora* Sieb. Zucc.) resistant clone progenies grown in the experimental site, at the Kazusa Experimental Station, Forestry Research Institute, Chiba Prefectural Agriculture and Forestry Research Center (35°21'N, 140°2'E). The experimental site was established in a flat area (0.05 ha) in 2002 with 17 open-pollinated families originated from resistant clones selected in the Kyushu district (except for Kagoshima), three open-pollinated families originated from resistant clones selected in Ehime, and one non-resistant family originated from Chiba (Endo and Fukuhara, 2017). The trees were planted

using the single-tree mixture model, with 1.8- × 1.8-m initial spacings. Growth characteristics, namely the stem diameter at 1.3 m above the ground and tree height, were measured in December 2018 and January 2019. Unfortunately, after the measurements, the trees were damaged by a typhoon in September 2019. After the typhoon, sample logs (40 cm in length) and disks (3 cm in thickness) were collected at approximately 1.3 m above the ground level from 40 trees with no serious damage (on examination with naked eyes) that were selected from among 11 open-pollinated resistant families (Table 1). Table 1 also lists the resistance ranking of the resistant clones (Toda, 2004) and survival rate of the 11 open-pollinated resistant families at the age of 15 years (Endo and Fukuhara, 2017). The resistance ranking (from 1 to 5, with 5 indicating the highest resistance) was determined on the basis of the results of inoculation tests for open-pollinated families of resistant clones by using the least-squares test (Toda, 2004).

Table 1

Resistance rankings and survival rates of the 11 open-pollinated resistant families used in the present study

Family	Resistance ranking	Survival rate at age of 15 years
A18	2	50.0
A50	4	83.3
B173	2	60.0
B186	4	63.6
B203	3	50.0
C142	3	71.4
D113	2	62.5
D170	3	62.5
E122	2	75.0
F7	2	42.9
G1	4	100.0

Note: Resistance ranking (from 1 to 5, with 5 indicating the highest resistance) was determined with the least-squares test using the results of the inoculation tests for open-pollinated families of resistant clones (Toda, 2004). Survival rate was calculated by dividing the number of survivor trees at the age of 15 years by the number of planted trees in 2002 (Endo and Fukuhara, 2017).

Bending properties and oven-dry density

Radial boards with pith (30 mm in thickness) were obtained from the sample logs. The radial boards were air-dried and then planed to 20 mm in thickness. Bending specimens (20 [R] × 20 [T] × 320 [L] mm) with a fourth annual ring from the pith at the center of the specimens in transverse sections were prepared from the radial boards in accordance with the Japanese Industrial Standard Z2101:2009 (Japanese Industrial Standard, 2009). The static bending test was performed using a universal testing machine (MSC-5/500-2, Tokyo Testing Machine, Tokyo, Japan) with a 280-mm span and 5-mm/min loading to the center of the radial surface of the specimens. The load and deflection data were recorded in a personal computer, and the modulus of elasticity (MOE) and modulus of rupture (MOR) were calculated. After the static bending test, small clear specimens (10 mm in longitudinal direction) were collected from

the bending specimens and then kiln-dried at 105°C. The weights and dimensions of the specimens were measured after oven drying with an electric balance (GX-300, A&D, Tokyo, Japan) and a digital caliper (CD-15, Mitutoyo, Kawasaki, Japan). On the basis of the oven-dry weight and dimension, the moisture content and oven-dry density at testing were calculated.

Latewood tracheid length and microfibril angle of the S₂ layer

Radial strips of 5-cm width and 1-cm thickness were prepared from sample disks. Small stick specimens were collected from the latewood of the third annual ring from the pith. The stick specimens were macerated with Schulze's solution (100 mL of 35% nitric acid containing 6 g of potassium chlorate) at 70°C for 2 hours. The macerated latewood tracheids were magnified with a profile projector (V-12B, Nikon, Tokyo, Japan) and measured with a digital caliper (CD-30C, Mitutoyo, Tokyo, Japan). The lengths of 30 latewood tracheids were measured in each radial position.

The microfibril angle (MFA) of the S₂ layer in the latewood tracheids was measured using the iodine method (Kobayashi, 1952). Small wood blocks with the third annual ring from the pith were collected from the radial strips. By using the wood blocks, radial sections (20 µm in thickness) were prepared with a sliding microtome (REM-710, Yamatokoki, Saitama, Japan). The sections were delignified with Schulze's solution for 15 min and then dehydrated with graded ethanol. The dehydrated sections were placed on grass slides and treated with 2 % iodine-potassium iodide and 35 % nitric acid. After mounting the coverslip, digital images of radial sections were captured with a digital camera (E-330, Olympus, Tokyo, Japan) and microscope (CX41, Olympus, Tokyo, Japan). The MFA of the S₂ layer in 20 latewoods in each radial position was measured using the ImageJ software (National Institute of Health, MD, USA).

Data analysis

Data analysis was performed using the R version 4.0.3 software (R Core Team, 2020). To calculate repeatability, the following linear mixed-effect model was developed using the lmer function in the lme4 packages (Bates et al., 2015):

$$y_{ij} = \mu + a_j + e_{ij}$$

where y_{ij} is the measured properties of the i th individual tree of the j th family, μ is the mean value, a_j is the random effect of the j th family, and e_{ij} is the residual. By using this model, the variance component was estimated.

The repeatability (R) was calculated using the following formula (Nakagawa and Schielzeth, 2010):

$$R = \delta_f^2 / (\delta_f^2 + \delta_e^2)$$

where δ_f^2 is the family variance component and δ_e^2 is the residual variance component.

To evaluate the relationships between the measured properties, Pearson's correlation coefficients were calculated using the data from the individual trees ($n = 40$). Families were

categorized by combining principal component analysis (PCA) and cluster analysis (Nezu et al., 2021). The measured characteristics with relatively higher repeatabilities, resistance rankings, and survival rates at the age of 15 years were used as variables for the PCA. The families were categorized into clusters according to their principal component scores calculated using the Euclidean distance and Ward's linkage methods.

Results and discussion

Mean values of growth characteristics and wood properties

Table 2 shows the growth characteristics of the sample trees. The mean stem diameter at 1.3 m above the ground and tree height of all the trees were 18.7 cm and 12.4 m, respectively. The family mean stem diameter and tree height ranged from 12.8 cm (C142) to 23.8 cm (D170), and from 11.2 m (D113) to 13.3 m (G1), respectively.

Table 2
Family mean values and repeatabilities of stem diameter and tree height

Family	<i>n</i>	Stem diameter (cm)		Tree height (m)	
		Mean	SD	Mean	SD
A18	5	17.5	2.5	12.2	0.9
A50	3	17.7	1.6	12.5	1.1
B173	3	15.7	1.1	12.8	0.6
B186	4	18.2	4.1	12.3	0.7
B203	3	16.1	3.4	11.5	0.4
C142	4	12.8	2.1	12.8	0.6
D113	3	15.2	1.4	11.2	0.6
D170	5	23.8	3.8	12.6	0.6
E122	4	23.1	6.9	12.6	0.9
F7	3	21.0	6.3	12.1	0.5
G1	3	22.5	4.3	13.3	1.3
Mean / Total	40	18.7	5.3	12.4	0.9
Repeatability		0.28		0.01	

Note: *n*, number of sample trees; SD, standard deviation. Stem diameter was measured at 1.3 m above the ground.

Table 3 shows the family mean values of the wood properties. The family mean oven-dry density ranged from 0.33 g/cm³ (B186 and G1) to 0.45 g/cm³ (F7), and that for all the trees was 0.37 g/cm³. The family mean latewood tracheid length and MFA of the S₂ layer in the latewood tracheids ranged from 1.46 mm (B173) to 2.18 mm (A50), and from 23.9° (D113) to 26.6° (C142). Those for all the trees were 1.91 mm and 25.2°, respectively. The mean MOE and MOR for all the trees were 5.50 GPa and 49.2 MPa, respectively. The minimum and maximum family mean values were, respectively, 4.03 GPa (B203) and 7.04 GPa (C142) for MOE, and 31.5 MPa (B203) to 69.0 MPa (F7) for MOR.

Table 3

Family mean values and repeatabilities of oven-dry density, tracheid length, microfibril angle, modulus of elasticity, and modulus of rupture

Family	n	OD (g/cm ³)		TL (mm)		MFA (degree)		MOE (GPa)		MOR (MPa)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
A18	5	0.35	0.03	1.89	0.35	25.7	0.8	5.03	1.65	43.6	11.0
A50	3	0.36	0.01	2.18	0.15	25.3	0.8	5.22	0.70	51.3	8.3
B173	3	0.38	0.02	1.46	0.07	25.8	0.6	6.09	1.21	47.9	17.2
B186	4	0.33	0.04	1.96	0.38	26.0	0.9	5.50	1.06	49.9	15.7
B203	3	0.36	0.02	1.59	0.08	24.1	1.8	4.03	1.31	31.5	16.7
C142	4	0.42	0.07	2.08	0.15	26.6	0.7	7.04	0.98	68.0	10.0
D113	3	0.36	0.03	2.09	0.21	23.9	1.3	4.97	0.80	35.0	3.0
D170	5	0.34	0.01	2.12	0.14	24.2	1.6	5.25	0.78	45.8	8.9
E122	4	0.42	0.02	1.80	0.33	25.4	1.6	5.55	1.37	57.2	5.5
F7	3	0.45	0.03	1.78	0.33	24.8	0.8	6.38	2.16	69.0	15.1
G1	3	0.33	0.02	1.85	0.14	25.0	1.0	5.43	0.40	39.0	3.5
Mean / Total	40	0.37	0.05	1.91	0.32	25.2	1.4	5.50	1.43	49.2	15.9
Repeatability		0.47		0.21		0.14		0.01		0.33	

Note: n, number of sample trees; SD, standard deviation; OD, oven-dry density; TL, latewood tracheid length in the third annual ring from the pith; MFA, microfibril angle of the S₂ layer in latewood tracheids in the third annual ring from the pith; MOE, modulus of elasticity; MOR, modulus of rupture.

Relationships between the measured characteristics

As shown in Figure 1, a weak positive correlation ($r = 0.335$, $p = 0.035$) was found between stem diameter and tree height, suggesting that the larger diameter trees, the higher height of trees. No significant correlations ($p > 0.05$) were found between the growth characteristics and the wood properties (Figure 2). Relationships between growth characteristics and wood properties have been investigated in coniferous plantation species (Tanabe et al., 2017; Takahashi et al., 2021). As shown in Figure 2, no significant correlations were obtained between growth characteristics and wood properties, indicating that wood properties are independent from growth characteristics. Thus, simultaneous selection is possible for *P. densiflora* trees with higher resistance to pine wilt disease, good growth characteristics, and higher wood quality for structural lumber production.

Figure 1

Relationship between the stem diameter at 1.3 m above the ground and tree height

Note: Number of sample trees = 40; r , correlation coefficient; p , probability; D, stem diameter at 1.3 m above the ground; TH, tree height. The correlation coefficients with bold emphasis have p values < 0.05 .

The positive correlation between MOE and MOR is well known, and these bending properties are affected by the wood density and MFA of the S₂ layer in latewood tracheids (Tanabe et al., 2017; Takahashi et al., 2021). Takahashi et al. (2021) reported that air-dry density and MFA significantly correlated with MOE and MOR, including the fourth annual ring from the pith in the specimens of *Cryptomeria japonica*. In the present study, oven-dry density significantly correlated with MOE ($r = 0.542$, $p < 0.001$) or MOR ($r = 0.673$, $p < 0.001$; Figure 3). MFA also significantly related with MOR ($r = 0.456$, $p = 0.003$; Figure 3). However, MFA showed no significant correlation with MOE ($r = 0.244$, $p = 0.130$) because the among-family variation in MFA in the third annual ring from the pith was not large enough. In addition, MOE of this species might be mainly correlated with wood density rather than MFA in juvenile wood. On the other hand, a positive high correlation coefficient was found between MOE and MOR ($r = 0.745$, $p < 0.001$; Figure 3). Thus, the MOR of wood from *P. densiflora* trees with pine wood nematode resistance can be nondestructively estimated on the basis of the MOE. In addition, wood density is a useful indicator for selecting trees with higher MOR from among *P. densiflora* trees with pine wood nematode resistance at a relatively younger age (around 5 years old).

Repeatability and classification of families

Mikami et al. (1989) estimated the narrow-sense heritability of stem diameter and tree height for *P. densiflora* trees from 18 families selected from Tohoku district and 14 families selected from Kansai district. The heritabilities of tree height were higher than those of stem diameter (Mikami et al., 1989). Our results are inconsistent with those obtained by Mikami et al. (1989) that showed that the repeatability of stem diameter ($R = 0.28$) was higher than that of tree height ($R = 0.01$; Table 2). The lower repeatability of tree height than that of stem diameter might

Figure 2
Relationships between growth characteristics and wood properties

Note: Number of sample trees = 40; r , correlation coefficient; p , probability; D, stem diameter at 1.3 m above the ground; TH, tree height; TL, latewood tracheid length in the third annual ring from the pith; MFA, microfibril angle of the S₂ layer in the latewood tracheid in the third annual ring from the pith; OD, oven-dry density; MOE, modulus of elasticity; MOR, modulus of rupture.

Figure 3
Relationships between wood properties

Note: Number of sample trees = 40; r , correlation coefficient; p , probability; TL, latewood tracheid length in the third annual ring from the pith; MFA, microfibril angle of the S_2 layer in the latewood tracheid in the third annual ring from the pith; OD, oven-dry density; MOE, modulus of elasticity; MOR, modulus of rupture. The correlation coefficients with bold emphasis have p values < 0.05.

be related with the experimental site conditions especially for flat areas.

Table 3 shows the repeatability of the wood properties. Oven-dry density had the highest repeatability ($R = 0.47$), followed by MOR ($R = 0.33$) and latewood tracheid length ($R = 0.21$). The lowest value ($R = 0.01$) was obtained for MOE. Hayatgheibi et al. (2017) reported that the narrow-sense heritability of average wood density was higher than those of MFA and MOE in *P. contorta*. A similar tendency was also found in *P. radiata* (Baltunis et al., 2007). The repeatabilities of oven-dry density, MFA, and MOE in the present study were similar to those obtained for *P. contorta* and *P. radiata* (Baltunis et al., 2007; Hayatgheibi et al., 2017). The repeatability of MOR was relatively higher than the other wood properties ($R = 0.33$). As shown in Figure 3, a significant relatively higher correlation coefficient was obtained between oven-dry density and MOR. The higher correlation between oven-dry density and MOR resulted in the relatively higher repeatability of MOR. On the other hand, the narrow-sense heritabilities of tracheid length, wood density, MFA, and MOE gradually increased with the cambial age of the *Pinus* species (Sugawa, 1989; Hong et al., 2015). Thus, the repeatabilities of the wood properties of the open-pollinated resistant family of *P. densiflora* might increase with cambial age.

Figure 4 shows the PCA results. The stem diameter, oven-dry density, MOR, resistance ranking, and survival rate at the age of 15 years were used as variables in the PCA. The first and second principal components (PC1 and PC2) accounted for 45.8 % and 26.3 %, respectively. The loading values are listed in Table 4. Although growth characteristics and survival rates are expected to be independent from each other, loadings graph showing the contribution of the stem diameter, resistance ranking, and survival rate at the age of 15 years to the PC1 and PC2 were similar (Figure 4). On the other hand, PC2 tended to show that wood properties are independent from growth characteristics and resistance to pine wilt disease (Figure 4). In the cluster analysis, 11 families were classified into three groups (Figure 5). One group (A50, B186, D170, and G1) consisted of the families with relatively higher resistance and growth rates and average wood properties. The second group (C142, E122, and F7) consisted of the families with average resistance and growth rates and higher wood properties. The third group was composed of the families with poor growth characteristics, wood properties, and resistance (A18, B173, B203, and D113). Removing such families (or genotypes) from seed orchards would be effective for obtaining better growth characteristics, wood properties, and resistance to the pine wilt disease in the tree breeding program. From our results, we conclude that genetic improvement of wood properties, especially wood density and MOR, can be achieved in resistant families of *P. densiflora*.

Figure 4
Principal component (PC) analysis scores of the selected properties

Note: Stem diameter (D), oven-dry density (OD), modulus of rupture (MOR), resistance ranking (RR), and survival rate (SR) were used as variables. Arrows with gray color indicate loading graph showing the contribution of the variables to the PC1 and PC2.

Conclusions

In this study, the repeatabilities of growth characteristics and wood properties were clarified for 11 open-pollinated resistant families of *P. densiflora*. The mean stem diameter, tree height, oven-dry density, latewood tracheid length, MFA, MOE, and MOR for all the trees were 18.7 cm, 12.4 m, 0.37 g/cm³, 1.91 mm, 25.2°, 5.50 GPa, and 49.2 MPa, respectively. Relatively higher repeatabilities were obtained for stem diameter ($R = 0.28$), oven-dry density ($R = 0.47$), latewood tracheid length ($R = 0.21$), and MOR ($R = 0.33$). Although no significant correlations were found between growth characteristics and wood properties, high positive correlations were obtained between MOE and MOR, and between oven-dry density and MOE or MOR. The 11 families examined were classified into three groups by PCA and cluster analysis. On the basis of the results, we conclude that *P. densiflora* trees for the breeding program can be simultaneously selected on the basis of three favorable characteristics for structural lumber production, namely higher resistance to pine wilt disease, good growth characteristics, and good wood quality.

Table 4

Loading values from the principal component analysis for the 11 open-pollinated resistant families

Variables	PC1	PC2
Stem diameter	0.136	0.468
Oven-dry density	-0.588	0.315
Modulus of rupture	-0.431	0.582
Resistance ranking	0.513	0.305
Survival ratio	0.432	0.501

Note: PC, principal component.

Figure 5
Dendrogram based on the principal component scores.

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