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Genetic architecture underlying the lignin biosynthesis pathway involves noncoding RNAs and transcription factors for growth and wood properties in *Populus*

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Summary

Lignin provides structural support in perennial woody plants and is a complex phenolic polymer derived from phenylpropanoid pathway. Lignin biosynthesis is regulated by coordinated networks involving transcription factors (TFs), microRNAs (miRNAs) and long noncoding RNAs (lncRNAs). However, the genetic networks underlying the lignin biosynthesis pathway for tree growth and wood properties remain unknown. Here, we used association genetics (additive, dominant and epistasis) and expression quantitative trait nucleotide (eQTN) mapping to decipher the genetic networks for tree growth and wood properties in 435 unrelated individuals of *Populus tomentosa*. We detected 124 significant associations ($P \leq$ 6.89E-05) for 10 growth and wood property traits using 30 265 single nucleotide polymorphisms from 203 lignin biosynthetic genes, 81 TF genes, 36 miRNA genes and 71 lncRNA loci, implying their common roles in wood formation. Epistasis analysis uncovered 745 significant pairwise interactions, which helped to construct proposed genetic networks of lignin biosynthesis pathway and found that these regulators might affect phenotypes by linking two lignin biosynthetic genes. eQTNs were used to interpret how causal genes contributed to phenotypes. Lastly, we investigated the possible functions of the genes encoding 4-coumarate: CoA ligase and cinnamate-4-hydroxylase in wood traits using epistasis, eQTN mapping and enzymatic activity assays. Our study provides new insights into the lignin biosynthesis pathway in poplar and enables the novel genetic factors as biomarkers for facilitating genetic improvement of trees.

Introduction

Lignin is a phenylpropanoid-derived phenolic polymer abundant in vascular plants and is a component of the secondary cell wall (Bonawitz and Chapple, 2010). Lignin provides mechanical strength and hydrophobicity to cell walls, enabling trees to grow to great heights and transport water and nutrients over long distances, and plays essential roles in protection from pathogens (Bhuiyan *et al.*, 2009; Ros, 1997). Lignin is also one of the main components of wood, which is the substantial character of perennial woody plants and provides the raw materials for industrial products and renewable energy (Novaes *et al.*, 2010).

The biosynthesis of the lignin monomer starts with the deamination of phenylalanine, resulting in the synthesis of three monolignols: coniferyl, sinapyl and *p*-coumaryl alcohols. In dicots, such as *Populus*, lignin polymers are composed of guaiacyl (G), syringyl (S) and low levels of *p*-hydroxyphenyl (H) units, which are processed from the three monomers, respectively (Campbell and Sederoff, 1996; Voxeur *et al.*, 2015). Some key genes in lignin biosynthesis pathway regulate the lignin content in dicots. For example, down-regulation of *4CL* (4-coumarate: CoA ligase) in hybrid poplar (*P. tremula* × *P. alba*) sharply decreased the amount of lignin and largely changed wood chemistry and wood metabolism (Voelker *et al.*, 2010). Furthermore, down-regulation of *C4H* (cinnamate-4-hydroxylase) in transgenic tobacco reduced the phenylalanine ammonia-lyase (PAL) enzymatic activity by feedback modulation (Blount *et al.*, 2000); phenylalanine concentration also increased the expression of *PAL*, *4CL*, *CCoAOMT* (caffoyl-CoA *O*-methyltransferase) and *CCR* (cinnamoyl-CoA reductase) in *Pinus taeda* (Anterola *et al.*, 2002). While these studies demonstrated the interactions of multiple lignin biosynthetic genes, other aspects of this network require further study, such as the patterns of genetic interaction within the lignin biosynthesis pathway and how the multigene coordinated network functions in wood formation.

Notably, growing evidence suggests that lignin biosynthesis pathway is regulated by various upstream genetic factors. Transcription factors (TFs) have important functions in the regulation of lignin biosynthesis. The presence of AC elements in the promoters of many lignin biosynthetic genes of *Pinus teada*, which can be recognized by MYB TFs, provides evidence of the involvement of common TFs in the regulation of lignin biosynthesis (Patzlaff *et al.*, 2003). Recently, regulation by noncoding RNAs (ncRNAs), such as microRNAs (miRNAs) and long noncoding RNAs (lncRNAs), has also attracted considerable attention. For example, overexpression of miR397a in *P. trichocarpa* down-regulated the expression of 17 *laccase* (*LAC*) genes, resulting in a reduction in lignin content (Lu *et al.*, 2013). In *P. tomentosa*, eight lncRNAs exhibited epistatic effects with 15 phenylpropanoid biosynthesis genes, which contributed to the