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ORIGINAL ARTICLE

Identification and expression analysis of the *PtGATL* genes under different nitrogen and carbon dioxide treatments in *Populus trichocarpa*

Juanfang Suo[1,2] · Shuang Zhang[1,2] · Caifeng Xu[3,4] · Ruhui Chang[1,2] · Xiuyue Xu[3,4] · Guanjun Liu[3,4] · Chuanping Yang[1,2] · Zhiru Xu[1,2,3] · Chunpu Qu[3,4,5]

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Abstract

Pectin is one of the most important components of the plant cell wall. Galacturonosyltransferase-like (GATL) is an important enzyme involved in forming pectin in *Arabidopsis thaliana*. In this study, 12 *PtGATL* genes were identified and characterized based on the *Populus trichocarpa* genome using bioinformatics methods. The results showed that the PtGATLs contained four typical motifs, including DXD, LPPF, GLG, and HXXGXXKPW. According to phylogenetic analysis, PtGATLs were divided into six groups. Chromosome distribution and genome synteny analysis showed that there were 11 segmental-duplicated gene pairs with repeated fragments on chromosomes 2, 5, 7, 8, 10, and 14. Tissue-specific expression profiles indicated that these *PtGATLs* had different expression patterns. The transcription level of *PtGATLs* was regulated by different carbon dioxide and nitrogen concentrations. In conclusion, the identification and analysis of *PtGATL* genes in poplar provide important information on the gene function.

Keywords Bioinformatics analysis · Expression pattern · Galacturonosyltransferase-like · Poplar

Introduction

The cell wall is a semi-rigid structure surrounding plant cells, mainly composed of cellulose, hemicellulose, and pectin, and plays an important role in plant growth and development (Showalter 1993; Willats et al. 2000; Debra

Zhiru Xu and Chunpu Qu contributed to the work equally and should be regarded as co-corresponding authors.

✉ Zhiru Xu
xuzhiru2003@126.com

✉ Chunpu Qu
qcp_0451@163.com

Juanfang Suo
243672692@qq.com

Shuang Zhang
zs18846795221@163.com

Caifeng Xu
xucaifeng0610@126.com

Ruhui Chang
crh1107@126.com

Xiuyue Xu
15663593162@163.com

Guanjun Liu
liuguanjun2013@nefu.edu.cn

Chuanping Yang
yangcp@nefu.edu.cn

[1] Key Laboratory of Saline-Alkali Vegetation Ecology Restoration (Northeast Forestry University), Ministry of Education, Harbin 150040, People's Republic of China

[2] College of Life Science, Northeast Forestry University, Harbin 150040, People's Republic of China

[3] State Key Laboratory of Tree Genetics and Breeding (Northeast Forestry University), School of Forestry, Northeast Forestry University, Harbin 150040, People's Republic of China

[4] School of Forestry, Northeast Forestry University, Harbin 150040, People's Republic of China

[5] College of Forestry, Guizhou University, Guiyang 550025, People's Republic of China

2008). Many genes are involved in plant cell wall synthesis, including glycosyltransferase (GT) (Kong et al. 2011). Galacturonosyltransferase-like (GATL) is a member of the GT family. GATL is similar to α-1, 4-galacturonidase-transferase, which transfers galacturonic acid from uridine 5'-diphosphogalacturonic acid to the pectic polysaccharide homogalacturonan (Sterling et al. 2001). According to previous reports, *GATLs* might be involved in the biosynthesis of cell wall polysaccharides (Yin et al. 2010). Multiple sequence alignment (MSA) analysis showed that the amino acid sequences of GATLs in many species were highly similar, and the genomic sequences almost contained no introns (Cheng et al. 2018). GATL contained several conserved domains, including motifs related to nucleoside diphosphate (NDP)-sugar donor binding (DXD) (Wiggins and Munro 1998), as well as enzyme catalytic sites (HXXGXXKPW, LPP) and GLG motifs (Sterling et al. 2006).

In *Arabidopsis thaliana*, nine of ten *AtGATL* genes (except *AtGATL4*) were expressed in roots, stems, leaves, and flowers (Kong et al. 2011). However, *AtGATL4* was only expressed in flowers. Previous research suggested that *AtGATL1* was involved in xylan synthesis (Brown et al. 2007; Lee et al. 2007; Kong et al. 2009). The contents of GalA (galacturonic acid) in the cell wall of the *atgatl3*, *atgatl6*, and *atgatl9* mutant plants decreased, suggesting that *AtGATLs* were involved in the process of cell wall biosynthesis (Kong et al. 2011). *GhGATLs* could regulate plant growth and fiber elongation through the synthesis of pectin in *Gossypium hirsutum* (Zheng et al. 2020).

In *Glycine max* and *Oryza sativa*, many abiotic stresses could induce the expression of *GATL* genes (Liu et al. 2016). *SaGATL9* was induced by oxidative stress in *Sedum alfredii* (Han et al. 2016). Most *EgGATLs* were induced by drought, temperature, and abscisic acid (ABA) in *Eucalyptus grandis* (Cheng et al. 2018). In *Populus deltoides, PdGATL1.1* and *PdGATL1.2*, the closest orthologs to the *Arabidopsis GATL1* gene, had also been proven related to xylan synthesis (Kong et al. 2009).

Exogenous nitrogen (N) had an effect on the biosynthesis of plant cell walls (Euring et al. 2012; Lu et al. 2019). High nitrogen conditions could lead to increase in cell wall polysaccharide synthesis (Pitre et al. 2007, 2010). Carbon dioxide (CO_2) assimilated by photosynthesis was the primary source of carbon in cell wall polysaccharides (Delmer and Haigler 2002). The synthesis of the cell wall was regulated by the carbon supply (Fujimoto et al. 2015). Although the concentration of exogenous N and CO_2 affected the synthesis of polysaccharides in cell wall, as well as the role of *GATL* in regulating plant cell wall polysaccharide content had been studied, there were no relevant reports on the relationship between *PtGATL* genes and N and CO_2 concentration. In this study, we identified 12 *PtGATL* genes in *Populus trichocarpa* genome. We characterized their phylogenetic relationships and analyzed the gene structure, chromosomal location, tandem repeats, and expression patterns. Finally, we investigated the *PtGATL* expression patterns in response to different concentrations of N and/or CO_2 by RT-qPCR. The results laid a theoretical foundation for the study of the function of *PtGATL* genes in synthesis of cell wall polysaccharide in *P. trichocarpa*.

Materials and methods

Identification and analysis of *P. trichocarpa* GATL family members

The HMM (hidden Markov model) file Glyco_transf_8 (PF01501) of *PtGATL*s was obtained in the Pfam database (Finn et al. 2006). The poplar GATLs were searched with AtGATL sequences (obtained from the *Arabidopsis* TAIR database) as a reference using the hmmsearch command of the HMMER v3.1 software (Potter et al. 2018). The basic characteristics of PtGATL amino acids were analyzed with an online ExPASy program (Wilkins et al. 1999). The subcellular localization of these proteins was predicted with the online tool WOLF PSORT (Horton et al. 2007). Gene ontology (GO) annotation of *PtGATLs* was conducted using Blast2GO v5.2 software. All PtGATL protein sequences were uploaded to Blast2GO and BLAST in the NCBI database. After drawing and annotating, GO results and visualization images were downloaded. All procedures were conducted with parameters as default.

Gene structure, MSA and phylogenetic analysis

The genomic and coding sequence (CDS) of *PtGATL* genes were downloaded from Phytozome and the distribution patterns of introns and exons were analyzed using GSDS (Hu et al. 2015). The conserved motifs of PtGATL protein sequences were predicted using MEME software (Bailey et al. 2009). The PtGATL amino acid sequences were aligned and the conserved motifs of DXD, LPPF, GLG and HXXGXXKPW were checked via Clustal X (Thompson et al. 1997). The phylogenetic tree was constructed using the GATL sequences of *P. trichocarpa*, *A. thaliana* (At3g06260, At1g13250, At1g19300, At3g50760, At1g02720, At3g62660, At3g28340, At1g24170, and At1g70090), and *O. sativa* (Os03g18890, Os03g24510, Os07g45260, Os04g44850, Os02g50600, Os03g47530, and Os06g13760) via neighbor-joining (NJ) algorithm with MEGA v7.0.14 software (Kumar et al. 2016).

Table 1 Parameters of the 12 identified *PtGATL* genes and deduced polypeptides present in the *P. trichocarpa* genome

Gene name	Locus name phytozome v3.0	NCBI ID	Amino acid no	Molecular weight (Da)	Isoelectric points	Aliphatic index	GRAVY	Chromosome location	Cellular localization
GATL1	Potri.002G132900	XP_002302469.1	353	39,582.20	5.53	90.68	−0.01	Chr02:9905736..9907441(−)	Chloroplast
GATL2	Potri.002G200200	XP_002302739.1	367	41,896.16	8.7	90.3	−0.083	Chr02:16118679..16121875(+)	vacuole
GATL3	Potri.005G128000	XP_006383257.1	352	39,335.35	7.62	92.05	0.008	Chr05:10115658..10116864(+)	Chloroplast
GATL4	Potri.007G031700	XP_002310780.1	352	39,016.82	7.15	89.26	0.017	Chr07:2407677..2409311(+)	Vacuole
GATL5	Potri.008G018100	XP_002311936.2	347	39,593.80	9.08	93.54	−0.103	Chr08:964665..965731(−)	Chloroplast
GATL6	Potri.008G116900	XP_002312381.1	346	39,939.00	9.22	90.72	−0.14	Chr08:7499038..7500651(−)	Extracellular
GATL7	Potri.008G192600	XP_002311789.1	378	42,986.23	8.67	86.56	−0.147	Chr08:13407056..13409029(+)	Vacuole
GATL8	Potri.010G038300	PNT14564.1	383	43,555.81	7.63	87.99	−0.194	Chr10:6514461..6516051(−)	Chloroplast
GATL9	Potri.010G129400	XP_002314884.1	342	39,035.83	9.03	90.99	−0.111	Chr10:14463092..14464803(+)	Extracellular
GATL10	Potri.010G242300	XP_002315420.1	348	39,947.29	9.26	95.49	−0.088	Chr10:21882013..21883143(−)	Chloroplast
GATL11	Potri.014G040300	XP_006375055.1	362	40,441.26	5.36	91.96	0.02	Chr14:3287281..3289072(−)	Extracellular
GATL12	Potri.014G125000	XP_002320324.1	368	42,205.55	8.93	92.17	−0.086	Chr14:9634408..9636377(+)	Chloroplast

Chromosome distribution and gene duplication of *PtGATL* genes

The chromosomal location of *PtGATLs* was retrieved from Phytozome and PopGenIE (Sjodin et al. 2009), which were used to construct the chromosome distribution map of *PtGATLs* using the MG2C 2.0 tool. Multicollinearity scanning toolkits (Krzywinski et al. 2009) were used to analyze gene duplication events on a Linux system.

Plant materials and treatments

The plant material *P. trichocarpa* was obtained from the State Key Laboratory of Tree Genetics and Breeding (Northeast Forestry University, Harbin, China). The seedlings, which were almost 15 cm in height, were cultured for 21 days after being rooted in hydroponic culture. Then, the seedlings were moved into a hydroponic box filled with modified 1/2 nitrogen-free Hoagland nutrient solution (Liu et al. 2015). The culture was carried out in a greenhouse under the condition of a 16 h light/8 h darkness cycle and a stable temperature of 25 °C. Finally, the seedlings were treated for 28 days and the methods were as follows: 0.1 or 5 mM NH_4NO_3 was added to Hoagland nutrient solution (Ehlting et al. 2007; Euring et al. 2014); at the same time, the CO_2 concentration was adjusted to 400 ppm or 800 ppm under each nitrogen concentration treatment (Klaiber et al. 2013; Caldera et al. 2017). During this period, the 1 mM NH_4NO_3 and 400 ppm CO_2 served as the control (Liu et al. 2015; Zhang et al. 2021), and the nutrient solution was renewed every 3 days. The poplar roots, stems, and leaves were sampled. The collected tissue samples were immediately frozen in liquid nitrogen and stored in a − 80 °C freezer for further analysis. The biological replicates were repeated in triplicate to ensure the reliability of the results.

Analysis of *PtGATLs* expression patterns

The tissue-specific expression patterns of *PtGATLs* in mature leaves, young leaves, roots, nodes, and internodes were retrieved from PopGenIE (Sjodin et al. 2009), and the visual images were constructed. The bar graphs of *PtGATL* genes in *P. trichocarpa* were generated by RT-qPCR data. Expression values of roots (R), stems (S), and leaves (L) were normalized to the relative expression of *PtUBQ7*.

Total RNA was extracted from roots, stems, and leaves of *P. trichocarpa* seedlings using the OMEGA kit (Plant RNA Kit; OMEGA). The RNA concentration was measured by spectrophotometer (NanoDrop 2000/2000c) and the RNA quality was examined using 1% agarose gel electrophoresis. cDNA was synthesized using extracted RNA (1 μg) with Prime Script™ RT kit (including RNase-free DNase I) (Takara Bio, Dalian, China). Based on the full-length cDNA sequence of *PtGATLs*, *PtUBQ7* and *PtCDC2* in *P. trichocarpa* genome database, oligonucleotide primers were designed by Primer Premier 5.0 software. The primer sequences used in this study for RT-qPCR are presented in Table S1. As the expression profiles of *PtUBQ7* and *PtCDC2* were stable, the *PtUBQ7* was selected as the reference gene (Wu et al, 2015). Based on the SYBR Green fluorescence program, the RT-qPCR experiment was performed using the UltraSYBR Mixture reagent (CWBIO, Beijing, China). The total reaction volume was 20 μL. The specific reaction systems were as follows: 95 °C for 10 min, then 95 °C for 15 s, 60 °C for 1 min for 45 cycles. Meanwhile, each reaction was repeated in triplicate (Chen et al. 2020a, b; Leng et al. 2021). The $2^{-\Delta\Delta CT}$ method was used to analyze the RT-qPCR amplification data (Livak and Schmittgen 2001). TBtools (Chen et al. 2020a, b) was used to generate heat maps of gene expression.

Results

Identification and analysis of the *PtGATL* gene members in *P. trichocarpa*

To identify the *GATL* genes in *P. trichocarpa*, we used *GATLs* of *A. thaliana* as probe sequences. 12 *PtGATLs* were identified in *P. trichocarpa* genome database. *PtGATL1–PtGATL12* were named in terms of their positions on the chromosome. The physical and chemical properties of the 12 *PtGATL* genes were analyzed (Table 1). The results showed that the putative PtGATL protein sequences contained 342 ~ 383 aa. The isoelectric points ranged from 5.36 to 9.26. According to the GRAVY scores, 3 (PtGATL3, PtGATL4, PtGATL11) of 12 PtGATLs were hydrophobic proteins, while the others were hydrophilic. The molecular weight of GATL proteins ranged from 39 to 44 kDa. The amino acid sequences similarity of the PtGATL family members was from 48.40 to 91.16% (Table S2), while PtGATL11 had the highest similarity with PtGATL1. The predicted proteins were localized in chloroplasts, vacuoles, and the extracellular spaces (Table 1). MSA of the 12 PtGATLs was performed and the conserved motifs were confirmed. The PtGATL family contained four conserved motifs, including DXD, LPPF, GLG and HXXGXXKPW (Fig. 1). In addition, all PtGATLs contained a conserved serine residue (Ser308)

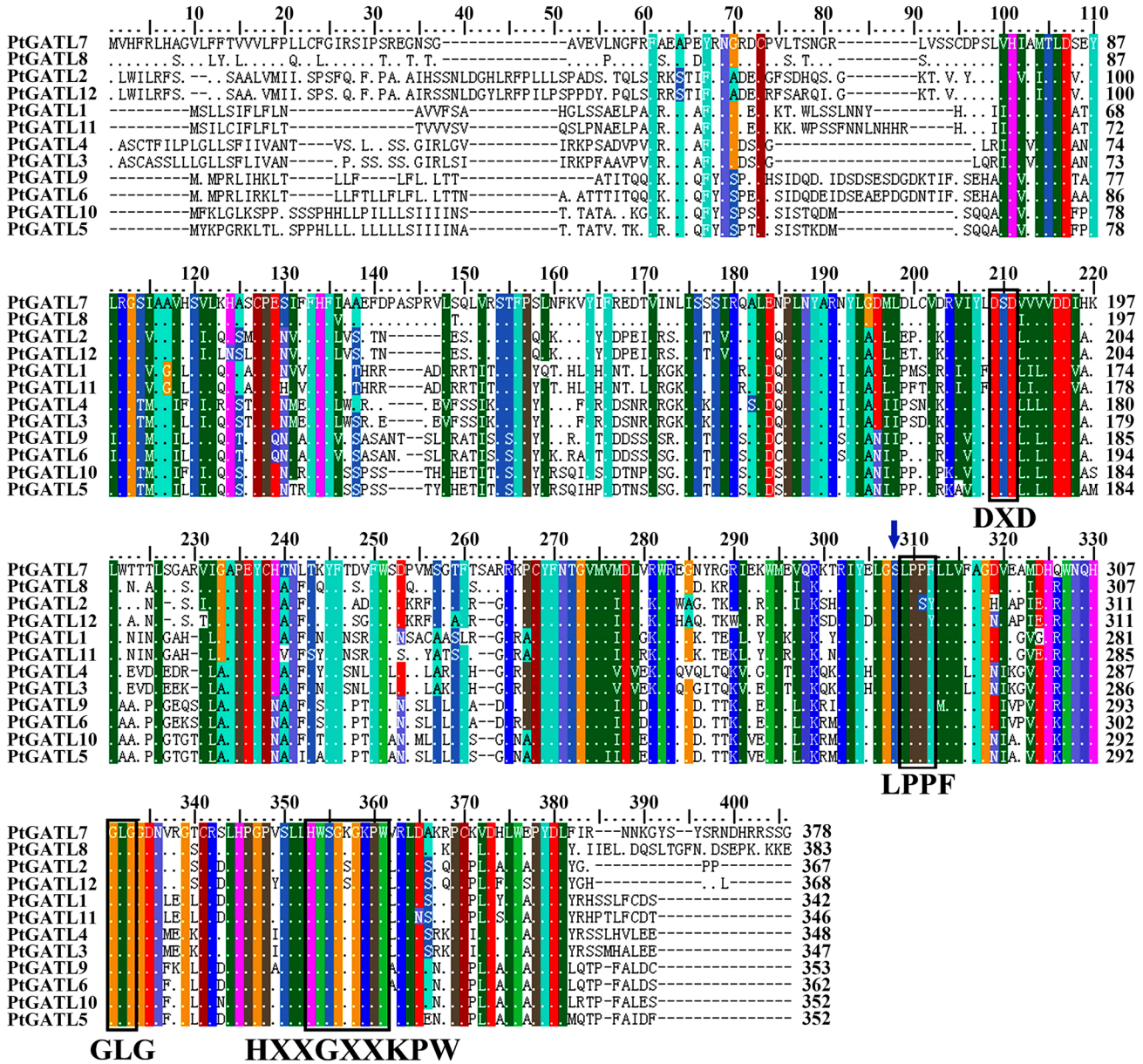


Fig. 1 Multiple alignments of the 12 identified PtGATL proteins. Conserved DXD, LPPF, GLG, and HXXGXXKPW motifs are represented by boxes and the blue arrow represents the putative serine (Ser308)

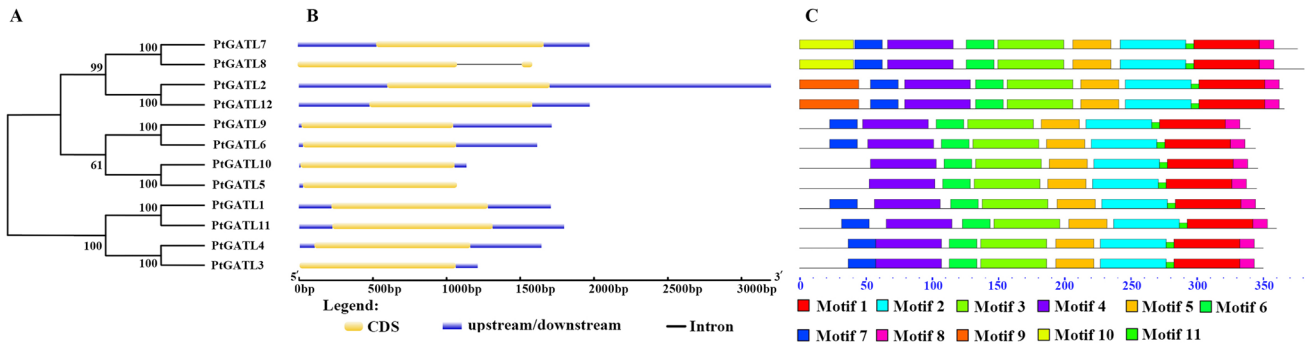


Fig. 2 Phylogeny and structure analysis of the *PtGATLs* in *P. trichocarpa*. **A** The phylogenetic tree was generated based on the full-length sequences of PtGATLs using the neighbor-joining method. **B** Structure of the corresponding *PtGATLs*. CDSs and the upstream/downstream sequences are represented by the yellow and blue lines, respectively. **C** Motifs in PtGATLs sequences predicted by online MEME tool

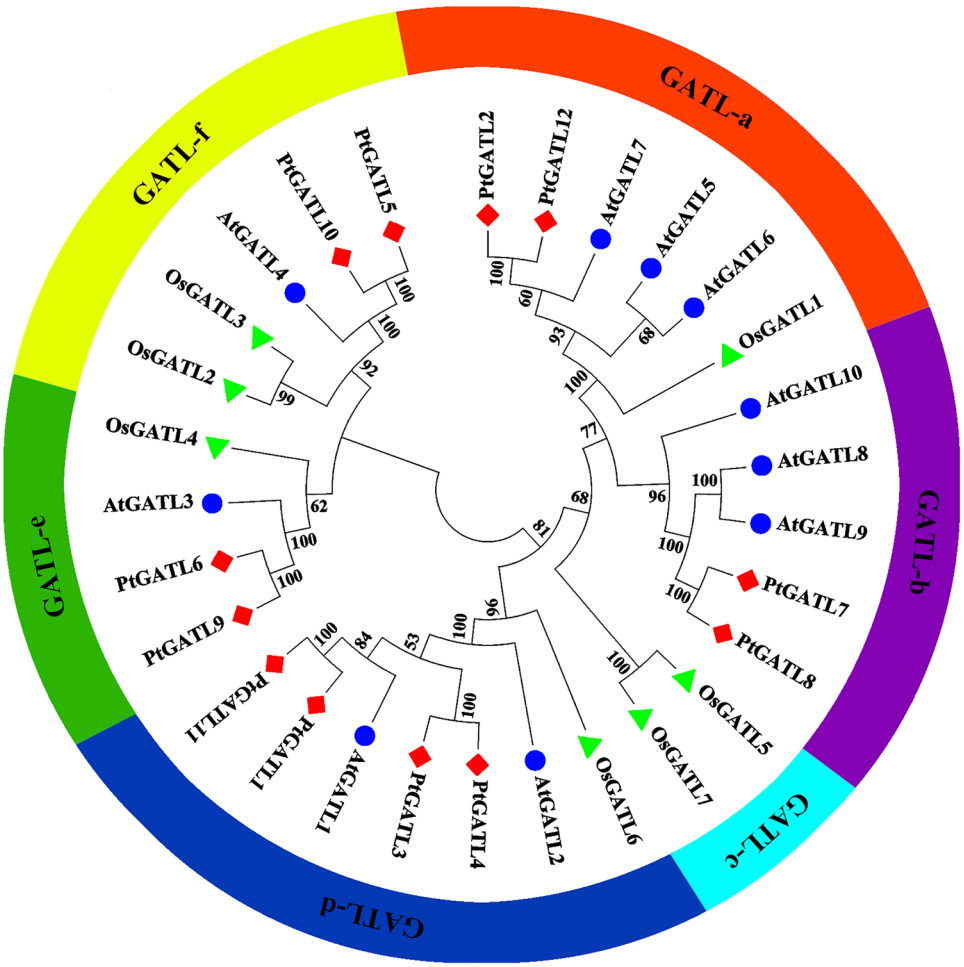


Fig. 3 Phylogenetic analysis of GATL proteins in *P. trichocarpa*, *O. sativa*, and *A. thaliana*. The phylogenetic tree was divided into six distinct subclasses. The red diamond represents PtGATLs, the blue dot represents AtGATLs, and the green triangle represents OsGATLs

upstream of the LPPF motif. However, the function of the GLG motif was not yet clear and required further experimental investigation.

The GO annotations were divided into three categories: cellular components, molecular functions, and biological processes. Detailed information about the GO annotation of the PtGATL proteins was provided in Table S3. For cellular components, 12 proteins were located in the cytoplasm, intracellular anatomical structure, organelle, and endomembrane. Four proteins were located in the membrane and

intrinsic component of the membrane. In the molecular function category, all PtGATLs family members participated in transferase activity (Fig. S1). These results indicated that the PtGATLs might play a significant role in transferase activity in poplar. However, there was no information about biological processes in GO annotations of *PtGATL* genes.

Gene structure, MSA and phylogeny analysis of the *PtGATL* genes

To further understand the evolutionary relationships, we analyzed the PtGATL family members in *P. trichocarpa* using phylogenetic analysis (Fig. 2A). In addition, all members (except *PtGATL8*) had no intron structures and the gene lengths were short (Fig. 2B), which was similar to the absence of introns in the *AtGATL* genes. Eleven conserved motifs were analyzed in PtGATL protein sequences using MEME (Fig. 2C; Table S4), and all PtGATL proteins had the same 11 motifs. The conserved motifs GLG and HXXGXXKPW were identified in motif 1. The conserved motifs LPPF and DXD were found in motifs 2 and 3, respectively. The nucleophilic receptor Ser308 was identified in motif 2. These results indicated that the conserved structural sequences were highly consistent with predicted conserved motifs of PtGATL proteins.

The GATLs of *P. trichocarpa*, rice, and *Arabidopsis* were analyzed, and a phylogenetic tree was constructed (Fig. 3). All these GATL proteins were divided into six subclasses: GATL-a, GATL-b, GATL-c, GATL-d, GATL-e and GATL-f. The number of members in each subclass ranged from two to seven. Among these, GATL-c only included two OsGATLs and GATL-d included four PtGATLs, while the rest of the subclasses included two PtGATLs.

Chromosome distribution and synonymy analysis of *PtGATL* genes

To understand the evolutionary process of the *PtGATL* genes, their chromosome locations were mapped in the poplar genome (Figure S2) and the *PtGATL* duplication was analyzed using MCScanX (Fig. 4). The results showed that 12 *PtGATL* genes were mapped onto six chromosomes (chr2, 5, 7, 8, 10, and 14) in *P. trichocarpa*. However, tandem duplications were not identified in these genes. The segmental duplication analysis results showed that 11 segmental

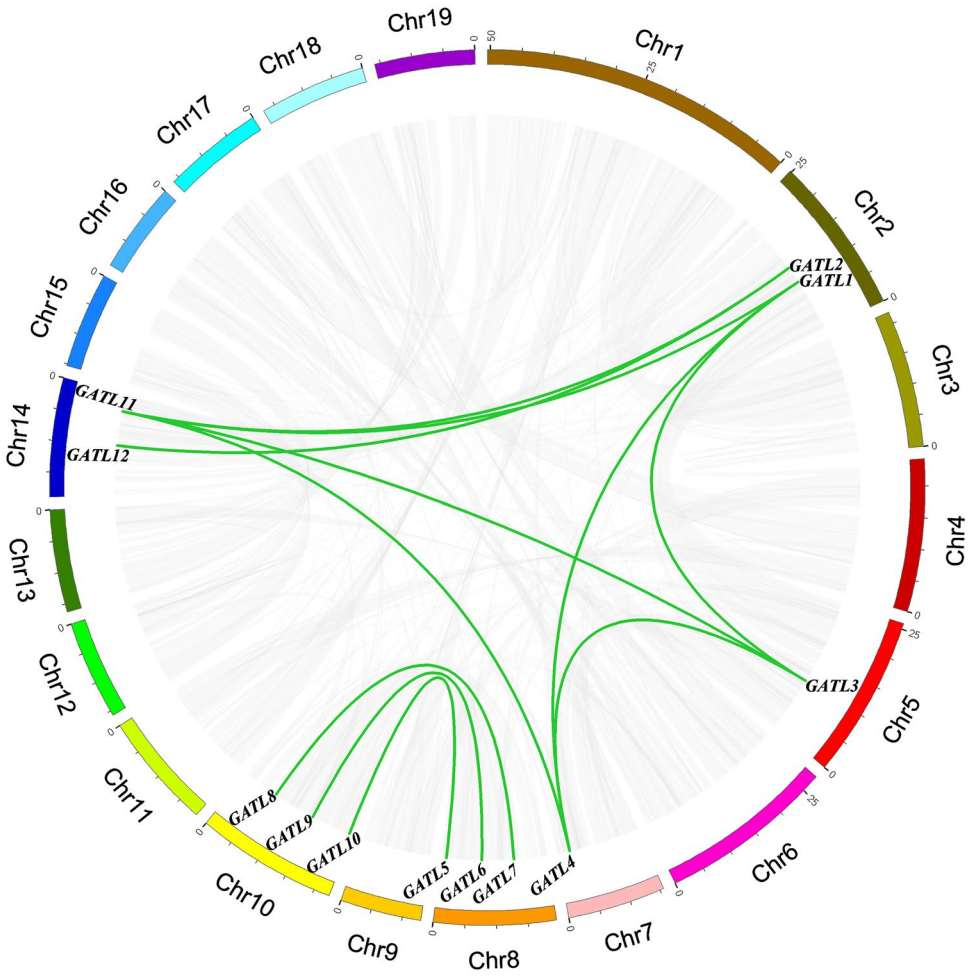


Fig. 4 Schematic representations for segmental duplications of *PtGATL* genes. Gray and thick green lines indicate all syntenic blocks between each chromosome in *P. trichocarpa* genome and the duplicated *PtGATL* gene pairs, respectively. The black font at the end of the green line and the scale bar marked on the chromosomes represent the genes name and the length of the chromosome (Mb), respectively. Chromosome numbers are shown at the bottom of each chromosome

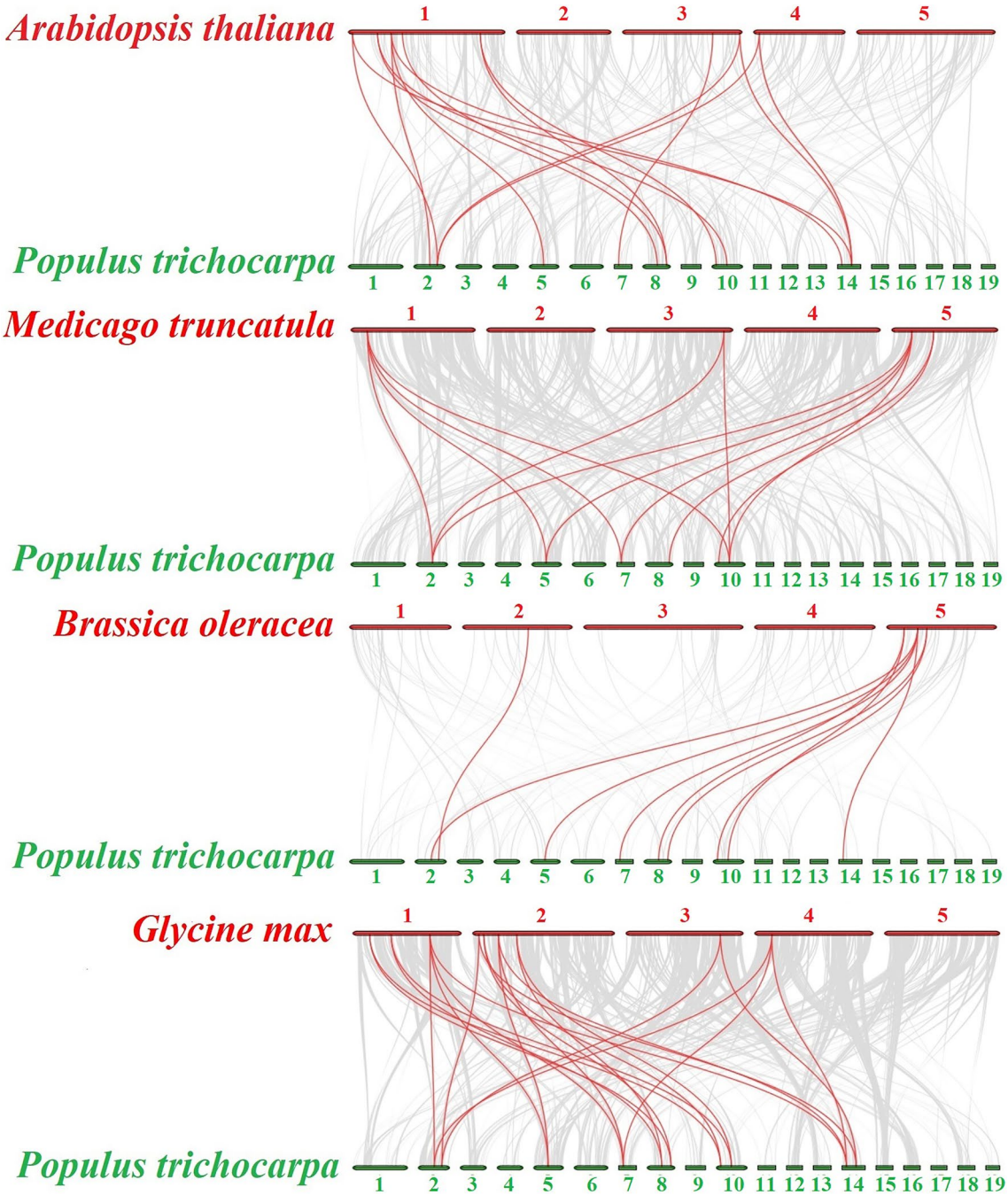
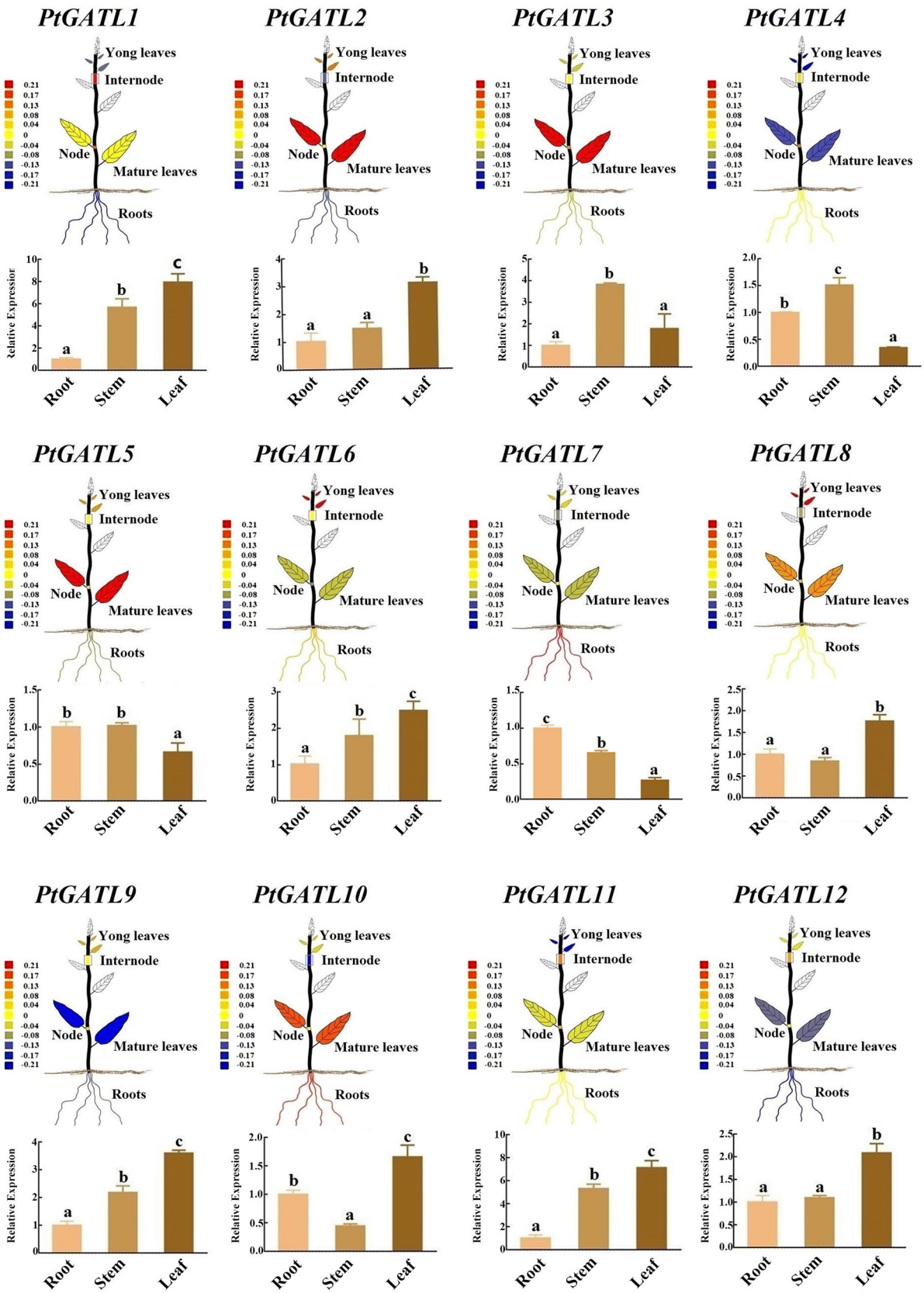


Fig. 5 Synteny analysis of *GATLs* between *P. trichocarpa, A. thaliana*, *G. max*, *B. oleracea*, and *M. truncatula*. Gray lines in the background and red lines represent collinear blocks among *P. trichocarpa* and other plant genomes, as well as *GATL* gene pairs, respectively. Red or green lines represent chromosomes which are marked with the chromosome number at the top or bottom. The species names are on the left

duplications of the 12 *PtGATL* genes were located on six of the chromosomes. For example, *PtGATL1/12, PtGATL1/4, PtGATL1/3, PtGATL2/11, PtGATL2/12, PtGATL3/11, PtGATL3/4, PtGATL4/11, PtGATL5/10, PtGATL6/9* and *PtGATL7/8* had segmental duplications on Chr2/14, Chr2/7, Chr1/5, Chr2/14, Chr2/14, Chr5/14, Chr5/7, Chr7/14, Chr8/10, Chr8/10 and Chr8/10, respectively. These results suggested a part of *PtGATL*s expansion through segmental duplication, which was the main way of duplication of *PtGATL* genes.

In addition, we constructed four comparative syntenic maps of the *GATL* gene family of *P. trichocarpa* associated


PtGATL1
PtGATL2
PtGATL3
PtGATL4
PtGATL5
PtGATL6
PtGATL7
PtGATL8
PtGATL9
PtGATL10
PtGATL11
PtGATL12
Yong leaves
Internode
Node
Mature leaves
Roots
Relative Expression
Root
Stem
Leaf

◂**Fig. 6** Visual images of the tissue-specific expression traits of *PtGATL* genes. Tissue-specific expression data of roots, internodes, nodes, mature leaves, and young leaves derived from PlantGenIE. Tissue-specific expression analysis on roots, stems, and leaves was performed using RT-qPCR and the bar graphs were generated. The expression level of each gene in plant roots was normalized to 1.0, and significant differences in gene expression level are indicated by letters

with four dicotyledonous plants (*A. thaliana*, *Glycine max*, *Medicago truncatula*, and *Brassica oleracea*) (Fig. 5). Most *PtGATL* genes showed a syntenic relationship with those four species. The numbers of genes with syntenic relationships between *P. trichocarpa* and the other four dicotyledonous plants were ten (*A. thaliana*), six (*M. truncatula*), nine (*B. oleracea*), and ten (*G. max*), respectively.

Tissue-specific expression analysis of *GATL* genes in the *P. trichocarpa* genome

To analyze the possible roles of the *PtGATLs* in the developmental processes of *P. trichocarpa*, tissue-specific expression of the *PtGATL* genes was analyzed (Fig. 6). We used the RT-qPCR method to further verify the previous microarray data (Fig. 6). The results from these two analyses were roughly the same. The expression traits of *PtGATL2/8/12* were similar, with high expression in leaves. On the other hand, *PtGATL7* had the lowest expression in roots but higher level in leaves. The expression of *PtGATL1/2/6/8/9/10/11/12* was higher in leaves, while *PtGATL3/4* were higher in stems. The expression level of *PtGATL5* was higher in roots and stems.

To study the responses of *PtGATL* genes to different concentrations of N and CO_2, the expression patterns of *PtGATLs* were analyzed under different treatments (Fig. 7). The expression levels *PtGATL1/2/9/10/11/12* were significantly up-regulated, while *PtGATL3/4* were significantly down-regulated under ambient CO_2 and low N treatments in roots. Under high CO_2 and low N treatments, the expression of only three genes (*PtGATL1/2/12*) was up-regulated significantly in roots. Under ambient CO_2 and high N treatments, the transcription levels of *PtGATL3/4/10* were significantly down-regulated in roots. Under high CO_2 and high N treatments, almost all *PtGATLs*, except *PtGATL2/9*, were significantly down-regulated in roots. The expression of *PtGATL5/6* was significantly induced only under ambient CO_2 and high N treatments in roots. The expression of *PtGATL3/4* was significantly down-regulated, and *PtGATL2* was significantly up-regulated under all treatments in roots. In stems, the expression of *PtGATL7/10* was significantly decreased only under high N treatments. The expression of *PtGATL1/2* was significantly increased and the expression patterns were similar under high CO_2 and low N, as well as high CO_2 and high N treatments. The transcription level of *PtGATL3* was significantly down-regulated under the four treatments in stems. In leaves, the transcription level of *PtGATL1* was significantly down-regulated under high CO_2 treatments. Under ambient CO_2 and low N conditions in leaves, the expression of only *PtGATL6* was significantly down-regulated.

Discussion

Nitrogen could affect the biosynthesis of plant cell walls (Aerts et al. 1995; Euring et al. 2012; Lu et al. 2019). The cell wall, as the plant's largest carbon pool, is also regulated by carbon supply (Showalter 1993; Delmer and Haigler 2002; Verbancic et al. 2018). Under the co-treatment of CO_2 and N, carbon distribution in the plant and the cell wall structure was changed (Luo et al., 2005, 2010). In this study, we identified 12 *PtGATL* genes via bioinformatics methods and studied the changes of their transcription levels under different concentrations of exogenous N and CO_2. Our results provided important information about the *PtGATL* genes expression by N and CO_2 treatments, and provided new insights into how carbon and nitrogen affected polysaccharides synthesis in the cell wall during plant developments.

MSA showed that the conserved structure sequences of the PtGATLs included DXD, LPPF, GLG, and HXXGXXKPW (Fig. 1, Table S4). The DXD motif and HXXGXXKPW motif were believed to interact with Mn^{2+} and bind to NDP-sugar donors (Persson et al. 2001; Gibbons et al. 2002; Yin et al. 2010). LPPF was a motif unique to GATL, which might have some important roles in maintaining the integrity of the binding pocket and/or in catalysis. All PtGATL proteins contained a conserved serine residue (Ser308), which might be the nucleophilic receptor for GATL proteins (Yin et al. 2010). GLG motif might be involved in protein–protein interactions during dimer formation (Persson et al. 2001; Gibbons et al, 2002).

Whole-genome analysis showed that the 12 genes located on the six chromosomes had segmental duplication to varying degrees and without tandem duplication (Fig. 4, S2). These results indicated that segmental duplication was the main driving force for the expansion of the *PtGATL* genes. The synonymous map of the *GATLs* of *P. trichocarpa* and the four dicot species showed that at least six genes were collinear with other species (Fig. 5), suggesting that these *PtGATL* genes might have played an essential role in the evolution of the *GATL* gene family in poplar.

Previous studies had found that there might be a signal peptide in the amino acid sequence of OsGATL (Sterling et al. 2001; Liu et al. 2016). Therefore, we performed sequence analysis on the members of PtGATL proteins (Table S6), and the results showed that all 12 PtGATLs

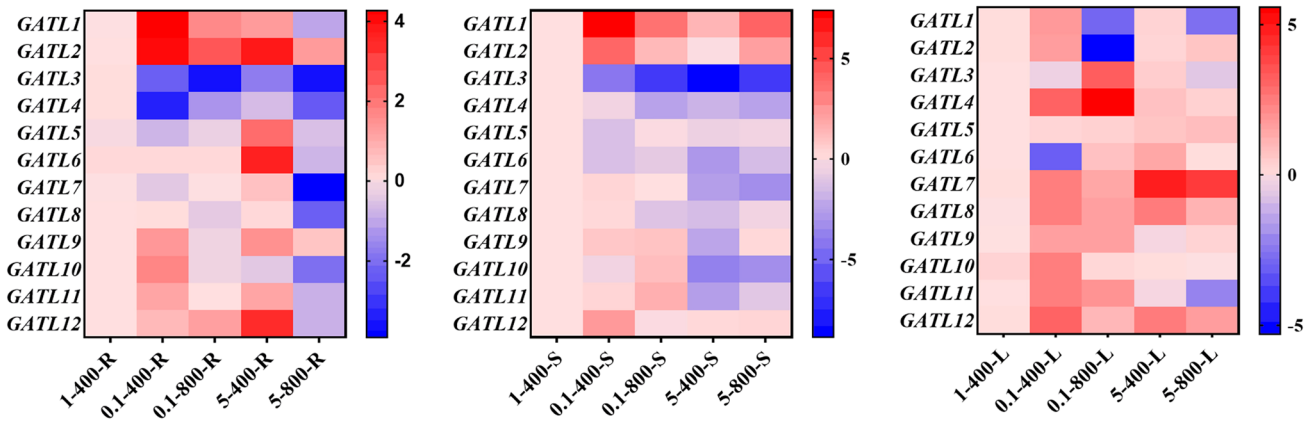


Fig. 7 Expression patterns of *PtGATL* genes in different tissues under different nitrogen and CO_2 treatments. Expression patterns of *PtGATL* genes in roots, stems, and leaves are shown. The concentration of N treatment is 0.1 mM NH_4NO_3 (low nitrogen concentration) and 5 mM NH_4NO_3 (high nitrogen concentration), and the concentration of CO_2 treatment is 400 ppm (adequate carbon concentration) and 800 ppm (high carbon concentration). The 1 mM NH_4NO_3 and 400 ppm CO_2 served as the control. The $2^{-\Delta\Delta CT}$ method was used to calculate the transcription level of *PtGATLs*, and the $\log_2$ (sample/control) value of each *PtGATL* was used to indicate its relative expression level. In heat map, the right side is a scale bar, and different colors indicate that the gene expression level in the treated sample is up-regulated or down-regulated compared to the control

contained signal peptides at the N-terminus. Previous research had shown that *AtGATL3* was related to cell wall polysaccharide synthesis in *Arabidopsis* (Kong et al. 2011). *AtGATL5* might play a role in regulating rhamnogalacturonan I synthesis (Kong et al. 2013). *AtGATL6* was involved in the initiation of primary cell wall synthesis or secondary cell wall synthesis, and also related to pectin synthesis (Kong et al. 2011). Phylogenetic analysis showed that *PtGATL6/9* and *AtGATL3*, *PtGATL2/12* and *AtGATL5/6/7* belonged to the same subclass, respectively, and their expression patterns were similar (Figs. 3, 6). Therefore, *PtGATLs* might be related to the synthesis of cell wall polysaccharides, and the relationship still need experimental verification. *AtGATL1*, *PdGATL1.1* and *PdGATL1.2*, which were homologous genes, involvement in xylan synthesis had been previously confirmed (Brown et al. 2007; Lee et al. 2007; Kong et al. 2009). The two genes, *PtGATL1* and *PtGATL11*, were in the same subclass as *AtGATL1*. We, therefore, deduced that the genes might be related to the synthesis of xylan.

The carbon sequestration capacity of trees was limited by the availability of soil nitrogen under higher CO_2 concentrations (Oren et al. 2001; Sigurdsson et al. 2001). Previous research reported that a few genes played an important role in C/N balance, and many physiological and molecular studies had been conducted (Zheng 2009). In this study, we found that the expression of *PtGATLs* respond to CO_2 and N conditions. For example, under all treatment conditions, the transcription levels of *PtGATL3/4* were significantly inhibited in roots and stems (Fig. 7). The transcription level of some genes did not change significantly when only C or N concentration was modified. For example, under ambient CO_2 treatments, the expression of *PtGATL8* was not change significantly, but it was significantly down-regulated under high C and high N concentration in roots. We speculated that *PtGATL8* might play an important function in the C/N balance.

Conclusion

We identified 12 *PtGATL* genes via bioinformatics methods with similar gene structures and conserved motifs. Phylogenetic analysis showed that *PtGATLs* were divided into six classes. The chromosomal locations and duplication of *PtGATL* genes were predicted. We found that under different N and CO_2 treatments, the expression patterns of *PtGATL* genes were different. This study provided a better understanding of the evolution of the *PtGATL* genes, and laid the foundation for further detailed analysis of the gene family.

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Author contributions ZX and CQ conceived and designed the study, JS performed most of the experiments, SZ, CX and RC conducted the sampling, SZ, and JS performed bioinformatics calculations, CX and

RC processed and analyzed the data, and JS, CQ, GL, C Y and ZX wrote the manuscript.

Declarations

Conflict of interest The authors declare no competing financial interests.

References

Aerts R, van Logtestijn R, van Staalduinen M, Toet S (1995) Nitrogen supply effects on productivity and potential leaf litter decay of carex species from peatlands differing in nutrient limitation. Oecologia 104:447–453. https://doi.org/10.1007/BF00341342

Bailey TL, Boden M, Buske FA et al (2009) MEME SUITE: tools for motif discovery and searching. Nucleic Acids Res 37:W202-208. https://doi.org/10.1093/nar/gkp335

Brown DM, Goubet F, Wong VW, Goodacre R, Stephens E, Dupree P, Turner SR (2007) Comparison of five xylan synthesis mutants reveals new insight into the mechanisms of xylan synthesis. Plant J 52:1154–1168. https://doi.org/10.1111/j.1365-313X.2007.03307.x

Caldera HI, De Costa WA, Woodward FI, Lake JA, Ranwala SM (2017) Effects of elevated carbon dioxide on stomatal characteristics and carbon isotope ratio of *Arabidopsis thaliana* ecotypes originating from an altitudinal gradient. Physiol Plant 159:74–92. https://doi.org/10.1111/ppl.12486

Chen C, Chen H, Zhang Y, Thomas HR, Frank MH, He Y, Xia R (2020a) TBtools: an integrative toolkit developed for interactive analyses of big biological data. Mol Plant 13:1194–1202. https://doi.org/10.1016/j.molp.2020.06.009

Chen J, Qu C, Chang R, Suo J, Yu J, Sun X, Liu G, Xu Z (2020b) Genome-wide identification of *BXL* genes in *Populus trichocarpa* and their expression under different nitrogen treatments. 3 Biotech 10:57. https://doi.org/10.1007/s13205-020-2061-5

Cheng LJ, Zheng M, Sun LJ, Wang XF, Tong ZK (2018) Expressional characterization of galacturonosyltransferase-like gene family in *Eucalyptus grandis* implies a role in abiotic stress responses. Tree Genet Genom 14:81. https://doi.org/10.1007/s11295-018-1294-5

Debra M (2008) Pectin structure and biosynthesis. Curr Opin Plant Biol 11:266–277. https://doi.org/10.1016/j.pbi.2008.03.006

Delmer DP, Haigler CH (2002) The regulation of metabolic flux to cellulose, a major sink for carbon in plants. Metab Eng 4:22–28. https://doi.org/10.1006/mben.2001.0206

Ehlting B, Dluzniewska P, Dietrich H et al (2007) Interaction of nitrogen nutrition and salinity in grey poplar (*Populus tremula* x *alba*). Plant Cell Environ 30:796–811. https://doi.org/10.1111/j.1365-3040.2007.01668.x

Euring D, LöFke C, Teichmann T, Polle A (2012) Nitrogen fertilization has differential effects on N allocation and lignin in two Populus species with contrasting ecology. Trees 26:1933–1942. https://doi.org/10.1007/s00468-012-0761-0

Euring D, Bai H, Janz D, Polle A (2014) Nitrogen-driven stem elongation in poplar is linked with wood modification and gene clusters for stress, photosynthesis and cell wall formation. BMC Plant Biol 14:391. https://doi.org/10.1186/s12870-014-0391-3

Finn RD, Mistry J, Schuster- Böckler B et al (2006) Pfam: clans, web tools and services. Nucleic Acids Res 34:D247-251. https://doi.org/10.1093/nar/gkj149

Fujimoto M, Suda Y, Vernhettes S, Nakano A, Ueda T (2015) Phosphatidylinositol 3-kinase and 4-kinase have distinct roles in intracellular trafficking of cellulose synthase complexes in *Arabidopsis thaliana*. Plant Cell Physiol 56:287–298. https://doi.org/10.1093/pcp/pcu195

Gibbons BJ, Roach PJ, Hurley TD (2002) Crystal structure of the autocatalytic initiator of glycogen biosynthesis, glycogenin. J Mol Biol 319:463–477. https://doi.org/10.1016/S0022-2836(02)00305-4

Han X, Yin H, Song X et al (2016) Integration of small RNAs, degradome and transcriptome sequencing in hyperaccumulator *Sedum alfredii* uncovers a complex regulatory network and provides insights into cadmium phytoremediation. Plant Biotechnol J 14:1470–1483. https://doi.org/10.1111/pbi.12512

Horton P, Park KJ, Obayashi T, Fujita N, Harada H, Adams-Collier CJ, Nakai K (2007) WoLF PSORT: protein localization predictor. Nucleic Acids Res 35:W585-587. https://doi.org/10.1093/nar/gkm259

Hu B, Jin J, Guo AY, Zhang H, Luo J, Gao G (2015) GSDS 2.0: an upgraded gene feature visualization server. Bioinformatics 31:1296–1297. https://doi.org/10.1093/bioinformatics/btu817

Klaiber J, Dorn S, Najar-Rodriguez AJ (2013) Acclimation to elevated CO_2 increases constitutive glucosinolate levels of *Brassica* plants and affects the performance of specialized herbivores from contrasting feeding guilds. J Chem Ecol 39:653–665. https://doi.org/10.1007/s10886-013-0282-3

Kong Y, Zhou G, Avci, et al (2009) Two poplar glycosyltransferase genes, *PdGATL1.1* and *PdGATL1.2*, are functional orthologs to *PARVUS/AtGATL1* in *Arabidopsis*. Mol Plant 2:1040–1050. https://doi.org/10.1093/mp/ssp068

Kong Y, Zhou G, Yin Y, Xu Y, Pattathil S, Hahn MG (2011) Molecular analysis of a family of *Arabidopsis* genes related to galacturonosyltransferases. Plant Physiol 155:1791–1805. https://doi.org/10.1104/pp.110.163220

Kong Y, Zhou G, Abdeen A et al (2013) GALACTURONOSYLTRANSFERASE-LIKE5 is involved in the production of *Arabidopsis* seed coat mucilage. Plant Physiol 163:1203–1217. https://doi.org/10.1104/pp.113.227041

Krzywinski M, Schein J, Birol I et al (2009) Circos: an information aesthetic for comparative genomics. Genom Res 19:1639–1645. https://doi.org/10.1101/gr.092759.109

Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 33:1870–1874. https://doi.org/10.1093/molbev/msw054

Lee C, Zhong R, Richardson EA, Himmelsbach DS, McPhail BT, Ye ZH (2007) The *PARVUS* gene is expressed in cells undergoing secondary wall thickening and is essential for glucuronoxylan biosynthesis. Plant Cell Physiol 48:1659–1672. https://doi.org/10.1093/pcp/pcm155

Leng X, Wang H, Zhang S, Qu C, Yang C, Xu Z, Liu G (2021) Identification and characterization of the *APX* gene family and its expression pattern under phytohormone treatment and abiotic stress in *Populus trichocarpa*. Genes (basel) 12:334. https://doi.org/10.3390/genes12030334

Liu B, Rennenberg H, Kreuzwieser J (2015) Hypoxia affects nitrogen uptake and distribution in young poplar (*Populus* × *canescens*) trees. PLoS One 10:e0136579. https://doi.org/10.1371/journal.pone.0136579

Liu J, Luo M, Yan X, Yu C, Li S (2016) Characterization of genes coding for galacturonosyltransferase-like (GATL) proteins in rice. Genes Genom 38:917–929. https://doi.org/10.1007/s13258-016-0436-0

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ Method. Methods 25:402–408. https://doi.org/10.1006/meth.2001.1262

Lu Y, Deng SR, Li ZR, Wu JT et al (2019) Competing endogenous RNA networks underlying anatomical and physiological characteristics of poplar wood in acclimation to low nitrogen availability. Plant Cell Physiol 60:2478–2495. https://doi.org/10.1093/pcp/pcz146

Luo ZB, Langenfeld-Heyser R, Calfapietra C, Polle A (2005) Influence of free air CO_2 enrichment (EUROFACE) and nitrogen fertilisation on the anatomy of juvenile wood of three poplar species after coppicing. Trees 19:109–118. https://doi.org/10.1007/s00468-004-0369-0

Luo ZB, Calfapietra C, Liberloo M, Scarascia-Mugnozza G, Polle A (2010) Carbon partitioning to mobile and structural fractions in poplar wood under elevated CO_2 (EUROFACE) and N fertilization. Global Change Biol 12:272–283. https://doi.org/10.1111/j.1365-2486.2005.01091.x

Oren R, Ellsworth DS, Johnsen KH et al (2001) Soil fertility limits carbon sequestration by forest ecosystems in a CO_2-enriched atmosphere. Nature 411:469–472. https://doi.org/10.1038/35078064

Persson K, Ly HD, Dieckelmann M, Wakarchuk WW, Withers SG, Strynadka NC (2001) Crystal structure of the retaining galactosyltransferase LgtC from Neisseria meningitidis in complex with donor and acceptor sugar analogs. Nat Struct Biol 8:166–175. https://doi.org/10.1038/84168

Pitre FE, Cooke JEK, Mackay JJ (2007) Short-term effects of nitrogen availability on wood formation and fibre properties in hybrid poplar. Trees-Struct Funct 21:249–259. https://doi.org/10.1007/s00468-007-0123-5

Pitre FE, Lafarguette F, Boyle B et al (2010) High nitrogen fertilization and stem leaning have overlapping effects on wood formation in poplar but invoke largely distinct molecular pathways. Tree Physiol 30:1273–1289. https://doi.org/10.1093/treephys/tpq073

Potter SC, Luciani A, Eddy SR, Park Y, Lopez R, Finn RD (2018) HMMER web server: 2018 update. Nucleic Acids Res 46:W200–W204. https://doi.org/10.1093/nar/gky448

Showalter AM (1993) Structure and function of plant cell wall proteins. Plant Cell 5:9–23. https://doi.org/10.1105/tpc.5.1.9

Sigurdsson BD, Thorgeirsson H, Linder S (2001) Growth and dry-matter partitioning of young *Populus trichocarpa* in response to carbon dioxide concentration and mineral nutrient availability. Tree Physiol 21:941–950. https://doi.org/10.1093/treephys/21.12-13.941

Sjodin A, Street NR, Sandberg G, Gustafsson P, Jansson S (2009) The populus genome integrative explorer (PopGenIE): a new resource for exploring the Populus genome. New Phytol 182:1013–1025. https://doi.org/10.1111/j.1469-8137.2009.02807.x

Sterling JD, Quigley HF, Orellana A, Mohnen D (2001) The catalytic site of the pectin biosynthetic enzyme alpha-1,4-galacturonosyltransferase is located in the lumen of the Golgi. Plant Physiol 127:360–371. https://doi.org/10.1104/pp.127.1.360

Sterling JD, Atmodjo MA, Inwood SE, Kumar Kolli VS, Quigley HF, Hahn MG, Mohnen D (2006) Functional identification of an *Arabidopsis* pectin biosynthetic homogalacturonan galacturonosyltransferase. Proc Natl Acad Sci 103:5236–5241. https://doi.org/10.1073/pnas.0600120103

Thompson J, Gibson T, Plewniak F, Jeanmougin F, Higgins DG (1997) The Clustal_X Windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tool. Nuclc Acids Res 25:4876–4882. https://doi.org/10.1093/nar/25.24.4876

Verbancic J, Lunn JE, Stitt M, Persson S (2018) Carbon supply and the regulation of cell wall synthesis. Mol Plant 11:75–94. https://doi.org/10.1016/j.molp.2017.10.004

Wiggins CA, Munro S (1998) Activity of the yeast MNN1 alpha-1,3-mannosyltransferase requires a motif conserved in many other families of glycosyltransferases. Proc Natl Acad Sci 95:7945–7950. https://doi.org/10.1073/pnas.95.14.7945

Wilkins MR, Gasteiger E, Bairoch A, Sanchez JC, Williams KL, Appel RD, Hochstrasser DF (1999) Protein identification and analysis tools in the ExPASy server. Methods Mol Biol 112:531–552. https://doi.org/10.1385/1-59259-584-7:531

Willats W, Steele-King CG, Mccartney L, Orfila C, Marcus SE, Knox JP (2000) Making and using antibody probes to study plant cell walls. Plant Physiol Biochem 38:27–36. https://doi.org/10.1016/S0981-9428(00)00170-4

Wu X, Yang H, Qu C et al (2015) Sequence and expression analysis of the *AMT* gene family in poplar. Front Plant Sci 6:337. https://doi.org/10.3389/fpls.2015.00337

Yin Y, Chen H, Hahn MG, Mohnen D, Xu Y (2010) Evolution and function of the plant cell wall synthesis-related glycosyltransferase family 8. Plant Physiol 153:1729–1746. https://doi.org/10.1104/pp.110.154229

Zhang S, Cao L, Yu J et al (2021) Genome-wide analysis of *UGDH* genes in *Populus trichocarpa* and responsiveness to nitrogen treatment. 3 Biotech 11:149. https://doi.org/10.1007/s13205-021-02697-9

Zheng ZL (2009) Carbon and nitrogen nutrient balance signaling in plants. Plant Signal Behav 4:584–591. https://doi.org/10.4161/psb.4.7.8540

Zheng L, Wu H, Qanmber G et al (2020) Genome-wide study of the GATL gene family in *Gossypium hirsutum* L. reveals that GhGATL genes act on pectin synthesis to regulate plant growth and fiber elongation. Genes (basel). https://doi.org/10.3390/genes11010064