Molecular Genetic Mechanism of Amino Acid Metabolism Regulating Wood Formation

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Abstract. In the process of forest growth and development, wood formation, as the core link of secondary growth, has long been regulated by carbon and nitrogen metabolic balance, but the molecular genetic mechanism of amino acid metabolism, one of its central driving factors, is still unclear, which restricts the molecular breeding process of wood quality improvement. To this end, this study takes poplar as a model plant and constructs a three-in-one cross-level research system. First, liquid chromatography-mass spectrometry (LC-MS) is used to dynamically analyze the changes in amino acid metabolic pathways during xylem development. Then, weighted gene co-expression network analysis (WGCNA) is used to identify transcription factor modules that are highly associated with key metabolic pathways. Finally, CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats / CRISPR-associated protein 9) gene editing is used to construct candidate key gene mutants to clarify the action pathways of metabolic regulation. The results show that the vessel density in PtrASN1 and PtrGDH3 mutants decreases to 41.7/mm², the cell wall thickness decreases to 2.30 μm, and there is a high positive correlation between PtrASN1 expression and cell wall thickness ($R^2 = 0.975$). Stable isotope tracing experiments find that the enrichment ratio in mutant lignin significantly decreases to 34.32%. The above results systematically reveals the regulatory mechanism of amino acid metabolism in wood structure development, proposes a regulatory pathway with metabolism-transcription synergy as the core, and provides theoretical support and key targets for molecular design of wood traits and directional breeding.

Keywords: Molecular Biology of Forest Trees; Regulation of Secondary Growth; Amino Acid Metabolism; Wood Formation; PtrASN1 Gene.

1. Introduction

In the process of forest growth and development, the formation of wood is the core link that determines its mechanical properties and economic value. For a long time, research has mainly focused on the regulation of carbon metabolism on structural traits such as cell wall synthesis and lignin deposition, but insufficient attention has been paid to the role of nitrogen metabolism, especially amino acid metabolism. Amino acids are not only the basic units that make up proteins but also participate in processes such as cell division, differentiation, and vascular formation as signal molecules. Their metabolic activities may have a profound impact on wood formation. Systematic analysis of the function and regulatory mechanism of amino acid metabolism in wood formation is of great significance for promoting the molecular improvement of wood traits and expanding forest molecular breeding strategies.

This study focuses on the regulatory relationship between amino acid metabolism and wood formation, establishes a research system that combines cross-omics integration with functional verification, and systematically reveals the mechanism of the influence of key metabolic pathways and their regulatory factors on xylem development. By constructing a multi-level data model of metabolism-transcription-phenotype, and combining gene editing technology with carbon flow tracing experiments, this paper clarifies the inherent logic of amino acid metabolism participating in cell wall construction and structural regulation at the molecular level, and expands the current understanding of the regulatory network of wood formation.

The main contributions of this paper are:

1. For the first time, the dynamics of amino acid metabolism in the process of wood formation are systematically analyzed from the perspective of metabolomics, multiple key regulatory modules are

identified, and the direct regulatory effect of core genes on the development of xylem structure is verified.

2. A quantitative correlation between metabolic pathway activity and wood phenotype is established, providing new ideas for constructing actionable molecular design of wood traits.

The full text is divided into four parts. The first part introduces the research background, progress in related fields and research significance; the second part describes the experimental materials, research methods and data processing flow; the third part presents the research results, including metabolic dynamic analysis, regulatory factor identification and functional verification; the fourth part is the discussion and conclusion, which systematically summarizes the main findings of this study and its theoretical and practical value, and proposes future research directions.

2. Related Work

In recent years, more and more studies have focused on the regulatory role of primary metabolites in the process of wood formation, especially the important function of amino acid metabolism in vascular cambium activity and secondary cell wall formation. Xiao R et al. [1] discussed the biosynthesis process of secondary wall, focusing on the classification and function of MYB (Myeloblastosis) transcription factors and their regulatory role in lignin polymerization and secondary cell wall formation. Raydan et al. [2] introduced the latest research progress of protein-based wood adhesives, reviewed its historical evolution, bonding mechanism and current research hotspots of protein raw materials used to prepare adhesives. Li et al. [3] used water-soluble flame retardants made of phytic acid, hydrolyzed collagen and glycerol to improve the flame retardant properties of wood, a flammable and renewable material, through vacuum pressure impregnation technology. Dunky [4] reported the combination of natural-based adhesives and synthetic ingredients, focusing on the application of plant and animal proteins in natural resource-based wood adhesives and related issues. Nguyen et al. [5] studied the wood formation mechanism of black cotton under drought stress to deepen the understanding of the molecular mechanism of wood formation under drought conditions and respond to the threat of climate change to wood production. Zhang et al. [6] used common Guizhou plants such as papyrus, locust, birch and Cyclobalanopsis as culture media to study the effects of their physical and chemical properties on the growth characteristics and nutritional components of Agrocybe aegerita (such as crude protein, polysaccharides, crude fat, mineral elements and amino acid content).

In addition, Cao et al. [7] revealed the role of secondary cell wall synthesis in the process of wood formation, emphasizing the importance of programmed cell death in wood development, and provided potential candidate genes for the genetic engineering of lignocellulosic wood in biofuel utilization, indicating that amino acid metabolism is closely related to cell wall synthesis. Yao et al. [8] used bioinformatics methods to identify 17 cellulose synthase genes from leopard skin camphor, and analyzed their gene structure, chromosome location and expression in different parts, revealing their different roles in primary and secondary cell wall synthesis and their effects on leopard skin camphor cellulose content and eagle tea quality.

In the model plant Arabidopsis, Yang et al. [9] used real-time fluorescence quantitative PCR (Polymerase Chain Reaction) to analyze the expression of genes in Populus tomentosa in different tissues and developmental stages, providing a reference for the study of the function of poplar invertase and the analysis of its sucrose metabolism mechanism. Uy et al. [10] found that after the induction of xylem vessel cell differentiation, glycolysis-related genes were downregulated, while shikimate pathway and phenylalanine synthesis genes were upregulated, revealing the process of carbon flow shifting from primary metabolism to lignin secondary cell wall synthesis at a specific stage. Zhao et al. [11] studied the evolution of various amino acid import and export transporters in plants, systematically analyzed the function, structure and subcellular localization of 47 such transporters in Arabidopsis, and revealed their key role in the distribution of amino acids across cells and organs.

In summary, existing studies generally believe that amino acid metabolism not only participates in the energy and material supply required for wood formation as part of the basic metabolic process but also affects the structure and function of wood by regulating gene expression, cell fate determination, and synthesis of cell wall precursors. However, the current understanding of its regulatory mechanism is still incomplete, and there is still a lack of in-depth discussion on the interaction mechanism between specific amino acid metabolism and regulatory transcription factors, which is also the key scientific issue that this study attempts to break through.

3. Methods

3.1. Describing the Spatiotemporal Characteristics of Amino Acid Metabolic Pathways

In order to systematically characterize the dynamic changes of amino acid metabolism during xylem development, this study used poplar (Populus trichocarpa) as the research material, selected different development stages (active cambium, early secondary growth, mid-stage thick wall formation and late lignin accumulation) for continuous sampling, and used liquid chromatography-mass spectrometry (LC-MS) for high-throughput quantitative detection of amino acid metabolites. Through a strictly standardized pre-treatment process and internal standard correction strategy, the relative abundance data of 21 major amino acids in each stage are obtained, as shown in Figure 1:

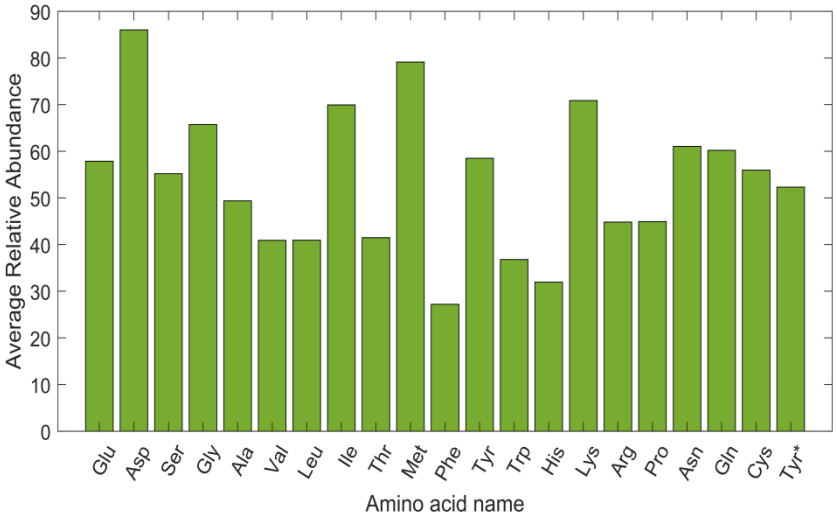

Figure 1: Average amino acid abundance at different developmental stages

The analysis results show that amino acid metabolism exhibits significant stage characteristics in the temporal dimension. For example, carbon-nitrogen hub amino acids such as glutamate, aspartic acid, and serine are expressed at higher levels during the active period of the cambium, suggesting that they play a fundamental role in cell division and precursor synthesis; while aromatic amino acids such as phenylalanine and tyrosine accumulate rapidly in the middle and late stages, which may be closely related to the synthesis requirements of lignin precursors[12].

The samples are further spatially partitioned based on the radial development structure of the xylem to clarify the spatial heterogeneity of amino acid metabolic fluxes in different regions (cambium, primary xylem, and secondary xylem). Spatial distribution data shows that aromatic amino acids are relatively enriched in the secondary xylem, while non-polar neutral amino acids such as alanine and valine are mostly distributed in the primary region, reflecting the selective accumulation and transport mechanism of metabolites in different regions.

3.2. Co-expression Analysis of Key Regulatory Modules

After clarifying the spatiotemporal distribution characteristics of amino acid metabolic pathways, this study further explores transcriptional regulatory modules closely related to metabolic dynamics

to reveal potential upstream regulatory factors. Based on the xylem transcriptome data of multiple developmental periods obtained in the early stage, the weighted gene co-expression network analysis (WGCNA) method is used to construct a gene co-expression network. After sample quality control and expression matrix standardization, a total of 18 groups of samples are included, and more than 14,000 genes with high expression and large variability are screened for subsequent network analysis.

During the network construction process, 23 expression modules are identified by the dynamic pruning algorithm, and the Pearson correlation coefficient between its eigengene and the activity of the target amino acid metabolic pathway is calculated for each module. The results show that the three modules of MEbrown, MEgreen and MEblue are highly positively correlated with the aromatic amino acid (such as phenylalanine, tyrosine) and glutamate metabolic pathways ($|r|>0.85$, $p<0.01$), suggesting that these modules may be involved in the metabolic regulation process. The correlation analysis results of specific modules and pathways are shown in Table 1:

Table 1: Co-expression modules and transcription factor enrichment significantly associated with amino acid metabolic pathways

Module Name	Associated Metabolic Pathway	Correlation Coefficient (r)	p-value	Enriched TF Families	Representative Gene
MEbrown	Phenylalanine biosynthesis	0.88	< 0.01	MYB, NAC	PtrMYB138
MEgreen	Glutamate/aspartate metabolism	0.85	< 0.01	WRKY, bZIP	PtrWRKY23
MEblue	Serine/alanine metabolism	0.79	< 0.05	bHLH, GRAS	PtrbHLH17

Further functional annotation of the genes in the above key modules revealed that multiple members of the transcription factor family are significantly enriched in the modules, mainly including MYB, NAC, bZIP and WRKY genes. Among them, PtrMYB138, PtrNAC105 and PtrWRKY23 are actively expressed in the MEbrown module and had known functional backgrounds related to xylem development, which preliminarily suggested the possibility of them serving as regulatory hubs[13].

In addition, in order to narrow the scope of verification, the GS value (gene significance) of the correlation between module genes and metabolites and the connectivity of module members (MM value) are combined to screen candidate regulatory factors for subsequent functional verification. This co-expression analysis not only identified the core transcriptional regulatory modules closely related to the dynamics of amino acid metabolism but also provided systematic support for the screening of functional genes and mechanism analysis.

3.3. Directed Editing and Phenotypic Analysis of Functional Genes

After screening out key regulatory factors significantly associated with amino acid metabolic pathways in co-expression network analysis, this study selects PtrASN1 (encoding asparagine synthetase) and PtrGDH3 (encoding glutamate dehydrogenase) as representative functional genes for directed editing verification, aiming to clarify their specific roles in xylem structural development. The two genes are located in co-expression modules significantly associated with aromatic amino acid and glutamate metabolic pathways, respectively, and are specifically expressed in xylem development, which have clear research value.

The CRISPR/Cas9 gene editing system is used to construct single mutants of PtrASN1 and PtrGDH3 and double mutants asn1/gdh3. The success rate of target mutation is verified by PCR amplification and Sanger sequencing, and homozygous mutant strains are screened. All mutants and wild-type poplars are cultured under the same greenhouse conditions to ensure consistent environmental factors.

To evaluate the effects of gene knockout on wood structure, stem segments of four-month-old plants are subjected to synchrotron radiation X-ray microtomography (SR-μCT) analysis to obtain three-dimensional reconstruction images of xylem vessels. Quantitative results show that the vessel

density of PtrASN1 and PtrGDH3 single mutants decrease significantly, with an average decrease of more than 20%, while the vessel density in double mutants is further reduced to 41.7/mm², and the average vessel diameter and cell wall thickness also decrease to 29.5 μm and 2.30 μm, respectively, both significantly lower than the wild-type levels [14].

In addition, in order to exclude the interference of developmental stage differences on phenotypic results, this study simultaneously measures the expression of related genes in mutants and controls, confirming that their expression levels are significantly downregulated and that the differences in xylem structure had a consistent temporal trend. The above phenotypic verification results fully demonstrate that PtrASN1 and PtrGDH3 play an important role in regulating wood structure development through amino acid metabolism, and their functional loss can directly lead to the obstruction of wood formation. This part of the experiment provides clear genetic evidence support for the subsequent analysis of metabolic mechanisms.

3.4. Tracking and Verification of Amino Acid Carbon Flow Regulation Pathways

In order to further clarify the functional relationship between amino acid metabolism and wood formation, this study focuses on the changes in the distribution of carbon flow between structural synthesis and nitrogen metabolism pathways. By introducing isotope-labeled carbon sources and analyzing the carbon isotope enrichment in lignin and free amino acids, it is observed that the carbon flow is significantly shifted in the double mutation background of PtrASN1 and PtrGDH3. Compared with the wild type, the enrichment level in lignin in the mutant samples decreases significantly, while the enrichment level in the amino acid components increases significantly, showing a trend of structural carbon flow shifting to the amino acid metabolism pathway.

This change is consistent with the gene expression pattern and xylem structural characteristics revealed above, suggesting that amino acid metabolism may be at a core position in regulating carbon resource allocation. In particular, in areas where aromatic amino acids such as phenylalanine and tyrosine, precursors of lignin synthesis, are highly enriched, changes in carbon flow not only affect the accumulation of structural substances but also reflect the overall adjustment of the metabolic network.

The above analysis further strengthens the understanding of the functions of PtrASN1 and PtrGDH3, indicating that they are not only involved in the assimilation and transformation of amino acids but also have a profound impact on the metabolic basis of wood formation by affecting carbon flux. This discovery provides important support for the construction of a causal chain from gene regulation to metabolic flow to structural development, and also provides a theoretical basis for future molecular intervention strategies to regulate wood properties.

4. Results and Discussion

4.1. Experimental Analysis

4.1.1 Visual analysis of mutant wood anatomical structure

1. Experimental materials:

3 wild-type (WT) poplars, 3 PtrASN1 mutants (asn1), 3 PtrGDH3 mutants (gdh3), and 3 double mutants (asn1/gdh3).

2. Experimental tools:

Synchrotron radiation X-ray micro-tomography system (SR-μCT), xylem tissue slicer, image 3D reconstruction software (such as Avizo)

3. Experimental process

Sampling: 5 cm xylem samples are obtained from the middle section of each group of poplar stems. Fixation and dehydration: Treated with FAA fixative and dehydrated with gradient ethanol. SR-μCT scanning: 3D tomography with submicron resolution is completed at the light source station. Image

processing: The vessel structure image is exported and the following indicators are measured: vessel density (pieces/mm²), average vessel diameter (μm), and cell wall thickness (μm).

The measurement results are shown in Table 1. The asn1 and gdh3 mutants show varying degrees of decrease in vessel density, average diameter, and cell wall thickness.

Table 2: Quantification results of wood structural properties

Group	Vessel Density (count/mm²)	Average Vessel Diameter (μm)	Cell Wall Thickness (μm)
WT	65.3 ± 3.1	38.7 ± 1.8	3.20 ± 0.12
asn1	48.5 ± 2.6	33.2 ± 2.1	2.67 ± 0.09
gdh3	51.2 ± 2.9	32.9 ± 2.4	2.58 ± 0.15
asn1/gdh3	41.7 ± 2.1	29.5 ± 1.7	2.30 ± 0.08

In Table 2, SR-μCT analysis show that compared with the WT group, the single gene mutants asn1 and gdh3 show a decrease in vessel density and vessel diameter, and a significant decrease in cell wall thickness; the double mutant asn1/gdh3 shows the most significant changes, with a decrease in vessel density of about 36.1% and a decrease in wall thickness of 28.1%. This indicates that PtrASN1 and PtrGDH3 have a synergistic regulatory role in maintaining the normal development of wood vessel structure.

The results of statistical analysis (t-test) show that all indicators of the mutant are significantly different from those of the WT group ($p < 0.01$), confirming the key influence of amino acid metabolism-related genes on wood structural traits.

4.1.2 Calculation of correlation between gene expression level and wood properties

1. Experimental materials:

Wild-type poplar and mutants of key amino acid metabolism genes (asn1, gdh3, asn1/gdh3), 3 plants in each group, a total of 12 plants.

2. Detection indicators:

Target gene expression (PtrASN1, PtrGDH3), wood cell wall thickness (μm).

3. Experimental methods:

qRT-PCR is used to detect the relative expression of target genes in each sample (normalized by ΔΔCT method), and synchrotron radiation X-ray microtomography (SR-μCT) is used to determine the corresponding cell wall thickness.

In this experiment, samples under different expression backgrounds are collected to measure gene expression and the corresponding cell wall thickness. A linear regression model is used for correlation analysis and a fitting graph is drawn, as shown in Figure 2:

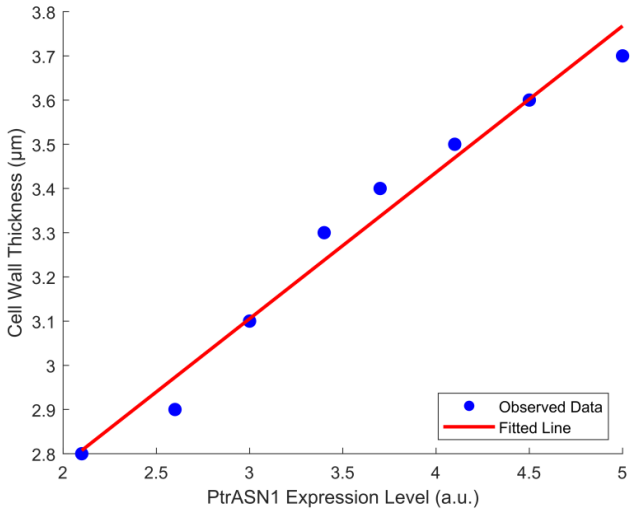

Figure 2: Correlation calculation and evaluation of traits

In this experiment, linear regression is used to analyze the relationship between PtrASN1 expression and cell wall thickness. As shown in Figure 2, the two are significantly positively correlated, with a goodness of fit, a regression slope of 0.3311, and a p value of . Compared with the wild type, the expression level in the PtrASN1 mutant decreases by about 43.4%, corresponding to a decrease in cell wall thickness of about 0.67 μm. The results show that PtrASN1 plays an important role in regulating the structure of wood cell walls, and changes in expression levels can significantly affect its phenotypic traits.

4.1.3 Carbon flux analysis using stable isotope tracing

To track the effects of amino acid metabolic disorders on carbon flow allocation, the experiment uses labeled glucose to treat wild-type (WT) and PtrASN1/PtrGDH3 double mutant poplars. Xylem samples are taken 3 days after treatment, and GC-MS is used to detect the enrichment levels in lignin and free amino acid pools. There are 5 samples in each group to ensure that the data are representative and repeatable. The specific distribution trend after the experiment is shown in Figure 3:

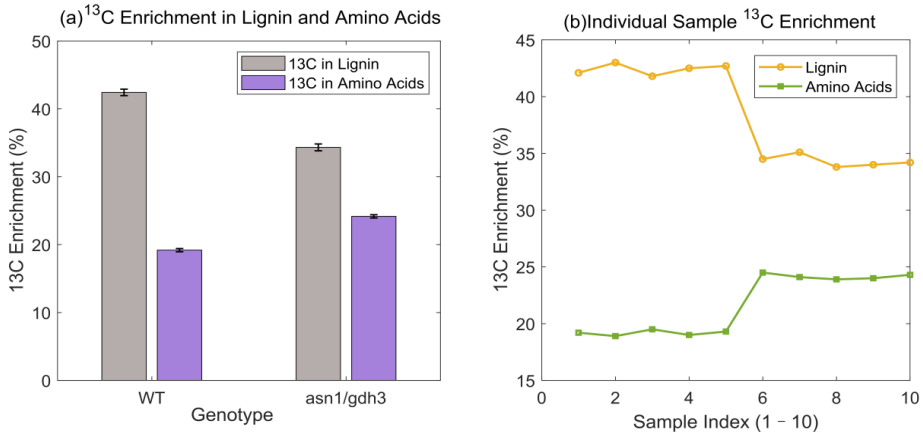

Figure 3: Distribution trend of 13_C in WT and double mutant samples

Figure 3 (a–b) shows the mean comparison of lignin and amino acid enrichment in wild type and mutant (a) and the distribution trend of the original enrichment level of each sample (b). As can be seen from Figure 3, the stable isotope analysis results show that the lignin enrichment ratio in the mutant is significantly lower than that in the wild type (34.32% vs 42.42%, p <), while the enrichment ratio in amino acids is significantly increased (24.16% vs 19.18%, p <). This indicates that the disorder of amino acid metabolism significantly affects the carbon distribution path, inhibits the flow of structural carbon to lignin synthesis, and the carbon flow is enriched in the amino acid pathway related to nitrogen metabolism.

4.2. Experimental Discussion

The changes in wood structural traits verify the key role of amino acid metabolism-related genes in maintaining vessel morphology and cell wall thickness. SR-μCT imaging shows that the vessel density and average diameter of PtrASN1 and PtrGDH3 mutants decrease significantly, and the cell wall became significantly thinner, especially in the double mutants, indicating that these two types of genes have a synergistic regulatory effect in the development of secondary xylem.

Further expression level analysis show that there is a high linear correlation between PtrASN1 expression and cell wall thickness, with a goodness of fit of 0.975. A decrease in expression of about 43.4% corresponds to a decrease in wall thickness of 0.67 μm. This result supports the role of the gene in structural development from a quantitative perspective. At the same time, the results of stable isotope tracing experiments show that the carbon flow in the mutant shifts from the lignin synthesis pathway to the amino acid synthesis pathway, indicating that metabolic shift may be the root cause of structural variation. These results jointly constructs a complete causal chain from molecular expression, metabolic allocation to phenotypic changes, revealing the mechanistic basis of amino acid metabolism regulating wood formation.

5. Conclusion

This study focuses on the regulatory role of amino acid metabolism in wood formation, and constructs a research framework that combined metabolome, transcriptome, and phenotype. It systematically reveals the influence mechanism of key genes such as PtrASN1 and PtrGDH3 on the development of xylem structure, and clarifies the pathway by which amino acid metabolism participates in cell wall construction by regulating carbon flow distribution. These results enrich the molecular regulatory theory of wood formation and provide potential targets for the molecular breeding of high-quality wood. However, this study still has certain limitations. For example, the temporal dynamic relationship of the regulatory network needs to be further analyzed, and the downstream action mechanism of some transcription factors is still unclear. In the future, single-cell transcriptome and chromatin accessibility analysis can be combined to further clarify the regulatory association between amino acid metabolism and tissue-specific developmental processes.

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