MINISTRY OF EDUCATION AND SCIENCE OF UKRAINE
KYIV NATIONAL UNIVERSITY OF TECHNOLOGIES AND DESIGN
Faculty of Chemical and Biopharmaceutical Technologies
Department of Biotechnology, Leather and Fur

QUALIFICATION THESIS

on the topic **Whole genome identification and expression analysis of AUX/IAA gene family in Populus wilsonii**
First (Bachelor's) level of higher education
Specialty 162 "Biotechnology and Bioengineering"
Educational and professional program "Biotechnology"

Completed: student of group
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Mengyao Zhang

Scientific
supervisor Iryna VOLOSHYNA,
Ph.D., As. prof.

Reviewer Olga IUNGIN
Ph.D., As. prof.

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APPROVE
Head of Biotechnology, Leather and Fur Department, Professor, Dr. Sc., Prof.
_____Olena MOKROUSOVA
«___»_____2025

ASSIGNMENTS
FOR THE QUALIFICATION THESIS
Zhang Mengyao

1. Thesis topic **Whole genome identification and expression analysis of AUX/IAA gene family in Populus euphratica**

Scientific supervisor Ph.D., Asos. Prof. Iryna VOLOSHYNA

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2. Initial data for work: assignments for qualification thesis, scientific literature on the topic of qualification thesis, materials of Pre-graduation practice

3. Content of the thesis (list of questions to be developed): literature review; object, purpose, and methods of the study; experimental part; conclusions

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WORK CALENDAR

№	The name of the stages of the qualification thesis	Terms of performance of stage	Note on performance
1	Introduction	until 11 April 2025	
2	Chapter 1. Literature review	until 20 April 2025	
3	Chapter 2. Object, purpose, and methods of the study	until 30 April 2025	
4	Chapter 3. Experimental part	until 11 May 2025	
5	Conclusions	until 15 May 2025	
6	Draw up a bachelor's thesis (final version)	until 25 May 2025	
7	Submission of qualification work to the supervisor for feedback	until 27 May 2025	
8	Submission of bachelor's thesis to the department for review (14 days before the defense)	28 May 2025	
9	Checking the bachelor's thesis for signs of plagiarism (10 days before the defense)	01 June 2025	Similarity coefficient ____% Citation rate ____%
10	Submission of bachelor's thesis for approval by the head of the department (from 7 days before the defense)	04 June 2025	

I am familiar with the task:

Student _____ Zhang Mengyao

Scientific supervisor _____ Iryna VOLOSHYNA

SUMMARY

Zhang Mengyao. Whole genome identification and expression analysis of AUX/IAA gene family in Populus euphratica – Manuscript.

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This study will use bioinformatics technology and combine genomic data of Populus wilsonii to systematically investigate the characteristics of members within the AUX/IAA gene family of Populus wilsonii. Based on Hidden Markov Model (HMM) and homologous sequence alignment, 35 AUX/IAA gene members (named PwiAUX/IAA1 to PwiAUX/IAA35) were identified from the entire genome of Populus wilsonii. Chromosomal localization analysis reveals that members exhibit uneven distribution across 12 chromosomes..

By employing the adjacency method to construct a phylogenetic tree, members of the family can be categorized into four subfamilies (Group A-D). Among them, the structural domain of Group B gene (such as Motif 10 representing specificity) suggests its special function in response to abiotic stress. Conservative motif analysis showed that all members carry core functional domains involved in auxin signaling (Motif 2 and Motif 3), while subfamily specific motifs (such as Motif 4/8 enriched in Group A associated with hormone response and Motif 6 enriched in Group C associated with light response) reveal the molecular basis of functional differentiation. 20 types of cis acting elements were identified through promoter region analysis, covering important pathways such as plant hormone regulation, stress response, and growth and development.

Organizational expression profiling analysis showed that PwiAUX/IAA14 and PwiAUX/IAA31 exhibited sustained high expression in multiple tissues such as xylem and phloem. The expression levels of PwiAUX/IAA6 and PwiAUX/IAA15 in root meristem tissue were significantly increased compared to other tissues. Protein interaction network analysis shows that core genes (such

as PwiAUX/IAA1, PwiAUX/IA9) form regulatory hubs through "one to many" or "many to many" interaction patterns, while the interaction range of edge genes is limited, suggesting that their functions may be tissue-specific.

This study comprehensively analyzed the phylogenetic relationships, spatiotemporal expression patterns, and protein interaction networks of the AUX/IAA gene family in Populus wilsonii for the first time, providing important theoretical basis for elucidating the molecular mechanisms of this family in auxin signaling, tissue differentiation, and stress adaptation. It has reference value for the study of forest gene families and genetic improvement of Populus wilsonii.

Key words: Populus wilsonii, AUX/IAA gene family;, Phylogenetic analysis; Expression pattern; Functional Differentiation

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INTRODUCTION

This study focuses on Populus wilsonii and conducts the first genome-wide systematic study of the AUX/IAA gene family using bioinformatics methods.

The study will rely on the whole genome database of Populus wilsonii, comprehensively using BLASTP sequence similarity retrieval (E-value threshold ≤ 1e-5) and InterProScan conservative domain annotation (integrating databases such as Pfam and SMART), combined with HMM hidden Markov model search, to accurately identify family members and systematically analyze their molecular characteristics, including gene structure (distribution of exons and introns, length variation of UTR regions), sequence conservation of conserved functional domains (such as Domain I/LxLxL motifs, Domain II/GWPPV motifs), and chromosome localization features. Based on this, the adjacency method will be used to construct a phylogenetic tree.

In addition, the study integrated the analysis of cis acting elements in the promoter region with multi tissue transcriptome data, identified key candidate genes significantly associated with xylem development, and analyzed their spatiotemporal expression patterns and regulatory networks in different tissues and developmental stages.

The research results will provide key technical support for molecular breeding of Populus wilsonii: by identifying AUX/IAA genes involved in wood stem development, precise gene editing targets will be provided for improving wood fiber quality.

At the same time, through the deep integration of basic theoretical innovation and application technology breakthroughs, not only can the understanding of the functional differentiation mechanism of the core gene family of the auxin signaling pathway in woody plants be deepened, but also a systematic analysis of the genomics framework can be provided as a reference methodology for the research of other forest gene families, which has important

scientific significance and application value in the fields of forest molecular breeding and woody plant functional genomics.

Chapter I
LITERATURE REVIEW

1.1Introduction to Populus wilsonii

Populus wilsonii is an important pioneer afforestation tree species in temperate and warm temperate regions of China. It has irreplaceable ecological and economic value in barren mountain greening, soil and water conservation, and artificial forest construction. This tree species has significant environmental adaptation advantages, not only can it quickly become a forest in poor soil, arid and semi-arid areas, but its wood fiber length and density also meet the raw material requirements of the papermaking and biomass energy industries.

However, for a long time, the genetic improvement process of Populus wilsonii has been constrained by two major bottlenecks: firstly, the lack of high-quality genomic data, and secondly, the lag in the analysis of growth, development, and stress response molecular mechanisms, resulting in a lack of key targets for targeted breeding.

With the development of high-throughput sequencing technology, the completion of the whole genome sequencing project of Populus wilsonii (such as the 2022 release of the Populus wilsonii genome map) provides key support for breaking through the above bottlenecks[1]. The AUX/IAA (Auxin/Indole-3-Acetic Acid) gene family, as the core regulatory factors of the auxin signaling pathway in plants, plays a crucial role in the secondary growth regulation of Populus species.

According to existing research, it has been found that AUX/IAA genes in closely related species such as P. trichocarpa can participate in stem thickening and wood quality formation by regulating vascular cambium activity and xylem cell differentiation[2].

As one of the most widely distributed wild species in the Populus genus[3] (distributed across the mountainous areas of North China, Northwest China, and

Southwest China), the Populus wilsonii has long faced complex environmental stresses such as drought, low temperature, and soil salinization[4]. Its AUX/IAA gene family may have formed unique adaptive evolutionary characteristics under long-term natural selection, such as responding to specific habitat pressures through gene replication, structural variation, or expression pattern changes.

1.2 Overseas and Domestic Research Status

1.2.1 Current situation abroad

AUX/IAA is a key gene family in plants, and transcription inhibitory factors interact with ARF (auxin response factor) proteins through conserved domains (such as Domain II), playing a switch role in the auxin signaling pathway[5]. Auxin can promote the ubiquitination degradation of AUX/IAA proteins, inhibit their inhibition of ARF, activate downstream gene expression, regulate plant growth and development, and help plants adapt to the environment[6].

The study of the AUX/IAA gene family began in the 1990s with genetic analysis of Arabidopsis mutants. With the advancement of molecular biology technology, its functional analysis has formed a relatively complete theoretical system, and the research scope has gradually expanded from model plants to forest species[7], showing the following characteristics: the systematic identification of the AUX/IAA gene family has been completed in more than 30 plants, such as Arabidopsis (29 members), rice (31 members) and other model plants[8]. It can be seen that all members of this family have typical structural features: the N-terminus contains conserved Domain I (LxLx motif, mediating protein interaction to inhibit ARF activity) and Domain II (GWPPV motif, responsible for ubiquitination degradation), and The C-end sequence exhibits high variability[9].

In recent years, the focus of related research has gradually shifted towards woody plants, especially the discovery and confirmation of 35 PtIAA genes in Populus trichocarpa[10]. Populus tremula in Europe found that PttIAA3 affects wood density by regulating secondary cell wall thickening[11].

In functional research, by utilizing gene editing and protein interaction techniques, the study of AUX/IAA genes has deepened from phenotype observation to molecular mechanism exploration, while also advancing towards tissue specificity and molecular interaction. In terms of organ development regulation, Arabidopsis IAA12/BDL regulates embryonic apical basal axis polarity by inhibiting ARF5/MP activity[12]; IAA17/AXR3 affects lateral root formation by regulating the polarity localization of PIN proteins[13]. In terms of secondary wall synthesis in woody plants, PtIAA14 and PtARF7 form regulatory modules in Populus tomentosa, which regulate secondary growth by affecting the expression of lignin synthase genes (such as PtCCoAOMT)[14].

The research methods have been upgraded to multi omics integration and spatiotemporal dynamic analysis, and the application of new technologies such as single-cell transcriptomics and chromatin accessibility analysis (ATAC seq) has promoted AUX/IAA research into a high spatiotemporal resolution stage[15]. For example, in 2024, it was reported that single-cell sequencing technology was used to analyze the cell type specific expression pattern of maize IAA gene in root tip meristematic tissues[16].

In addition, the protein structure prediction technology based on AlphaFold2 has successfully resolved the three-dimensional conformational changes of the IAA-ARF complex[17], providing structural biology evidence for elucidating the dynamic regulation mechanism of auxin signaling.

1.2.2 Domestic situation

In terms of domestic research status, Chinese scholars have made certain progress in the field of AUX/IAA gene research. In terms of rice, OsIAA11 affects tiller angle by regulating auxin polarity transport[18]; The Journal of

Botany has revealed the molecular mechanism of the interaction between OsIAA9 and OsARF19 in regulating grain filling[19]. In cotton research, it has been identified that GhIAA16 enhances resistance to Verticillium wilt by regulating the JA signaling pathway[20], and woody plant research has focused on a few species. Among them, the team from Zhejiang Agriculture and Forestry University used CRISPR/Cas9 technology to knock out the PtIAA14 gene in Populus tomentosa and found that it affects the activity of the cambium by regulating the expression of WOX4[21]. The CRISPR-dCas9 transcriptional activation system was revealed in Populus tomentosa to regulate the involvement of NAC transcription factors in lignocellulosic development[22].

In addition, the genome data of Populus wilsonii has been made public, but systematic research on its gene family is still in its infancy. Existing research has mostly focused on genetic diversity analysis (such as SSR marker development) and physiological ecological investigations[23], with a lack of molecular mechanism studies. In terms of genomics research, significant breakthroughs have been made in China's poplar genomics research in recent years, with research still focusing on cultivated varieties. For example, in 2020, the Chinese Academy of Forestry and Beijing Forestry University jointly released the chromosome level genome of Populus tomentosa, which was the first species in the Populus genus to complete T2T (telomere to telomere) assembly[24]. This genome has been widely used in disease resistance gene mining and wood formation mechanism research.

Secondly, domestic teams mainly conduct comparative genomics research on Populus tomentosa based on the current version of the genome[25]. For example, Huazhong Agricultural University revealed through collinearity analysis that the expansion of the NAC gene family in Populus tomentosa is related to the evolution of drought adaptation.

Finally, relevant database websites have publicly released the reference genome of Yiyang, but due to the lack of systematic analysis of the genome[26],

especially in cutting-edge fields such as transposon (TE) mediated genomic plasticity and chromatin three-dimensional structure regulation, further exploration is still needed.

In terms of tissue-specific expression profiles, in 2018, Nanjing Forestry University constructed a full-length transcriptome database (Iso Seq) for the roots, stems, leaves, and cambium of Populus tomentosa, identifying 2143 tissue-specific alternative splicing isoforms. Among them, the high expression genes of the cambium were significantly enriched in the lignin synthesis pathway[27]. Secondly, regarding the protein interaction network, China Agricultural University constructed a network map containing 327 AUX/IAA interacting proteins through screening of the Populus tomentosa yeast library. Among them, the interaction between PtIAA12 and MAPK kinase PtMPK6 was confirmed to be involved in ROS signaling regulation[28].

Summary of the chapter I

1.Introduction to Populus wilsonii: Populus wilsonii is a key pioneer tree in China's temperate zones, providing ecological (soil conservation) and economic (papermaking) value via suitable wood fibers. Genetic improvement is limited by scarce genomic data and unclear growth/stress mechanisms. The 2022 genome release aids progress. AUX/IAA genes regulate poplar secondary growth; related species show they influence stem thickening via cambium activity. Adapted to harsh environments, P. wilsonii may have unique AUX/IAA traits from natural selection.

2.Overseas Research Status: AUX/IAA genes repress auxin signaling, degraded by auxin to activate growth/stress responses. Studied in 30+ species (e.g., 35 PtIAA in P. trichocarpa) since 1990s, featuring conserved N-domains and variable C-termini. Woody plant research (e.g., P. tremula's PttIAA3 for wood density) uses gene editing. Advanced methods include multi-omics/single-cell analysis and AlphaFold2 for protein structure insights.

3.Domestic Research Status: Chinese research focuses on crops (rice/cotton) and woody plants (CRISPR in P. tomentosa links PtIAA14 to cambium activity via WOX4). P. wilsonii’s genome is public but understudied, with most work on genetics/physiology. Key advances: P. tomentosa’s 2020 genome links NAC genes to drought adaptation. Gaps exist in transposon/chromatin research for P. wilsonii.

Chapter II

OBJECT, PURPOSE, AND METHODS OF THE STUDY

2.1 Experimental materials

2.1.1 Tools and databases used

Table 2-1 Tools/databases used for data analysis and their purposes

Tool/Database Name	**Purpose**
FigShare	Populus wilsonii Genome and Annotation Data Download
Phytozome	Arabidopsis and Populus tomentosa AUX/IAA protein sequence alignment database
HMMER	Gene Family Screening Based on Hidden Markov Model
NCBI BLAST+	Homologous gene sequence verification
GSDS 2.0	Visualization of gene structure
Trimal	Conservative segment extraction
MEGA 11	Construction of phylogenetic tree
PlantCARE	Prediction of promoter cis acting elements
STRING	Protein interaction network prediction
Cytoscape	Visualization of protein-protein interaction network

2.2 Identification and analysis methods of AUX/IAA gene family in Populus wilsonii

2.2.1 Hidden Markov Model (HMM) screening and homologous sequence validation of AUX/IAA gene family in Populus wilsonii

Using HMMER v3.3.2 software, download the seed file of the AUX/IAA domain (PF02309) from the Pfam database, scan the whole genome protein sequence of Populus wilsonii, obtain AUX/IAA candidate family members, remove genes with low reliability, and then record the obtained candidate genes into the Pfam database to verify and confirm the genes in the AUX/IAA domain. After comparing the full length of the AUX/IAA domain, delete genes with less than 70% coverage in the domain, and finally obtain genes with high reliability[29].

Afterwards, candidate genes for AUX/IAA in the Populus wilsonii genome were screened using BLASTP (NCBI BLAST+v2.13.0) with Arabidopsis and Populus tomentosa AUX/IAA protein sequences as templates, and the intersection results were obtained through integration.

2.2.2 Gene Structure and Conservative Domain Analysis

This study first extracted the genomic coordinates of the exon, intron, and untranslated region (UTR) of the target AUX/IAA gene from the genome annotation file of Populus wilsonii, and converted them into standard formats such as BED or GFF. Subsequently, using the GSDS 2.0 tool（http://gsds.gao-lab.org）to upload the gene structure file, select the matching reference genome version, and set visualization parameters such as exon color and intron connection style. Finally, generate a gene structure diagram with annotated exon number, intron length, and UTR region distribution.

In structural comparative analysis, by comparing the exon intron composition of different family members, if most genes contain three exons and the intron insertion positions are consistent, it indicates that the gene family

structure is conserved[30]; If there is a deletion of exons or a difference in intron length, it indicates the possibility of functional differentiation.

In the conservative domain prediction stage, the FASTA format amino acid sequences encoded by AUX/IAA genes were first extracted from the Populus wilsonii protein sequence library, and the InterProScan tool was used to integrate authoritative databases such as Pfam and SMART for joint functional annotation. Then use MEME tool（http://memesuite.org/tools/meme）to analyze conservative motifs and generate visualization results, and finally standardize the motif diagram using Tbtools software.

2.2.3 System Evolution Analysis

This study used MEGA 11 software (Molecular Evolutionary Genetics Analysis, Version 11.0.12) to construct a phylogenetic tree. The specific process is as follows: after aligning the amino acid sequence with ClustalW, low-quality regions with a gap ratio of over 50% and sites containing missing data are removed to ensure the reliability of the alignment.

Based on the maximum likelihood method (ML), a JTT model suitable for eukaryotic protein sequence analysis was selected using the Akaike Information Criterion (AIC) through the ModelFinder module to correct for heterogeneity in amino acid substitution rates between sites[31].

When constructing a phylogenetic tree, 1000 bootstrap resampling tests are set up to evaluate branch reliability, and the nearest neighbor interaction algorithm (NNI) combined with heuristic search is used to optimize the tree structure to stability. The generated tree quantifies the genetic distance based on branch length[32], with node annotations indicating a support rate of ≥ 50% for self expansion. After visualization by TreeView, family members are divided into four subfamilies, Group A-D, based on branch structure, and collinearity is verified with gene structure features and conservative motif distribution.

2.2.4 Analysis of cis acting elements on AUX/IAA gene promoters

Based on the genome annotation file of Populus wilsonii, confirm the transcription start site (TSS) coordinates of each AUX/IAA gene, and designate the upstream 2000bp region of TSS as the core regulatory region of the promoter. Subsequently, the promoter sequence was extracted from the FASTA file of the Populus wilsonii genome using Bedtools software. The next step can refer to the PlantCARE website（http://bioinformatics.psb.ugent.be/webtools/plantcare/html/）Batch analysis and comparison of the promoter sequences in FASTA format can be performed, and tissue specific elements such as meristem specific elements (CAT box, GCCACT), vascular bundle specific elements (TELO box, AAACCCTA), and other types of elements can be identified through systematic recognition.

Afterwards, in the process of data statistics, the Pandas library in Python can be used to calculate the frequency of occurrence of various types of components, which facilitates the screening of high-frequency conservative components appearing in family members. Then, TBtools can be used to input a rectangular file with gene ID as the row, component type as the column, and component quantity as the value, and then draw a heatmap with color gradient to represent frequency.

2.3 Analysis of Organizational Expression Patterns and Construction of Protein Interaction Networks

2.3.1 Analysis of Organizational Expression Patterns

This study is based on RNA seq data analysis of the tissue expression patterns of the AUX/IAA gene family in Populus wilsonii. Firstly, the original FPKM data is subjected to Log2 (FPKM+1) standardization to reduce the bias of highly expressed genes and correct for differences in sequencing depth between samples.

Subsequently, gene and tissue expression matrices were constructed using TBtools software, and gene expression similarity was calculated using Euclidean distance. Hierarchical clustering analysis was performed using the complete linkage method to identify significantly differentially expressed clusters based on the maximum distance criterion between classes. The final generated heatmap is graded by red and blue colors (red represents high expression, blue represents low expression).

2.3.2 Construction of protein interaction network

When constructing a protein interaction network for the AUX/IAA gene family, the AUX/IAA protein sequence (FASTA format) of Populus wilsonii is first uploaded to the STRING database. Afterwards, the closely related species Populus tomentosa can be selected as the reference species, and a high confidence threshold of comprehensive score ≥ 0.7 is set. Multiple sources of evidence such as experimental verification, co expression analysis, and text mining are integrated, and a TSV format interaction list containing interaction types (such as physical and genetic interactions) and confidence scores is downloaded.

Afterwards, using Cytoscape v3.9.1 to import interaction data and visualize the network, the node size needs to be set based on the degree of connectivity, with higher degrees of connectivity resulting in larger node volumes. Among them, the color of the edges needs to be colored according to the gradient of confidence ratings (where gray represents low confidence and red represents high confidence).

Afterwards, the centrality index of nodes can be calculated using the CytoHubba plugin to screen for core genes that play a critical role in the network properties, and then the MCODE plugin can be used to identify closely interacting sub networks.

Summary of chapter II

1.Gene screening and validation: HMMER software was used to screen candidate genes in the Populus wilsonii genome based on AUX/IAA domains. After domain integrity filtering, the homologous sequences of Arabidopsis and Populus tomentosa were subjected to secondary screening using BLASTP, and the results were integrated to determine high reliability family members.

2.Structural and conservative domain analysis: Extract gene structural coordinates, visualize exon intron composition using GSDS tools, and analyze family structural conservation; Using multiple databases to jointly annotate conservative structural domains, analyze conservative motifs through MEME and plot them.

3.System Evolution Analysis: Based on MEGA software, a phylogenetic tree is constructed. Through sequence alignment, model screening (JTT model), and self expansion testing, family members are divided into four subfamilies, and evolutionary relationships are verified by combining gene structure and motif distribution.

4.Initiator element analysis: Extract the 2000bp sequence upstream of the transcription start site, identify tissue-specific cis acting elements through PlantCARE, count element frequencies, and visualize the distribution of conserved elements using a heatmap.

5.Expression pattern analysis: Standardize RNA seq data, construct expression matrices and cluster analysis, and display differential expression patterns of genes in different tissues through heat maps.

6.Construction of protein interaction network: Based on STRING database, high confidence interaction relationships were screened, and Cytoscape visualization network was used to screen core genes and interaction sub networks by combining node connectivity and confidence.

Chapter III
EXPERIMENTAL PART

3.1 Identification and physicochemical properties analysis of AUX/IAA gene family in Populus wilsonii

Research has shown that the AUX/IAA gene family plays a crucial role in regulating and controlling plant growth and development[33]. To systematically analyze the biological characteristics of the PwiAUX/IAA gene family in Populus wilsonii, this study will conduct comprehensive identification and analysis of its members based on genomic data. After integrating the sequence HMM and BLAST results, a total of 35 PwiAUX/IAA genes were screened (Table 3-1).

After analyzing the chromosomal localization, it can be found that these genes are distributed on multiple chromosomes, which can explain their widespread distribution in the genome. At the same time, there are significant differences in the length of encoded amino acid sequences among family members, such as the 34.5% difference in sequence length between PwiAUX/IAA1 (220 amino acids) and PwiAUX/IAA25 (245 amino acids).

The molecular weight distribution range of the encoded protein is 19.21-40.07 kDa, with an isoelectric point between 5.25-9.26, indicating significant diversity in its physicochemical properties.

Table 3-2 Characteristics of AUX/IAA Genes in Populus wilsonii

Sequence ID	Gene ID	Chromosome	Startup	Endpoint	Number of amino acids	Molecular weight	Isoelectric point

Pwi01G015940.1	PwiAUX/IAA1	Chr01	16447171	16449318	220	24470.58	5.25
Pwi01G015950.1	PwiAUX/IAA2	Chr01	16452592	16456332	189	21213.1	6.19
Pwi01G016670.1	PwiAUX/IAA3	Chr01	17527623	17531111	353	38018.46	8.42
Pwi01G017020.1	PwiAUX/IAA4	Chr01	18290485	18294065	337	37066.88	8.87
Pwi02G004160.1	PwiAUX/IAA5	Chr02	3070845	3073783	237	26273.07	8.52
Pwi02G004170.1	PwiAUX/IAA6	Chr02	3083056	3085011	199	22260.2	8.32
Pwi02G009780.1	PwiAUX/IAA7	Chr02	8357579	8363151	368	40067.5	6.14
Pwi02G016600.1	PwiAUX/IAA8	Chr02	15132399	15133463	174	19280.62	4.86
Pwi02G022810.1	PwiAUX/IAA9	Chr02	25535888	25541675	290	30999.98	8.86
Pwi03G005110.1	PwiAUX/IAA10	Chr03	9269124	9272503	335	36871.8	8.54
Pwi03G005370.1	PwiAUX/IAA11	Chr03	9802203	9805849	351	37884.5	8.58
Pwi03G005920.1	PwiAUX/IAA12	Chr03	10754727	10760581	183	20652.57	8.33
Pwi05G004520.1	PwiAUX/IAA13	Chr05	3782542	3784162	201	22855.76	5.22
Pwi05G004530.1	PwiAUX/IAA14	Chr05	3792926	3796464	238	25822.18	5.77

Pwi05G020200.1	PwiAUX/IAA15	Chr05	24273066	24274938	199	22176.97	5.49
Pwi05G020210.1	PwiAUX/IAA16	Chr05	24287047	24290938	237	26250.91	8.68
Pwi06G005810.1	PwiAUX/IAA17	Chr06	5122328	5123989	233	26626.9	5.73
Pwi06G014170.1	PwiAUX/IAA18	Chr06	15618851	15622390	319	34497.75	6.45
Pwi06G014800.1	PwiAUX/IAA19	Chr06	17862077	17863781	175	19280.82	6.97
Pwi06G020660.1	PwiAUX/IAA20	Chr06	25203985	25207719	332	37112.99	8.56
Pwi06G022430.1	PwiAUX/IAA21	Chr06	26723249	26724670	229	26260.52	6.13
Pwi08G014330.1	PwiAUX/IAA22	Chr08	11302085	11304302	226	24918.17	5.7
Pwi08G014340.1	PwiAUX/IAA23	Chr08	11319541	11322141	279	31243.02	8.66
Pwi08G015330.1	PwiAUX/IAA24	Chr08	12187012	12190190	313	32839.95	9.26
Pwi09G010980.1	PwiAUX/IAA25	Chr09	11389861	11390830	145	16223.6	6.63
Pwi10G005100.1	PwiAUX/IAA26	Chr10	8760839	8764039	303	32136.39	8.98
Pwi10G006320.1	PwiAUX/IAA27	Chr10	9866621	9869446	277	30521.95	8.69
Pwi10G006330.1	PwiAUX/IAA28	Chr10	9896832	9898447	192	21460.32	6.03

Pwi10G010010.1	PwiAUX/IAA29	Chr10	13219158	13220981	194	21944.66	5.68
Pwi13G003910.1	PwiAUX/IAA30	Chr13	3306157	3308013	203	22893.94	6.77
Pwi13G003920.1	PwiAUX/IAA31	Chr13	3325352	3328377	246	26767.52	7.57
Pwi14G008570.1	PwiAUX/IAA32	Chr14	7552376	7553485	175	19561.93	5.04
Pwi18G004720.1	PwiAUX/IAA33	Chr18	5839088	5842807	341	37848.65	8.42
Pwi18G007730.1	PwiAUX/IAA34	Chr18	12154282	12155921	175	19276.63	6.51
Pwi18G010590.1	PwiAUX/IAA35	Chr18	14788064	14789595	233	26231.42	5.72

3.2 Chromosome Localization of AUX/IAA Genes in *Populus wilsonii*

Chromosomes, as the main carriers of genetic information, have important genetic mapping features that serve as a basis for analyzing gene functions and evolutionary mechanisms[34].

This study analyzed the chromosomal distribution of the AUX/IAA gene family, and the results showed that the 35 PwiAUX/IAA members of the family were distributed on 11 chromosomes (Chr01, Chr02, Chr03, Chr05, Chr06, Chr08, Chr09, Chr10, Chr13, Chr14, Chr18), exhibiting non-uniform distribution characteristics in the genome (Figure.3.1). The systematic analysis of the family's chromosomal localization characteristics and distribution patterns was conducted. Similar to the pattern of the AUX/IAA gene family in Arabidopsis, which is concentrated on five chromosomes and has tandem repeat clusters[35], the AUX/IAA genes in Populus wilsonii show a local clustering trend

on chromosomes such as Chr01, Chr08, and Chr10, and the overall gene cluster also exhibits tandem repeat characteristics.

From a specific distribution perspective, there are significant differences in the degree of gene enrichment among different chromosomes. The PwiAUX/IAA7, PwiAUX/IA8, and PwiAUX/IA9 of Chr02 are scattered and do not form a tight gene cluster. Some chromosomes also exhibit a single gene distribution: Chr09 and Chr14 only contain one family member.

The annotation information of homologous gene pairs in the map indicates significant chromosomal heterogeneity in the AUX/IAA gene family of Populus wilsonii. PwiAUX/IAA1 and PwiAUX/IAA2 on Chr01, PwiAUX/IA5 and PwiAUX/IA6 on Chr02, PwiAUX/IA13 and PwiAUX/IA14, PwiAUX/IA15 and PwiAUX/IA16 on Chr05, PwiAUX/IA22 and PwiAUX/IA23 on Chr08, PwiAUX/IA27 and PwiAUX/IA28 on Chr10, and PwiAUX/IA30 and PwiAUX/IA31 on Chr13 all form serial repeats. According to collinearity analysis, no chromosomes with collinear gene pairs were found.

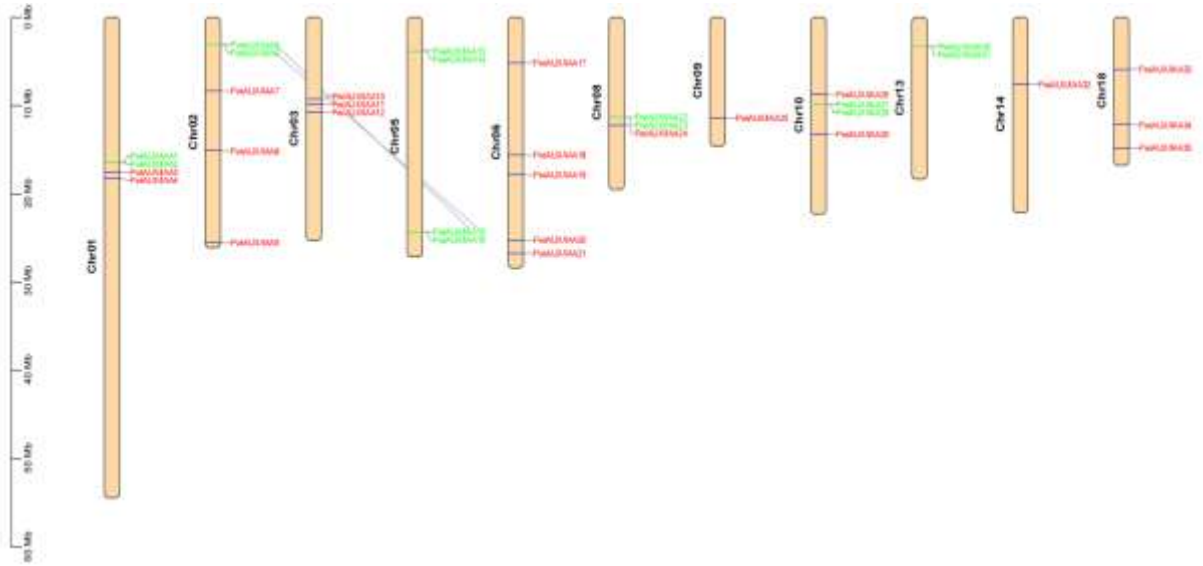

Figure. 3-1 Chromosome position and fragment duplication of AUX/IAA genes in Populus wilsonii

3.3 Phylogenesis of Populus wilsonii AUX/IAA

This study constructed a phylogenetic tree of the AUX/IAA gene family in Populus wilsonii (3-4Figure.) to analyze its phylogenetic characteristics. Based on evolutionary relationships, the genes of this family are clearly divided into four groups (Group A-D), with significant evolutionary differentiation between groups.

Group A includes members such as PwiAUX/IAA17 and PwiAUX/IAA19, whose node self expansion support rate is high (bootstrap value ≥ 0.95), indicating strong gene conservation in this group. It is speculated that this may be due to recent gene replication events and maintain functional conservation. Group B, represented by PwiAUX/IAA9 and PwiAUX/IA24, forms independent branches with low cross group support (<0.60), suggesting that this group of genes may form unique functional modules through domain recombination or forward selection. It is worth noting that Group C only contains PwiAUX/IAA2 and PwiAUX/IA12, and its long branch length (>0.8) combined with high self expansion support rate (0.98) suggests that it may have experienced special selection pressure or functional innovation. Group D members such as PwiAUX/IAA6 and PwiAUX/IAA15 showed moderate support rates (0.85-0.95), and the cross species conserved motif distribution pattern was highly similar to that of dicotyledonous homologous genes, further confirming the functional stability of this group of genes in evolution.

This study elucidated the evolutionary trajectory of the AUX/IAA gene family in Populus wilsonii from a phylogenetic perspective, and its grouping characteristics and functional differentiation patterns provided evolutionary biology basis for analyzing the regulatory network of this family in the growth and development of Populus wilsonii[36]. Subsequently, the functional differentiation hypothesis can be further validated by combining the gene expression patterns and phenotype data of each group, deepening the understanding of the regulatory mechanism of this gene family.

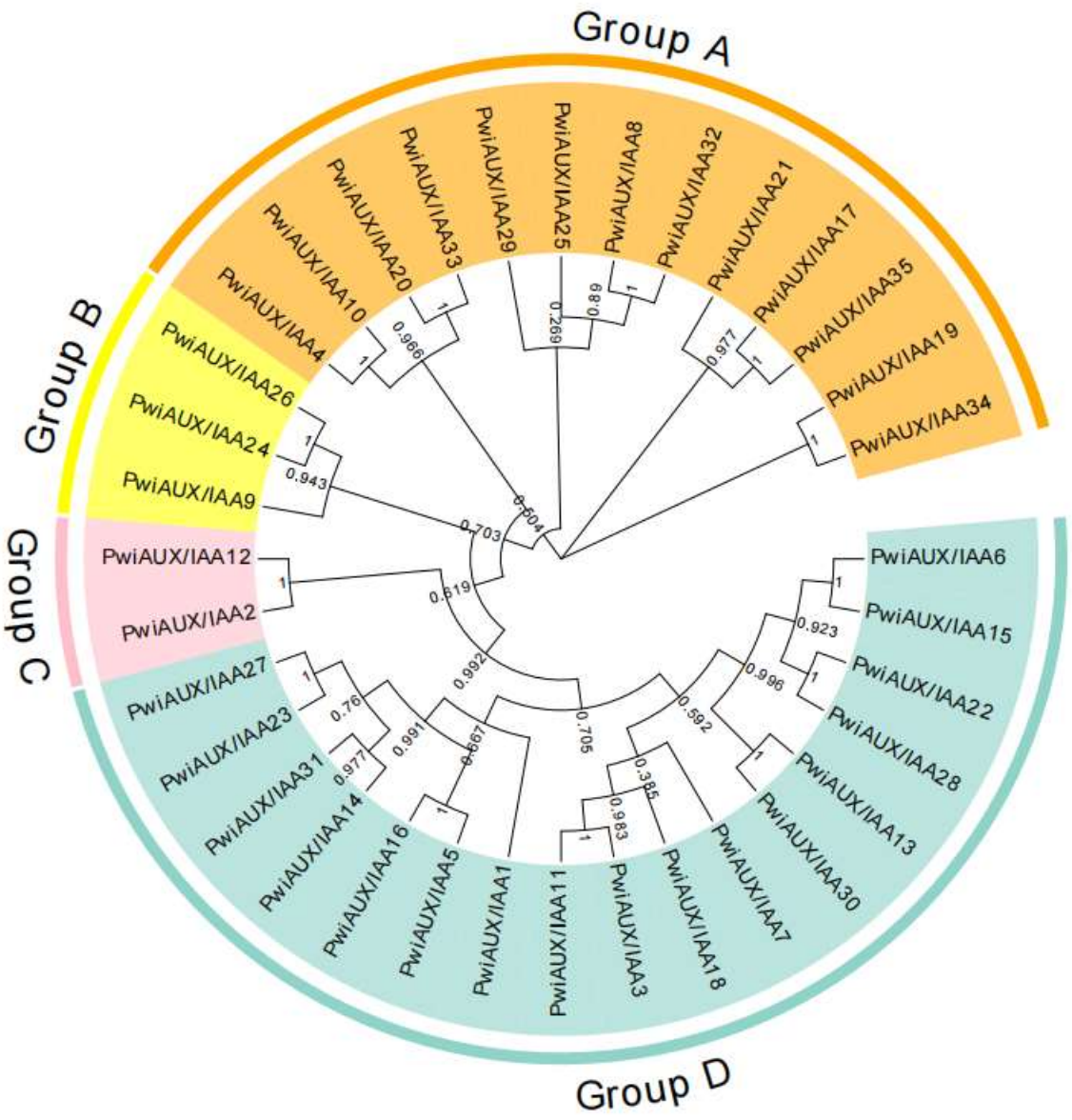

Figure. 3-2 Phylogenetic relationship of AUX/IAA in four groups of Populus wilsonii trees

3.4 Conservative domain and motif analysis of AUX/IAA genes in Populus wilsonii

This study is based on bioinformatics methods to analyze the conserved motifs and cis acting elements of the AUX/IAA gene family in Populus wilsonii (3-3, Figure). Through MEME software and Pfam database retrieval, 10 conserved motifs (Motif 1-10, Figure 3-4) and multiple functionally related cis acting elements were identified among 35 PwiAUX/IAA genes. The distribution

characteristics of these motifs and elements are significantly correlated with phylogenetic grouping. All family members contain core functional domains involved in auxin signaling response (Motif 2 and Motif 3), indicating that the family has a basic conserved function in plant hormone regulatory pathways.

The motif composition of different subfamilies shows specific differentiation: Group A (such as PwiAUX/IAA1, PwiAUX/IA7) is enriched in Motif 4 and Motif 8, both of which are related to hormone response, suggesting that this group of genes plays an important role in auxin signaling; Group B (such as PwiAUX/IAA9, PwiAUX/IA24) specifically contains Motif 10, while Group C (such as PwiAUX/IA2, PwiAUX/IA12) lacks the conserved motif Motif 5, but significantly enriches the motif Motif 6 related to light response.

Gene structure analysis shows that there are differences in the distribution of UTR and CDS regions among various genes. Some genes have significant changes in UTR length, while CDS regions exhibit a relatively conservative coding framework[37]. This structural feature may be closely related to gene expression regulation and functional diversity.

The analysis of the promoter region shows that this family of genes contains multiple cis acting elements, whose functions involve hormone response, stress response, and growth and development regulation[38]. PwiAUX/IAA14 and PwiAUX/IAA31 both contain auxin responsive elements (TGA elements) and drought inducing elements (MBS), suggesting their dual function in auxin mediated drought tolerance regulation; PwiAUX/IAA6 and PwiAUX/IAA15 contain more gibberellin responsive elements (GARE motif) and meristem specific elements (CAT box), suggesting their involvement in regulating stem apical meristem development. In addition, low expression genes such as PwiAUX/IAA25 may lose their transcriptional activity or develop special functions during evolution due to the loss of core motifs and a significant reduction in cis elements.

The motif distribution pattern is highly consistent with the phylogenetic grouping: genes within the same evolutionary branch have similar motif combinations (such as Group A containing Motif 1-3, 4, 8), while the motif composition of genes across groups differs significantly (such as Group C lacking key motifs). This consistency suggests that the acquisition, loss, or variation of motifs may be the molecular basis for the functional differentiation of AUX/IAA genes.

In summary, this study reveals the characteristics of "core functional conservation and subfamily specific differentiation" in the AUX/IAA gene family of Populus wilsonii. The conservation of the core motif supports its fundamental role in the auxin signaling pathway, while the differences in specific motifs and cis elements provide molecular evidence for functional differentiation.

Conserved amino acid analysis shows that specific amino acids within the motif are strictly conserved in evolution and are crucial for maintaining the structure and function of the motif. Some hydrophobic or polar amino acids may play a key role in protein interactions or hormone binding[39].

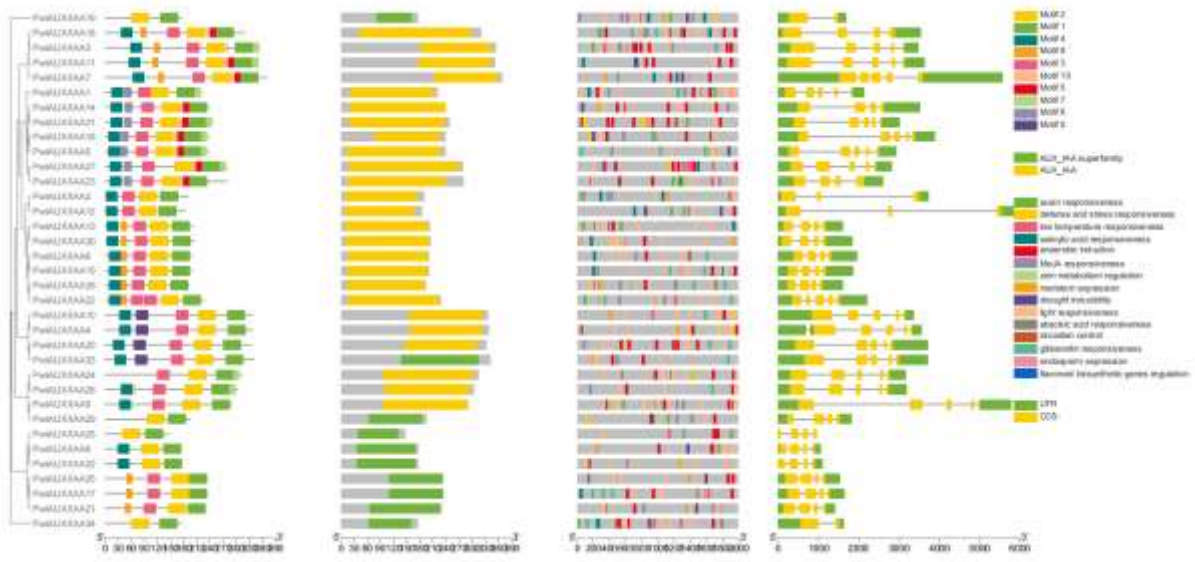

Figure 3-3 Evolutionary tree, Motif analysis, conserved domains, promoters, and gene structure of PwiAUX/IAA genes (from left to right)

Figure 3-4 Conserved amino acid sequences of some motifs in PwiAUX/IAA protein

3.5 Tissue-specific expression of AUX/IAA genes in Populus wilsonii

This study revealed the potential functional differences in the growth and development of the Populus wilsonii AUX/IAA gene family by analyzing their tissue-specific expression patterns (Figure 3-5). Heat map analysis showed that PwiAUX/IAA14 and PwiAUX/IAP31 exhibited sustained high expression in the

xylem, phloem, and meristematic tissues (red to orange regions), indicating that they may participate in multi tissue development regulation by regulating basic physiological processes; The expression levels of genes such as PwiAUX/IAA25 in most tissues are below the detection threshold (gray to blue area), indicating that their functions may be specifically regulated by specific environmental signals or developmental stages.

It is worth noting that some genes, such as PwiAUX/IAA6 and PwiAUX/IAA15, have significantly increased expression levels (>5-fold) in root tip meristematic tissues compared to other tissues, indicating that they may participate in root development by regulating cell division activity. In addition, about 35% of genes (such as PwiAUX/IAA9, PwiAUX/IAA24) exhibit significant enrichment characteristics in specific tissues such as xylem.

At the same time, it can be demonstrated that the widely expressed AUX/IAA genes may maintain basic plant growth regulatory functions, while tissue-specific genes precisely regulate specific developmental processes through spatiotemporal expression. These findings provide important evidence for analyzing the functional differentiation of the AUX/IAA gene family in Populus wilsonii and its molecular mechanisms in organ construction[40]. Subsequently, its biological function can be further validated through gene silencing or overexpression experiments, combined with tissue-specific promoter analysis.

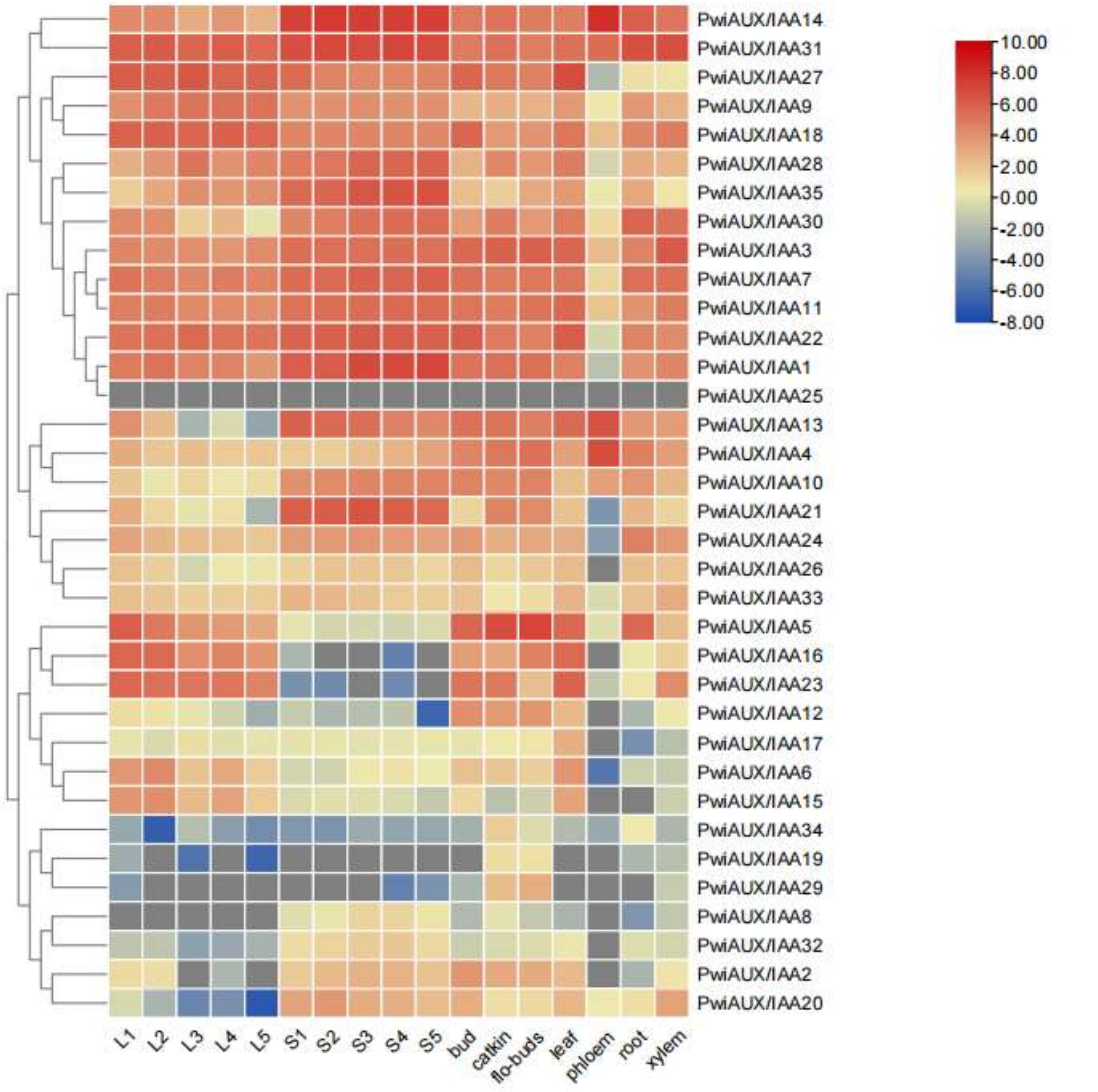

Figure 3-5 RT qPCR analysis results of PwiAUX/IAA in multiple groups

3.6 Interactive protein network of AUX/IAA gene family in Populus wilsonii

This study constructed a protein interaction network of AUX/IAA genes in Populus wilsonii (Figure 3-6-6) to elucidate the mechanism of action of this family of genes in the regulatory network. In a network diagram[41], nodes represent genes, and their size is positively correlated with interaction frequency or network centrality, while lines represent protein-protein interactions.

Analysis shows that core nodes such as PwiAUX/IAA1, PwiAUX/IAA2, and PwiAUX/IA9 have high-frequency interaction characteristics, among which PwiAUX/IAA1 forms multi-channel connections with PwiAUX/IA4, PwiAUX/IA26, etc., indicating its pivotal role in regulating the growth of Populus wilsonii. In contrast, edge nodes such as PwiAUX/IAA6 and PwiAUX/IAA14 have a narrower range of interaction, indicating that their functionality may be limited to specific organizations or conditions.

Network topology analysis shows that the interaction modes are mainly "one to many" and "many to many"[42]. For example, PwiAUX/IAA9 connects genes such as PwiAUX/AA10 and PwiAUX/AA29 in a "one to many" mode, indicating its ability to synergistically regulate multiple downstream targets; The core node group (such as PwiAUX/IAA1, PwiAUX/IAA2, PwiAUX/IA4) forms a complex regulatory network through "many to many" interactions, which may synergistically play a role in hormone signal integration.

This study reveals the functional hierarchy of AUX/IAA genes in Populus wilsonii: core genes coordinate multiple physiological processes through extensive interactions, and specific interaction patterns reflect the division and collaboration of gene functions. These findings lay a structural foundation for elucidating the regulatory mechanisms of this family of genes. Subsequently, key interactions can be validated through yeast two hybrid or immunoprecipitation experiments[43], and their specific mechanisms in the growth and development of Populus wilsonii can be elucidated using genetic methods.

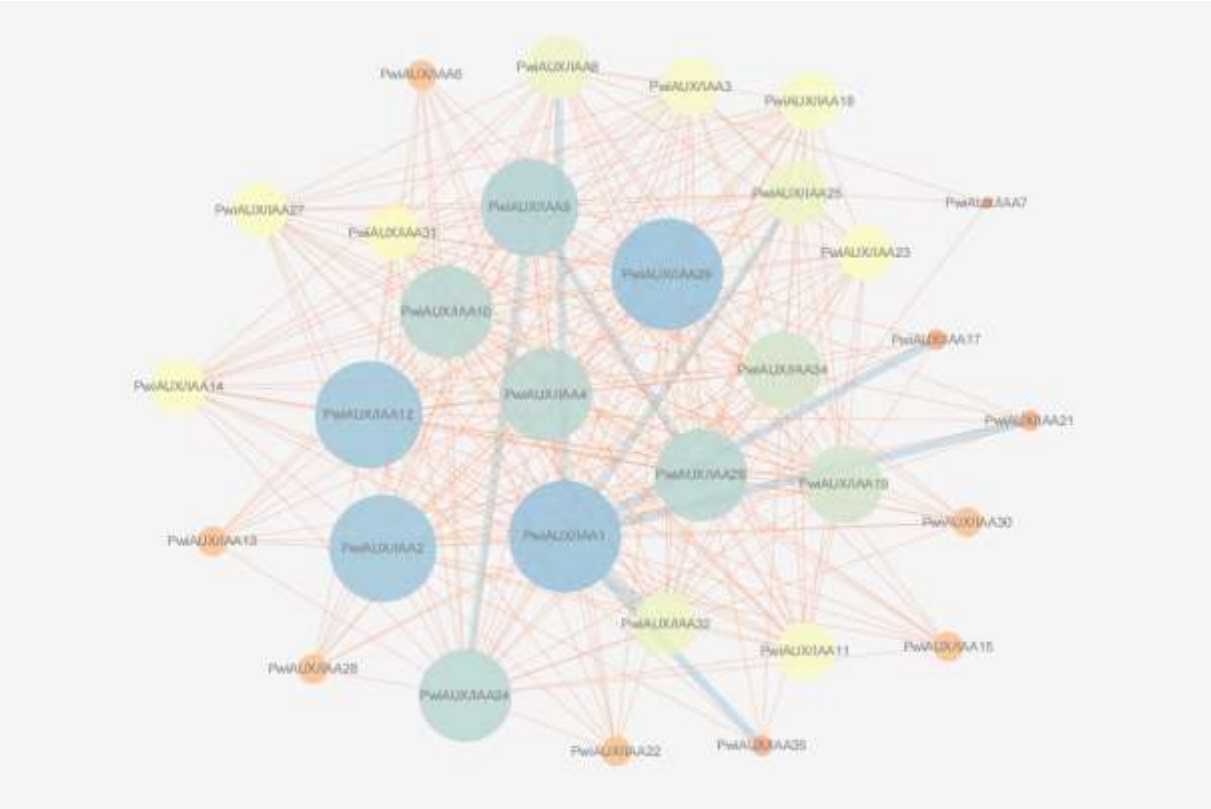

Figure 3-6 Construction of the Interacting Protein Network of AUX/IAA Gene Family in *Populus wilsonii*

Summary of chapter III

1.Gene identification and physicochemical properties: 35 AUX/IAA genes were screened using HMM and BLAST, distributed on 12 chromosomes, with significant differences in amino acid length[44], molecular weight (19.21-40.07 kDa), and isoelectric point (5.25-9.26) among members.

2.Chromosome Localization: 35 genes are distributed on 11 chromosomes, showing a non-uniform distribution[45]. Some chromosomes (such as Chr01 and Chr08) exhibit locally clustered tandem repeats, with no collinear gene pairs[46].

3.Systemic Development: The family is divided into four subfamilies (Group A-D): Group A is highly conservative, Group B is functionally specialized, Group C undergoes special selection, and Group D is functionally stable[47].

4.Conservative structural domains and motifs: All members contain the core motif of auxin signaling (Motif 2, 3), and subfamilies have specific motifs (such as Group A hormone response motif); The promoter contains 20 types of components, involving hormones[48], stress, and developmental regulation.

5.Organizational expression patterns: PwiAUX/IAA14/31 is highly expressed in multiple tissues[49], while PwiAUX/IAA6/15 is significantly highly expressed in root meristematic tissue, reflecting the characteristics of extensive expression and tissue-specific binding[50].

6.Protein Interaction Network: Core genes (such as PwiAUX/IAA1/9) form regulatory hubs, while edge genes have limited interaction ranges and tissue-specific functions[51]. The network topology reflects the hierarchical regulation of genes[52].

CONCLUSION

1.Genetic Family Characteristics and Evolutionary Mechanisms: 35 AUX/IAA genes were identified from the genome of Populus wilsonii, and their chromosomal localization showed non-uniform distribution, significantly different from the tandem repeat pattern in Arabidopsis. Research suggests that family expansion is mainly driven by chromosome fragment duplication or translocation, while dispersed distribution and local aggregation promote functional differentiation and collaborative expression, respectively, reflecting the optimization strategy of gene regulatory networks under natural selection.

2.Relationship between Systemic Development and Functional Differentiation: Phylogenetic analysis divides the family into four subfamilies (Group A-D), each exhibiting specific differentiation in conserved motifs, cis acting elements, and expression patterns. Group B: Its structural domain recombination features imply special functions in response to abiotic stress; Group C: Enriched photoresponsive elements, highly consistent with the evolutionary strategy of poplar photoperiod regulation; Core motif conservation: supports the fundamental function of the family in the auxin signaling pathway, while subfamily specific motifs reveal the molecular basis of functional differentiation.

3.Functional hierarchy of spatiotemporal expression and interaction networks: Tissue-specific expression: PwiAUX/IAA14 and PwiAUX/IAA31 are consistently highly expressed in multiple tissues such as xylem, while PwiAUX/IAA6 and PwiAUX/IAA15 are specifically highly expressed in root meristematic tissue, revealing a dual functional pattern of extensive regulation and tissue specialization;

Protein interaction network: Core genes (such as PwiAUX/IAA1, PwiAUX/IA9) form regulatory hubs through "one to many" or "many to many" interaction patterns, while the interaction range of edge genes is limited, indicating tissue specificity in their function.

4.Research Value and Application Prospects: This study provides the first systematic analysis of the evolutionary characteristics and regulatory network of the AUX/IAA gene family in Populus wilsonii, providing important theoretical

basis for elucidating the molecular mechanisms of auxin signaling, tissue differentiation, and stress adaptation. The key genes identified through research can provide precise gene editing targets for molecular breeding of forest trees, such as improving wood fiber quality. Its systematic analysis framework provides a methodological reference for the study of other forest gene families.

5.Research Limitations and Future Directions: The research has not yet been combined with experimental verification (such as gene editing, protein interaction verification) of gene function. In the future, it is necessary to further clarify the regulatory mechanisms of key genes and deepen the understanding of the molecular mechanisms of the auxin signaling pathway in woody plants through technologies such as tissue-specific expression validation, yeast two hybrid, and immunoprecipitation.

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