

Supplemental Text 1

We compare the predictions from our model of *P. trichocarpa* with three transgenic studies in the literature for hybrid poplar [1, 2] and tobacco [3].

4CL knockdowns

In hybrid poplar (*(P. tremula × alba)*) a *4CL3* (referred to as *4CL1* in [1]) knockout mutant resulted in a decrease in lignin content, S monolignol subunits, and S/G ratio [1]. A small decrease in lignin content was also predicted in *4CL3* knockdown simulations using our model (Fig S9), and a larger decrease is predicted in knockdowns of both of the *4CL* genes (Fig S8-S9). Our model also predicted a small decrease in the S subunits (Fig S8-S9), but not to the same degree as seen in hybrid poplar [1]. The decrease in S/G ratio observed in hybrid poplar, was not observed in previous studies of transgenic *4CL* knockdown *P. trichocarpa*, where the S/G ratio remained at wildtype levels [4]. However, our model predicted an increase in S/G ratio, which is inconsistent with both what was observed in hybrid poplar [1] and with the predicted S and G subunits, which suggest wildtype S/G ratios (Fig S8-S9). Since we predict S/G ratio separate from the S and G monolignol predictions, there can be cases where the predicted values are inconsistent due to limited amounts of training data. Unfortunately, we did not have any wood composition properties quantified using NMR for the *4CL P. trichocarpa* transgenics, which could explain this inconsistency. This is an area of future improvement for our model.

Tsai et al. also measured a decrease in $\beta - \beta$ linkages and an increase in the *p*-hydroxybenzoates in the *4CL1* knockout hybrid poplar mutants [1]. In knockdown simulations of *4CL3*, our model predicted a similar increase in *p*-hydroxybenzoate, but did not predict a change from wildtype for the $\beta - \beta$ linkages (Fig S9). At knockout levels of both *4CL* genes, however, our model did predict a decrease in $\beta - \beta$ linkages (Fig S8).

AldOMT and CAD single and combinatorial knockdowns

In hybrid poplar (*P. tremula × alba*), wildtype levels of lignin content were measured for *AldOMT* (referred to as *COMT* in [2]) knockdowns, while slight decreases in lignin content were measured in the *CAD* and the combinatorial *AldOMT* and *CAD* gene knockdowns to $\sim 90\%$ and $\sim 85\%$ of wildtype levels respectively [2]. Our model predicted a similar decrease in severe *CAD* knockdowns to $\sim 90\%$ of the wildtype lignin content (Fig S16-S17, S31A). Our model also predicted a slight decrease in the *AldOMT2* knockdown to $\sim 85\%$ of the wildtype lignin content (Fig S29, S31A). Both of these predictions are in good agreement with the measured lignin contents in *P. trichocarpa* (Figs S16-S17, S29). In simulations of the combinatorial knockdown of these genes, our model predicted similar levels of decrease as in the single knockdowns (Fig S31A).

Lapierre et al. observed a severe decrease in $\beta - \beta$ linkages in the *AldOMT* individual and the *CAD* and *AldOMT* combinatorial knockdown [2]. Our model predicted wildtype levels of $\beta - \beta$ linkages in the *AldOMT2* knockdown simulations (Fig S29, S31B), which is inconsistent with what was seen in [2]. The wood composition properties measured using NMR, including the $\beta - \beta$ and $\beta - 1$ linkages, were not measured for the *AldOMT2* transgenic *P. trichocarpa*, which could explain this inconsistency. This is a possible area of future improvement for our model. For severe knockdowns of the *CAD* genes our model predicted a decrease in the number of $\beta - \beta$ linkages, which aligns with the measured values in *P. trichocarpa* (Figs S16-S17, S31B). For the combined *AldOMT2* and *CAD* knockdown, our model predicted a more severe decrease in $\beta - \beta$ linkages than predicted for either of the individual gene knockdowns (Fig S31B), similar to the trend observed in hybrid poplar [2].

In hybrid poplar, *CAD* knockdowns resulted in an increase in $\beta - 1$ linkages, while the measurements from the combined *AldOMT* and *CAD* suggested some decrease in $\beta - 1$ linkages [2]. Our model predicted a similar increase in $\beta - 1$ linkages when *CAD* was knocked down (Figs S16-S17, S31C). A decrease in $\beta - 1$ linkages was predicted for the combined *AldOMT2* and *CAD* knockdown simulations (Fig S31C) consistent with the trend in [2].

***AldOMT* and *CCoAOMT* single and combinatorial knockdowns**

The combined knockdown of the *AldOMT* (referred to as *COMT* in [3]) and *CCoAOMT* genes in tobacco resulted in a decrease in lignin content to ~65% of wildtype levels [3]. This was a greater decrease than observed in either of the individual *CCoAOMT* or *AldOMT* knockdowns which had down to ~70% and ~76% of wildtype lignin content respectively [3]. Our model predicted a similar trend for *P. trichocarpa* (Fig S32A). Individually knocking down the *CCoAOMT* and *AldOMT2* genes resulted in predicted lignin contents down to ~82% and ~87% of wildtype levels respectively. While the combined knockdown had a predicted lignin content down to ~79% of wildtype levels.

Our model also predicted a negative impact to height for the combined and individual knockdowns of the *AldOMT2* and *CCoAOMT* genes (Fig S32B), however, Zhao et al. did not observe any negative impacts to tobacco growth in their transgenics [3]. Changes in the amount of the S and G monolignols were also observed in the individual *CCoAOMT* and *AldOMT* tobacco transgenics, while only small or no changes from wildtype were predicted for *P. trichocarpa* using our model (Fig S19-S22, S29). Changes in the S and G monolignol contents similar to those observed in tobacco have also been observed in hybrid poplars [5, 6]. This is an area for future improvement of our model as we were not able to train the random forests on NMR wood composition data obtained from the *AldOMT2* transgenics or for the *CCoAOMT* gene family transgenics other than the *CCoAOMT1* knockdowns.

References

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