

Wood chemical and mechanical responses to modified lignin composition in upright and inclined hybrid poplar

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Abstract

Lignin is an important phenolic polymer in the secondary cell walls. These walls are responsible for the mechanical and some physiological features of the woody tissue in plants. In angiosperms, the predominant monomers of lignin are syringyl and guaiacyl. The ratio of these monomers was found to change in response to external stimuli such as wind or gravity. The latter induces the formation of special type of wood called tension wood. The impact that results from artificially modifying the lignin monomeric ratio was investigated in three types of wood tissues in one-year old hybrid poplar clone 717 (*Populus tremula* x *P. alba*). The clone was transformed to overexpress the F5H/Cald5H gene to different levels resulting in increased syringyl to guaiacyl ratio (S:G). Wild type poplars and the transgenic lines were left upright or inclined 45° from vertical for three months to induce the gravitropic responses including tension wood formation. Wild type poplar stems had 6.4% and 7.6% increase in percent syringyl in tension wood side than normal or opposite wood, respectively. Increasing syringyl formation increased percent acid soluble lignin 2.3 folds. Cell wall crystallinity was also higher in tension wood than the other types of wood tissues. Both tension and opposite woods had higher percent total sugars. Interestingly, in tension wood a 19.1% increase in percent syringyl led to 3.6% decrease in percent total sugars and 4.1% decrease in percent glucose. Percent galactose in tension wood was also higher but dropped 0.1% in response to the lignin monomer modification in the same tissue. Xylose and rhamnose were lower in tension wood than normal wood in wild type stems. Mechanically, the stems modulus of elasticity (MOE) did not change with increased syringyl when tested with 4-point bending or under compression. A decrease in the stems modulus of rupture (MOR) in response to increased S:G ratio was detected. Trees with increased S:G ratio seemed to adjust their stems to gravity faster after inclination. Evaluating the response of these lines to inclination will improve our understanding of the role lignin monomeric composition plays in altering xylem chemical composition and mechanical properties of normal and tension wood.

Introduction

Research is intensifying towards modifying woody plants to meet the increasing demand for an alternative source of biological ethanol. Poplar is considered a relatively faster renewable resource for bulk cellulose. To increase the efficiency to utilize the cellulose in the woody tissues millions of dollars are being put into research to modify genes that control cell wall structure and components.

Lignin is an important phenolic polymer in the secondary cell walls ranking second in abundance on earth behind cellulose. It acts as a cementing material in xylem tissues of trees providing support and insulation (Hacke *et al.*, 2001), thus is a major mechanical and physiological feature of the woody tissues in plants. Lignin is made up of three monomers; syringyl, guaiacyl and *p*-hydroxyphenyl. The latter occurs at higher concentrations in softwoods (Harris, 2005). In hardwoods the predominant monomers of lignin are syringyl and guaiacyl (Mosha and Goring, 1975). To understand the function of each monolignol in tree mechanics and physiology, several studies have looked at the differing ratios of monomers in different tissues or in different species (Yoshinaga *et al.*, 1992). The ratio of these monomers was also found to change in response to external stimuli such as wind (Koehler and

Telewski, 2006) or gravity as presented in this study. The latter induces the formation of special type of wood called tension wood. In the case of inclined trunks, it is formed only on the upper side of the stem while the other side is termed opposite wood. The major anatomical characteristic of tension wood is a unique fiber with an additional inner layer of gelatinous appearance (gelatinous layer), which is mostly composed of cellulose. In this layer the highly crystalline microfibrils are oriented almost parallel to the longitudinal axis of growth or what is referred to as approaching zero degrees (Pilate *et al.*, 2004). The combined mechanical strength of lignin in the opposite wood and potentially the cellulose in the gelatinous fibers of tension wood is necessary for trees to perform gravitropic responses.

Investigating the impact of lignin composition in transgenic lines of the same species will minimize possible bias by other anatomical parameters introduced when different plant species or different growth forms of the same species are compared. Successful attempts have been made to genetically alter the ratio of the monomers keeping lignin levels almost the same in poplar xylem tissues. Ferulate 5-hydroxylase (F5H) is the enzyme that acts downstream in the lignin biosynthetic pathway. It converts the intermediates for the synthesis of the monolignol guaiacyl into others that make the monolignol syringyl (Chen *et al.*, 2000). Up-regulation of the enzyme drives the lignin biosynthetic pathway towards producing more syringyl over guaiacyl without changing the overall lignin content. The purpose was to investigate the impact of modified lignin composition in transgenic hybrid poplar, as a model for woody perennial angiosperm, expressing different levels of syringyl and guaiacyl on cell wall biochemical components in tension, opposite and normal wood.

Materials and Methods

This study was conducted on wild type hybrid poplar (*Populus tremula* x *P. alba*) clone 717 as well as on transgenic lines that were altered to express F5H gene at different levels resulting in higher syringyl to guaiacyl ratio from 68.5% in the wild type to 93.4% in the over-expression lines.

The plant material used in this study was propagated using root sprouts over multiple generations. The level of expression of the F5H gene in the different lines was proven to remain stable over time. Trees with 50-60 cm long stems were inclined 45° from vertical to induce the gravitropic responses including tension wood formation and were grown for 3 months along with uninclined controls. The stem samples were taken 15 cm from soil level to the center of the segments. They were debarked and air-dried at 60°C until constant weight. Stem segments from the inclined trees were longitudinally cut to separate tension wood from opposite wood for independent chemical analyses.

Using an Instron, with 500 N load cell and 20 mm/min crosshead speed, 14 cm stem segments were four-point bended to an extent enough to enable the calculation of flexural stiffness (EI) and the modulus of elasticity (MOE) within the elastic range. Same stems were dried and loaded again to rupture in order to calculate modulus of rupture (MOR). For calculation of the second moment of area (*I*), an idealized oval shape was assumed for the cross sections. Modulus of elasticity was also measured through testing the stems under compressive forces. Cylindrical debarked stem segments, twice as long as their diameter, were exposed to compressive force parallel to the grain using an Instron with 500N load cell at 3 mm/min crosshead speed. The same wood samples tested above were air dried and sent to University of Vancouver at British Columbia, Canada for testing using thioacidolysis technique to determine the syringyl to guaiacyl ratio (S:G) and total lignin content in the various poplar lines. Carbohydrates, cell wall crystallinity and microfibril angle were also quantified in the same samples.

Results and Discussion

Impact on lignin. In this study an increase in syringyl to guaiacyl ratio was found to result from inclining the trees to force the formation of tension wood. The wild type poplar had an average of 6.4% increase in syringyl content in tension wood than normal wood and an average of 7.6% increase than opposite wood. Similar increase in the ratio was also reported on the same type of hybrid poplar trees as a result of mechanically perturbing them to simulate wind sway (Koehler and Telewski, 2006).

The effect of inclination on S:G ratio diminished with increasing level of expression of the F5H gene in the modified trees. Tension wood of the trees with highest expression level had an insignificant 0.6% increase in syringyl compared to tension wood of wild type trees.

When the F5H gene was over-expressed to increase percent syringyl, trees with normal wood had slight, yet significant, decrease in their percent total lignin when compared to the unmodified control group. In another study using 13 different poplars, an evidence was presented showing a strong negative correlation between S:G ratio and the lignin content in those trees (Bose *et al.*, 2009). Percent total lignin (soluble and insoluble) in tension wood was significantly lower than normal or opposite wood. On the other hand and due to enhanced syringyl in the upright trees, percent acid insoluble lignin decreased 5.5%. Percent acid soluble lignin went up 2.3 folds in the same tissue. The increase in the percent soluble lignin was an expected outcome in this investigation since syringyl is considered more reactive than guaiacyl and a positive correlation existed between lignin solubility and syringyl monomer content which resulted in more than 60% reduction in pulping time and less use of hazardous chemicals in the process (Huntley *et al.*, 2003).

Impact on cellulose microfibril angle (MFA) and crystallinity. The wild type as well as the genetically modified trees that were displaced with regard to gravity expressed a typical gravitropic response by producing tension wood and returned to the vertical orientation. In those trees, MFA decreased (more parallel with the stem axis) in tension wood whereas cellulose crystallinity increased which goes along with previous reports (Boyd, 1977; Pilate *et al.*, 2004). However, altering S:G ratio did not impact MFA or crystallinity in the different wood tissues. During this experiment, preliminary data indicated a higher speed of recovery to the vertical position in poplar trees with increasing S:G ratio.

Impact on wood carbohydrate content. Tension and opposite woods had higher percent total sugars. Interestingly, there was a slight, yet significant, interaction between percent syringyl and percent total sugars in tension wood; a 19.1% increase in percent syringyl led to 3.6% decrease in percent total sugars and 4.1% decrease in percent glucose. A syringyl molecule is 16.7% heavier than a guaiacyl molecule. And since glucose is an essential carbon source for lignin biosynthesis, building of syringyl is a higher glucose requiring process than building guaiacyl (Amthor, 2003). Percent galactose in tension wood was also higher than normal wood but then dropped 0.1% in response to the lignin modification. Rhamnose significantly decreased on both sides of the stem (i.e. tension and opposite wood) due to the inclination, but it did not change with elevated syringyl. Both types of treatments, inclination and elevated syringyl, did not seem to have an effect on mannose or arabinose percentages.

Tension wood had significantly lower xylose (16.6%) when compared to opposite or normal wood to the contrary of a previous study by Andresson-Gunnerås *et al.* (2006) who reported that xylose was more abundant in poplar tension wood. They also reported a reduction in hemicellulose in the same tissue as was reported by Timell (1969), hence reduction in xylans and mannans (for review refer to Lerouxel *et al.*, 2006). The syringyl modification did not seem to have an impact on percent xylose in this study.

Compensatory regulation was mentioned to exist between lignin and cellulose deposition representing tree adaptation to sustain mechanical strength when lignin was reduced (Hu *et al.*, 1999) and in tension wood in response to gravitational and mechanical stimuli (Timell, 1986). Hu *et al.* (1999) observations suggested that cellulose synthesis is substrate limited and that the carbon, originally used to build lignin, is instead directed towards cellulose and hemicellulose synthesis when lignin synthesis was suppressed in woody plants, or towards building the gelatinous layer of tension wood (Schubert, 1965). This competition for carbon may explain the reduction in total sugars and glucose percentages in this study when the heavier syringyl was enhanced.

Impact on biomechanics. The stems modulus of elasticity (MOE) did not change with increased syringyl when tested with 4-point bending or under compression. A decrease in the stems modulus of rupture (MOR) in response to increased S:G ratio was detected. Lignin rich in syringyl is considered less condensed due to less branching of the lignin polymer (Abreu *et al.*, 2009), this may explain the lower MOR of the stems in all three poplar lines over-expressing F5H gene.

Conclusion

In addition to the currently used over-expression lines, RNAi-suppressed F5H poplars (*Populus tremula* x *P. alba*) clone 717 were generated in our lab to reduce S:G ratio. After propagating these new lines we will select a range of S:G ratio in those trees to extend our study on the influence of altered S:G on the trees mechanical, physiological and chemical properties. In addition, evaluating the response of these lines to inclination will improve our understanding of the role lignin monomeric composition plays in normal as well as in tension wood.

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