

Genetic variations and miRNA–target interactions contribute to natural phenotypic variations in *Populus*

Jinhui Chen^{1,2}, Jianbo Xie^{1,2}, Beibei Chen^{1,2}, Mingyang Quan^{1,2}, Ying Li^{1,2}, Bailian Li^{1,2,3} and Deqiang Zhang^{1,2}

¹National Engineering Laboratory for Tree Breeding, College of Biological Sciences and Technology, Beijing Forestry University, No. 35 Qinghua East Road, Beijing 100083, China; ²Key

Laboratory of Genetics and Breeding in Forest Trees and Ornamental Plants, Ministry of Education, College of Biological Sciences and Technology, Beijing Forestry University, No. 35

Qinghua East Road, Beijing 100083, China; ³Department of Forestry, North Carolina State University, Raleigh, NC 27695-8203, USA

Author for correspondence:

Deqiang Zhang

Tel: +86 10 62336007

Email: DeqiangZhang@bjfu.edu.cn

Received: 24 March 2016

Accepted: 28 April 2016

New Phytologist (2016) **212**: 150–160

doi: 10.1111/nph.14040

Key words: association genetics, epistatic interaction, microRNA (miRNA), *Populus*, single-nucleotide polymorphism (SNP), target gene.

Summary

- Variation in regulatory factors, including microRNAs (miRNAs), contributes to variation in quantitative and complex traits. However, in plants, variants in miRNAs and their target genes that contribute to natural phenotypic variation, and the underlying regulatory networks, remain poorly characterized.
- We investigated the associations and interactions of single-nucleotide polymorphisms (SNPs) in miRNAs and their target genes with phenotypes in 435 individuals from a natural population of *Populus*.
- We used RNA-seq to identify 217 miRNAs differentially expressed in a tension wood system, and identified 1196 candidate target genes; degradome sequencing confirmed 60 of the target sites. In addition, 72 miRNA–target pairs showed significant co-expression. Gene ontology (GO) term analysis showed that most of the genes in the co-regulated pairs participate in biological regulation. Genome resequencing found 5383 common SNPs (frequency ≥ 0.05) in 139 miRNAs and 31 037 SNPs in 819 target genes. Single-SNP association analyses identified 232 significant associations between wood traits ($P \leq 0.05$) and SNPs in 102 miRNAs and 1387 associations with 478 target genes. Among these, 102 miRNA–target pairs associated with the same traits. Multi-SNP associations found 102 epistatic pairs associated with traits. Furthermore, a reconstructed regulatory network contained 12 significantly co-expressed pairs, including eight miRNAs and nine targets associated with traits. Lastly, both expression and genetic association showed that miR156i, miR156j, miR396a and miR6445b were involved in the formation of tension wood.
- This study shows that variants in miRNAs and target genes contribute to natural phenotypic variation and annotated roles and interactions of miRNAs and their target genes by genetic association analysis.

Introduction

Emerging evidence indicates that variants that alter gene expression, rather than variants that alter protein sequences, act as the primary cause of natural variation in complex traits; for example, a recent study in humans found that > 95% of single-nucleotide polymorphisms (SNPs) identified by previous genome-wide association studies (GWASs) were mapped to noncoding sequences (Maurano *et al.*, 2012). Moreover, regulatory variants can affect all known types of transcripts and processes (Gaffney, 2013). Thus, increasing numbers of studies have aimed to show that genetic variants that affect gene expression occur widely in natural populations using systems genetics approaches to explore the effects of regulatory variation on complex traits and disease (Civelek & Lusis, 2014; Albert & Kruglyak, 2015). Many studies of regulatory variation have centered on transcriptional elements, such as promoters and long-range enhancers (Albert & Kruglyak,

2015). However, GWASs of noncoding transcripts, such as microRNAs (miRNAs) (Civelek *et al.*, 2013) and large intergenic noncoding RNAs (Kumar *et al.*, 2013), have revealed that genetic variation in noncoding RNAs also contributes to phenotypic variation of quantitative and complex traits.

Among the noncoding RNAs, miRNAs are single-stranded RNAs of *c.* 21–24 nucleotides. miRNAs act in gene regulation at the post-transcriptional level, playing important roles in cell proliferation, cell differentiation, apoptosis and metabolism (Bartel, 2009). The levels of a mature miRNA can influence a large number of targets and, in turn, affect phenotypes (Todesco *et al.*, 2012), as increased miRNA levels generally associate with decreased target mRNA levels. Recent studies have supported the idea that SNPs can alter primary miRNA (pri-miRNA) processing or maturation, and influence the expression of target genes and the resulting phenotypes (Werner *et al.*, 2010; Todesco *et al.*, 2012). For example, in *Arabidopsis thaliana*, a natural SNP in