J Wood Sci (2002) 48:106–113

ORIGINAL ARTICLE

Katsuhiko Takata · Yukio Teraoka

Genotypic effects on the variation of wood quality and growth traits in plantation forest made by cutting cultivars of Japanese cedar

Received: July 25, 2000 / Accepted: May 16, 2001

Abstract We studied the effect of genotypes of planting stocks regarding the variation of the modulus of elasticity of tree trunks on standing trees (trunk-MOE), tree height (TH), and diameter at breast height (DBH) in a 19-year-old Japanese cedar plantation made with root cuttings. Trunk-MOE was assessed nondestructively using a tree-bending method. Genotypes of individual trees were detected using the random amplified polymorphic DNA (RAPD) technique. RAPD analysis revealed that the sampled plantation consisted of 14 genotypes. Genotypic effects on DBH and TH were unclear, and there was no significant difference among genotypes. This result indicated that an acquired variation should have more influence than an inherited variation on DBH and TH. For trunk-MOE, there were significant differences among the four largest genotypes at the 5% level. However the coefficient of variation in trunk-MOE of each genotype ranged from 7.5% to 26.8%. It seems reasonable to assume that the wide variation in trunk-MOE in a sampled plantation may depend on the environmental effect within a clone as well as on the genetic origin of clones. We therefore conclude that the use of multiple planting stocks from different cuttings for which the wood quality is unknown contributed strongly to the wide variation in trunk-MOE in the plantation of Japanese cedar.

Key words Nondestructive test · Genotypic effect · Trunk-MOE · RAPD · Japanese cedar

K. Takata[1] (✉)
Department of Forest Biosciences, Division of Forest and Forest Products Sciences, Faculty of Agriculture, Graduate School, Kyushu University, Fukuoka 812-8581, Japan

Y. Teraoka
Department of Environmental Sciences and Technology, Faculty of Agriculture, Kagoshima University, Kagoshima 890-0065, Japan

Present address:
[1]Institute of Wood Technology, Akita Prefectural University, 11-1 Kaieizaka, Noshiro 016-0876, Japan
Tel. +81-185-52-6987; Fax +81-185-52-6975
e-mail: katsu@iwt.akita-pu.ac.jp

Introduction

Cryptomeria japonica D. Don (Japanese cedar), one of the most important conifers for timber production in Japan, has been planted widely in mountainous areas. Especially in the Kyushu region, many cutting cultivars of this species have been developed by private foresters and utilized for reforestation.[1] Because of the huge amount of wood stock in plantation forests and its favorable wood properties, interest in its management and utilization is at an all-time high in Japan.

To have a clear understanding of the wood characteristics of cutting cultivars, many studies have been carried out on the variation of wood properties[2–7] and wood anatomical features.[8–10] These studies have demonstrated that most of the fundamental wood characteristics differ significantly among cutting cultivars and suggested that these differences are derived mainly from differences in the genetic makeup among and within each cultivar. Despite the continuously increasing number of studies on the variation of wood characteristics, relatively little is known about the genetic variation among and within them or about the genotypic effects on the variation of wood quality and growth traits.

Trunk modulus of elasticity (trunk-MOE) indicates the stiffness of the tree trunk and is an important variable for predicting the wood quality of green log specimens and evaluating timber strength.[11] Consequently, it has been widely studied, especially in relation to radial growth rate. Several results, sometimes contradictory, have been reported on the relation between the two variables. Koizumi et al.[12] found no correlation between diameter at breast height (DBH) and trunk-MOE of plus-tree clones of Japanese larch, so parent trees with suitable performance for growth and mechanical properties can be selected for propagation. In contrast, negative and weak correlations between DBH and trunk-MOE were reported for an 18-year-old progeny test of Japanese larch.[13] Takata et al.,[14] also working on Japanese larch, explained the different results for the correlation of the two variables in several prov-

enance trial stands by the environmental effects and genotype–environment interaction. Furthermore, Takata[15] found a moderate negative correlation between DBH and trunk-MOE in spruce [*Picea glehnii* (Fr. Schm.)] and fir [*Abies sachalinensis* (Fr. Schm.) Mast.] in several plantations in Hokkaido.

Recent progress in molecular methods based on the polymerase chain reaction (PCR) has provided new types of DNA markers that are easier to produce and analyze than other genetic markers. The random amplified polymorphic DNA (RAPD) technique[16,17] is one novel DNA-marker system. The RAPD technique is thought to have a number of advantages over other DNA-based marker systems: The technique produces abundant loci; it is relatively inexpensive; and the technology is readily accessible to nonspecialists.[18] Although the markers are dominant and there have been doubts about their reliability and reproducibility,[19,20] this technique and its modifications have become an increasingly common tool in genetic studies, especially for identifying and discriminating plant cultivars and clones.[21–28] These studies have shown that this technique can provide reproducible and unique RAPD profiles for various genotypes. The primary objective of the present study was to investigate nondestructively the variation of trunk-MOE as a wood quality trait and two growth traits of Japanese cedar plantation forest prepared with several unknown root cuttings. The study focused on the following three issues: (1) the relations between wood quality and growth traits in an actual plantation forest; (2) the genetic makeup of planting stocks for the sampled stand; and (3) the genotypic effect on the variation of growth traits and wood quality traits.

Materials and methods

Plant materials

The study was carried out at a plantation forest prepared with several cutting cultivars of Japanese cedar in 1976. The stand, located in Shiiba village in Miyazaki prefecture, is an experimental forest of Kyushu University. Two experimental plots, I and II, were established within the stand. Plot I was located on a hill slope site, and plot II was settled on a gentle ridge at a higher elevation than plot I. Two growth traits, tree height (TH) and the DBH, were measured for all individuals within the plots. The MOE of the tree trunk of standing trees was evaluated nondestructively only for healthy individuals in the plots.

From 1995 to 1999, three phenotypic traits were investigated continually to check the reproducibility of and annual change in these three traits. In November 1995 trunk-MOE_{1995}, DBH_{1995}, and TH_{1995} were evaluated, and in May 1997 DBH_{1997} and TH_{1997} were measured. In November 1999, DBH_{1999} and TH_{1999} for all trees and the trunk-MOE_{1999} for the selected 35 healthy individuals were remeasured. Genotypes of individual trees were detected using RAPD markers. The details of study plots and number of sample materials are described in Table 1.

Table 1. Outline of the sample stand

Plot no.	Area (ha)	Exposure of slope	Slope angle	No. of trees
I	0.02	North	20°	37 (30)
II	0.04	North	5°	71 (41)

Numbers in parentheses are the number of trees measured for trunk-MOE

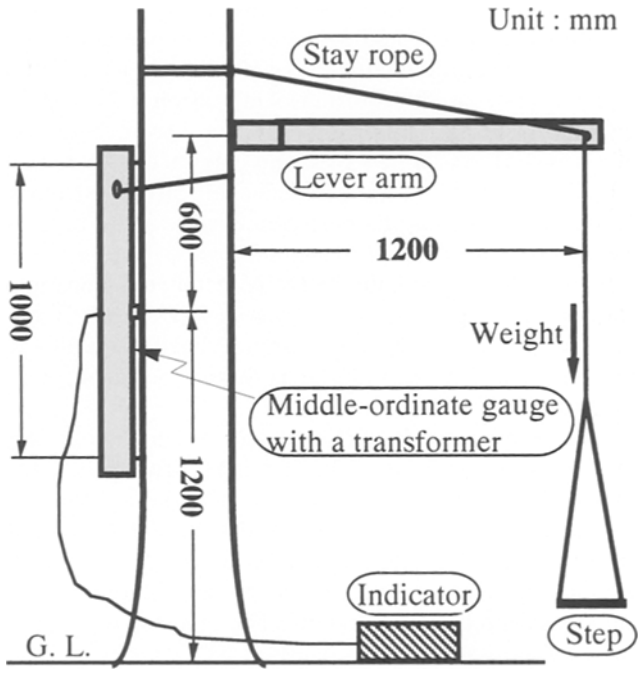

Fig. 1. Setup of the tree bending test

Evaluation of Trunk-MOE, DBH, and TH

A tree-bending test developed by Koizumi and Ueda[29] was applied to the evaluation of trunk-MOE. The setup of the tree-bending test is shown in Fig. 1. When an operator stood on a step on the end of a lever arm, his or her weight was converted to a bending moment acting on a stem. The deflection caused by the bending moment was measured by the middle-ordinate gauge with a transformer of 1-μm sensitivity, which was placed on the opposite side of the stem at breast height. This nondestructive measurement was made twice per tree in two directions at right angles to each other. The obtained values were averaged to compensate for the error caused by the uneven shape of the cross section. The trunk-MOE was calculated based on the applied moment and the moment inertia of the trunk determined from the girth and bark thickness at breast height. Theoretical and experimental studies indicated that the error in the trunk-MOE evaluation caused by the irregular form of the stem and other factors is negligible.[11,15]

The DBH was calculated from the girth of the tree trunk at breast height with bark, which was obtained in evaluating the trunk-MOE. TH was measured visually with a measuring pole.

DNA Extraction and Amplification

For the RAPD analysis, total DNA was extracted from about 150 mg of fresh needles. A modified version of the DNA extraction procedure of the cetyltrimethyl-ammonium bromide (CTAB) method[30] was used.[31] Fresh needles were ground to a fine powder with a pestle in liquid N_2 and were completely homogenized with 1.0 ml extraction buffer [100 mM Tris-HCl, pH 9.0, 2% CTAB, 2% polyvinyl pyrrolidone (PVP), 0.1% β-mercaptoethanol, 1.4 M NaCl, 20 mM EDTA]. The homogenate was incubated for 60 min at 65°C with gentle mixing every 10 min, extracted with an equal volume of chloroform/isoamyl alcohol 24:1 (CIA), and centrifuged at 12000 rpm for 10 min. The aqueous phase was transferred to a new tube and extracted with an equal volume of CIA and centrifuged again at 12000 rpm for 10 min. The aqueous phase was removed to a new tube, and an equal volume of isopropanol and 20 μl of sodium acetate were added to precipitate the DNA. After centrifugation at 15000 rpm for 5 min, the pellet was washed with 70% ethanol and dried for 3 min under vacuum. The DNA pellet was suspended in 300 μl of sterile H_2O and stored further at −20°C until RAPD analysis.

The reaction for RAPD analysis was carried out under the following conditions: 50 mM Tris-HCl (pH 8.5), 5.0 mM $MgCl_2$, 500 μg/ml BSA, 0.5 mM each dNTP, 2.0% Ficoll, 4.0 mM tartrazine, 0.1 mM EDTA, 0.4 unit *T-th* DNA polymerase, 0.25 μM primer, and 10 ng total DNA for a total volume of 10 μl. Air Thermo-Cycler 1605 (Idaho Technology) was conditioned as follows: The first step was 1 min at 93°C followed by 60 cycles of 10 s at 93°C, 30 s at 36°C, and 1 min at 72°C. The final step was 2 min at 72°C.

The amplification products were electrophoresed in 1.2% agarose gels in 0.5 × TBE buffer with a DNA standard (100 basepairs ladder, GIBCO-BRL and New England BioLabs). Fragments were visualized under ultraviolet (UV) transillumination after staining with ethidium bromide. The reproducibility of the amplification products was tested at least three times for each sample and primer.

Data scoring and analysis for genotype identification

The arbitrary primer kits OPA to OPY (Operon Technologies, Alameda, CA, USA) were purchased and an additional 10 primers (FB-1 to FB-10) were synthesized in the Laboratory of Forest Botany, Department of Forest Products, Faculty of Agriculture, Kyushu University. Based on the results of screening of a part of these primers (data not shown), four primers were selected for preclassification. The sequences of the primers were 5′-GTGACGTAGG-3′ (OPA-08), 5′-TTTGCCCGGA-3′ (OPB-16), 5′-TGTCTGGGTG-3′ (OPC-10), and 5′-ACGTAGCGTC-3′ (FB-07). The seven loci derived from four primers, called by the primer used and the molecular weight in basepairs, were scored as band presence (1) or absence (0) for all individuals.

After all individuals were divided into groups according to their band profiles at the seven loci, DNA fingerprinting analysis was carried out for all groups to check the genomic identity of the individuals included in the same group. Six primers were selected for DNA fingerprinting analysis based on the rich polymorphism and good reproducibility. The sequences of the primers used for fingerprinting analysis were 5′-GTGACGTAGG-3′ (OPA-08) 5′-CAATCGCCGT-3′ (OPA-11), 5′-GTCCGTACTG-3′ (OPN-19), 5′-CCTCCAGTGT-3′ (OPO-08), 5′-CACCCCCTTG-3′ (OPS-04), and 5′-CCACCGCCAG-3′ (FB-01). For this fingerprinting analysis, all loci produced through the same PCR procedure and visualized on the same gel were used for genotype identification.

Results

Variation of trunk-MOE and growth traits

Mean values, standard deviations (SD), and coefficients of variation (CV) for three traits are shown in Table 2. The mean value of trunk-MOE_{1995} at age 19 reached 5.42 GPa (range 3.73–6.96 GPa). The mean trunk MOE_{1999} was 5.39 GPa and had almost the same range of variation as those for the trunk-MOE_{1995}. The DBH averaged 16.0 cm in

Table 2. Trunk-MOE, DBH, and tree height

Trait	Mean	SD	CV (%)
Trunk-MOE (GPa)			
1995			
Total	5.42	0.78	14.5
Plot I	5.45	0.73	13.4
Plot II	5.39	0.83	15.3
1999			
Total	5.39	0.88	16.4
Plot I	5.66	0.96	17.1
Plot II	5.25	0.82	15.7
DBH (cm)			
1995			
Total	16.0	3.2	20.6
Plot I	17.4	2.3	13.3
Plot II	15.3	3.4	22.4
1997			
Total	16.8	3.4	23.2
Plot I	18.2	2.4	13.3
Plot II	16.1	3.5	22.1
1999			
Total	17.9	3.6	20.1
Plot I	19.4	2.7	13.7
Plot II	17.1	3.8	22.0
Tree height (m)			
1995			
Total	8.7	1.4	15.6
Plot I	9.9	0.9	9.4
Plot II	8.2	1.2	14.4
1997			
Total	9.2	1.4	15.2
Plot I	10.3	0.9	9.1
Plot II	8.6	1.2	13.9
1999			
Total	11.0	1.7	15.3
Plot I	12.4	1.3	10.3
Plot II	10.3	1.4	13.3

SD, standard deviation; CV, coefficient of variation; MOE, modulus of elasticity; DBH, diameter at breast height

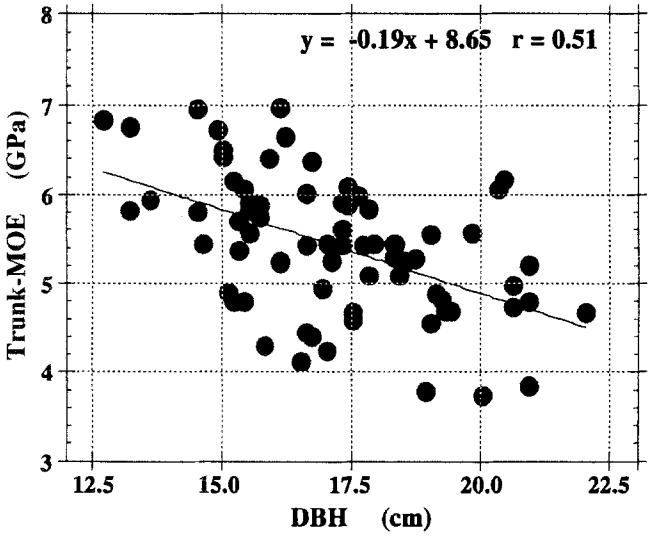

Fig. 2. Relation between diameter at breast height (*DBH*) and trunk modulus of elasticity (MOE)

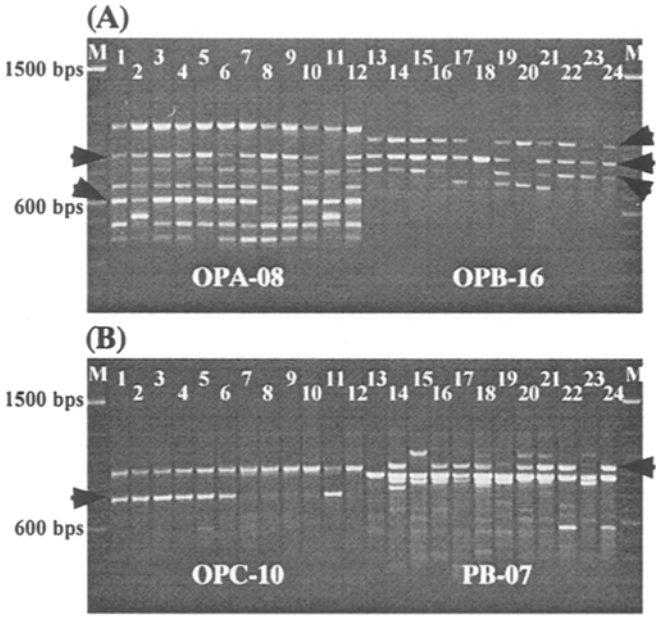

Fig. 3. Seven specific fragments utilized to identify DNA types. In each panel the first and last lanes (*M*) are molecular weight markers (100 base ladders, GIBCO-BRL). *Arrows* indicate the seven specific polymorphic fragments. **A** Five fragments: A08-610 and A08-920 with OPA-08 (lanes 1–12) and B16-810, B16-910, and B16-1100 with OPB-16 (lanes 13–24). **B** Two fragments, C10-800 with OPC-10 (lanes 1–12) and PB07-1090 with PB-07 (lanes 13–24)

1995, 16.8 cm in 1997, and 17.9 cm in 1999, with ranges of 6.1–22.0 cm, 6.5–23.2 cm, and 6.8–24.8 cm, respectively. The mean values for TH_{1995}, TH_{1997}, and TH_{1999} were 8.7, 9.1, and 10.9 m, respectively. The CV for each trait was rather large: for trunk-MOE and TH 15% and for DBH 20%. The SD and CV values in plot II were considerably larger than those in plot I for DBH and TH. A comparison of the data of the two plots shows significant differences for DBH and TH ($P < 0.01$) but no significant difference for trunk-MOE.

A classical relation between wood property trait and radial growth trait was found at the individual level: trunk-MOE was negatively correlated with DBH (Fig. 2). Trunk-MOE dropped from 6.96 to 3.73 GPa as the DBH increased from 12.7 to 22.0 cm. The range of trunk-MOE at the same DBH, however, was wide: At about 16.0 cm DBH the trunk-MOE varied from 4.0 to 7.0 GPa.

The relation between the MOEs measured in 1995 and 1999 was highly significant ($r^2 = 0.71$) at $P < 0.01$. This result agrees with the positive and significant correlation between trunk-MOEs measured at different ages by Koizumi[11] and Takata[15] in a forest prepared with seedlings of Japanese larch [*Larix kaempferi* (Lamb.) Sarg.]. These results also indicate that trunk-MOE is highly reproducible and is a reliable index for evaluating wood quality of conifer species when they reach maturity.

For two growth traits, the correlation between the value in 1995 and the values in 1997 and 1999 show apparently statistical significance. No apparent correlation between TH and trunk-MOE was found, nor was there a significant correlation between TH and DBH.

Genetic makeup of planting stock in a sample stand

The seven loci derived from four primers are shown in Fig. 3. These loci, which were reproducible and clearly distinguishable, were effective for classifying all samples. After classification based on the band profiles at the seven loci, DNA fingerprinting analysis was carried out for all groups to check the genomic identity of the individuals included in the group. An example of the fingerprinting profiles generated by the primer of OPS-14 for 15 individuals classified into genotype C is presented in Fig. 4. Every individual shared the same RAPD band profile. RAPD analysis revealed that the sample plantation forest consisted of at least 14 genotypes based on the presence or absence of seven loci. In Table 3 these 14 genotypes are denoted A through N, in order of population size. The biggest population was genotype A with 31 individuals; six genotypes (I, J, K, L, M, N) consisted of one individual. In the Kyushu region, we have surveyed the genetic makeup and checked the representative genotypes for 84 cutting cultivars using RAPD markers.[24] Fingerprinting analysis for all individuals with the representative genotypes of 84 cutting cultivars revealed that two genotypes detected in the present study were historical, famous cutting cultivars called "Tanoaka" and "Obiaka", which have been utilized in the Kyushu region for a long time (data not shown).

The mean values and CVs of the three traits for six genotypes are shown in Table 4. Three major genotypes, (A, B, C) accounted for about 70% of all sampled trees. The CVs for DBH of genotypes B, D, and F were relatively smaller than the others. Compared with other genotypes, genotype D showed good performance in radial growth. The mean values for TH among six genotypes were generally similar. Genotype E had a wide variation in TH and DBH. As for trunk-MOE, genotype D had a rather low

value compared to those of other genotypes. Four genotypes, (A, B, C, D) had a smaller CV values than the CV calculated for all samples. On the other hand, the CVs of genotypes E and F were somewhat larger. The CV of genotype E, in particular, was about twice as large as that of the overall samples.

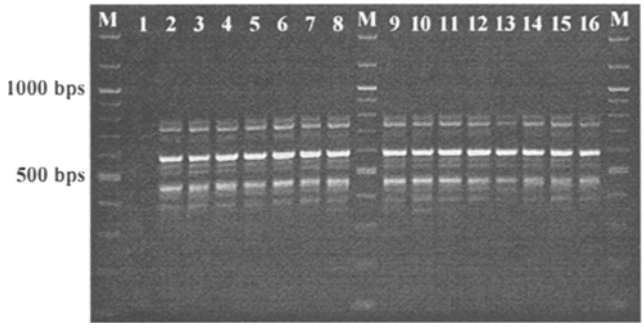

Fig. 4. Fingerprinting analysis for 15 samples of genotype C. Lane 1, negative control; lanes 2–16, individuals classified into genotype C. The first and last lanes (*M*) are molecular weight markers (100 base ladders, New England BioLabs). Primer is OPS-04 (5′-CACCCCCTTG-3′)

Discussion

Relation between radial growth and trunk-MOE

The relation between radial growth and MOE is an important factor to consider for the wood quality of Japanese cedar. In the present study, the correlation between DBH and trunk-MOE was negative and weak (Fig. 2). Judgments about the correlation between radial growth and MOE have differed, however, according to species,[15,32] origin of the seedlings of the forests tested,[33] and site conditions.[32] At the same time, the magnitude of the MOE value in *Larix* spp. was well explained by the variation in ring density and latewood percentage, which varied over a wide range with a change in ring width.[34] Therefore, to understand the relation between DBH and trunk-MOE in Japanese cedar it may be useful to look more closely at some of the more important features of the relation between ring width and wood density and the latewood percentage in Japanese cedar.

In general, Japanese cedar is known to exhibit a gradual transition in wood density between earlywood and latewood similar to that seen in fir (*Abies* spp.) or spruce (*Picea*

Table 3. Band profiles of 14 genotypes

Genotype	No. of trees	Fragment pattern						
		OPA08-610	OPA08-920	OPB16-810	OPB16-910	OPB16-1100	OPC10-800	PB07-1090
A	31 (12, 19)	1	1	1	1	1	1	1
B	26 (10, 16)	1	1	1	1	1	0	1
C	15 (6, 9)	0	1	1	1	1	0	1
D	9 (5, 4)	0	1	1	0	1	1	1
E	8 (3, 5)	1	1	1	1	0	1	1
F	6 (0, 6)	0	1	1	1	0	1	1
G	3 (0, 3)	0	1	1	0	0	0	1
H	3 (0, 3)	0	1	1	0	1	0	1
I	1 (0, 1)	0	1	0	1	1	0	1
J	1 (0, 1)	0	1	1	1	1	1	1
K	1 (0, 1)	1	0	1	0	0	1	1
L	1 (0, 1)	1	1	0	1	1	1	1
M	1 (0, 1)	1	1	1	1	0	0	1
N	1 (0, 1)	1	1	1	1	1	0	1

Numbers in parentheses are the number of trees in plots A and B, respectively

Table 4. DBH, tree height, and trunk-MOE for six major genotypes

Genotype	No. of trees	DBH_{1999} (cm)		TH_{1999} (m)		$Trunk\text{-}MOE_{1995}$ (GPa)	
		Mean	CV (%)	Mean	CV (%)	Mean	CV (%)
A	31 (16)	17.4	23.1	10.9	15.8	5.53	12.2
B	26 (20)	18.5	14.4	11.4	13.4	5.38	11.2
C	15 (11)	17.5	20.7	11.3	14.3	5.68	11.0
D	9 (7)	20.2	16.3	11.5	17.5	4.81	7.5
E	8 (6)	17.6	27.4	10.8	22.8	5.69	26.8
F	6 (4)	17.8	13.8	10.7	7.3	5.39	18.8
Overall	108 (71)	17.9	20.1	11.0	15.3	5.42	14.5

TH, tree height
Numbers in parentheses are the samples for measurements of trunk MOE

spp.).[35] There is fairly general agreement about the relations between latewood percentage and ring width, and wood density: The latewood percentage decreases with increasing ring width,[3,4,36,37] and wood density increases with an increasing latewood percentage.[3–6] Hirakawa and Fujisawa[6] explained these phenomena as follows: Many trees with low density and a good radial growth rate have wide rings and tend to have a smaller latewood percentage than those with high density. In contrast to these relations, the relation between ring width and wood density is not clear. Fukazawa[38] reported that the relation between these traits was in inverse proportion, although exceptionally there were some cultivars with a proportional relation. Several researchers[3,4] have presented a similar view. Oda et al.[3] pointed out that ring width basically had an effect on wood density through the latewood percentage, although it is necessary to pay attention to the exceptional cultivars with no effect of wing width on wood density. These results indicate that the genetic origin of sample trees would be an important key to a clear understanding of the effect of ring width on wood density.

Hirakawa and Fujisawa[6] summarized the relation between DBH and MOE from genotypes of trees regarding this point of view. That is, in the case of using monoclones MOE generally decreases with increasing DBH, as the wood density decreases with increasing radial growth. In contrast, in the case of testing multiclones, MOE and DBH are characteristically independent of each other, as the microfibril angle of the S_2 layer and wood density affects the MOE value of each clone. In, previous reports using multiclones of plus tree, Fujisawa et al.[33] showed that there was no significant correlation between MOE and DBH for the total clones, which is explained by the difference in the regression coefficient between DBH and MOE per clone. Recently, several reports showed that the variation in MOE for logs among cutting cultivars was explained well by the characteristics of the microfibril angle of the S_2 layer in each cultivar.[6,39] This concept seems to have considerable validity, although it is based on the assumption that the microfibril angle of the S_2 layer is controlled genetically per clone. Regression coefficients for DBH and trunk-MOE of six genotypes detected in this study are shown in Fig. 5. Here the length of the regression lines indicates the range for each trait. Roughly, the six genotypes can be divided into two types according to the regression coefficient: one with a gentle regression and another with a rather steep regression. Thus the negative and weak correlations between DBH and trunk-MOE observed in this study can be explained as the result of the gathering of individuals from several genotypes that have different regression coefficients.

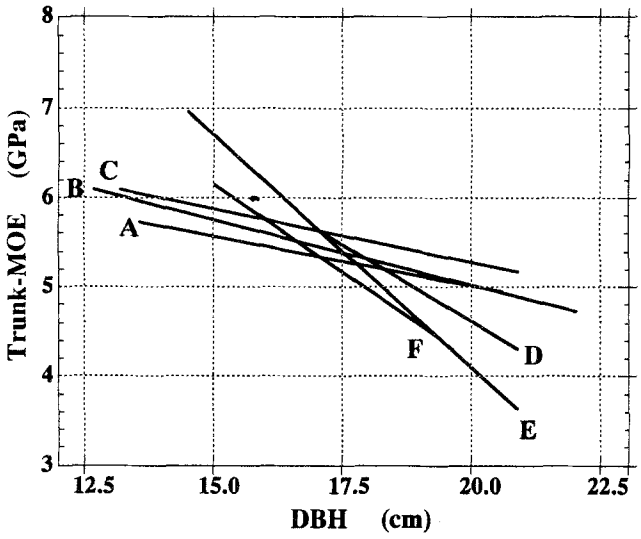

Fig. 5. Difference in regression coefficients between DBH and trunk-MOE for six clones

Genotypic effects on the variation of DBH, TH, and trunk-MOE

Using a DNA molecular marker, we showed that the sample plantation forest consisted of at least 14 genotypes. We also showed that there were three major genotypes that accounted for about 70% of all sampled trees (Table 3). The variation in trunk-MOE, DBH, and TH within a sample plot, however, was notably wide (Table 2). Furthermore, trunk-MOE dropped from 6.96 GPa to 3.73 GPa as the DBH increased, and the range was wide at the same DBH (Fig. 2). We assume that there were two causes, with the interaction between them accounting for the wide variation in the three investigated traits. The first was the genotypic effect, which was caused by the difference in the genetic origin of the planting stocks. The second was the environmental effect, which was the difference in site conditions (topology at the growing place) and the difference of microenvironment (suppression and competition among adjacent individuals). We discuss the variation in the three traits in detail using the genotypic effect and two types of environmental effect mentioned above.

The mean DBH varied from 17.4 to 20.2 cm, and the variations among the data within the same genotype were rather wide except for genotypes B and F. There was no significant difference among the four genotypes (A–D) or among six genotypes (A–F), and we cannot find a clear genotypic effect on DBH (Table 5). In earlier work based on 12 clones from progeny tests of plus tree of Japanese cedar, Fujisawa at al.[33] documented that the CV of the clone average for DBH was in the range of 12.5%–15.0% in progeny trial stands. The CV within the genotype in the present study is about 2.0 times as large as the CV in their data. Fujisawa et al.[33] studied samples planted in a randomized complete block design, replicated three times, with 50 trees per plot at three locations; collected only three trees with an average diameter per plot for research. In our study, all healthy trees were sampled for measuring the DBH, so the difference in CVs for DBH between the two studies might be due to the different sampling methods. Furthermore, trees of each genotype were planted at random in the forest. Considering these aspects, the wide variation in DBH within a genotype observed in this study may be due to the

Table 5. Results of ANOVA on DBH, TH, and trunk-MOE

No. of genotypes	*F*	*P*
DBH		
4	2.01	0.1193
6	1.18	0.3280
Tree height		
4	0.64	0.5910
6	0.53	0.7547
Trunk-MOE		
4	3.33	0.0267
6	1.44	0.2248

site condition effect between plots A and B (Table 2) and to the microenvironmental effects between adjacent individuals. It seems reasonable to suggest that unclear differences in DBH among genotypes may be mainly due to these two environmental effects.

The genotypic effect on TH was also unclear, and there was no significant difference among genotypes (Table 5). Wide variation was observed within a genotype except for genotype F, and a small difference was shown between genotypes for the variation in TH (Table 4). For Japanese cedar, Koizumi et al.[32] suggested that the influence of local site conditions (e.g., wind and soil) would be greater on growth trials than on wood quality traits. As noted above, plot II was located at a windier site than plot I. Thus, it seems reasonable to assume that the difference in the local site conditions (e.g., the wind condition) could cause the observed difference in mean TH. These results suggest that an acquired variation is more influential than an inherited variation on growth traits.

There were significant differences in trunk-MOE (*F* value 3.33) among the four genotypes at the 5% level; there were no significant differences among the six genotypes (Table 5). Fujisawa et al.,[33] working on several progeny trials with plus trees of Japanese cedar, reported that the dynamic-MOE of green logs had a small variation within a clone and a greater broad-sense heritability (0.597–0.857). The CV of the clone average ranged from 7.5% to 10.0%. For plus trees of Japanese larch, a narrow variation in trunk-MOE within the same clone was also observed at several progeny trial forests, and the CV of the clone average for trunk-MOE was about 7.0%.[12,13] The CV of the genotype average for trunk-MOE in this study ranged from 7.5% to 26.8% (genotypes A–F), and these values are relatively larger than those of earlier research on plus trees. In the earlier studies on plus trees, samples were selected by the diameter of the tree trunk, and the trees with an average diameter per plot or per plus tree were used. We presume that the intention of these sampling methods was to reduce the size effect caused by the difference in radial growth rate on the measurement of MOE. In this study, the variation in DBH of the trees for measuring trunk-MOE was wide (Fig. 2) because we tried to check the trunk-MOE of all healthy trees in the sample plots to study variation in a plantation forest. As mentioned, the MOE decreases with increasing DBH within a clone. Hence the variation of trunk-MOE in a sampled stand can be divided into two parts: one due to the genetic origin of clones and another due to the radial size effect on each tree within a clone after planting. In other words, there is an inherited variation and an acquired one, and we can recognize the wider variation in an actual plantation than in a progeny trial stand because of the acquired variation.

Fujisawa et al.[33] pointed out that if a plantation forest were composed of several genotypes the variation in MOE and DBH within the plantation would vary widely. In this study, we present for the first time an actual variation of trunk-MOE in the plantation forest that was dependent on the genotypes of the planted stocks. Our study goes a step further in showing practically why wide variation in wood quality occurs in a plantation forest. The results of the present study clearly warn against using multiple planting stocks from different cutting cultivars for which the wood quality is unknown. Our results also emphasize the importance of the genetic makeup of planting stocks for obtaining high-quality forest plantations.

We carried out the research at only one plantation forest in Miyazaki prefecture. It is thus important that future work include additional plantation forests in other prefectures which are planted with root cuttings of other cultivars. As demonstrated in this study using the tree-bending test and DNA molecular analysis, we can estimate the performance of a conifer plantation forest nondestructively. Continuing this nondestructive research on plantation forests could provide much information relevant to a better understanding of the genetic aspects of wood quality and growth traits of cutting cultivars of Japanese cedar.

Conclusions

Based on our analyses performed on a 19-year-old plantation forest composed of unknown cutting cultivars of Japanese cedar, the following can be concluded about the relations between DBH and trunk-MOE, and the genotypic effects on the variation of trunk-MOE, DBH, and TH.

1. There was a negative and weak correlation between DBH and trunk-MOE. This correlation would be based on the difference in the regression coefficient between DBH and trunk-MOE per clone in a plantation forest.
2. RAPD analysis revealed that the sampled plantation consisted of 14 genotypes. Genotypic effects on DBH and TH were unclear, and there was no significant difference among genotypes. This result indicated that an acquired variation should be stronger than an inherited variation on DBH and TH.
3. For trunk-MOE, there were significant differences among the four largest genotypes at the 5% level. However, the CV for trunk-MOE of each genotype ranged from 7.5% to 26.8%. It seems reasonable to assume that the wide variation in trunk-MOE at a sampled plantation may be divided into two parts: one due to the difference of genetic origin of clones and another due to the radial size effect on each tree in a clone.

Acknowledgments We thank the staff of Miyazaki Experimental Forest, Kyushu University Forests, for their help in the experiment. Our special thanks are due to Dr. Alfred E. Szmidt for reading the manuscript and making a number of helpful suggestions. We are grateful to the anonymous reviewers for their helpful comments on a previous version of the manuscript. Part of this study was supported by a grant-in-aid for Scientific Research from the Ministry of Education, Science, and Culture of Japan (no. 06556024).

References

1. Miyajima H (1989) *Cryptomeria japonica* and *Chamaecyparis obtusa* in Kyushu region (in Japanese). Kyushu University Press, Fukuoka, pp 34–56
2. Oda K, Koga S, Tsutsumi J (1988) Annual ring structure of Sugi (*Cryptomeria japonica*) cultivars for considering wood quality breeding (in Japanese). Bull Kyushu Univ For 58:109–122
3. Oda K, Hisada Y, Tsutsumi J (1989) Variation in wood properties within Sugi (*Cryptomeria japonica*) cultivars growing in the same stand (in Japanese). Bull Kyushu Univ For 60:69–81
4. Oda K, Watanabe E, Tsutsumi J (1990) Wood properties of Sugi (*Cryptomeria japonica*): An approach to the wood quality of twelve cultivars as structural members (in Japanese). Bull Kyushu Univ For 62:115–126
5. Koga S, Oda K, Tsutsumi J (1990) Variation in wood properties within Sugi (*Cryptomeria japonica* D. Don) cultivars (in Japanese). Bull Kyushu Univ For 62:101–113
6. Hirakawa Y, Fujisawa Y (1995) The relationships between microfibril angles of the S_2 layer and latewood tracheid lengths in elite sugi tree (*Cryptomeria japonica*) clones (in Japanese). Mokuzai Gakkaishi 41:123–131
7. Hirakawa Y, Yamashita K, Nakada R, Fujisawa Y (1997) The effects of S_2 microfibril angles of latewood tracheids and densities on modulus of elasticity variation of sugi tree (*Cryptomeria japonica*) logs (in Japanese). Mokuzai Gakkaishi 43:717–724
8. Fujisaki K (1985) On the relationship between the anatomical features and wood quality in the Sugi cultivars (I) (in Japanese). Bull Ehime Univ For 23:47–59
9. Fujisaki K, Shibuya M (1986) On the relationship between the anatomical features and wood quality in the Sugi cultivars (II) (in Japanese). Bull Ehime Univ For 24:61–70
10. Shibuya M, Fujisaki K (1987) Relationship between the anatomical features and wood quality in the Sugi cultivars (I) (in Japanese). Bull Ehime Univ For 25:149–158
11. Koizumi A (1987) Studies on the estimation of the mechanical properties of standing trees by non-destructive bending test (in Japanese). Res Bull Coll Exp For Hokkaido Univ 44:1329–1415
12. Koizumi A, Takata K, Ueda K, Katayose T (1990) Radial growth and wood quality of plus trees of Japanese larch. I. (in Japanese). Mokuzai Gakkaishi 36:98–102
13. Koizumi A, Takata K, Ueda K, Katayose T (1990) Radial growth and wood quality of plus trees of Japanese larch. II. (in Japanese). Mokuzai Gakkaishi 36:704–708
14. Takata K, Koizumi A, Ueda K (1992) Geographic variation in the moduli of elasticity of tree trunks among Japanese larch in provenance trial-stands (in Japanese). Mokuzai Gakkaishi 38:222–227
15. Takata K (1994) Estimation of wood quality of forest trees of Japanese larch by means of modulus of elasticity of tree trunk (in Japanese). Res Bull Coll Exp For Hokkaido Univ 51:115–166
16. Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18:6531–6535
17. Welsh J, McClelland M (1990) Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res 18:7213–7218
18. Rafalski JA, Tingey SV (1993) Genetic diagnostics in plant breeding: RAPDs, microsatellites and machines. Trends Genet 9:275–280
19. Weeden NF, Timmerman GM, Hemmat M, Kneen BE, Lodhi MA (1992) Inheritance and reliability of RAPD markers. In: Application of RAPD technology to plant breeding. Joint Plant Breeding Symposia Series, Minneapolis, pp 12–17
20. Penner GA, Bush A, Wise R, Kim W, Domier L, Kasha K, Laroche A, Scoles G, Molnar SJ, Fedak G (1993) Reproducibility of random amplified polymorphic DNA (RAPD) analysis among laboratories. PCR Methods Applications 2:341–345
21. Keil M, Griffin AR (1994) Use of random amplified DNA (RAPD) markers in the discrimination and verification of genotypes in *Eucalyptus*. Theoret Appl Genet 89:442–450
22. Van de ven WTG, McNicol RJ (1995) The use of RAPD markers for identification of Sitka spruce (*Picea sitchensis*) clone. Heredity 75:126–132
23. Schnell RJ, Ronning CM, Knight RJ Jr (1995) Identification of cultivars and validation of genetic relationship in *Mangifera indica* L. using RAPD markers. Theoret Appl Genet 90:269–274
24. Takata K, Shiraishi S (1996) Discrimination of Japanese cedar (*Cryptomeria japonica* D. Don) cutting cultivars in Kyushu region with RAPD markers (in Japanese). Bull Kyushu Univ For 75:1–14
25. Nesbitt KA, Potts BM, Vaillancourt RE, Reid JB (1997) Fingerprinting and pedigree analysis in *Eucalyptus globulus* using RAPDs. Silvae Genet 46:6–11
26. Goto S, Watanabe A, Ikeda K (1997) Use of RAPD markers for cultivar identification in *Rhus succedance* L (in Japanese). J Jpn For Soc 79:229–233
27. Goto S (1998) Genetic fingerprinting of nematode-resistant clones of Japanese black pine (*Pinus thunbergii* Parl.) using RAPD markers. J For Res 3:127–130
28. Goto S, Ieiri R, Miyahara F (1999) RAPD analysis of cutting cultivars and plus tree clones of sugi (*Cryptomeria japonica* D. Don) in Fukuoka Prefecture (in Japanese). J Jpn For Soc 81:187–193
29. Koizumi A, Ueda K (1986) Estimation of the mechanical properties of standing trees by non-destructive bending test (in Japanese). Mokuzai Gakkaishi 33:669–676
30. Murray MG, Thompson WF (1980) Rapid isolation of high molecular weight plant plant DNA. Nucleic Acids Res 8:6531–6535
31. Shiraishi S, Watanabe A (1995) Identification of chloroplast genome between *Pinus densiflora* Sieb. et Zucc. and *P. thunbergii* Parl. based on the polymorphism in *rbc*L gene (in Japanese). J Jpn For Soc 77:429–436
32. Koizumi A, Takata K, Ueda K (1989) The modulus of elasticity of tree trunks for plantation-grown conifers in Hiyama Experiment Forest (in Japanese). Res Bull Coll Exp For Hokkaido Univ 46:441–450
33. Fujisawa Y, Ohta S, Nishimura K, Tajima M (1992) Wood characteristics and genetic variations in Sugi (*Cryptomeria japonica*): clonal differences and correlations between locations of dynamic moduli of elasticity and diameter growths in plus-tree clones. Mokuzai Gakkaishi 38:638–644
34. Takata K, Hirakawa Y (1996) Variation of growth ring parameters among Japanese larch from different provenances. In: Donaldson LA, Singh AP, Bufferfield BG, Whitehouse LJ (eds) Recent advances in wood anatomy. New Zealand Forest Research Institute, Rotorua, 351–356
35. Core HA, Côté WA, Day AC (1979) Wood structure and identification. 2nd edition In: Côté WA (ed) Wood Science Series. Syracuse University Press, Syracuse, NY
36. Kano T (1960) On the wood quality of Sugi (report 1) Sugi (*Cryptomeria japonica* D. Don) grown at Kamabuchi district (in Japanese). Bull Gov For Exp Station 125:95–119
37. Kano T (1961) On the wood quality of Sugi (report 2) Sugi (*Cryptomeria japonica* D. Don) grown at Nishikawa district (in Japanese). Bull Gov For Exp Station 134:115–139
38. Fukazawa, K. (1967) The variation of wood quality within a tree of *Cryptomeria japonica* D. Don: characteristics of juvenile and adult wood resulting from various growth condition and genetic factors (in Japanese). Bull Gifu Univ For 25:47–127
39. Yamashita K, Hirakawa Y, Fujisawa Y, Nakada R (2000) Effect of microfibril angle and density on variation of modulu of elasticity of sugi tree (*Cryptomeria japonica*) logs among eighteen cultivars (in Japanese). Mokuzai Gakkaishi 46:510–522