Ultrastructure and Mechanical Properties of *Populus* Wood with Reduced Lignin Content Caused by Transgenic Down-Regulation of Cinnamate 4-Hydroxylase

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Several key enzymes in lignin biosynthesis of *Populus* have been down-regulated by transgenic approaches to investigate their role in wood lignification and to explore their potential for lignin modification. Cinnamate 4-hydroxylase is an enzyme in the early phenylpropanoid pathway that has not yet been functionally analyzed in *Populus*. This study shows that down-regulation of cinnamate 4-hydroxylase reduced Klason lignin content by 30% with no significant change in syringyl to guaiacyl ratio. The lignin reduction resulted in ultrastructural differences of the wood and a 10% decrease in wood density. Mechanical properties investigated by tensile tests and dynamic mechanical analysis showed a decrease in stiffness, which could be explained by the lower density. The study demonstrates that a large modification in lignin content only has minor influences on tensile properties of wood in its axial direction and highlights the usefulness of wood modified beyond its natural variation by transgene technology in exploring the impact of wood biopolymer composition and ultrastructure on its material properties.

Introduction

Lignin is a major polymer in plant secondary cell walls and, hence, wood. An important role of lignin in the wood cell wall is to function as a cross-linking matrix between moisture sensitive cellulose and hemicelluloses, and thereby, lignin contributes to the mechanical rigidity.[1−5] However, the importance of lignin content and composition highly depends on how loads are applied (i.e., loading mode) in combination with the structural organization of the cell walls. In cell walls with a small cellulose microfibril angle (MFA), longitudinal loads are to a large extent governed by the axially oriented cellulose fibrils and lignin may therefore function mainly as a rigidifying component,[6] which prevents the cellulose fibrils from buckling under compressive loads. In cell walls with a high MFA, such as compression wood of conifers or certain sclerenchyma fibres in palm trees, the lignin contributes to the shear stiffness and strength of the matrix and thereby stiffens the wood also under tension in its longitudinal direction.[7−9] However, quantitative measurements of the specific influence of lignin content and composition, based on the natural variability of lignin in different tissue types, is difficult because, like in the case of compression wood, not only the lignin content but also density, MFA, and so on vary between the tissue types.[10] In fact, only a weak impact of lignin content on the compression strength was found in a comparative study of different wood tissues including normal and compression wood.[11]

Concerning wood pulping, lignin constitutes an obstacle for fiber liberation generally requiring large amounts of chemicals and/or energy. Therefore, the prospect of using transgene technology to reduce and/or modify lignin has raised great interest. *Populus* has served as a model for such approaches, where the activity of many, but not all, of the key enzymes in lignin biosynthesis have been decreased and the resulting effect on lignin chemistry characterized.[12,13] In some studies, it has also been demonstrated that lignin modification in *Populus* result in improved pulping properties (e.g., reduction in Kappa number).[14] Transgenic lignin modification may also affect mechanical properties of wood. However, only few studies have investigated the impact of transgenic modifications of lignin content and structure on the mechanical and ultrastructural properties of the wood. Yet transgenic wood offers experimental material valuable to elucidate and quantify the specific functions of lignin in cell wall mechanics, because this material can be manipulated in its cell wall composition much beyond the variation found in nature. Kasal et al.[15] performed mechanical tests on wood from transgenic quaking aspen with reduced lignin due to down-regulation of 4-coumarate-CoA ligase and increased syringyl (S) to guaiacyl (G) ratio through overexpression of coniferaldehyde 5-hydroxylase. They showed that the transgenic wood possessed inferior mechanical properties in axial compression compared to the wild-type (wt) control. However, no difference in axial tensile strength or transverse bending was found. Horvath[16] used the same genotypes and found that a reduction in lignin content or S to G ratio reduced the (static

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and dynamic) modulus of elasticity and that a combination of the two had the largest effect.

The mechanical properties of wood reside at least in part in the ultrastructure of the fiber cell wall.[5] Indeed, transgenic modification of lignin content and composition has been observed to affect the architecture of fiber walls in several species. In *Populus* down-regulated in cinnamoyl-CoA reductase (CCR) activity, fiber walls showed concentric sub layering and a sometimes disorganized architecture.[17] Similarly, in both *Arabidopsis* and tobacco limited in CCR activity a looser structure, disordered cellulose microfibril organization, as well as thinner cell walls were observed in fibers and vessels.[18,19] Also, down-regulation of caffeoyl-coenzyme A *O*-methyltransferase (CCoAOMT) activities in flax resulted in collapsed xylem cells with thinner walls.[20]

In this study the effect of transgenic down-regulation of cinnamate 4-hydroxylase (C4H; a key enzyme in early phenylpropanoid pathway) on the wood ultrastructure and mechanical behavior was studied. Fourier transform infrared spectroscopy (FTIR), wide-angle X-ray scattering (WAXS), and atomic force microscopy (AFM) were used for characterization of the wood samples with respect to micro- and nanostructure. Static mechanical tests were performed along with dynamic mechanical analysis (DMA) as a function of humidity and temperature under water-saturated conditions with the intention of revealing hemicellulose and lignin softening, respectively.[21]

Experimental Section

Plant Material. C4H antisense construct was generated by cloning a partial cDNA (A060P68U, http://www.populus.db.umu.se) in a gateway compatible antisense binary vector, pK7GWIWG2(I).[22] The construct was then transformed into hybrid aspen (clone T89; *Populus tremula x tremuloides*), as described previously.[23] Kanamycin-resistant lines were clonally propagated in vitro and planted in the greenhouse. Transgenic and wt plants were grown in a greenhouse under a photoperiod of 18 h with natural light supplemented with metal halogen lamps. The temperature was 22/15 °C (day/night), and the trees were watered daily and fertilized once a week with a nutrient solution (Superba, Yara AB). Trees were grown to a height of 1.5 m. A 20 cm long stem section was collected 10 cm above the soil and stored in −70 °C until used for chemical, structural, and mechanical analysis. Induced tension wood was collected from wt trees that were tilted 45° for 3 weeks.

The abundance of C4H transcript was determined in wt and transgenic trees. Wood forming tissues (xylem and phloem scrapings) were sampled as described in Gray-Mitsumune et al.[24] Total RNA was extracted from powdered tissue using RNeasy plant mini kit (Qiagen, Crawley, U.K.). First-strand cDNA synthesis was carried out using iScript first strand cDNA synthesis kit (Bio-Rad Laboratories, Sundbyberg, Sweden). C4H levels were measured by quantitative RT-PCR using IQ SYBR Green SuperMix (Bio-Rad) on an IQ iCycler machine under the following conditions: 40 cycles of 95 °C for 10 s, 55 °C for 30 s, and 72 °C for 30 s. Resultant PCR product was analyzed by melt curve analysis. The sequence of the primers used was C4H forward 5′-GATCTTGGTCAACGCCTGGTGG-3′, C4H reverse 5′-ATTGCCGTTGGCCTCGAACCT-3′. Expression values were normalized to those of 26rRNA gene. Two independent lines, 1B and 3B, showed down-regulation of C4H levels to 23.5 ± 15% ($n = 3$) and 12.5 ± 4% ($n = 3$) of T89 control plants, respectively. These lines were selected for further analysis. Because there was no significant difference between the lines in C4H transcript abundance or any other property subsequently measured, the two lines were treated as one group in subsequent analysis.

Determination of Wood Components. Wet chemistry analysis was made according to Ona et al.[25] with some modifications. Stem samples were bark peeled, cleaved to remove the pith, and freeze-dried for 48 h. The dried wood was ground in a centrifugal mill providing a defined particle size of 0.5 mm (Z200, Retsch, Haan, Germany) and extracted for 7 h with 1:2 ethanol/toluene. Organic extractives were determined gravimetrically (AX205 Deltarange, Mettler-Toledo, Greifensee, Switzerland) after vacuum drying overnight. Organic extracted samples were flushed with deionized water (Milli-Q Advantage 10, Millipore AB, Solna, Sweden) and dried overnight, and the water extractives were determined gravimetrically. Holocellulose was produced by $NaClO_2$ delignification. The amount of α-cellulose was determined gravimetrically after 17.5% alkali dissolution of holocellulose. Klason lignin was determined on extractive free samples.

Pyrolysis Coupled to Gas Chromatography/Mass Spectrometry (Py-GC/MS). Small sample portions of extractive free wood fraction were ball-milled (MM400, Retsch, Haan, Germany) for 2 min at 30 Hz in stainless steel jars (1 mL) with one ball (diameter 7 mm). A total of 30−40 μg of powder was then applied to the online pyrolizer (Pyrola 2000, PyrolAB, Lund, Sweden) mounted on a GC/MS (7890A/5975C, Agilent Technologies AB Sweden, Kista, Sweden). Pyrolysis was conducted for 2 s at 450 °C. The pyrolysate was separated on a 30 m length, d 250 μm, 25 μm film thickness capillary column (J&W DB-5, Agilent Technologies Sweden AB, Kista, Sweden). The oven temperature program started with 40 °C at pyrolysis, followed by a temperature ramp: increase of 32 °C/min to 100 °C, then an increase of 6 °C/min to 118.75 °C, and then by 15 °C/min to 250 °C, and finally increased 32 °C/min to 320 °C. Total run time was 16 min and full-scan spectra were recorded in the range of 40−500 *m/z*. Peak detection, identification, and integration were automated with the instruments' software (Chemstation, Agilent Technologies Sweden AB, Kista, Sweden). Ratios between S- and G-type lignin as well as carbohydrate degradation products were calculated on integrated peak areas from selected *m/z* channels according to literature.[26]

Fourier Transform Infrared (FTIR) Measurements. FTIR spectra were measured in transmission in a Perkin-Elmer, Spectrum 100 FTIR equipped with a microscope, Spectrum Spotlight 400 FTIR imaging system. For each tree, one specimen was chosen, taken from the microtome sections used for dynamic mechanical measurement (see below), on which seven areas of 100 by 100 μm were defined and spectra were recorded, using nonpolarized mid-IR light. A liquid nitrogen-cooled Mercury Cadmium Telluride detector was used and spectra were recorded in the wavenumber interval of 700−4000/cm with a spectral resolution of 1/cm. The spectra were baseline corrected at 3764, 1842, and 776/cm. The difference in total absorbance (due to different thickness of the measured areas) was compensated for by a normalization of the spectra to the absorbance at 1424/cm.

Crystallinity Determination. The crystallinity was determined on hackled small pieces (using a sharp razor blade) from mechanically tested wood slices using an X-ray diffractometer (D8 advance, Bruker AXS, Germany) in the symmetrical reflection mode. Organosolv lignin (Sigma, U.S.A.) was chosen as an amorphous standard. The intensity was measured as a function of the scattering angle 2θ by $\theta-2\theta$ scan, with scanning angle range of 10−50° and step size of 0.2°.[27] The crystallinity index was calculated as $\mathrm{CrI} = (I_{002} - I_{am})/I_{002}$, where I_{002} is the maximum intensity of the peak at 2θ about 22° and I_{am} is the minimum intensity at 2θ about 18°.[28]

Microfibril Angle Measurements. Microfibril angle (MFA) measurements were performed on mechanically tested samples by wide-angle X-ray scattering (Nanostar, Bruker AXS, Germany) with a sample−detector distance of 4.9 cm using Cu Kα radiation (wavelength 0.154 nm). The diffraction patterns were collected with a two-dimensional (2D) position-sensitive (Hi-star) detector, with a measuring time of 1 h. The intensity was plotted against the azimuthal angle. MFA were determined at three points of each sample.

Atomic Force Microscopy (AFM) Measurements. Freeze-dried epoxy embedded wood cross sections were examined with a NanoScope IIIa Multimode AFM (Digital Instruments, U.S.A.). The specimens were scanned under TappingMode using sharp tapping mode probes with a

Table 1. Wet Chemical, FT-IR, and Py-GC/MS Analysis of Wood from Transgenic and Wild-Type Trees[a]

	Klason lignin [% dry weight]	α-cellulose [% dry weight]	FTIR lignin 1500 cm^{-1}/1424 cm^{-1}	FTIR lignin 1591 cm^{-1}/1424 cm^{-1}	Py-GC/MS S to G ratio	Py-GC/MS carbon to lignin ratio
transgenic	15.1 ± 1.2***	37.6 ± 3.2*	0.44 ± 0.03***	0.64 ± 0.04*	1.5 ± 0.1	9.1 ± 0.9***
wild-type	22.4 ± 3.2	32.8 ± 2.0	0.60 ± 0.01	0.74 ± 0.04	1.4 ± 0.2	4.4 ± 0.3

[a] FTIR relative absorbance intensity for lignin (1500 and 1591 cm^{-1}) related to the cellulose absorbance at 1424 cm^{-1}. Mean ± s.d., 3 wt and 6 transgenic biological replicates, respectively. Student's t-test was done between transgenic lines and wild-type. *$p = 0.05$, **$p = 0.01$, ***$p = 0.001$.

tip radius between 4.18 and 4.29 nm. The length of the cantilever was 125 μm, the spring constant was 40 N/m, and the resonant frequency was 300 kHz. The samples were scanned at ambient humidity at 25 °C. Images were taken in both height mode (where the deflection of the cantilever is directly used to measure the z position), and in the phase mode (where the phase lag is used to determine differences in material properties). Seven scans from different areas were done for each specimen.

An image processing software inspired by the watershed segmentation,[29] developed at the Centre for Image Analysis in Uppsala,[30] was used to evaluate the AFM images with regard to cellulose aggregate size, assuming a square cross-section. The enlargement due to the tip radius was calculated for each specimen to be between 0.5 and 2 nm. The widths of the pore and matrix lamella within the fiber wall were calculated using another image processing routine, where each AFM image was transformed into a binary form (black and white) with the pore and matrix components represented as white areas.

Density Measurements. The bulk density of the quasi-static loading samples was calculated on the basis of the oven-dry mass (Sartorius microbalances, precision of 0.01 mg) and the wet volume for all lines and saplings.

Quasi-Static Mechanical Tests. For the quasi-static mechanical tests wood blocks were embedded in polyethylene glycol (PEG) with molecular weight 2000 to preserve the material during cutting. About 80 μm thick samples were cut from the PEG embedded blocks with a rotating microtome in the longitudinal/radial direction. After cutting, the slices were washed with fresh water to dissolve the PEG and kept wet during the entire testing process. The dimensions of specimens were approximately 1.5, 0.08, and 20 mm in the radial (R), tangential (T), and longitudinal (L) direction. Measurements were performed in tension with specimens loaded parallel to grain until rupture of the specimen. The microtensile tester consisted of a motorized positioning system (Owis, Germany) equipped with a load cell with a capacity of 50 N (Honeywell, U.S.A.). The gauge length was ~10 mm and the strain rate was 2.5 μm/s. Force and elongation were monitored during the experiment and strain was calculated on machine path basis.

Dynamic Mechanical Analysis. Specimens with dimensions 0.07 (R) by 2 (T) by 20 mm (L) were prepared by microtome cuts from wet samples and placed in deionized water at +4 °C until mechanical testing. Viscoelastic measurements were performed in tension with the specimen loaded parallel to grain using a Perkin-Elmer dynamic mechanical DMA analyzer with a load capacity of 7 N (Perkin-Elmer). Two different types of experiments were conducted: a humidity scan (at constant temperature) succeeded by a temperature scan (where the specimen was submerged in water). Each specimen was slightly dried using a blotting paper to remove surplus of water and was thereafter mounted in the grips (span length ~ 12 mm). A small load (10 mN) was applied, and the specimen was left to dry for 1 h at a relative humidity (RH) of 5% and a temperature of 30 °C. The RH was then increased from 5 to 95% at a rate of 1% per minute and thereafter lowered in one step down to 5% RH and kept there for 4 h. This initial step was done to prevent buckling of the specimen and expose all specimens to a well-defined humidity cycling history. In the following step, a static load (F_{stat}) and a dynamic load (F_{dyn}) were applied at a frequency of 1 Hz and at an amplitude of 10 μm (F_{stat} was set to 150% of F_{dyn}). The RH was then raised from 5 to 95% (0.33% per minute) and thereafter lowered in one step down to 5% RH to dwell at this level for 4 h (this procedure was repeated once). Directly after, a temperature scan followed, where the test chamber was first filled with deionized water (30 °C). The scan utilized a heating rate of 0.5 °C/min from 30 to 90 °C. During measurements, the storage modulus (E') was recorded. The glass transition temperature (T_g) was determined from the temperature scans as the onset in the decreasing storage modulus.

Results and Discussion

C4H is a key enzyme in lignin biosynthesis acting in the early phenylpropanoid pathway.[12] RNAi was used to silence C4H expression, and two independent transgenic lines with about 80% reduced expression were selected. When grown under greenhouse conditions, the transgenic lines showed a slight reduction in height growth (transgenic 129 ± 7 cm, wt 144 ± 3 cm, mean ± s.d, $n = 6$ and 3, respectively) but were otherwise not different from the wt and exhibited normal wood anatomy when inspected under light microscopy.

Wet chemistry analysis showed that the reduced expression of C4H caused a decrease in relative proportion of Klason lignin content from 22% in wt trees to 15% in the transgenic lines, with a corresponding increase in cellulose content (Table 1). This change in cell wall composition was also confirmed by Py-GC/MS analysis showing a relative decrease in lignin and a corresponding increase in the cellulose/hemicellulose content, whereas no difference was observed in the S to G ratio (Table 1). Further, FT-IR measurements showed that absorption spectra of the transgenic lines had a noticeably lower lignin absorption at the wavenumbers 1500 and 1591 cm^{-1} (Table 1).

This is the first description of C4H silencing in a forest tree, and the strong reduction of lignin in the wood is consistent with earlier observation in *Arabidopsis*, tobacco, and alfalfa xylem tissues where C4H has been down-regulated.[31−34] This is not surprising because all phenylalanine-derived units have to be hydroxylated by C4H to be incorporated in lignin. The S to G ratio in previous studies was found to increase, decrease, and remain unchanged (as in the case here investigated) after reduced C4H expression and may, therefore, be species dependent.

It was further of interest to investigate if the reduced lignin had any effect on the ultrastructural architecture of the wood fiber wall. This has earlier been observed in both tobacco and *Arabidopsis* with reduced lignin contents,[19] and could be expected to influence the mechanical properties of wood. As a first approach the bulk density, which is an indicator of structural changes in the wood as a whole, was determined. The transgenic trees had a significantly lower density compared to the wt (~260 ± 30 kg/m^3 and ~296 ± 6 kg/m^3, respectively; Figure 1a). Therefore, cell wall ultrastructure was examined in more detail using atomic force microscopy (AFM). The microstructural images from the AFM data suggested that the transgenic trees had smaller cellulose aggregates (aggregated microfibrils) and larger size of hemicellulose/lignin lamellae separating the aggregates, while the frequency of these lamellae did not differ significantly (Table 2, Figure 2). Although the absolute size of aggregates may be affected by sample preparation,[35,36] the AFM measurements indicate a more loose arrangement of the aggregates in the cell wall of the transgenic trees as compared to

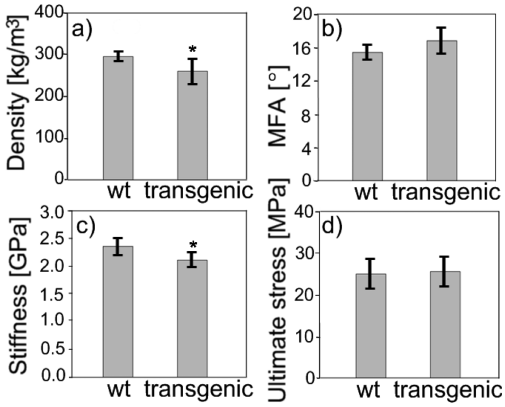

Figure 1. Mechanical and anatomical features of wild-type and transgenic trees; mean ± s.d.; (a) density; (b) cellulose microfibril angle of $n = 4$ biological replicates; (c) stiffness; (d) ultimate stress of $n = 5$ biological replicates; *significant difference between biological replicates of wild-type and transgenic trees at $p = 0.05$ (*t*-test).

Table 2. Atomic Force Microscopy of Wood from Transgenic Tree and Wild-Type[a]

sample	aggregate size [nm]	lamellar size [nm]	No. of lamellas per μm
transgenic	22 ± 2	13 ± 6*	18 ± 5
wild-type	25 ± 3	6 ± 2	14 ± 8

[a] Mean ± s.d, $n = 7$. Students *t*-test was done between transgenic lines and wild-type. *$p = 0.05$.

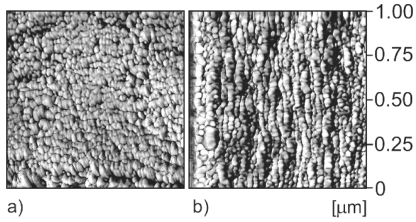

Figure 2. Atomic force microscopy images of cell wall cross sections: (a) wild-type and (b) transgenic aspen.

wt trees, which may affect cell wall density and wood density accordingly. Loosening of cell wall structure has earlier been observed as an effect of reduced lignin in genetically modified *Populus* and *Arabidopsis* xylem fibers,[17,19] but in *Arabidopsis* it was related to lignin composition rather than to its content per se.[18] A correlation between lignin content and aggregate size has also been found in a natural population of *Populus*.[37]

No difference in cellulose crystallinity was found between transgenic and wt trees, as examined from FTIR spectra by the ratio of the peaks 1372 and 2900/cm[38,39] and for the wood of one wt tree and one transgenic tree in X-ray diffraction (data not shown). This suggests that cellulose crystallinity was not affected, although additional measurements would be required to be conclusive on this point.[28,40]

X-ray measurements further showed no significant difference in MFA between transgenic and wt trees (Figure 1b). Therefore, it seems reasonable to assume that the cellulose orientation was not affected by the genetic modification. Ruel et al.[19] performed X-ray diffraction measurements on *Arabidopsis* cell walls and reported on less oriented microfibrils in CCR1 mutants reduced in lignin. However, Ruel[19] and co-workers showed only the diffraction diagrams and did not present calculated data, which makes it difficult to interpret this inconsistency.

Taken together, the down-regulation of C4H and the resulting reduction in lignin content significantly affected wood density

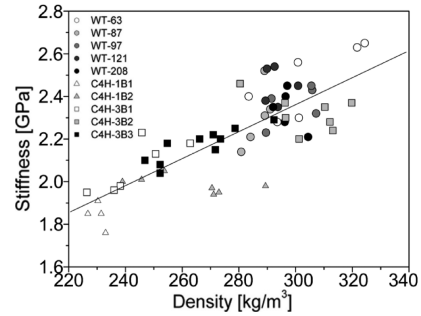

Figure 3. Correlation between density and stiffness in wild-type and transgenic trees. Technical replicates from five wt and five transgenic trees; $r^2 = 0.65$.

and cell wall ultrastructure, whereas no considerable effects on lignin S to G ratio, cellulose microfibril orientation, and cellulose crystallinity was found.

Quasi-static mechanical tests of transgenic trees can help to elucidate how the changes in ultrastructure and chemical composition influence the mechanical performance of wood. Tensile tests showed a slight but significant decrease in stiffness (i.e., Young's modulus) for the transgenic lines, compared to the wt control (Figure 1c), whereas tensile strength (i.e., ultimate stress) was not altered (Figure 1d).

Density and cellulose microfibril angle are known to influence the elastic properties of wood. High-density wood is stiffer than low-density wood and a small MFA in the cell wall contributes to a high stiffness of the material.[5,41,42] For the samples studied here, the MFA of the transgenic trees was not significantly different from that of the wt trees. Hence, cellulose microfibril orientation is not the structural parameter that can explain the deceased stiffness of the wood with reduced lignin content. However, when stiffness was plotted against bulk density for all wt and transgenic trees, a good correlation was found (Figure 3), suggesting that the difference in stiffness between transgenic and wt trees can, at least partly, be caused by the difference in wood density. A similar correlation between static stiffness in three-point bending and (green specific) density was observed by Horvath.[16]

One might have expected that a reduction in lignin content by 30% would have altered the mechanical tensile strength more severely. However, the mechanical tests were performed in uniaxial tension on samples with rather small microfibril angles. Under these conditions, the cellulose fibrils are the main load bearing component and lignin content and composition may play a minor role.[41,42] Moreover, the absolute cellulose content per wood volume was literally unchanged between the wt and the transgenic trees (97 and 98 kg/m^3, respectively), thus, explaining the preserved tensile strength in the transgenic material.

For wood samples with a higher microfibril angle, or under different loading conditions (such as axial compression or bending) it seems reasonable to assume that lignin reduction could have a higher impact on the mechanical performance. However, macroscopic bending and compression tests are not well suited for mechanical tests on small saplings with a limited amount of wood material due to the strong influence of sample size and shape. Therefore, uniaxial tensile test on thin wood sections was the method of choice.

Dynamic mechanical tests are often used for characterization of a material under influences of a change in temperature and/or moisture. In this context, the glass transition temperature (T_g) of the material is an important parameter because it describes in which temperature (and moisture) region a transition of the

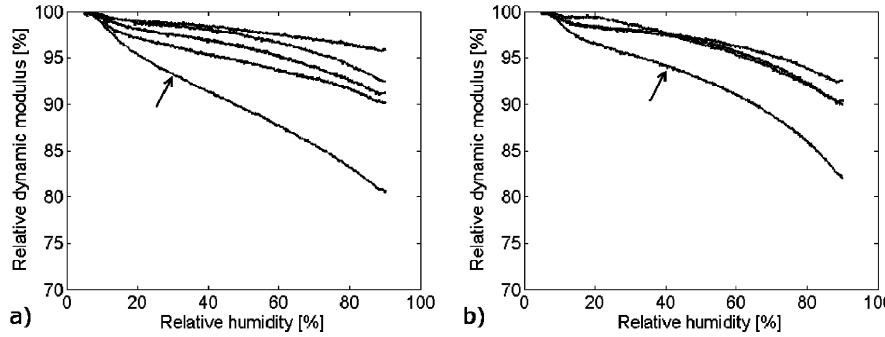

Figure 4. Relative dynamic modulus as a function of relative humidity in transgenic and wild-type trees. (a) Five independent wild-type trees and (b) four independent transgenic trees. The arrows indicate samples containing tension wood.

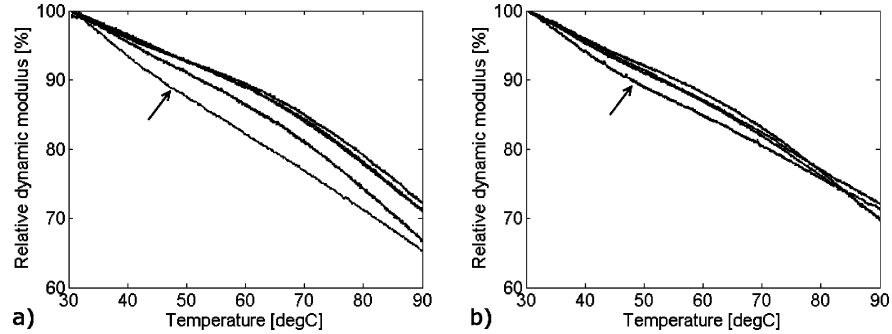

Figure 5. Relative dynamic modulus as a function of temperature in transgenic and wild-type trees. (a) Five independent wild-type trees and (b) four independent transgenic trees. The arrows indicate samples containing tension wood.

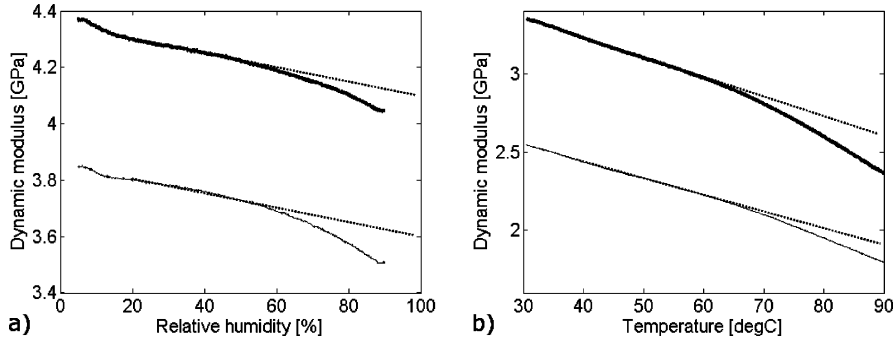

Figure 6. Dynamic modulus of wild-type (thick line) and transgenic (thin line) trees as a function of (a) relative humidity, RH, and (b) temperature. The dotted lines indicate the onset of xylan (a) and lignin softening (b), respectively, as the point where the modulus deviates from this line. Curves are constructed from all sample curves (excluding outliers) of the modulus development against RH or temperature.

material from a solid into a more rubbery or flowable state occurs. The RH and the temperature scans showed consistent (dynamic) property trends. The curves from the humidity scans revealed a similar softening behavior with increasing RH both for transgenic and wt samples (Figure 4). The major transition observed at about 80% RH is believed to originate from softening of hemicelluloses.[43−45] The temperature scans indicated a softening region around 70 °C for both the transgenic and the wt samples (Figure 5). This is due to lignin softening, which occurs at this relatively lower temperature in hardwood (70−90 °C) as compared to softwood (80−100 °C).[43−46]

The absolute dynamic moduli of constructed average curves were considerably lower in wood from the transgenic trees as compared to the wt trees (Figure 6). From the RH-scans (Figure 6a), it is apparent that the hemicellulose softening was larger for the transgenic trees than for the wt trees. In a similar way, the degree of lignin softening during temperature increase under wet conditions was larger for the wt trees as compared to the transgenic trees (Figure 6b). Both of these observations are in line with the observed lower lignin content in the transgenic specimens.

From the temperature scans, the T_g, determined as the offset of the curves, was 67 ± 0.5 and 69 ± 0.0 °C for wood samples from the transgenic and wt trees, respectively. The difference was not significant (*t*-test, 0.05 level). Earlier observations of a number of wood species including *Populus tremula* suggest that increasing S to G ratio renders a lowering of the lignin T_g.[45] Moreover, Baumberger et al.[47] noticed a difference in T_g between lignin originating from transgenic poplars with modified lignin and wt poplars. The difference was ascribed to the structural features of the lignin polymer, that is, the number of lignin units involved in C−C bonds and lignin molecular weight. Thus, the lack of difference in softening temperature of lignin observed here for the transgenic wood is in line with the chemical analysis that the reduced lignin did not result in any major difference in lignin S to G ratio (Table 1).

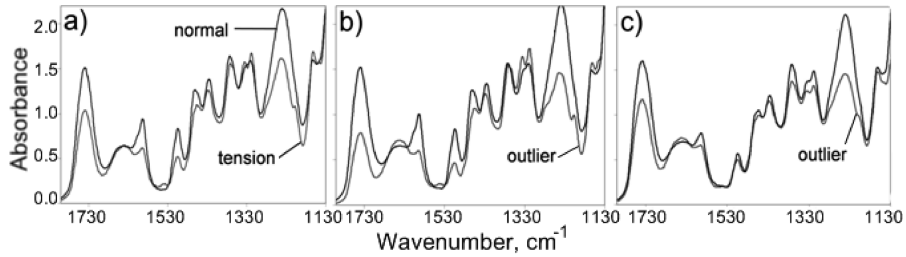

Figure 7. FTIR spectra of wood samples from *Populus* trees: (a) spectra from wild-type trees with tension wood induced by tilting and from wild-type normal wood, (b) spectra from wild-type with normal wood and from the tension wood containing outlier sample, and (c) spectra from transgenic trees with normal wood and from the tension wood containing outlier sample.

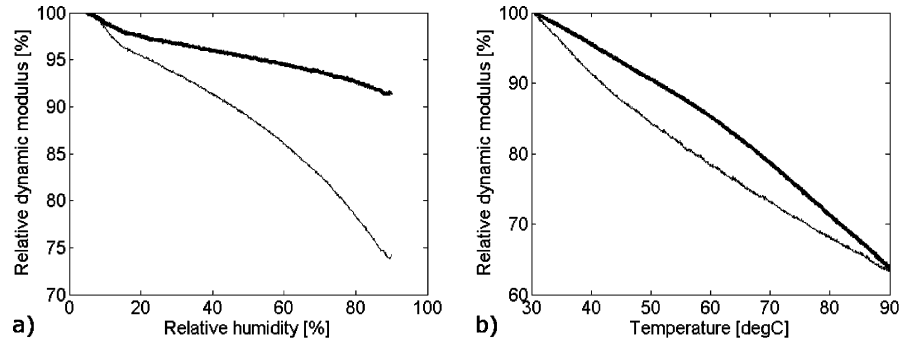

Figure 8. Relative dynamic modulus of normal wood (thick line) and tension wood obtained from tilted trees (thin line) as a function of (a) relative humidity and (b) temperature from an independent wild-type tree.

One of the replicate trees from both the wt and transgenic group showed a significantly different behavior for the relative dynamic modulus (indicated with arrows in Figures 4 and 5). FTIR measurements of these specimens clearly revealed that they contained tension wood displayed as lower absorption peaks at the wavenumbers 1735 and 1240/cm corresponding to carboxylic groups and C−O xylan, respectively (Figure 7). These outliers were, to a greater extent, influenced by the increase in RH and showed a less pronounced lignin softening in the wet temperature scans; both comparable with measurements of an independent hybrid aspen sample where tension wood had been induced by leaning (Figure 8). The dynamic modulus of tension wood was much more sensitive to moisture but less sensitive to temperature compared to normal wood. In our experience the occurrence of arcs of tension wood is frequent in young greenhouse grown *Populus*. The results in this study demonstrate that this may result in outlier behavior when small-sized samples are used unless care is taken.

Conclusions

This study shows that a major reduction in Klason lignin (30%) in *Populus* wood, with no significant change in S to G ratio, caused a small but significant reduction in axial stiffness (Young's modulus), but did not affect axial tensile strength (ultimate stress) or glass transition temperature. These results were explained by a difference in wood density and cell wall structure rather than differences in cellulose MFA or crystallinity. The study highlight the need to document not only processing properties but also mechanical behavior of wood for new tree genotypes intended for commercial use. The study also shows the value of transgenic technology to produce model wood materials with altered structure and polymer composition to evaluate components crucial for mechanical behavior. The fact that tension wood was not easily detected by visual inspection but occurred in the test specimens, points out that care must be taken when comparing properties from small greenhouse specimens often used in studies of transgenic trees.

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