Genome-wide identification of auxin-responsive microRNAs in the poplar stem

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Abstract

**Background** Wood (secondary xylem) of forests is widely used in construction, paper making, furniture, and as a feedstock for biofuels. Wood development is strictly regulated by the phytohormone auxin and MicroRNAs (miRNAs). Currently, the regulatory mechanisms of auxin-induced miRNAs during wood formation in tree species remain unclear.

**Methods and results** Here, we investigated the effect of auxin application on wood development in poplar and identified miRNAs in the stems treated with different concentrations (0 mg/L, CK; 5 mg/L, Low; 10 mg/L, High) of indol-3-butyric acid (IBA). High-throughput sequencing showed that the 24-nt sRNAs were the most abundant classes in three types of small RNA libraries. A total of 118 known miRNAs and 134 novel miRNAs were obtained. Sixty-nine unique developmental-related miRNAs exhibited specific expression patterns in response to auxin, which was consistent with the influence of auxin on wood development. 269 target genes of 69 auxin-responsive miRNAs were predicted. Of them, three novel miRNAs had the most number (≥9) of target genes, which correspondingly belong to SPL, GRF and ARF families. The evolutionary relationships and tissue expression patterns of these target genes were further analyzed. The relative expression levels of four representative miRNAs and their partial target genes were confirmed by using RT-qPCR.

**Conclusions** The present study provides new insights into auxin-responsive miRNAs during wood development in trees.

Introduction

The stem of a plant is responsible for mechanical supporting and transporting water, minerals, and glucose from the source organs to the sink organs. For tree species, woody stem contains large amounts of lignocellulosic biomass, which is used as the materials for the industrial production of pulp, paper and biofuels. Stem development in trees undergoes a series of successive and dynamic events, which involve multiple levels of control including (post-)transcriptional regulation and protein-protein interaction [1]. The understanding of the mechanisms underlying wood development is essential for genetic manipulation of wood biomass and its properties.

MicroRNAs (miRNAs) in plant and animals are a class of 20–24 nucleotides (nts) non-coding small RNAs that are generated from hairpin structures by the RNaseIII type enzyme Dicer. Generally, miRNAs control the expression of target genes at the post-transcriptional level through direct cleavage or inhibition of translation in the cytoplasm [2]. Some miRNAs are shown to function in chromatin modification by mediating DNA methylation and also regulate gene expression by targeting their promoters [3, 4]. Plant miRNAs play key roles in almost all aspects of growth and development, ranging from embryogenesis, vegetative growth, organ formation, juvenile-to-adult transition, flowering time determination and senescence and death [5–7].
In the woody model plant poplar, a number of miRNAs are shown to regulate secondary wall biosynthesis during wood formation. In *Populus tomentosa*, the usage of high-throughput sequencing identifies 15 vascular cambium (VC)-related miRNAs in differentiating xylem tissues [8]. Integration analysis of association genetics and expression quantitative trait nucleotide (eQTN) shows that 36 miRNAs are associated with lignin biosynthesis during wood formation [9]. Some miRNAs and their targets are functionally validated. For instance, miR397a from *P. tomentosa* suppresses the expression of lignin-biosynthesis genes *LACs* and lignin accumulation in stems [10]. In hybrid aspen (*P. tremula × P. alba*), *Pta*-miR165/166-targeted *POPCORONA* regulates cell differentiation of xylem and phloem fibers during secondary growth [11]. Overexpression of miR6443 in *P. tomentosa* specifically suppresses the expression of its target gene, *FERULATE 5-YDROXYLASE 2 (F5H2)*, resulting in a significant decrease in S lignin level [12]. *PtoTCP20* is a target gene of miR319a and promotes xylem differentiation via interactions with *PtoWOX4* and *PtoWND6* in *P. tomentosa* [13].

Auxin plays a central role in regulating the initiation and proliferation of vascular cambium and differentiation of the cambial derivative cells during wood formation [1]. A subset of miRNAs are shown to target genes involved in auxin homeostasis and signaling [14]. In Arabidopsis, miR393 and miR847 enhance auxin signaling by cleaving the mRNAs of *TRANSPORT INHIBITOR RESPONSE 1/AUXIN SIGNALING F-BOX PROTEIN 2, 3 (TIR1/AFB2, 3)* and *INDOLE ACETIC ACID 28 (IAA28)*, respectively [15, 16]. MiR160 and miR167 inhibit auxin signaling by cleaving the mRNAs of *AUXIN RESPONSE FACTOR 10, 16, 17 (ARF10, 16, 17)* and *ARF6, 8*, respectively [17]. MiR390 directs the production of trans-acting small interfering RNAs (tasiRNAs) from the mRNA of *TRANS-ACTING SIRNA 3 (TAS3)* to suppress *ARF2, 3, 4* expression [18]. Moreover, multiple feedback points are present in the miR160-miR167/ARF or miR390-TAS3-tasiARF regulatory network. In addition, miR165, 166 are shown to regulate auxin level through activation of *YUC1, 4, 10*, three auxin biosynthesis genes [19]. In rice, OsmiR156 guides the cleavage of *SQUAMOSA PROMOTER BINDING PROTEIN-LIKE 7 (OsSPL7)*, repressing the expression of *OsGH3.8* that catalyzes the ATP-dependent formation of IAA-amino acid conjugates [20]. Compared with Arabidopsis and rice, few studies on auxin-responsive miRNAs were identified in trees.

In this study, we analyzed the influence of exogenous auxin application on wood development and miRNAs expression in the stems of poplar. Sixty-nine unique developmental-related miRNAs exhibited specific expression patterns in response to auxin. Our results may be useful for clarifying the regulatory mechanism of wood formation by auxin in trees.

**Materials And Methods**

**Plant materials and IBA treatment**

*Populus deltoides × P. euramericana* cv “nanlin895”, a widely cultivated poplar in China, was used as the materials. Plantlets with three young leaves were cut from the tips of one-month-old plants and then grown for another month on half-strength MS (Murashige-Skoog) medium supplemented 0, 5 or 10 mg/L of IBA. The growth conditions were as follows: 16/8 h (day/night), 2000 lux, and 25–27°C.
Microscopy

Basal stems (0.5 cm section) of one-month-old poplars were cut and fixed in 4% paraformaldehyde at 4°C, and then embedded in Paraplast following the method described previously [21]. Transverse sections were obtained by using a Leica RM 2235 microtome (Leica). Sections were adhered to microscope slides (Thermo Fisher) overnight at 37°C and stained with 0.1% toluidine blue O (Sigma) for light microscopy (Olympus DX51).

Total RNAs isolation and sRNA sequencing

Basal stems (0.5 cm section) of one-month-old poplars were sampled for miRNA sequencing and real-time quantitative RT-qPCR validation. For each treatment, ten plants were pooled for sampling and three biological replications were set. Total RNAs were isolated according to the method adopted in our lab [21]. RNA quality was analyzed using a NanoDrop spectrophotometer (Thermo Fisher Scientific). Small RNAs (sRNAs) cDNA libraries were constructed and sequenced on an illumina HiSeq 2500 system at the honortech company (Beijing, China). The construction of the cDNA libraries consisted of two steps: (1) Total RNAs were purified using electrophoresis and RNA molecules in a size range of 18–35 nt were enriched. 18- and 26-nt labeled RNA oligonucleotides were used as size standards; and (2) The isolated sRNAs were 5’ and 3’ RNA adapter-ligated, reverse transcribed into cDNA, and amplified by PCR.

Small RNA analysis and miRNAs prediction

Raw reads were transformed into clean reads by removing low-quality reads, adapter reads, contaminants, poly (A) tails, and reads smaller than 18 nt. The remaining sequences were used for searching against Rfam v11 (http://rfam.xfam.org) and miRbase release 21 (http://www.mirbase.org) databases. Then, the abundance of each type RNAs was counted. After discarding the sequences that matched to non-coding rRNAs, tRNAs, snRNAs, and snoRNAs, the high-quality reads were mapped to the *P. trichocarpa* genomic sequence (Phytozome v13, http://www.phytozome.net/poplar) using the SOAP software (http://soap.genomics.org.cn). Known miRNAs were determined by searching the above sequences against miRbase release 21. Novel miRNAs were identified using Mirdeep2 with plant parameters in miREvo. MFE (minimal folding free energies) of precursor miRNA secondary structure was predicted using RNAfold.

Differential expression analysis of miRNAs

To determine the differential expression of miRNAs among different treatments, raw expression data were normalized, log2-transformed and identified with the R package edgeR [22]. Parameters were set as |logFC (fold change)| > 1 and adjusted *p* < 0.05 (Benjamini-Hochberg adjustment).

Target genes prediction and GO and KEGG pathway analyses

Target genes of differentially expressed miRNAs were predicted using patmatch software. Functional annotations of these target genes were analyzed based on the JGI v2.0 database. Gene Ontology (GO)
enrichment analysis was conducted to compare the enrichment rank for each term. All targets were mapped to the terms in the database (http://www.geneontology.org/) and the number of targets for each GO term was calculated with a corrected $p \leq 0.05$. A Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis was performed to make out metabolic pathways with significant enrichment using the calculation formula and parameters in GO analysis [23].

**Quantitative real time PCR (RT-qPCR)**

Reverse transcription of mRNA and miRNA was performed using Evo M-MLV RT Mix Kit and miRNA 1st Strand cDNA Synthesis Kit (Accurate Biotech), respectively. The expression levels of miRNAs and their target genes were detected by RT-qPCR assays using PerfectStart Green qPCR SuperMix Kit (Transgen) on the Applied Biosystems QuantStudio™ Real-Time PCR detected system (Thermos Fisher). *U6* and *UBQ* genes were used as reference genes. Primers are listed in Supplemental Table 4. The relative transcript expression was determined by the $2^{-\Delta\Delta CT}$ method.

**Results**

**Stem development of poplar under different concentrations of IBA treatment**

To investigate how auxin treatment affects stem development in poplar, we analyzed one-month-old plants grown on 1/2 MS medium with 0 (control, CK), 5 (low concentration treatment, LT) or 10 (high concentration treatment, HT) mg/L of IBA. Compared with CK plants, LT plants were higher but HT plants were shorter (Supplemental Fig. 1). Observation of stem sections revealed that both CK and HT plants had a discontinuous cambium, which was occasionally seen in LT plants (Fig. 1). Furthermore, IBA treatment led to a significant increase in xylem width of LT plants but a reduction in xylem width of HT plants, which was correlated with the alterations in stem diameter of these plants. These results indicated that stem development of poplar was promoted by low concentration of IBA treatment but inhibited by high concentration of IBA treatment.

**Deep sequencing of sRNAs in IBA-treated poplar stems**

To examine the changes of miRNAs in the poplar stem after IBA treatment, we generated and sequenced three sRNA libraries that were derived from the stems treated with three concentrations of IBA. Raw read totals of 11553428, 11366870 and 11708291 from CK, LT and HT, respectively, were obtained. After the removal of the adaptors, contaminants, polyA sequences and sequences smaller than 18 nt, 1984978 (CK), 1851509 (LT) and 2001996 (HT) clean reads remained for further analysis. Of the 18 to 32 nt sRNA clean reads, the majority of them were in the range of 21 to 25 nt in length (Fig. 2). 24-nt sRNAs were the most abundant classes in each library, comprising 48.4% (CK), 46.9% (LT) and 49.3% (HT) of the total number of sRNAs. The proportion of 21-nt sRNAs in the stems of HT plants and 24-nt sRNAs in the stems of LT plants were visibly smaller than that in the stems of CK plants (Fig. 2). Using various RNA databases, sRNA sequences that matched to the *Populus* genome were classified as miRNAs, tRNAs, rRNAs, small nucleolar RNAs (snoRNAs) and small nuclear RNAs (snRNAs) (Fig. 3). Of them, non-
annotated small RNAs varied from 62.9 to 69.0% of the total reads and from 90.8 to 92.4% of the unique reads in the IBA-treated stems. These non-annotated small RNAs were used for prediction and identification of candidate novel miRNAs.

Identification of known and novel miRNAs in IBA-treated poplar stems

Among the *P. trichocarpa* sequences in the miRBase 21 and Rfam v11 databases, 118 mature miRNAs belonging to 87 miRNA families were identified in the three sRNA libraries (Supplemental Table 1). The largest two miRNA families were Ptr-let7 (8 members) and Ptr-miR30 (5 members). The let7 genes play diverse roles in biological processes, including cell proliferation, growth, development and immune [24]. Highly expressed let7 family members in the poplar stem suggested functional constraint and may be involved in multiple biological activities. Notably, a majority (57.6%) of miRNA families had only one member. The Mirdeep2 software was employed to screen novel miRNAs from candidates by exploring the secondary hairpin structure, the Dicer cleavage site and the minimum folding free energy index (MFEI, > 0.85). As a result, 134 novel miRNAs were obtained in the three samples (Supplemental Table 1). The average MFEI value of novel miRNAs in each library was 0.98 ± 0.19. These miRNAs displayed diverse responses to auxin. For instance, the expression levels of ptr-novel-miR-30-3p stayed basically unchanged with or without IBA, while ptr-novel-miR53-5p and ptr-novel-miR85-3p were induced by IBA treatment. Further, different members of a given miRNA family (e.g. ptc-miR169ac) displayed different expression patterns in response to auxin (Supplemental Table 1). U (uridine) is the dominant biased base of miRNAs recognized by the AGO1 protein [25]. We here showed that more than 50% of conserved or novel miRNAs began with a 5’ U, and this ratio was lower in novel miRNAs than in conserved miRNAs.

Differentially expression analysis of miRNAs among IBA-treated poplar stems

To determine the effect of auxin treatment on miRNA expression in the poplar stem, the relative abundances of IBA-treated (CK, LT and HT) miRNA sequences were compared. MiRNAs with adjusted *p* < 0.05 less than 0.05 and fold changes less than 0.5 or greater than 2 were considered to be differentially expressed miRNAs (DE-miRNAs). Compared with CK plants, 16 miRNAs showed altered expression levels in the stems of LT plants, with half of them being upregulated (Fig. 4a). Sixty-eight DE-miRNAs were identified in LT versus CK plants, and 59 DE-miRNAs were identified in HT versus LT plants. Of the 59 DE-miRNAs, 50 miRNAs showed altered expression in HT compared with CK plants. Notably, 5 common DE-miRNAs (ptr-miR1, ptr-miR451, ptr-miR486, ptr-novel-miR32-5p and ptr-novel-miR50-5p) were found among the three types of DE-miRNAs (Fig. 4b).

Sixty-nine unique miRNAs with altered expression levels among the IBA-treated stems were normalized and clustered. The heatmaps revealed that nearly all of known miRNAs in the stems of CK and LT plants showed similar expression patterns, and 94.2% of them had lower expression levels than those in HT plants (Fig. 5a). Among these DE-miRNAs, members of let7 family ptr-let7b, ptr-let7d, ptr-let7e, ptr-let7f, and ptr-let7g in HT plants were upregulated relative to CK and LT plants. In contrast, a majority of novel miRNAs in CK had similar expression to those in HT plants, whose expression patterns are opposite to those of novel miRNAs in LT plants (Fig. 5b). Of them, 4 auxin-responsive and development-related
miRNAs were further analyzed, including ptr-novel-miR24-5p (a homolog of Arabidopsis miR156), ptr-novel-miR39-3p (a homolog of maize miR396), ptr-novel-miR53-5p (a homolog of poplar miR169) and ptr-novel-miR28-3p (a homolog of Arabidopsis miR408). MiR156 is well known for controlling the meristem cell fate transition in Arabidopsis [26] and lignin content change in poplar [27]. In our study, higher expression levels of ptr-novel-miR24-5p were detected in the stems of LT plants than in those of CK and HT plants. The poplar miR169 and miR396 are most highly expressed during the vascular cambium differentiation stage [8]. Overexpression of miR396 in switchgrass leads to reduced plant height and lignin content by targeting *GROWTH-REGULATING FACTOR1, 3, 9* [28]. We showed that the expression level of ptr-novel-miR39-3p was repressed, while the expression of ptr-novel-miR53-5p was induced with IBA treatment. MiR408 post-transcriptionally regulates the expression of laccase-like multicopper oxidase family members *LAC3, LAC12, and LAC13*, which are responsive for lignin polymerization [29]. We here found lower expression level of ptr-novel-miR28-3p in the stems of HT plants than in those of CK plants.

**Prediction of auxin-responsive miRNA targets in the poplar stem**

To examine the functions of miRNAs with altered expression among CK, LT and HT, we predicted their putative targets using patmatch software (Supplemental Table 3). A total of 59 unigene sequences were predicted to be the targets of 10 DE-miRNAs in LT versus CK, 68 unigene sequences to be the targets of 19 DE-miRNAs in HT versus CK, and 123 unigene sequences to be the targets of 27 DE-miRNAs in HT versus LT. The number of predicted targets varied from 1 to 26 per miRNA and most had two to three targets. Of them, 60 target genes for 12 DE-miRNAs sequences were functionally annotated (Table 1). These target genes involved multiple developmental processes, such as cell wall formation, signal transduction, and transcriptional regulation. We found that ptr-novel-miR23-5p, ptr-novel-miR50-5p and ptr-novel-miR117-5p had lower expression levels in the stems of HT plants than in those of CK plants. They had the most number (≥ 9) of target genes, which correspond to SPL, GRF and ARF families. Members of the SPL family have emerged as pivotal regulators of diverse biological processes in plants, including the timing of vegetative and reproductive phase change, leaf development, tillering/branching, plastochron, panicle/tassel architecture, fruit ripening, fertility and biomass [30]. GRFs function as key regulators of multiple biological processes including flower and seed formation, stem and leaf development, and the coordination of growth processes under adverse environmental conditions [31]. ARFs are the components of the auxin signaling pathway and they participate in almost all developmental processes in plants [32]. These findings implied the diverse roles for the ptr-novel-miR23-5p-targeted *SPL*, ptr-novel-miR50-5p-targeted *GRF* and ptr-novel-miR117-5p-targeted *ARF* modules in poplar.
Table 1
Target prediction and annotation of partial miRNAs with altered expression among IBA-treated poplar stems.

<table>
<thead>
<tr>
<th>miRNA</th>
<th>Target genes</th>
<th>Arabidopsis homolog</th>
<th>Annotations</th>
<th>Samples</th>
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<td>ptr-miR-320d</td>
<td>Potri.001G404600</td>
<td>AT1G52870</td>
<td>peroxisomal membrane 22 kDa family protein</td>
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<tr>
<td></td>
<td>Potri.008G182500</td>
<td>AT1G13190</td>
<td>RNA-binding family protein</td>
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<td></td>
<td>Potri.006G032500</td>
<td>AT4G15560 (CLA1)</td>
<td>1-deoxyxylulose 5-phosphate synthase</td>
<td>HT/CK, HT/LT</td>
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<td>ptr-miR-451</td>
<td>Potri.004G019500</td>
<td>AT1G61820 (BGLU46)</td>
<td>Phenylpropanoid biosynthesis</td>
<td>LT/CK, HT/CK, HT/LT</td>
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<td>AT1G76130 (AMY2)</td>
<td>1,4-alpha-D-glucan glucanohydrolase</td>
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<td>ptr-novel-miR−23−5p</td>
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<td>Squamosa promoter binding protein-like transcription factor</td>
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Note: CK, plants treated with 0 mg/L IBA; LT, plants treated with 5 mg/L IBA; HT, plants treated with 10 mg/L IBA.
<table>
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Note: CK, plants treated with 0 mg/L IBA; LT, plants treated with 5 mg/L IBA; HT, plants treated with 10 mg/L IBA.
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Note: CK, plants treated with 0 mg/L IBA; LT, plants treated with 5 mg/L IBA; HT, plants treated with 10 mg/L IBA.

To gain insight into the tissue expression patterns of the 41 SPL, GRF and ARF genes, a comprehensive analysis was conducted based on Populus microarray data [33]. Nine genes do not have the corresponding probe sets in the microarray dataset, and the expression profiles of remaining 32 genes were thus studied (Fig. 6). Most genes demonstrated distinct tissue specific expression patterns except
for mature leaves, where all have low transcriptional levels. Sixteen paralogous pairs (six in SPLs or GRFs and four in ARFs) were identified in the 41 target genes. These gene pairs displayed two distinct expression patterns. In the first category which covered two gene pairs (Potri.003G172600/Potri.001G055900 and Potri.003G065000/Potri.003G065000), two gene duplicates shared almost identical expression patterns with respect to the tissues examined. The second category contained eight gene pairs. The expression patterns of the two counterparts in each gene pair were partially overlapping but different.

**Validation of partial miRNAs and their target gene expression**

To confirm the expression of identified miRNAs and their target genes in response to auxin, ptr-miR451 (target gene: Potri.004G019500), ptr-novel-miR23 (target gene: Potri.012G100700), ptr-novel-miR50 (target gene: Potri.006G115200) and ptr-novel-miR117 (target gene: Potri.010G223200) were selected for RT-qPCR analysis. The results revealed that the four miRNAs had different expression patterns after IBA treatment and each miRNA exhibited opposite auxin response to its target gene (Fig. 7). Compared with CK plants, the expression of ptr-miR451 decreased in the stems of LT plants but increased in those of HT plants. The expression levels of ptr-novel-miR23 stayed unchanged in LT plants and decreased in HT plants, while the expression of ptr-novel-miR50 and ptr-novel-miR117 increased in LT plants but decreased in HT plants. These results were in agreement with our sequencing data.

**Discussion**

In tree species, a number of miRNAs have been identified in tissues or under certain environmental conditions [6]. However, little research has been conducted on auxin-responsive miRNAs during wood formation on a genome-wide scale. In this study, we revealed that in poplar wood development was promoted by low concentration of auxin application but inhibited by high concentration of auxin. We further investigated the expression patterns of conserved and novel miRNAs subjected to auxin treatment during wood formation. Approximate 2 million unique sRNAs reads were obtained from three IBA-treated stem samples using high-throughput sequencing technology. The 24-nt sRNAs constituted the most abundant class, which was in agreement with previous finding that the xylem tissue during active growth has a major peak at 24 nt in poplar [34, 35]. Similarly, in Arabidopsis, rice, peanuts and alfalfa 24-nt sRNAs were substantially more abundant than 21-nt sRNAs [36–39]. Among the *P. trichocarpa* sequences in the miRBase 21 and Rfam v11 databases, we identified 118 conserved miRNAs and 134 novel miRNAs in the poplar stem.

Auxin is a predominant hormone that induces cambium formation and xylem differentiation during wood formation [1]. Of 252 miRNAs identified, the expression levels of 69 miRNAs were significantly altered in response to auxin. We focus on 4 auxin-responsive miRNAs (ptr-novel-miR24-5p, ptr-novel-miR39-3p, ptr-novel-miR53-5p and ptr-novel-miR28-3p). The homologs (miR156, miR396, miR169 and miR408) of the four miRNAs in poplar or other species are associated with cambium activity and lignin accumulation. In
poplar, miR169-targeted *PagHAP2-6* regulates cambium dormancy in response to ABA [40], implying the crosstalk regulation of cambium activity by auxin- and ABA-induced miR169. Overexpression of miR156 in poplar results in a 30% decrease in lignin content in stems, with lower syringyl (S)-to-guaiacyl (G) monolignol ratio [27]. In switchgrass, overexpression of miR396 leads to reduced plant height and lignin content [28]. In Arabidopsis, overexpression of miR408 results in diminished lignification of the vascular bundle [29]. Since auxin regulates lignin biosynthesis during secondary cell wall formation [41], it is speculated that these poplar miRNAs might be related to auxin-induced lignin biosynthesis during wood formation.

Sixty target genes for 12 auxin-responsive miRNAs were annotated in this study. Of them, we further analyzed ptr-novel-miR23-5p that targeted SPL family members, ptr-novel-miR50-5p that targeted GRF family members and ptr-novel-miR117-5p that targeted ARF family members, because they had the most number of target genes. Some of SPLs, GRFs and ARFs are reported to play key roles in lignin biosynthesis during secondary wall formation. For instance, in switchgrass suppression of *PvSPL2* activity by miR156 increases biomass yield and reduces lignin accumulation [42]. MiR396-targeted *PvGRF1*, 9 regulates plant height and G-lignin content [28]. In poplar, the PtoAUX/IAA9-PtoARF5-PtoHB7/8 module regulates xylem expansion, vessel formation and lignin biosynthesis during wood development [43]. Suppression of *PtrARF2.1* expression by RNAi upregulates the expression of most of lignin biosynthesis genes [44]. We here showed that several poplar *SPL*, *GRF* and *ARF* genes exhibited high expression in stems, implying the potential roles of these gene mediated by miRNAs in secondary wall biosynthesis. This speculation need to be further investigated.

**Conclusion**

In the present study, we investigated how wood development is affected by different concentrations of IBA treatment, and identified known and novel auxin-responsive miRNAs in the poplar stem. We found that sixty-nine unique developmental-related miRNAs exhibited specific expression patterns in response to auxin. The target genes of the 69 miRNAs were predicted, and the relative expression levels of four miRNAs and their partial target genes were confirmed by RT-qPCR. These results add to our knowledge of auxin-responsive miRNAs during wood formation and may be useful for improving wood biomass in trees.

**Declarations**

**Acknowledgements**

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Author contribution

LY, WL and SS performed the experimental work. TP, QW and WL contributed the bioinformatic analyses. GC gave some critical suggestions on this manuscript. YB and YC designed the study and drafted the manuscript. All authors read and approved the final manuscript.

Data archiving statement

The small RNA raw sequence data can be available at the NIH Short Read Archive database (http://www.ncbi.nlm.nih.gov/sra) under the BioProject accession number PRJNA850678.

Competing interest

The authors declare that they have no conflict of interest.

Ethical statement

This article does not contain any studies with human participants or animals performed by any of the authors.

References


Figures

Figure 1

Effects of auxin treatment on stem development in poplar. Stem sections were sampled from the poplars grown in 1/2 MS with 0 (control, CK), 5 (low concentration, LT) or 10 (high concentration, HT) mg/L IBA. Xy, Xylem. Bar= 200 μm.
Figure 2

Size distribution of unique small RNAs (sRNAs) sequences in IBA-treated poplar stems. CK: plants treated with 0 mg/L IBA; LT: plants treated with 5 mg/L IBA; HT: plants treated with 10 mg/L IBA.
Figure 3

Sequence classification of total and unique reads in IBA-treated poplar stems. tRNA, transfer RNA; snoRNA, small nucleolar RNA; snRNA, small nuclear RNA; rRNA, ribosomal RNA; miRNA, microRNA. CK: plants treated with 0 mg/L IBA; LT: plants treated with 5 mg/L IBA; HT: plants treated with 10 mg/L IBA.
Figure 4

Differentially expressed miRNAs (DE-miRNAs) among IBA-treated poplar stems. a miRNAs with up-regulated and down-regulated expression in LT (plants treated with 5 mg/L IBA) versus CK (plants treated with 0 mg/L IBA), HT (plants treated with 10 mg/L IBA) versus CK or HT versus LT. b Venn diagram of DE-miRNAs in (a).
Figure 5

Heatmap of known and novel miRNAs in IBA-treated poplar stems. a, b The expression levels of known (a) and novel (b) miRNAs were normalized to total RPM. High (red) or low (green) expression levels were established based on normalized data generated using Genesis 1.8.1. Color scale at the top of the dendrogram represents log\(^{2}\) expression values. CK: plants treated with 0 mg/L IBA; LT: plants treated with 5 mg/L IBA; HT: plants treated with 10 mg/L IBA.
Figure 6

Tissue expression patterns of target genes of three representative miRNAs. Evolutionary analyses were conducted using the Neighbor-Joining method in MEGA7. Heatmap shows the expression profiles of target genes in different tissues based on ePlant software (http://bar.utoronto.ca/eplant_poplar/). ML, mature leaves; YL, young leaves; Rt, roots; DS, Dark-grown seedling; DSL, Dark-grown seedling, exposed to light for 3hr; LS, Continuous light-grown seedling; FC, female catkins; MC, male catkins; XL, differentiating xylems.
Figure 7

RT-qPCR validation of four miRNAs and their target genes in IBA-treated stems. The expression levels of miRNAs and target genes in CK plants were arbitrarily set to 1. Each experiment was performed with three replicates. Error bars represent the standard deviation of three replicates. Reference genes were *U6* for miRNAs and *UBQ* for target genes.
Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementalFigure1.tif
- SupplementalTable1.xlsx
- SupplementalTable2.xlsx
- SupplementalTable3.xlsx
- SupplementalTable4.xlsx