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NOTE

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Clonal variation of carbon content in wood of *Larix kaempferi* (Japanese larch)

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Abstract Variations in carbon content in wood among 102 clones, selected from almost the entire natural distribution area, were investigated in *Larix kaempferi*. The average carbon content was 50.50%, 50.94%, and 50.80% in sapwood, heartwood, and whole wood, respectively. The difference in carbon content between clones was significant. The clonal repeatabilities were 0.46, 0.38, and 0.44 in heartwood, sapwood and whole wood, respectively. The coefficients of variation in the clonal mean carbon content were only 0.43%, 0.42%, and 0.41% in heartwood, sapwood, and whole wood, respectively. This small genetic variation and resulting small relative genetic gain of carbon content indicate that the genetic improvement of carbon content by selection has a small effect on the genetic improvement of carbon sequestration capacity by selection in *L. kaempferi*.

Key words Carbon content · *Larix* · Japanese larch · Clonal variation · Genetic variation

Introduction

Carbon content in wood (C-content) has been considered to be approximately 50% (w/w), irrespective of species, and this value has been used to estimate carbon storage in the wood part of trees and forests.[1,2] Recent reports,[3–8] however, indicate that there is a large variation in C-content between species. Elias and Potvin[3] measured the C-content in woods of 32 tropical broad-leaved trees and found C-content ranging from 44.4% to 49.4%. Lamlom and Savidge[4] analyzed the heartwood of 19 conifers and 22 broad-leaved trees. They reported that conifers (47.21%–55.20%) had a somewhat higher C-content than broad-leaved species (46.27%–49.97%). These reports suggest that variations in C-content between species should be taken into account for accurate evaluation of carbon sequestration in trees and forests.

Larix kaempferi (Lambert) Carrière (Japanese larch) is one of the most important tree species in Japan, covering 10.2% of the plantation forest area,[9] and the genus *Larix* is widely distributed across the cooler regions of Northern Hemisphere. C-contents of some species of *Larix* have already been reported: 47.21% in *Larix laricina* and 47.60% in *Larix occidentalis*.[4] It is important to determine the C-content in *L. kaempferi* to assess interspecific variations in the genus *Larix* and to more accurately estimate the amount of carbon storage in Japanese larch forests.

In many species, knowledge of the intraspecific genetic variations in C-content is very limited. Forests are expected to serve as a carbon sink to mitigate the increase in atmospheric carbon dioxide, a greenhouse gas contributing to global warming.[10] Hence, genetic improvement of the carbon sequestration capacity is an attractive objective in forest tree breeding. The carbon sequestration capacity of a tree depends on many traits, such as growth rate, wood density, and C-content. C-content will be more important for genetic improvement of the carbon sequestration capacity if there are large genetic variations in C-content and higher heritability in this characteristic. To determine whether C-content is an important selection trait for genetic improvement of the carbon sequestration capacity, genetic variations in C-content should be examined. Tamura et al.[7] reported that the clonal variation in C-content in *Cryptomeria japonica* (sugi) is very small (0.3% coefficient of variation). Fukatsu et al.[8] reported that C-content varies little between half-sib families (0.23% coefficient of variation) in *Chamaecyparis obtusa* (hinoki). To our knowledge, the intraspecific variations in other species, including *L. kaempferi*, have not yet been studied.

In the present study, variations in C-content were measured in 102 plus-tree clones of *L. kaempferi* to obtain fundamental information for both evaluating carbon storage in

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larch forests and for determining suitable traits for genetic improvement of the carbon sequestration capacity.

Materials and methods

Plant materials and sample preparation

Larix kaempferi clones planted in clonal archives at the Nagano Breeding Material Management Garden of the Forest Tree Breeding Center in Komoro, Nagano Pr efecture, Japan (36°21′ N, 138°25′ E), were used for C-content measurements. The clonal archives were established in 1960–1965 under a row plot design, in which there are ten ramets in each line without replication. The clones planted in the clonal archives were selected as plus trees from plantation and natural forests of *L. kaempferi* on Honshu Island for better growth and stem straightness in the 1950s and 1960s. We selected 102 clones, which covered almost all the natural distribution area of *L. kaempferi* from the clonal archives for this study (Fig. 1, adapted from Hayashi[11]). Sample trees were harvested from these 102 clones (one to three ramets per clone, 2.71 on average, 277 trees in total) in 1997. The average diameter at breast height (coefficient of variation) in the sample trees was 26.6 cm (19.9%). The discs obtained at breast height from each tree were cut into strips from bark to pith (radial direction) from two directions with a 5-mm thickness and a width (tangential direction) of 3–5 cm. The heartwood ratio in the radius was measured in each strip. The strips were divided into heartwood and sapwood. Each part was milled into powder with a sample mill (CSM-F1, Shizuoka Seiki, Shizuoka, Japan).

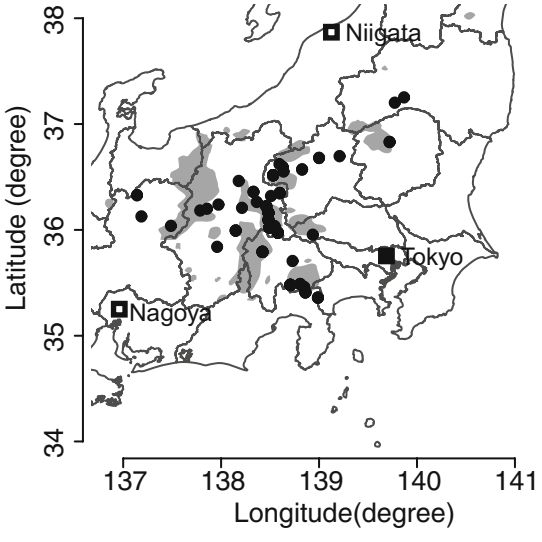

Fig. 1. The natural distribution region of *Larix kaempferi* and the locations where sample clones were selected. The *gray regions* are the natural distribution regions of *L. kaempferi* (from Hayashi[11]). *Filled circles* indicate the location where sample clones used in this research were selected. Clones selected from the same municipalities are depicted in the same filled circle

Measurement of C-content

Each sample powder (4–6 mg) was placed in a preweighed tin boat and oven-dried at 70°C for 72 h. Each tin boat was then cooled to room temperature in a small glass bottle half-filled with silica gel in a desiccator to prevent the reabsorption of moisture. The tin boats containing sample were sealed and then weighed with an ultra-micro electrical balance (MT5, Mettler-Toledo, Columbus, OH, USA) immediately after cooling. Net sample weights were then calculated by subtracting the tare weights. The C-content (w/w) of each sample powder was measured in a gas chromatographic elemental analyzer (Vario EL III, Elementar, Hanau, Germany). Calibration curves were based on acetanilide ($C_6H_5NHCOCH_3$) standards. Approximately 5 mg of acetanilide was measured after every 20 sample measurements to compensate for measurement error due to atmospheric pressure fluctuation. The measurement for each sample was replicated at least three times. Measurements were repeated until the standard deviation for the C-content in the replications was smaller than 0.3%. The mean of the replications for each sample was used for statistical analysis. The order of measurements for samples and replications was randomized to avoid systematic errors.

The C-content of whole wood was calculated for each tree as the weighted mean of the C-content of sapwood and heartwood. The proportions of heartwood area to sapwood area, which were calculated from the heartwood ratio in the radius, were used as the weighting factors.

Statistical analysis

One-way analysis of variance (ANOVA) was used to estimate the clonal effect on the variation in C-content. Repeatability was calculated as an indicator of heritability as follows:

$$R^2 = \sigma_c/(\sigma_c + \sigma_e) \quad (1)$$

where R is the repeatability, σ_c is the variance due to interclonal differences, and σ_e is the error variance. Each variance was derived from the result of the ANOVA. Because each clone was planted in only one line with several ramets, there was no environmental replication. Therefore, it was not possible to separate the variance due to genotype from variance due to that portion of environmental effects that might have varied orthogonal to row direction in the clonal archive. Estimates of clonal repeatability were thus based on the assumption of environmental uniformity at the site, at least in terms of environmental factors that might influence the C-content. The relative genetic gain in the genetic improvement by selection is calculated as follows:[12]

$$G_r = i \cdot R^2 \cdot \sigma_{Pr} \quad (2)$$

where G_r is the relative genetic gain based on the average value of preselected population, σ_{Pr} is the relative phenotypic standard deviation (coefficient of variation), i is the intensity of selection.

The significance of the differences between sapwood and heartwood was examined by the Wilcoxon matched-pairs

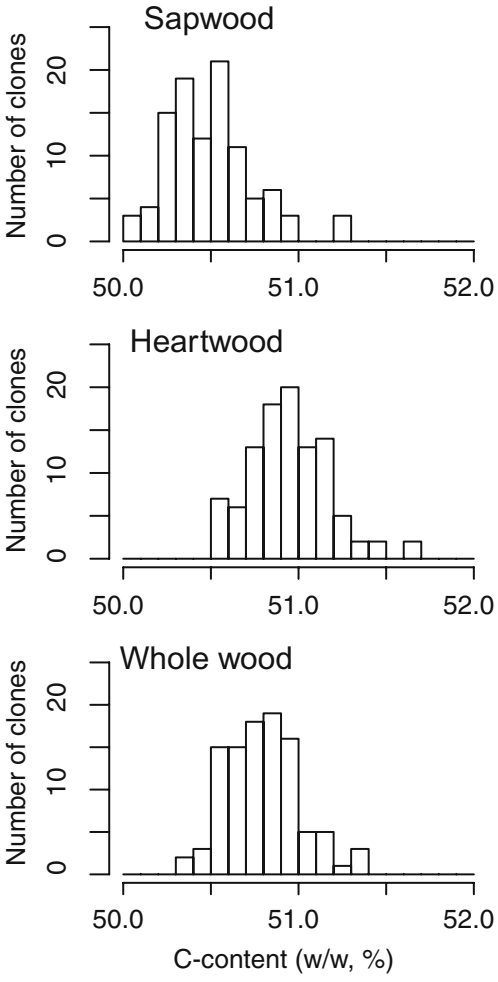

Fig. 2. Frequency distribution of carbon content (*C-content*) of sapwood (*top*), heartwood (*middle*), and whole wood (*bottom*). Clonal means were used. $n = 102$

signed-ranks test. The correlation in C-content between sapwood and heartwood was examined by Pearson's correlation coefficient. The normality of the C-content distribution was tested by the Kolmogorov-Smirnov test. All statistical analyses were performed with the statistical package *R*.[13]

Results

The clonal means of the C-content were distributed as shown in Fig. 2, and the average C-content for all clonal means was 50.50%, 50.94%, and 50.80% in sapwood, heartwood, and whole wood, respectively. Departures from the normal distribution in the clonal means of C-content were not significant ($P = 0.24$ in sapwood, $P = 0.69$ in heartwood, and $P = 0.96$ in whole wood), as indicated by Kolmogorov-Smirnov tests. The C-content differed significantly between sapwood and heartwood ($P < 0.001$, Wilcoxon matched-pairs signed-ranks test). In almost all clones, the C-content was higher in the heartwood than in the sapwood (Fig. 3). The coefficient of variation was 0.42% in both the sapwood

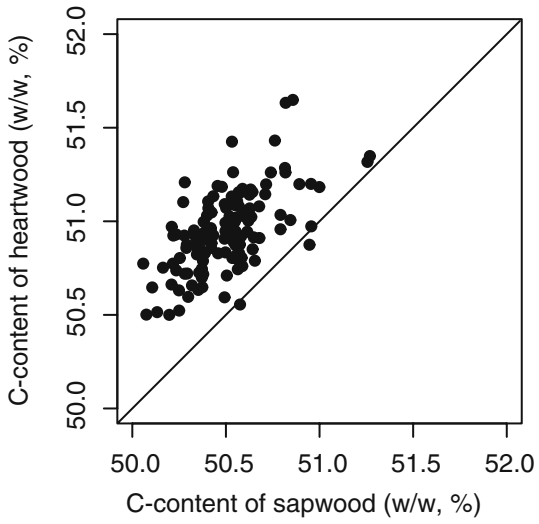

Fig. 3. Relation between C-content of sapwood and C-content of heartwood. All 102 clonal means are plotted. The *diagonal line* shows $x = y$

and heartwood. There was a significant correlation in the clonal mean C-content between sapwood and heartwood (Fig. 3, $r = 0.63$, $P < 0.001$).

One-way ANOVA revealed a significant difference ($P < 0.001$) in C-content between clones in both sapwood and heartwood. The clonal repeatability in C-content was higher in heartwood ($R^2 = 0.46$) than in sapwood ($R^2 = 0.38$) and in whole wood ($R^2 = 0.44$). The relative genetic gain of C-content was 0.16%, 0.19%, and 0.18% in sapwood, heartwood, and whole wood, respectively, when the intensity of selection is 1.0.

Discussion

The natural distribution of *Larix kaempferi* is limited to the central part of the mainland (Honshu) in Japan, except for the relict population in the northern part. The origin of the plus-tree clones sampled in this study covers almost the entire area of the *L. kaempferi* natural distribution (Fig. 1). The results of the present study are therefore representative of the genetic variations in C-content of *L. kaempferi*.

The average C-content in *L. kaempferi* was in the range reported for other coniferous species.[1,4,5,7,8] We summarize the average C-content and its variation in Table 1. The average C-content of the two other main plantation species in Japan, *Cryptomeria japonica* and *Chamaecyparis obtusa*, is 51.7%[7] and 51.6%,[8] respectively; *L. kaempferi* has a slightly lower C-content (50.80%). Knowledge of the C-content in wood is necessary for estimation of the carbon storage in woody biomass of forest stands, in addition to the stem volume and wood density.[1] The amount of stem volume of the major coniferous species in the forest of Japan is already estimated from the database of forest registers.[14] Information for the average wood density of each major species has also been compiled. There is no compiled information about the average C-content of the major plantation

Table 1. Carbon content and its variation in major coniferous species and in genus *Larix*

Tree species	Carbon content[a] (%)	Number of strains	Number of individuals	Reference
Major coniferous species				
Worldwide				
Abies amabilis	48.55 (NC)	–	1	4
Chamaecyparis nootkatenis	52.84 (NC)	–	1	4
Picea glauca	50.39 (NC)	–	1	4
Pinus strobus	49.74 (NC)	–	1	4
Japan				
Cryptomeria japonica	51.7 (0.3)	47[b]	336	7
Chamaecyparis obtusa	51.6 (0.23)	27[c]	162	8
Genus *Larix*				
Larix laricina	47.21 (NC)	–	1	4
Larix occidentalis	47.60 (NC)	–	1	4
Larix kaempferi	50.8 (0.41)	102[b]	277	This study

NC, not calculated
[a] Data given as mean with coefficient of variation in parentheses
[b] Number of clones
[c] Number of half sibs

species in Japan obtained from various genotypes. The results of the present study joined with former studies[5,7,8] provide C-content of major plantation species, occupying 79% of the plantation forest area,[9] and allow us to estimate the amount of carbon storage more reliably in a large part of the plantation forests in Japan. Lamlom and Savidge[6] reported that the C-content in *Larix laricina* and *Larix occidentalis* is 47.21% and 47.60%, respectively. These two *Larix* species have the lowest C-content of the 21 coniferous species that they analyzed.[6] The C-content in *L. kaempferi* in the present study is near the midpoint of their range. Our findings indicate that there is a relatively large intergeneric variation in C-content in the genus *Larix*.

In *L. kaempferi*, the C-content in heartwood was significantly higher than that in sapwood. Lamlom and Savidge[6] studied the radial variations in C-content in *Sequoiadendron giganteum* (giant sequoia) and reported that the C-content in heartwood has little radial variation but is higher in heartwood than in sapwood. Similarly, Tamura et al.[5] reported that the C-content in *C. japonica* is higher in the heartwood than in the sapwood. Our findings in *L. kaempferi* were consistent with these previous results. In addition, the difference in the C-content between heartwood and sapwood was determined by analyzing 102 clones, suggesting the generality of this finding through the various genotypes. The high C-content in heartwood is associated with the heartwood extractives.[5,6] The genus *Larix* has a high extractive content in the heartwood and the extractives affect several wood properties, such as wood density,[15] decay resistance,[16] and strength.[17] The high C-content in the heartwood in *L. kaempferi* might also be due to its relatively high extractive content.

The clonal variation in C-content in *L. kaempferi* was very small, similar to that reported for *C. japonica*[7] and *C. obtusa*[8] (Table 1), whereas the repeatability was relatively high. The carbon storage in the trunk of a tree is calculated as the product of the volume of the trunk, the average wood density, the C-content, and some constants. The relative genetic gain of C-content is directly proportional to the genetic gain of carbon storage in the trunk when the other two traits are not improved. The small relative genetic gain in C-content therefore means that the genetic improvement of C-content by selection has a fairly small effect in the improvement of carbon storage capacity. Nakada et al.[18] obtained coefficients of variation of 8% for wood density and 21% for diameter at breast height between clones in *L. kaempferi*. Because of the higher coefficients of variation, these other two traits would show higher relative genetic gain and have higher effect on the improvement of carbon storage capacity than the C-content accordingly. These findings suggest that a genetic improvement in C-content by selection is not efficient for the improvement of CO_2 sequestration capacity in *L. kaempferi*.

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