Altered Lignin Content and Composition in Transgenic *Populus trichocarpa* Results in a Decrease of Modulus of Elasticity

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Transgenic *Populus trichocarpa* wood was compared to the corresponding wild-type material. The static modulus of elasticity in three-point bending was measured and the chemical composition among the specimens were compared, including the glucose, xylose, and lignin contents as well as the S/G ratio. Changes in chemical composition, created by genetic manipulations of the lignin biosynthetic pathway, affect the mechanical properties of young small-diameter transgenic trees. There are indications that a decrease in lignin content causes severe reductions in mechanical properties. Changes in lignin structure, either from an increased S/G ratio or structural lignin modifications, also negatively influence the mechanical properties.

*Keywords:* CAD; C3H; C4H; Lignin; Glucose; Xylose; Modulus of Elasticity

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**INTRODUCTION**

Wood, a mix of three major polymers (cellulose, lignin, and hemicelluloses in roughly a 2:1:1 ratio), is utilized for fibers, building materials, pulp, and as a feedstock for biofuels and biochemicals. Cellulose microfibrils impart tensile strength in the cell walls, with hemicelluloses and lignin supporting the microfibrils along the axis (Salmén et al. 2012). Lignin, a complex polymer with phenylpropanoid subunits of syringyl (S), guaiacyl (G), and hydroxyphenyl (H) types (Whetten et al. 1998), makes up approximately 20 to 30% of the dry weight of wood (Pettersen 1984). The amount of the lignin subunits and the coupling between them are vital to the mechanical strength of wood. However, lignin must be removed at tremendous economic, chemical, and environmental cost in order to access the fibers and cellulose within the wood. Genetic engineering is therefore being utilized to grow healthy trees that develop wood with a modified composition (S/G ratio) or reduced lignin amount. Properties of these trees that are critical for their survival could be severely affected, however, because lignin confers compressive support and stiffness to the cell walls. Mechanical testing of transgenic trees with variable lignin contents and compositions is extremely important to determine whether the changes result in a degraded wood structure and further reduce the survivability of these trees.

Lignin is produced by the phenylpropanoid pathway along with other metabolites such as flavonoids, tannins, and esters (Bonawitz and Chapple 2010). Shuford *et al.* (2012) published an understanding of the monolignol biosynthesis starting from phenylalanine and progressing to the H, G, and S units.
Cinnamate 4-hydroxylase (C4H) hydroxylates cinnamic acid to form p-coumaric acid, the most direct precursor to H monolignols (Russel 1971). C4H is also involved in secondary metabolism pathways, since p-coumarate is also an intermediate for the biosynthesis of many secondary compounds. P-Coumaric acid 3-hydroxylase (C3H) hydroxylates p-coumaroyl ester derivatives at the ring-3 position, leading to coniferaldehyde and G monolignols (Schoch et al. 2001).

Cinnamyl alcohol dehydrogenase (CAD) is an enzyme that facilitates the last step of lignin biosynthesis. CAD reduces cinnamaldehydes into cinnamyl alcohols and potentially reduces sinapaldehydes into sinapyl alcohols before oxidative polymerization as well (Sibout et al. 2005), suggesting two different CAD genes (CAD-C and CAD-D) that regulate cinnamyl and sinapyl alcohols. In studies conducted by Lapierre et al. (1999) and Lapierre et al. (2000), transgenic poplars downregulated for CAD showed no difference in growth; however, an increased proportion of aldehyde compounds were found within the lignin from these trees. CAD down-regulated transgenic tobacco plants had xylem with a significant reddish coloration, possibly caused by polymerized cinnamaldehydes (Yahiaoui et al. 1998).

Primary or secondary methods used for mechanical property testing of wood call for large materials. However, standard size specimens are unobtainable in young specimens grown for genetic modification purposes. Using micromechanical testing, evaluation procedures have been developed to reliably determine the mechanical properties of small diameter trees (Kasal et al. 2007; Horvath et al. 2010) and small strips cut from trees (Burgert et al. 2003). These procedures have given researchers a tool to compare the modulus of elasticity (MOE) of small diameter transgenic trees with a control to better evaluate the properties of such specimens.

The objective of this study was to assess how different chemical contents and compositions, created by genetic manipulations of the lignin biosynthesis pathway, effect the elastic modulus, measured by three-point bending, of six-month-old small-diameter transgenic Populus trichocarpa grown in a greenhouse.

**EXPERIMENTAL**

**Materials**

Young *P. trichocarpa* (Nisqually-1) trees were used for this investigation, including one wild type as a control (PtrWT-1) and the following seven different transgenic groups with low (L), mid (M), and high (H) levels of expression called “lines”:

- **PtrC3H3** - Genetic group with reduced PtrC3H3; 3 genetic lines, (lines 02-H, 05-L, and 10-M)
- **PtrCAD1** - Genetic group with reduced PtrCAD1; 3 genetic lines (lines 02-M, 05-L, and 10-H)
- **PtrCAD2** - Genetic group with reduced PtrCAD2; 3 genetic lines (lines 06-M, 08-L, and 19-H)
- **PtrCAD1&2** - Genetic group with reduced PtrCAD1&2; 3 genetic lines (line 01-L, 07-M, and 15-H)
- **PtrCAD1/CAD2/OMT** - Genetic group with reduced PtrCAD1 and increased PtrCAD2 (using OMT promoter); 3 genetic lines (lines 07-H, 11-M, and 16-L)
- PtrCAD1/CAD2/4CL - Genetic group with reduced PtrCAD1 and increased PtrCAD2 (using 4CL promoter); 3 genetic lines (line 07-H, 21-M, and 30-L)
- PtrC3H3/C4H1&2 - Genetic group with reduced PtrC3H3 PtrC4H1 and PtrC4H2; 3 genetic lines (line 04-L, 10-H, and 13-M)

Sample trees were propagated through rooted cuttings and grown in the greenhouse of the Forest Biotechnology Group at North Carolina State University (Song et al. 2006; Wang et al. 2018). A total of 174 stems, between three and 12 stems from each line, were harvested in July 2012, after 6 months of growth. The lower part of each stem was cut for this investigation, placed in plastic bags, and kept in a freezer until measured to maintain a green condition and prevent fungal degradation.

**Methods**

**Lignin determination and sugar analysis**

The detailed protocol is published elsewhere (Sluiter et al. 2010). Transgenic lines were selected for sugar and lignin analyses, based on the mechanical testing results, and were compared to the wild-type specimens. For each genetic line, approximately three biological replicates (trees) were milled and combined into one sample, and thus three combined samples were obtained from each line. The samples were extracted with benzene/ethanol and hydrolyzed with 3% H$_2$SO$_4$ at room temperature for 1.5 h. The mixture was diluted with deionized water and autoclaved at 121 °C for 1.5 h. The mixture was then filtered through a fine coarseness crucible, and acid-insoluble lignin was determined gravimetrically. The filtrate was used to determine acid-soluble lignin content by UV-VIS absorption. The concentration of sugars (glucose and xylose) in the filtrate was quantified with high performance liquid chromatography (HPLC) using a Shodex sugar SP0810 column, deionized (DI) water as the eluent, flow rate of 0.5 ml/min, injection volume of 20 μL, a column temperature of 80 °C, and refractive index detector at 55 °C. Arabinose and mannose were measured as the sum of the combined concentrations due to the fact that the peaks coeluted. Monomeric sugars were converted to polymeric sugars using the correction factor of 0.9 for glucose and 0.88 for xylose. These analyses were performed in duplicate.

**NMR spectroscopy**

The S/G ratio was reported as shown by Wang et al. (2018) using NMR spectra that were acquired on a Bruker DRX-360 instrument (Karlsruhe, Germany). Acetylated lignins were dissolved in 0.4 mL of acetone; unacetylated lignins were dissolved in acetone and deuterium oxide. The central acetone solvent peak was used as internal reference.

**Static mechanical testing**

A modified ASTM D143 standard method (Kasal et al. 2007; Horvath et al. 2010) was used to determine the static MOE using a three-point bending test on an MTS Alliance RF/300 mechanical testing machine (Eden Prairie, MN, USA) with a crosshead speed of 1.27 mm/min. An MTS Testworks 4 system was used to obtain the load-deflection curve. The three-point bending mechanism’s supports consisted of fixed rollers to allow for primarily vertical reaction forces at the ends of the specimens. The span was adjusted based on a span-to-diameter ratio of 15 and a specially designed bearing block was used to avoid surface crushing of the specimens. As the pith contributes no significant mechanical properties to the stem, the diameter of the pith was measured at the point of loading and
subtracted from the diameter of the specimen. To observe and minimize rotation of the specimen while loaded, a small modified paper clip was attached to the specimen and a small white board with a center mark was placed behind the clip (Horvath et al. 2010). The specimen was repositioned until no rotation was observed.

Static MOE was calculated as follows,

$$\text{Static MOE} = \frac{s(4L^3)}{3\pi(D^4 - d^4)}$$

(1)

where Static MOE is the static modulus of elasticity (MPa), \(s\) is the slope of the linear portion of the load-deflection diagram (N/mm), \(L\) is the span or distance between the two supports (mm), \(D\) is the diameter of the specimen (mm), and \(d\) is the diameter of the pith (mm).

**Experimental data analysis**

Analysis of variance (ANOVA) was used to test the effect of the transgenic modifications on the mechanical and chemical properties of transgenic black cottonwood. The analysis was performed using SAS software (SAS 2009), and Dunnett’s multiple range test at alpha 0.05 was used to compare the mechanical and chemical properties to those of the wild-type.

**RESULTS AND DISCUSSION**

The chemical analyses results are shown in Table 1. Across genetic lines, lignin values ranged from 9.9% for PtrC3H3-05-L to 24.2% for wild-type PtrWT. Lignin values were significantly lower in PtrC3H3-05-L (9.9%), PtrC3H3-10-M (13.5%), PtrCAD1&2-07-M (15.8%), PtrC3H3/C4H1&2-04-L (11.7%), and PtrC3H3/C4H1&2-13-M (13.4%) compared to the wild type. Expressed as a percentage of reduction from the wild-type, the highest reduction was 59% for PtrC3H3-05-L.

Genetic lines with low expression levels of PtrC3H3 and PtrC3H3/C4H1&2 had lower lignin contents than the medium expression in that genetic construct. Unexpectedly, the medium expression of PtrCAD1&2 had a low lignin content, but the low expression level did not.

S/G ratios ranged from 1.86 for PtrCAD2-08-L to 9.93 for PtrC3H3-05-L, with an S/G ratio of 2.67 for wild-type specimens. S/G ratios were large for both the low and middle expression levels in PtrC3H3 construct, PtrCAD1&2-07-M, and PtrC3H3/C4H1&2-13-M.

Glucose values in the genetic lines ranged from 40.8% in PtrCAD1-05 to 53.6% in PtrC3H3/C4H1&2-04-L, with wild-type glucose values of 45.9%. Glucose content was significantly higher in the PtrCAD1&2-L, PtrCAD1&2-07-M, and PtrC3H3/C4H1&2-04-L genetic lines, while all the other genetic lines had no significant change compared to the wild-type.
### Table 1. Percent and Standard Error of Sugars, S/G ratio, Total Lignin, Cinnamaldehyde, and Standard Deviation of MOE of Selected Wild-type and Transgenic Black Cottonwood Clones by Gene Construct

<table>
<thead>
<tr>
<th>Sample</th>
<th>Glucose (%)</th>
<th>Xylose (%)</th>
<th>S/G Ratio</th>
<th>Total Lignin (%)</th>
<th>CA (%)</th>
<th>MOE (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PtrWT-01 (Wild Type)</td>
<td>45.9 ± 0.9</td>
<td>16.3 ± 0.4</td>
<td>2.67</td>
<td>23.2 ± 0.9</td>
<td>4.8</td>
<td>4315 ± 455</td>
</tr>
<tr>
<td>PtrC3H3-02 (H)</td>
<td>49.8 ± 0.5</td>
<td>16.6 ± 1.6</td>
<td>2.27</td>
<td>22.7 ± 2.3</td>
<td>0</td>
<td>4086 ± 443</td>
</tr>
<tr>
<td>PtrC3H3-05 (L)</td>
<td>53.6 ± 0.1*</td>
<td>20.0 ± 0.1</td>
<td>9.93†</td>
<td>9.9 ± 0.1*</td>
<td>0</td>
<td>485 ± 101*</td>
</tr>
<tr>
<td>PtrC3H3-10 (M)</td>
<td>49.8 ± 0.3</td>
<td>17.7 ± 0.5</td>
<td>3.73†</td>
<td>13.5 ± 0.2*</td>
<td>0</td>
<td>1831 ± 372*</td>
</tr>
<tr>
<td>PtrCAD1-02 (M)</td>
<td>43.8 ± 1.3</td>
<td>15.6 ± 0.1</td>
<td>2.67</td>
<td>21.3 ± 0.3</td>
<td>14.9</td>
<td>3752 ± 224</td>
</tr>
<tr>
<td>PtrCAD1-05 (L)</td>
<td>40.8 ± 1.5</td>
<td>16.6 ± 0.3</td>
<td>2.45</td>
<td>21.8 ± 0.2</td>
<td>29.2</td>
<td>1452 ± 133*</td>
</tr>
<tr>
<td>PtrCAD1-10 (H)</td>
<td>42.6 ± 2.3</td>
<td>14.7 ± 0.2</td>
<td>2.26</td>
<td>21.8 ± 1.0</td>
<td>10.3</td>
<td>3347 ± 302*</td>
</tr>
<tr>
<td>PtrCAD2-06 (M)</td>
<td>44.9 ± 3.0</td>
<td>16.3 ± 0.3</td>
<td>2.38</td>
<td>22.4 ± 0.4</td>
<td>8.7</td>
<td>4487 ± 539</td>
</tr>
<tr>
<td>PtrCAD2-08 (L)</td>
<td>42.7 ± 1.3</td>
<td>15.6 ± 0.9</td>
<td>1.86</td>
<td>23.5 ± 0.3</td>
<td>9.4</td>
<td>3487 ± 441</td>
</tr>
<tr>
<td>PtrCAD2-19 (H)</td>
<td>48.0 ± 0.6</td>
<td>14.4 ± 0.0</td>
<td>---</td>
<td>21.5 ± 0.2</td>
<td>10.0</td>
<td>2780 ± 718*</td>
</tr>
<tr>
<td>PtrCAD1&amp;2-01 (L)</td>
<td>42.9 ± 0.5</td>
<td>18.5 ± 0.1*</td>
<td>2.00</td>
<td>20.9 ± 0.8</td>
<td>22.8</td>
<td>2117 ± 336*</td>
</tr>
<tr>
<td>PtrCAD1&amp;2-07 (M)</td>
<td>45.9 ± 0.1</td>
<td>19.8 ± 0.1*</td>
<td>3.02†</td>
<td>15.8 ± 1.7*</td>
<td>30.4</td>
<td>598 ± 198*</td>
</tr>
<tr>
<td>PtrCAD1&amp;2-15 (H)</td>
<td>45.1 ± 0.4</td>
<td>16.2 ± 0.1</td>
<td>---</td>
<td>22.0 ± 0.3</td>
<td>---</td>
<td>2470 ± 373*</td>
</tr>
<tr>
<td>PtrCAD1/CAD2/OMT-07 (H)</td>
<td>42.5 ± 0.6</td>
<td>18.3 ± 0.5</td>
<td>2.49</td>
<td>22.7 ± 0.6</td>
<td>12.3</td>
<td>4110 ± 726</td>
</tr>
<tr>
<td>PtrCAD1/CAD2/OMT-11 (M)</td>
<td>47.5 ± 0.7</td>
<td>14.9 ± 0.6</td>
<td>2.30</td>
<td>20.2 ± 1.9</td>
<td>14.3</td>
<td>2941 ± 1140*</td>
</tr>
<tr>
<td>PtrCAD1/CAD2/OMT-16 (L)</td>
<td>42.1 ± 0.0</td>
<td>16.8 ± 0.0</td>
<td>1.90</td>
<td>23.0 ± 0.3</td>
<td>21.0</td>
<td>3076 ± 333*</td>
</tr>
<tr>
<td>PtrCAD1/CAD2/4CL-07 (H)</td>
<td>42.9 ± 0.5</td>
<td>18.5 ± 0.2</td>
<td>1.98</td>
<td>22.5 ± 0.8</td>
<td>---</td>
<td>2140 ± 307*</td>
</tr>
<tr>
<td>PtrCAD1/CAD2/4CL-21 (M)</td>
<td>45.9 ± 0.1</td>
<td>18.9 ± 0.0*</td>
<td>2.15</td>
<td>23.2 ± 0.3</td>
<td>---</td>
<td>2015 ± 238*</td>
</tr>
<tr>
<td>PtrCAD1/CAD2/4CL-30 (L)</td>
<td>43.8 ± 0.6</td>
<td>17.6 ± 0.1</td>
<td>---</td>
<td>23.5 ± 2.1</td>
<td>---</td>
<td>2480 ± 169*</td>
</tr>
<tr>
<td>PtrC3H3/C4H1&amp;2-04 (L)</td>
<td>52.1 ± 4.1*</td>
<td>18.1 ± 1.5</td>
<td>2.77</td>
<td>11.7 ± 0.4*</td>
<td>0</td>
<td>1090 ± 334*</td>
</tr>
<tr>
<td>PtrC3H3/C4H1&amp;2-10 (H)</td>
<td>51.4</td>
<td>17.7</td>
<td>2.55</td>
<td>---</td>
<td>0</td>
<td>4364 ± 322</td>
</tr>
<tr>
<td>PtrC3H3/C4H1&amp;2-13 (M)</td>
<td>51.3 ± 2.4</td>
<td>18.4 ± 1.1</td>
<td>3.06†</td>
<td>13.4 ± 0.1*</td>
<td>0</td>
<td>1755 ± 224*</td>
</tr>
</tbody>
</table>

**Note:** Asterisk (*) indicates statistically significant result from the wild type using a Dunnett’s comparison test at α=0.05 level. Sugar and lignin components were determined by analytical chemistry for genetic lines. The values for chemistry are means ± standard error of three samples/measurements from each genetic line. †S/G ratio values were compared to those found for natural populations of *Populus trichocarpa* (Huda et al. 2011) of 1.0 to 3.0. CA=Cinnamaldehyde % (Wang et al. 2018). The MOE values are means ± standard deviation of three samples/measurements from each genetic line. --- means that there is no data at this point. (L/M/H) : Low/Middle/High expression level for each construct.

Three biological replicates (stems) were combined into one sample with a total of three samples for each genetic line. One measurement was taken for each sample.

Cinnamaldehyde levels measured for all *PtrCAD* transgenic lines were higher compared to the levels found in the wild-type (~4%) with a high of 30.4% in the PtrCAD1&2-07-M line. Wang *et al.* (2018) observed coniferaldehyde products coupling into the lignin polymer using 2D-NMR data.
Average MOE values of transgenic black cottonwood trees ranged from 485 MPa to 4,487 MPa, while the wild-type MOE was 4,315 MPa (Table 1). Based on statistical analysis, across genetic lines, MOE values were not significantly lower than the wild-type for only six genetic lines: PtrC3H3-02-H, PtrCAD1-02-M, PtrCAD2-06-M, PtrCAD2-08-L, PtrCAD1&2/OMT-07-H, and PtrC3H3/C4H1&2-10-H. Those with significantly reduced MOE values had a 22% to 89% reduction range compared to the wild-type.

The MOE values in this study were within the range of those found in the literature for similar poplar trees (Bendtsen and Senft 1986; Zhang 1997; Peszlen 1998; Xiang 2011; Ozparpucu et al. 2018). As expected, reported MOE values for mature black cottonwood tested at the green condition (FPL 1999) were higher than MOE values of the 6-month old trees in this study. However, Bendtsen and Senft (1986) analyzed individual growth rings of 30-year-old eastern cottonwood, and in the first year of growth found MOE values that were comparable to our results. In poplar downregulated for C4H, it has been suggested that a decrease in tensile stiffness has been associated with a decrease in density (Bjurhager et al. 2010); however, in a diffuse porous species, comparisons between MOE and density were not strongly correlated, as shown in a study by Zhang (1994). Ozparpucu et al. (2018) found lower MOE values measured in tension, ranging from 2,500 MPa in CAD reduced transgenic poplars to 3,000 MPa in the wild-type.

Few studies have investigated the impact of transgenic modifications of the structure and content of lignin on the mechanical properties of wood. Bjurhager et al. (2010) studied young transgenic hybrid aspen clones grown in the greenhouse downregulated for C4H and found a slight but significant reduction in dynamic MOE. Awad et al. (2012) analyzed hybrid poplar with antisense CAD grown in a greenhouse and found a 29% MOE reduction in the CAD poplars. Özparpucu et al. (2017) analyzed CAD reduced poplars and found the MOE to be reduced by 10 to 15% compared to the wild-type. In a separate study with a different type of genetic modification to the CAD gene, Özparpucu et al. (2018) found that CAD downregulated poplars resulted in no change in the MOE in tension even though both lignin content and composition were altered. Our study, using the same C4H and CAD genes, resulted in even higher MOE reductions (75% and 86%, respectively) compared to the wild-type.

Other studies have investigated poplar with lignin modifications using different genes in the lignin biosynthetic pathway. Kasal et al. (2007) evaluated the mechanical properties of one-year-old quaking aspen at about 12% moisture content (MC) with antisense 4CL gene (reduction in lignin) and 4CL+CAld5H (reduced lignin+increased S/G ratio) and found no significant differences in MOE compared to the wild-type. Horvath et al. (2010) also tested two-year-old quaking aspen with the same genotypes as Kasal et al. (2007), at a MC greater than 30% (green condition) but observed a 52% reduction in MOE compared to wild-types. Voelker et al. (2011) examined transgenic hybrid white poplar, with antisense 4CL genes, grown for one to two years in the field and found 62% reductions in MOE at 12% MC compared to the wild-type. Lin et al. (2016) studied the effects that an anionic peroxidase had on lignin and MOE in P. trichocarpa and found reductions up to 60% in comparison to the wild-type.

Even in investigations of xylose and glucose reduced poplars, mechanical properties were also severely reduced even though the lignin content was higher. Li et al. (2011) studied greenhouse grown poplars that exhibited xylose reductions of 60% and increases in lignin by 20% and found that the MOE was reduced by 34%. Xiang (2011) investigated greenhouse grown six-month-old transgenic poplars modified for reduced cellulose and found that it resulted in average glucose reductions of 62%, average lignin
increases of 40%, and an MOE reduction by 50 to 90% All the aforementioned studies on modifications to the lignin, hemicelluloses, or cellulose biosynthesis pathways reported reductions in MOE which indicate that these genetic modifications could affect the bonding of the major chemicals in the cell wall and thus the mechanical behavior of the wood.

Lignin contents of the wild-type specimens in this study were within the range of those values reported in the literature for wild-type P. trichocarpa. Thirty mature wild-type P. trichocarpa trees from three separate sites in British Columbia had lignin contents ranging from 20.27% in sapwood to 23.25% in heartwood by Swan and Kellogg (1986). Studer et al. (2011) found a large range of lignin contents, from 15.7% to 27.9%, after analyzing a total of 1,100 natural mature P. trichocarpa trees in a population from a large geographical distribution. Min et al. (2014) examined wild-type P. trichocarpa with lignin values close to 21%, glucose values of approximately 40%, and xylose values ranging from 10% to 17%. Li et al. (2011) studied wild-type P. trichocarpa trees grown in a greenhouse and reported lignin values ranging from 21% to 24% glucose values ranging from 43% to 45%, and xylose values around 16%.

For our transgenic poplars, the reductions to lignin caused some other major chemical components to have increased values. Decreases in lignin content from 23% to about 10% resulted in glucose contents to increase from 46% to approximately 54%, and increased xylose from 16% to 20%. Min et al. (2014) showed reduced lignin values from 22% to 10% caused glucose content to increase from 43% to 51% and xylose content to increase from 15% to 19% on antisense 4CL transgenic poplars. In a study by Li et al. (2011), transgenic trees with genetic reductions in xylose synthesis decreased xylose content from 16% to 10%, which caused a decrease in glucose content from 43% to 40% and an increase in lignin content from 21% to 30%.

The S/G ratios found in this study had a wide range, and the S/G ratio of 9.93 in PtcC3H3-05-L is the highest reported so far in the literature for P. trichocarpa. Studer et al. (2011) found a range of S/G ratios from 1.0 to 3.0 in a natural population of P. trichocarpa from a large geographical distribution across the northeast United States. Li et al. (2003) analyzed greenhouse grown quaking aspen and reported S/G ratios of 2.2 in the wild-type and 5.2 in transgenic aspen with the sense CAld5H gene. The distribution of phenolic units in lignin shows irregularities which can be traced back to factors influencing the lignin biosynthetic pathway (Lundquist and Parkås 2011). The S/G ratio in lignin is a good indicator of the degree and nature of cross-linking (Ferrer et al. 2008). Lignin that is abundant in G monolignols is highly cross-linked because of the greater proportion of carbon-carbon bonds, whereas S-rich lignin is not as condensed because of ether bonds at the 4-hydroxyl posit ion which are more labile. The higher the S/G ratio, the more easily lignin can be depolymerized because of less available reactive sites (Ziebell et al. 2010). Based on our results, it is plausible that this lower cross-linking within the lignin explains the severe reductions in MOE seen in the C3H genetic groups with high S/G ratios.

MOE value reductions were found in every genetic group with at least one genetic line; however, only nine genetic lines exhibited significant changes in major chemical constituents. PtcC3H3-05-L exhibited drastic reductions in lignin (57%) coupled with a large increase in S/G ratio that resulted in a severe 89% reduction in MOE. PtcC3H4-10-M to a lesser degree also resulted a 58% reduction MOE from a 42% reduction in lignin with an increase in S/G ratio. PtcC3H3/C4H1&2-04-L showed a significant 50% reduction in lignin leading to a large 75% reduction in MOE. It has been hypothesized that a decrease in lignin content could soften the hemicelluloses because of the increase in accessible hydroxyl groups (Kohler and Spatz 2002), which could result in a significantly lower MOE.
at the green condition in low lignin transgenic wood (Horvath et al. 2012). PtrC3H3/C4H1&2-13-M showed a significant 42% reduction in lignin and 59% MOE and an increased S/G ratio. PtrCAD1&2-07-M was the only CAD genotype that had significant reductions in lignin and increased S/G ratio but showed a large increase in cinnamaldehyde which also led to severe reductions in MOE. PtrCAD1&2-01-L displayed an increase in cinnamaldehyde which led to a decrease in MOE. Changes to the chemistry within these trees, such as reduction in lignin content or increases in glucose, xylose, S/G ratio, or cinnamaldehyde content, can significantly decrease the mechanical properties of the stem as a whole. It has been shown that downregulation of the CAD gene leads to increased incorporation of aldehyde groups into the lignin, which suggests that it could reduce lignin interaction with hemicellulose, leading to increased porosity of the cell walls (Carmona et al. 2015) or reduced cross-linking density (Hepworth and Vincent 1998).

CONCLUSIONS

This study used the three-point bending technique to investigate the effect of differing chemical contents and compositions on the elastic modulus of six-month-old small-diameter greenhouse-grown transgenic P. trichocarpa, generated by genetic manipulations of C3H, C4H, and CAD at different levels of expression.

1. Results suggest that a significant decrease in lignin content causes severe reductions in MOE.
2. Changes in lignin structure, through the manipulation of the S/G ratio with greater amounts of the syringyl units, also negatively influence the MOE.
3. Genetic modifications to CAD1 and CAD2 genes resulted in significant MOE reductions, even with no change in lignin content. Incorporation of cinnamaldehyde compounds within the lignin structure may lead to increased porosity and/or reduced cross-linking density within the lignin.

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