

Thermo-mechanical Behavior of Genetically Modified *Populus trichocarpa*

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Wood processing is often performed at elevated temperatures under moisture-saturated conditions; therefore, it is important to understand the impact of the lignin content and lignin chemical structure on the thermo-mechanical properties of wood. In this study, genetically modified *Populus trichocarpa* wood specimens with down-regulated cinnamyl alcohol dehydrogenase, cinnamate 3-hydroxylase, and cinnamate 4-hydroxylase with altered lignin contents and/or lignin structures were utilized to probe the relationship between the lignin content, lignin monomer composition, and thermo-mechanical properties of solid wood. The thermo-mechanical properties of these unique samples were measured using dynamic mechanical analysis and the nuclear magnetic resonance (NMR) spin-spin relaxation time. The results showed that the transgenic *P. trichocarpa* samples had decreased storage and loss moduli compared with the wildtype. The solid-state NMR revealed increased lignin molecular mobility in the reduced-lignin transgenic lines. Also, noticeably reduced glass transition temperatures (T_g) were observed in the transgenic lines with reduced lignin contents and altered lignin monomer compositions compared with the wildtype. The increased lignin molecular mobility and reduced T_g in these samples can probably contribute to wood utilization and processing, such as lignin removal for pulp and paper and biofuels production, as well as particle consolidation during wood composite manufacturing.

Keywords: Genetically modified poplar; Lignin; Viscoelastic properties; Glass transition temperature; Dynamic mechanical analysis; Cinnamyl alcohol dehydrogenase (CAD); Cinnamate 3-hydroxylase (C3H); Cinnamate 4-hydroxylase (C4H)

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INTRODUCTION

Populus trichocarpa has been extensively studied because of its beneficial qualities, such as fast growth rate, high biomass yield, and low lignin content, which makes it an attractive feedstock for pulp and paper, bioenergy, and biochemical applications (Adebayo *et al.* 2011; Mizrachi *et al.* 2012). Also, *P. trichocarpa* was the first tree species to have its entire genome sequenced and is easy to propagate, thus making it a good species for genetic modification (Tuskan *et al.* 2006). The recalcitrant nature of lignin in biomass has motivated research focused on reducing the lignin content and/or altering the lignin structure by genetic modification (Chen *et al.* 2001; Li *et al.* 2003; Li *et al.* 2014; Wang *et al.* 2018).

High temperature processes are commonly used to remove lignin in pulp and paper, and biofuels production, while many composite manufacturing systems use elevated temperatures and pressures to induce consolidation. Therefore, the thermo-mechanical behavior of wood is an important property worth exploring (Back and Salmén 1982; Irvine 1985; Baumberger *et al.* 2002; Hendriks and Zeeman 2009). An important parameter to consider when elevated temperatures are used for biomass processing is the glass transition temperature (T_g), which is defined as the temperature where an amorphous material changes from a glassy state to a rubbery state, allowing for relaxation and flow. The physical phenomena that are attributed to the T_g include thermal expansion and the concurrent increase in free volume at elevated temperatures, which results in molecular motion (Irvine 1985). Frequently, delignification or consolidation processes occur under moisture-saturated conditions. Increasing the moisture content lowers the T_g values of wood components because of the disruption and replacement of hydrogen bonding between water molecules and wood biopolymers (*i.e.*, cellulose, hemicelluloses, and lignin) (Irvine 1985; Kelley *et al.* 1987; Placet *et al.* 2007). This effect is most noticeable in carbohydrate components, which have a greater capacity for hydrogen bonding compared with other biopolymers. However, the amorphous cellulose fraction may be constrained to some degree because of the microfibril structure of cellulose in the crystalline and amorphous regions along the polymeric chain. The crystalline cellulose component of wood is expected to exhibit mainly elastic behavior (Olsson and Salmén 1992).

Under moisture-saturated conditions, the T_g values of amorphous cellulose and hemicelluloses are reduced to sub-ambient temperatures, while the T_g of lignin ranges from 90 °C to 100 °C (Salmén 1984). Therefore, the viscoelastic properties of lignin are hypothesized to control the viscoelastic behavior of the bulk wood when under moisture-saturated conditions and at or above ambient temperatures (Olsson and Salmén 1992). Thus, the chemical structure and perhaps even the relative lignin content determine the overall thermo-mechanical behavior of solid wood. However, measuring the T_g in solid wood is complex because of the nonuniform mixing of its three structural components (cellulose, hemicellulose, and lignin). Measurements are further complicated by the presence of polymer interactions, such as lignin carbohydrate complexes (LCC), and the anisotropic nature of the cell wall.

Comparative studies of the T_g of several hardwood and softwood species have attributed the lower T_g values of hardwoods to the presence of syringyl (S) subunits (which occurs in hardwood lignins), while higher T_g values in softwoods have been attributed to their lignin monomer composition being composed mainly of guaiacyl (G) subunits (Olsson and Salmén 1992; Olsson and Salmén 1997). Olsson and Salmén (1992) and Olsson and Salmén (1997) hypothesized that an increased number of methoxyl groups in S-unit-rich lignin reduced the number of carbon-carbon bonds associated with lignin crosslinking and limited the entanglement of the lignin polymer, which led to higher molecular mobility. Different results were reported by Horvath *et al.* (2011), who measured the T_g of aspen wood specimens which were genetically modified to have a reduced lignin content, high S/G ratio, or both a reduced lignin content and high S/G ratio. These authors reported decreased T_g values in low-lignin samples and concluded that the S/G ratio has no remarkable influence on the T_g of solid wood under moisture-saturated conditions (Horvath *et al.* 2011).

With the advent of genetic engineering, wood samples from a single species exhibiting a wide range of lignin properties can be produced, thus allowing more detailed investigations into the relationship between the lignin and thermo-mechanical properties

of solid wood. In this study, the thermo-mechanical properties of wood specimens from *Populus trichocarpa* down regulated in cinnamyl alcohol dehydrogenase (CAD), cinnamate 3-hydroxylase (C3H), and cinnamate 4-hydroxylase (C4H) were measured. These three enzymes, along with several others not discussed here, are responsible for catalyzing reactions in the monolignol biosynthetic pathway which converts the syringaldehyde precursor to one of three monolignols (Wang *et al.* 2014).

The genes controlling the lignin biosynthetic pathway have been identified and modified previously (Shi *et al.* 2010; Shuford *et al.* 2012; Wang *et al.* 2014, 2018), and there are several studies that have measured the thermo-mechanical properties of genetically modified wood species (Hepworth *et al.* 1998; Baumberger *et al.* 2002; Bjurhager *et al.* 2010; Horvath *et al.* 2011). However, more research is needed to obtain a comprehensive knowledge of the physical changes that occur in such gene-modified samples.

In this study, the unique properties of wood specimens with down-regulated lignin biosynthesis genes were utilized to assess the relationship between the chemical wood properties and their viscoelastic behaviors. This was accomplished by measuring the molecular mobilities and viscoelastic properties of various wood samples from wildtype and transgenic *P. trichocarpa*. The purpose of this research was to add to the current understanding of the relationship between the lignin structure, lignin content, and viscoelastic properties of solid wood.

EXPERIMENTAL

Samples

Populus trichocarpa was genetically modified by the Forest Biotechnology Group at NC State for decreased gene expression of cinnamyl alcohol dehydrogenase (CAD), cinnamate 3-hydroxylase (C3H), and cinnamate 4-hydroxylase (C4H). Both the wildtype and transgenic lines consisting of single and double CAD down-regulated, C3H down-regulated, and combined C3H and C4H down-regulated genetic lines were studied. The test samples were labelled as follows: WT (wildtype), CAD1 (single CAD gene knock-down), CAD1,2 (double CAD gene knock-down), C3H (C3H gene knock-down), and C3H:C4H (simultaneous C3H and C4H gene knock-down) (Wang *et al.* 2018). The WT tree lines were grown alongside the genetically modified tree lines to act as a control and were harvested from the greenhouse after six months of growth. The stems were debarked before use. Considering the small diameter of the stem and the fact that chemical analysis is destructive, separate sections of the stem were used in the study: the stem above 45 cm was used for the chemical analyses and the bottom 45 cm of the stem were used for all of the other analyses. Composite samples consisting of three replicates were used for the chemical analysis due to the fact that there is a large amount of variability between each individual stem (*i.e.* biological replicate). Three replicates of the composite sample were analyzed in order to measure the experimental variability in measured chemical composition data.

Chemical Composition

The chemical compositions of the wildtype and transgenic samples as measured by the Forest Biotechnology group were reported in a separate publication (Wang *et al.* 2018), and adopted for use in this study. Briefly, the lignin content was measured by following

the Klason lignin method (Lu *et al.* 2013), and the cell wall sugars in the acid soluble fraction were measured by neutralizing them with CaCO_3 , which was followed by quantification by high pressure liquid chromatography or gas chromatography with a flame ionization detector. The lignin monomer composition and S/G ratio of these samples were measured using a solution-state 2D nuclear magnetic resonance (NMR) method (Kim and Ralph 2010; Mansfield *et al.* 2012). Each compositional analysis was performed in duplicate, and the 2D NMR analysis was done as a single point measurement for each genetic line.

Dynamic Mechanical Analysis

Using a micro-circular saw, clear samples (*i.e.*, no knots or pith) at green condition were cut from the stem of each plant into sections with the dimensions of 25 mm (longitudinally) \times 4 mm to 6 mm (tangentially) \times 1 mm (radially) for the dynamic mechanical analysis (DMA). All of the samples were then dried under vacuum at 60 °C and saturated with ethylene glycol for a minimum of 3 d. The utility of using ethylene glycol as a plasticizer for wood was demonstrated by Wennerblom *et al.* (1996), who found a lower softening temperature of wood in ethylene glycol than in water because of the closer solubility parameter of lignin to that of the former. Other researchers (Laborie *et al.* 2004; Horvath *et al.* 2011) have used the solvent successfully in their DMA work. A 3-point bending submersion clamp was used with the DMA instrument (DMA Q800, TA Instruments, New Castle, USA), which was operated with a constant strain and heating rate of 2 °C/min from 35 °C to 95 °C. Preliminary experiments showed that the T_g of wood submerged in ethylene glycol is below 95 °C. A frequency of 0.1 Hz was used also based on preliminary testing. The samples were heated twice; the first heating was done to remove the thermal history and the second heating was performed to collect data. The storage modulus (E'), loss modulus (E''), and $\tan \delta$ were measured. The T_g was the temperature at which the peak in the $\tan \delta$ curve occurred. A 6th order polynomial equation was fitted to each of the $\tan \delta$ DMA measurements, and the peak on the curve generated by the equation was used to determine the T_g and maximum $\tan \delta$ values. The linear viscoelastic region (LVR) was approximately less than 0.1% strain. The LVR is defined as the strain at which the initial E' for a given temperature decreases by 10% as measured by the DMA (Sun *et al.* 2007). To ensure testing occurred within the LVR, a strain of 0.05% was used during dynamic testing. The DMA test was performed with at least three replicates for each genetic line.

Solid-state Nuclear Magnetic Resonance Relaxation Time

The solid-state NMR spin-spin relaxation times (T_2) were measured using the WT and transgenic wood samples as an indicator of molecular mobility. Wood samples were milled first and then sieved through a 40-mesh screen prior to analysis. The solid-state NMR spectra were acquired with a 200-MHz spectrometer equipped with a 4.7 T magnet (Bruker Avance III, Billerica, USA). The resonant frequencies used were a ^1H of 200.1 MHz and ^{13}C of 50.3 MHz. The cross polarization magic angle spinning (CPMAS) spectra were acquired with a 7-mm CPMAS probe and ZrO_2 rotors (Bruker Biospin K1921, Billerica, USA). The solid-state CPMAS T_2 NMR measurements were performed as described by Tekely and Vignon (1987). Each slice in the 2D acquisition was collected with a MAS of 5 kHz and recycle delay of 3 s. The samples were air-dried and 5000 scans were collected. The delay times for the samples were 3.5 μs , 5.5 μs , 7.5 μs , 9.5 μs , 12.5

μs , 15.5 μs , 18.5 μs , 20.5 μs , 25 μs , 30 μs , and 40 μs . This analysis was performed as a single point measurement on a composite sample for each genetic line.

Statistics

An analysis of variance (ANOVA) was performed using the statistical software SAS version 9.2 (SAS Institute, Cary, USA). A general linear model (Proc GLM) was used to test for significance of the genetic modifications against the measured response variables. Tukey's multiple comparison was utilized for mean separation with an α of 0.05 to determine the significant differences between the mean values of each genetic line for the viscoelastic properties (T_g , $\tan \delta$ at the T_g , E' , and E'').

RESULTS AND DISCUSSION

Dynamic Mechanical Analysis

The dynamic moduli (E' and E''), $\tan \delta$, and T_g (judged by the $\tan \delta$) were measured by DMA for the WT and transgenic lines CAD1, CAD1,2, C3H, and C3H:C4H. (Table 1; Fig. 1). The ANOVA analysis showed that the genetic modifications and resulting morphological changes had a significant effect on the measured thermo-mechanical properties. All of the transgenic lines demonstrated significantly decreased E' and E'' values compared with that of the WT. The transgenic samples CAD1,2, C3H, and C3H:C4H presented significantly reduced lignin contents, with content reductions ranging from 5% to 57% below that of the WT (Table 2). Additionally, CAD1 had significantly lower E' and E'' values compared with that of the WT, despite its similar lignin content. Figure 2 shows that there was a poor correlation between the lignin content and the E' and E'' values. Wood density (expressed as specific gravity) was highest in WT; however, there was no correlation between density and glass transition temperature in the wood specimens.

Previous work has shown that genetically modified aspen that has been down-regulated in 4CL and hybrid poplar down-regulated in C4H, both with reduced lignin contents, exhibited reduced E' and E'' values (Bjurhager *et al.* 2010; Horvath *et al.* 2011). Hepworth *et al.* (1998) reported a decreased E' value in CAD down-regulated tobacco, which had a similar lignin content to that of the control.

Genetic modification also had a significant effect on the S/G ratio in the transgenic lines, exhibiting both high and low S/G ratios at 2.5 in CAD1 and 9.9 in C3H compared with 2.6 in the WT (Table 2). However, no trend between the S/G ratio and E' or E'' was observed. This indicated that factors other than the lignin content and S/G ratio influence the mechanical properties of this genetic line. As an example of the perturbation of the lignin properties induced by genetic modification, previous researchers have reported that CAD reduction in poplar resulted in a decreased molecular weight and cross-linking density of the lignin (Hepworth and Vincent 1998; Baumberger *et al.* 2002).

The $\tan \delta$ is the ratio of the E' and E'' , and is a measure of energy dissipation in a material. The value of $\tan \delta$ is related to the viscoelastic response of wood. A higher $\tan \delta$ indicates more viscous behavior, while a lower $\tan \delta$ indicates more elastic behavior. The $\tan \delta$ measured at the T_g varied between the WT and transgenic lines. The two genetic lines with the lowest T_g , CAD1,2 and C3H, also had significantly increased $\tan \delta$ values, which indicated a more viscous behavior. Hepworth *et al.* (1998) also observed increased $\tan \delta$ values in CAD down-regulated tobacco.

Table 1. Viscoelastic Properties of the Wildtype and Transgenic *P. trichocarpa*

Sample	T_g (°C)	Tan δ at T_g	E' @ 40 °C (MPa)	E'' at 40 °C (MPa)	Wood Density (Specific Gravity) ³
WT	88 a ¹ (3) ²	0.16 cd (0.02)	3750 a (980)	450 a (120)	0.39 (0.05)
CAD1	82 ab (4)	0.14 d (0.01)	1440 b (130)	150 bc (20)	0.28 (0.03)
CAD1,2	68 c (1)	0.21 a (0.00)	160 d (30)	30 c (10)	0.26 (0.03)
C3H	52 d (5)	0.22 ab (0.02)	240 cd (130)	50 c (20)	0.32 (0.03)
C3H:C4H	73 bc (4)	0.18 bc (0.02)	1610 b (590)	250 b (80)	0.26 (0.04)

¹ Letters signify significantly different means between genetic groups ($\alpha = 0.05$)

² Values in parentheses indicate the standard deviation of the three biological replicates

³ Wood density data was adapted from Wang *et al.* (2018), and values in parentheses indicate the standard deviation of at least 2 biological replicates.

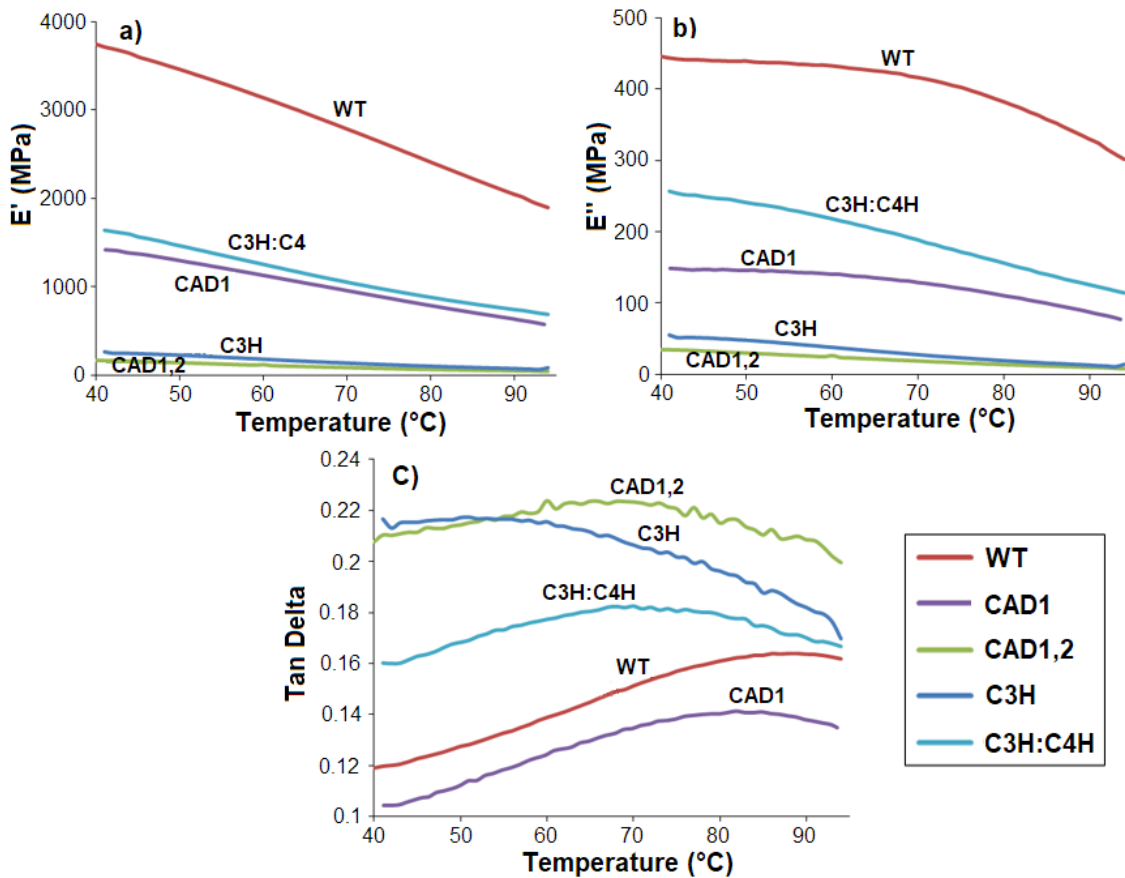


Fig. 1. Variations in the E' (a), E'' (b), and (c) tan δ with the temperature for the wildtype and transgenic *P. trichocarpa*

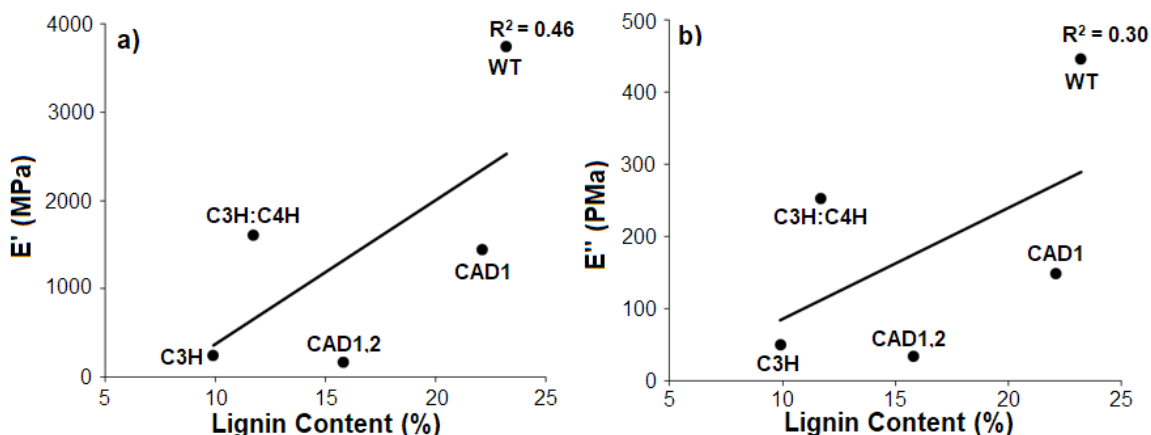


Fig. 2. Correlation plots between the (a) E' and (b) E'' vs. the lignin content (%) in the wildtype and genetically modified *P. trichocarpa*

Table 2. Structural Carbohydrates, Lignin Content, and S/G Ratio for the Wildtype and Transgenic *P. trichocarpa*

Sample	Glucose (%)	Xylose (%)	Galactose (%)	Mannose (%)	Total Lignin (%)	Lignin S/G	H-lignin (% of Total Lignin)
WT	46.1 (2.1) ¹	15.9 (0.3)	1.1 (0.3)	3.4 (0.2)	22.9 (1.9)	2.6	0.2
CAD1	45.6 (3.3)	19.7 (1.0)	1.4 (0.2)	2.4 (0.1)	21.7 (0.3)	2.5	0.3
CAD1,2	45.9 (0.2)	19.8 (0.2)	1.3 (0.1)	2.0 (0.2)	15.8 (2.9)	3.0	2.7
C3H	59.5 (0.1)	22.7 (0.1)	2.2 (0.0)	2.2 (0.4)	9.9 (0.1)	10.0	44.8
C3H:C4H	57.8 (7.9)	20.5 (2.9)	2.2 (0.4)	2.5 (0.6)	11.7 (0.7)	2.8	32.8

Data adapted from Wang *et al.* (2018); ¹ Values in parentheses indicate the standard deviation of two replicates

All of the transgenic lines, with the exception of CAD1, had a significantly lower T_g as measured by the peak in the $\tan \delta$ (Table 1). The highest T_g was measured with the WT at 88 °C, and the lowest was observed with the C3H at 52 °C. Previous research has reported that the T_g of poplar species saturated with water and ethylene glycol is in the range of 65 °C to 96 °C, which was in agreement with that of the WT samples in this study (Olsson and Salmén 1992; Olsson and Salmén 1997; Bjurhager *et al.* 2010; Horvath *et al.* 2011).

The CAD1,2 line had a significantly lower T_g at 68 °C compared with that of the WT. Baumberger *et al.* (2002) reported a remarkably higher T_g compared with the control in isolated lignin samples derived from CAD-deficient poplar. These authors attributed the increase in T_g to increased C-C bonding and decreased β -O-4 linkages in the lignin derived from CAD down-regulated samples. It is important to note that Baumberger *et al.* (2002) measured T_g values in isolated lignin samples, which may only represent a portion of the native lignin, whereas this research utilized solid wood samples. The use of solid wood samples provides a more realistic picture of the T_g of wood because there are no artifacts associated with lignin isolation, such as the hydrolysis of labile interunit linkages and lowering of the lignin molecular weight. Additionally, using solid wood samples for T_g measurements includes the morphological interactions between the lignin and cell wall

carbohydrates, *e.g.*, the LCC linkages. Baumberger *et al.* (2002) reported up to a 29% reduction in the molecular weight of lignin isolated from CAD-deficient poplar. It was possible that the similar effects of decreased molecular weight and increased free phenolic units that were described in CAD-deficient hybrid poplar by Baumberger *et al.* (2002), Lapierre *et al.* (1999), and Lapierre *et al.* (2004) may have contributed to the decreased T_g in the CAD1,2 genetic line.

The transgenic samples C3H and C3H:C4H exhibited significantly decreased T_g values, *i.e.*, 52 °C and 73 °C, respectively. These samples had low total lignin content, with a high proportion of *p*-hydroxyphenyl-lignin (H-lignin) (45% for C3H and 33% for C3H:C4H). Previous research has demonstrated that down-regulation of C3H and C4H can result in major changes to lignin content and lignin composition. For example, Ralph *et al.* (2012) reported that C3H down-regulated poplar presented a severe reduction in the lignin content, drastic changes in the lignin monomer composition (mainly because of the increase in H-units at the expense of G-units), increased S/G ratio, and only minor changes to interunit linkages. The down-regulation of C3H and hydroxycinnamoyl-coenzyme A shikimate hydroxycinnamoyl transferase in alfalfa resulted in lignin reductions of up to approximately 30% to 50% compared with the control (Chen *et al.* 2006; Chen and Dixon 2007). The H-unit content in these samples increased by approximately 50% to 75% as measured by thioacidolysis (Ziebell *et al.* 2010), a reduced methoxyl group content, and more C-C linkages (Ralph *et al.* 2006; Pu *et al.* 2009). Additionally, Ziebell *et al.* (2010) reported that the lignin in the C3H and C4H down-regulated alfalfa had significantly reduced molecular weights and increased extractability with NaOH. Reddy *et al.* (2005) reported that C4H down-regulation in alfalfa resulted in a decreased lignin content and reduced S/G ratio. Based on these previous studies on C3H and C4H down-regulated plants, it was possible that lignin interunit linkages were significantly different in the C3H and C3H:C4H genetic lines. Therefore, changes in the lignin structure, possible reductions in the lignin molecular weight, and decreased lignin content may have been responsible for the observed decrease in the T_g values in the transgenic poplar used in this study.

Spin-spin Relaxation Time

The NMR T_2 of lignin has been used as an indicator of molecular mobility in polymer systems (Cheng *et al.* 2011). In wood, T_2 is also related to the wood-water interactions and wood structure when measurements are conducted at a high moisture content (Gilardi *et al.* 1994; Foston and Ragauskas 2010). Gilardi (1994) concluded that the observed increase in the T_2 with increasing wood moisture content is because of the associated increase in the mobility of water molecules and possibly the cell wall components. It is important to note that the frequency employed by NMR is not similar to that used for DMA, and the NMR T_2 reported in this work was specific to lignin because of the ability to target specific carbon nuclei. Thus, the T_2 was specifically assigned to lignin.

A negative correlation between the lignin content and T_2 was observed (Fig. 3b), which indicated that the lignin polymer in wood with a reduced lignin content exhibited a higher molecular mobility. However, assessing the strength of this correlation is difficult because the measured T_2 values followed a non-linear distribution (*i.e.* the T_2 results fell into groups of high or low values without datapoint in between). Additionally, a negative correlation was also observed between the T_2 and T_g (Fig. 3a), which reinforced the argument that increased molecular mobility is related to the T_g . This was consistent with the results from Olsson and Salmén (1992), who demonstrated that increased crosslinking

in lignin through an acid pretreatment resulted in a higher softening temperature. The potentially contrasting effect of the lignin content and S/G ratio on the T_2 was difficult to determine because the T_2 values of the measured samples typically fell into high and low T_2 values.

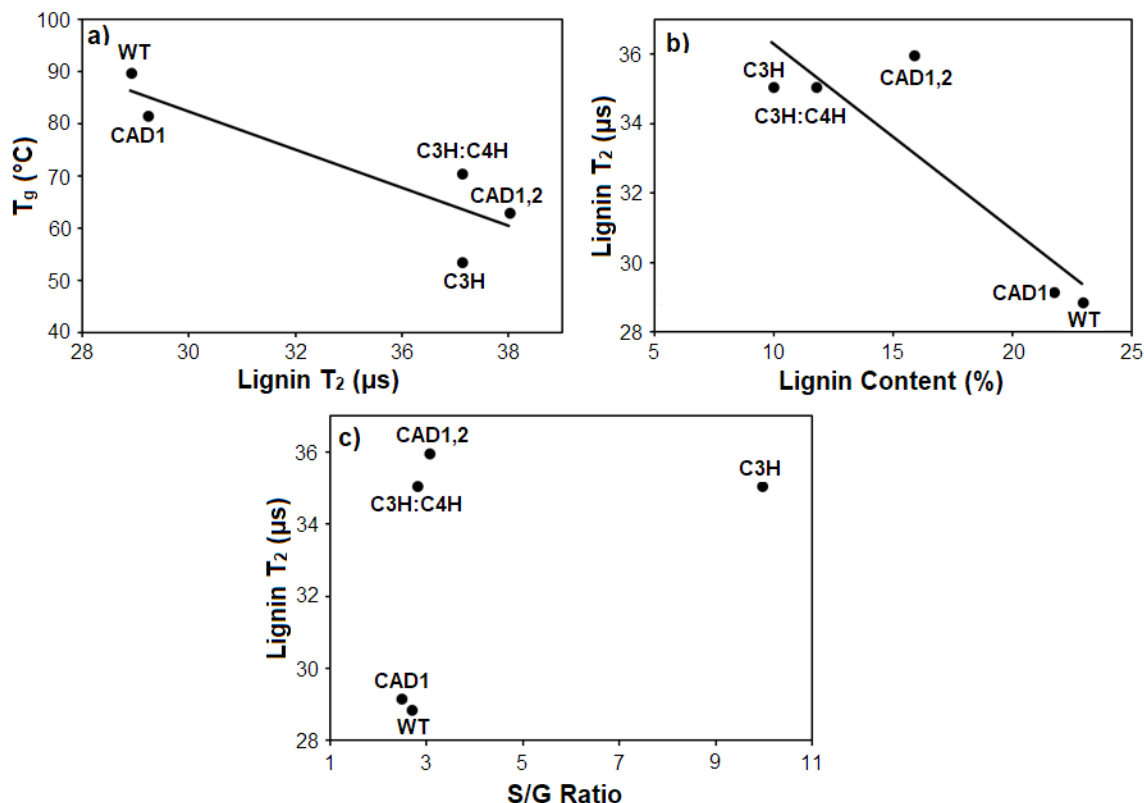


Fig. 3. Correlation plots between the (a) T_g , (b) lignin content, and (c) S/G ratio and the T_2 as determined by NMR for the wildtype and genetically modified *P. trichocarpa*

Lignin Properties and T_g of the Solid Wood

A positive correlation ($R^2 = 0.78$) was found between the T_g and lignin content (Fig. 4a), which indicated that the large range in the measured lignin contents (9.9% to 22.9%) directly influenced the T_g values. When the extremely high S/G ratio in the C3H sample and the lack of intermediate points were considered, it was difficult to determine the potential effect of the S/G ratio on the T_g (Fig. 4b). This observation was supported by Horvath *et al.* (2011), who tested genetically modified aspen with a reduced lignin content, increased S/G ratio, and both a low lignin content and high S/G ratio. Horvath *et al.* (2011) reported reduced T_g values in low-lignin transgenic samples, and there was no remarkable difference in the T_g of transgenic samples with increased S/G ratios compared with the wildtype. Furthermore, Bjurhager *et al.* (2010) reported no change in the T_g values measured in poplar with down-regulated C4H exhibiting a 30% reduction in the lignin content with no noticeable change in the S/G ratio. This dissimilarity in results may have been because of differences in the measurement techniques, different genetic modifications, and greater lignin reductions involved in this study than those reported by Bjurhager *et al.* (2010). Horvath *et al.* (2011) hypothesized that a decreased lignin content

in cell walls may lead to more free volume between polymers and greater molecular mobility, which would result in a reduced T_g .

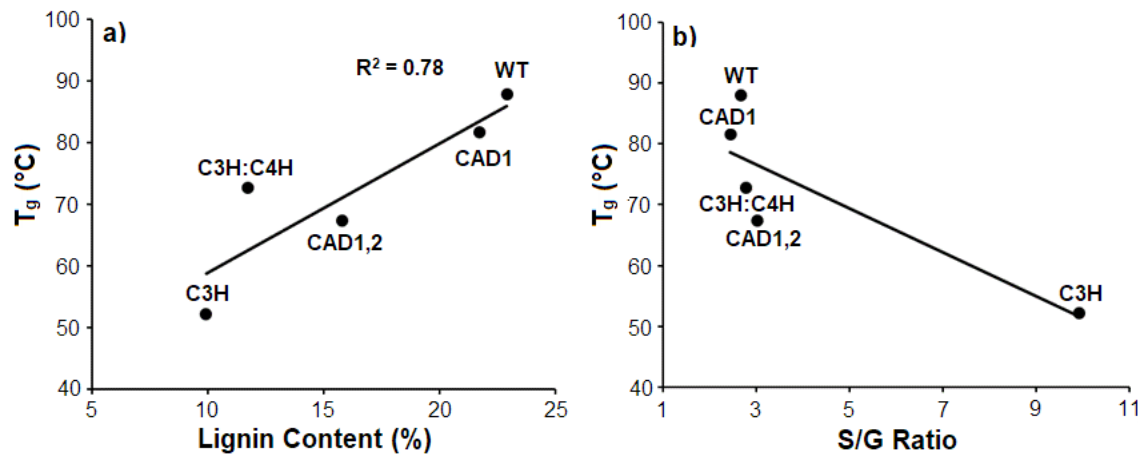


Fig. 4. Correlation between the T_g and the (a) lignin content (%) and (b) S/G ratio for the wildtype and genetically modified *P. trichocarpa* wood samples

The chemical structure, degree of cross-linking, and molecular weight have been reported to affect the T_g of lignin (Hatakeyama and Hatakeyama 2010); however, in solid wood, the relationship between the lignin properties and T_g may be more complicated. We hypothesize that changes in the lignin structure caused by the down-regulation of lignin synthesis enzymes, such as increased methoxyl groups, altered interunit linkages, reduced molecular weight, and increased free phenolic groups, are responsible for the observed decreased T_g under plasticizing conditions.

The second proposed mechanism is that low-lignin wood samples may have greater free volume between cell wall polymers, which would in turn result in greater molecular mobility and a corresponding decrease in glass transition temperature. However, due to the complex nature of thermal softening in a multicomponent system such as solid wood, in addition to the limited number of wood specimens which were tested in this study, more work is required to further explain the relationship between lignin content, lignin composition, and their effect on the thermo-mechanical properties of solid wood.

The thermo-mechanical properties of solid wood are important, considering that wood processing such as the lignin removal for pulp and biofuels production, and wood composite manufacturing rely on treatments under high temperature and water-saturated conditions. The transgenic poplar wood specimens with altered lignin properties used in this study exhibited increased lignin molecular mobility and reduced T_g values. This will likely have noticeable effects on the wood processing requirements, and a possible benefit includes the ability to decrease the processing severity (*i.e.*, reduced temperature, pressure, *etc.*).

CONCLUSIONS

1. Significant alterations to the viscoelastic properties, including reduced the storage and loss modulus (E' and E'') values and an increased value of their ratio ($\tan \delta$), were

measured by dynamic mechanical analysis (DMA) for all of the genetically modified six-month-old *P. trichocarpa* wood specimens with down-regulated CAD, C3H, and/or C4H.

2. Solid-state nuclear magnetic resonance (NMR) was used to measure the NMR spin-spin relaxation times (T_2) of lignin as an indicator of the molecular mobility. The results indicated that the genetically modified samples with a low lignin content exhibited an increased molecular mobility.
3. A significantly reduced glass transition temperature (T_g) was observed in the transgenic *P. trichocarpa* with a reduced lignin content and altered S/G ratio compared with the WT. There was a positive correlation between the lignin content and T_g of the solid wood, however, the influence of the S/G ratio was not clear.

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