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 **Effect of growth hormone signal transduction on biomass increase in *Cenchrus purpureus*.**

First (Bachelor's) level of higher education

Specialty 162 "Biotechnology and Bioengineering"

Educational and professional program "Biotechnology"

Completed: student of group BEBT-20
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Scientific supervisor
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Educational and professional program Biotechnology

APPROVE
Head of Department of Biotechnology, Leather and Fur, Professor,
Doctor of Technical Science
Olena MOKROUSOVA

_____
«___»_____2024

ASSIGNMENTS
FOR THE QUALIFICATION THESIS
Li Tianle

1. Thesis topic **Effect of growth hormone signal transduction on biomass increase in *Cenchrus purpureus*.**
Scientific supervisor Olena Okhmat, Ph.D., Assoc. Prof.

approved by the order of KNUTD "____"_____2024, №_____

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

4. Date of issuance of the assignments_____

EXECUTION SCHEDULE

№	The name of the stages of the qualification thesis	Terms of performance of stage	
1	Introduction	From 01 April 2024 to 11 April 2024	
2	Chapter 1. Literature review	From 06 April 2024 to 20 April 2024	
3	Chapter 2. Object, purpose, and methods of the study	From 21 April 2024 to 30 April 2024	
4	Chapter 3. Experimental part	From 01 May 2024 to 10 May 2024	
5	Conclusions	From 07 May 2024 to 12 May 2024	
6	Draw up a bachelor's thesis (final version)	From 20 May 2024 to 25 May 2024	
7	Submission of qualification work to the supervisor for feedback	From 25 May 2024 to 27 May 2024	
8	Submission of bachelor's thesis to the department for review (14 days before the defense)	27 May 2024	
9	Checking the bachelor's thesis for signs of plagiarism (10 days before the defense)	31 May 2024	
10	Submission of bachelor's thesis for approval by the head of the department (from 7 days before the defense)	03 June 2024	

I am familiar with the task:

Student _____ Tianle LI

Scientific supervisor _____Olena OKHMAT

SUMMARY

Tianle Li. Effect of growth hormone signal transduction on biomass increase in *Cenchrus purpureus*– Manuscript.

Qualification thesis on the specialty 162 «Biotechnology and Bioengineering». – Kyiv National University of Technologies and Design, Kyiv, 2024.

Elephant grass, also known as Napier grass, is important forage in Asia, Africa and the tropical and subtropical regions of the Americas, and is also a new type of energy plant. Elephant grass is widely used in agricultural production and industrial applications because of its well-developed root system, high light efficiency and high biomass. In order to reveal the genetic basis of its high biomass, we explored the auxin signal transduction pathway of elephant grass through bioinformatics methods. In this study, BlastP was used to identify 57 members of 6 gene families in the auxin signal transduction pathway of elephant grass cells. Chromosome localization analysis showed that 6 gene families were distributed on 14 chromosomes of elephant grass, and the B6 chromosome had the most genes. Protein molecular weight, isoelectric point, instability coefficient, fat solubility index and hydrophilic index of elephant grass were obtained by using TBtools Protein parameter calculation tool. Phylogenetic analysis showed that the homologous expression of biological functions of gene families in plants may be related to phylogenetic evolution. Selection pressure analysis showed that all gene families were not affected by positive selection, that is, no beneficial mutations occurred in the evolution process and were preserved by natural selection. Expression analysis showed that the expression levels of each gene were different in different tissues. Only a small amount of C3H family gene was detected in different tissues, and the other family genes were expressed in different developmental stages or different developmental parts of the roots and leaves, indicating that they participated in the whole development process of the roots and leaves of elephant grass, and played an important role in the regulation of growth and development of elephant grass. Protein interaction analysis showed that the genes

were related to each other, and the two linked genes had similar biological functions, among which AUX/IAA family was the main core gene of elephant grass gene family protein network. The analysis of the signal transduction pathway of grass cytokinin in this study provides an important reference for further understanding of the growth and development mechanism of elephant grass, and also lays a foundation for future research on related genetic improvement and growth regulation.

Key words: *Elephant grass; Cytokine signaling pathway; Chromosomal localization; Expression analysis; Phylogenetic tree analysis; Protein interaction analysis*

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INTRODUCTION

The relevance of the topic is Analysis and study of growth hormone signal transduction pathway in elephant grass.

The purpose of the study is the Elephant grass is a new type of energy plant and animal husbandry grass, and its growth and development characteristics have attracted much attention. In order to improve the planting efficiency of elephant grass and develop animal husbandry and industry in China, the growth hormone transduction pathway of object grass has been studied. This study intends to obtain all gene families of elephant grass growth hormone transduction pathway through bioinformatics analysis, and clarify the transmission process of elephant grass growth hormone signal and its key molecular mechanism through chromosome localization analysis, protein parameter analysis, whole genome replication analysis, phylogenetic tree analysis, expression analysis, protein interaction analysis and gene structure analysis.

The objectives of the study This will provide a theoretical basis for the regulation of plant growth and development. Through bioinformatics analysis of the regulatory factors of grass, key factors and regulatory networks related to growth hormone signal transduction pathways of elephant grass are obtained, which will provide an important reference for subsequent research on the mechanism of plant growth regulation and provide a deeper understanding and understanding of the biological functions of growth hormone and the regulatory mechanisms of plant growth and development. It is helpful to reveal the commonness and difference of growth hormone signal transduction pathways in elephant grass, which is of great significance to further explore the regulation of plant growth and development, and provide theoretical support for theoretical research in related fields.

The object of the study growth hormone signal transduction pathway in elephant grass.

The subject of the study elephant grass.

Research methods (1) By using the BlastP tool, the auxin pathway genes of elephant grass cells were searched, and the conditions were :identity>85 p<1e-9.

(2) Organize the elephant grass gene ID to be studied into the newly created txt document, and rename the required genes to another newly created document in turn. Sort out the LINK file of the desired gene in the elephant grass gene bank: find the tandem duplicate gene and set the appropriate RGB color against the txt document. Use the Gene Location Visualize from GTF/GFF tool of TBtools software to import the above sorted files successively and export the positioning analysis diagram.

(3) Find out the desired auxin gene protein sequence from the downloaded elephant grass protein sequence and organize it into fasta file. The MEGA-X tool was used to construct the phylogenetic tree by adjacency method for the protein sequences required by the object grass. Beautify the formed evolutionary tree with the FigTree tool.

(4) Transcriptome sequencing data or qPCR and other methods can be used to analyze the expression of auxin gene in different tissues and under different treatment conditions. Attention needs to be paid to the quality control of experimental design and data analysis, as well as the verification of results in conjunction with other experimental data. The obtained TPM gene expression data of elephant grass were sorted out, gene ids were renamed, and the heat map was introduced into TBtools' HeatMap to analyze the expression levels of different genes.

Due to the close relationship between elephant grass and purple elephant grass, the protein sequence set and protein interaction network of purple elephant grass were downloaded. TBtools' PPI Predict tool is used to introduce the complete protein sequence of elephangrass and the two downloaded files in turn. Multiple files are generated in the working directory. The output file after protein interaction analysis using TBtools can be directly used for filtering or visualization. Cytoscape tools were used to draw and beautify the coexpression network analysis diagram to facilitate the analysis of the interaction between genes.

The scientific novelty This paper reflects the analysis and research of plant hormone signal transduction pathway, experimental verification and data analysis of the growth hormone signal transduction pathway of grass.

The practical significance of the results obtained is It is expected to elucidate the transmission process of elephant grass growth hormone signal and its key molecular mechanisms, thus providing a theoretical basis for the regulation of plant growth and development, and it is expected to obtain the relevant key factors and regulatory networks of elephant grass growth hormone signal transduction pathway, which will provide an important reference for the subsequent research on the mechanism of plant growth regulation. It is expected that the biological function of this hormone and the regulation mechanism of plant growth and development will be more deeply understood and recognized, which will provide certain reference and inspiration for theoretical research in related fields.

CHAPTER 1
LITERATURE REVIEW

1.1 Overview of elephant grass

1.1.1 Introduction to elephant grass

Elephant grass (Pennisetum purpureum Schum) is a large, tufted perennial herbaceous plant of the gramineae family and the panicum family, often with underground stems. Elephant grass, also known as purple Wolftail, is native to tropical Africa and was introduced into China from India, Myanmar and other places in the 1940s. It has the advantages of rapid growth, high yield and good reproduction [1]. Purple elephant grass has the characteristics of easy planting, high yield, good palatability, strong stress resistance, high nutritional value, strong regeneration and high calorific value compared with other herbage, which is not only applicable to herbivorous herbage, but also applicable to industrial production and water and soil conservation, which is of great significance for the development of animal husbandry and industry in China [2].

1.1.2 Research progress of elephant grass

Elephant grass is A tropical herb with important economic value. It has 2n=4x=28 chromosomes and is a heterotetraploid organism, including two subgenomes with genotype A'A'BB [3]. Ye Jianjun analyzed the traits of 10 samples of Pennsylvania herbage, selected 10 measurement indicators, analyzed the differences among materials, and finally divided Pennsylvania herbage into three categories. Through RAPD marker and ISSR marker analysis, it was found that the genome of purple elephant grass was quite different from that of other varieties and became a single category [4]. Wu Juanzi et al. isolated and cloned another CCoAOMT gene from elephant grass, named it CpCCoAOMT2 and carried out bioinformatics analysis. The analysis results showed that CpCCoAOMT2 gene and its encoded protein had homology with CCoAOMT gene and CCoAOMT protein of

various plants at nucleotide and amino acid levels, which provided theoretical basis for studying the function of CCoAOMT gene and its encoded protein in the lignin metabolism of elephant grass. It provides the basis for the lignin improvement of elephant grass [5]. Lin Xiongjie et al. carried out ITS and chloroplast matK analysis on six strains of the genus Pyrrhiza, through which the six strains could be well distinguished and the phylogenetic relationships between elephant grass and other strains could be studied by constructing corresponding phylogenetic trees. However, in the phylogenetic tree constructed by matK, only elephant grass (MQ) could be distinguished from the other five strains of the genus Pyrrhiza with a relatively long genetic distance [6]. Zhu Qionghua et al. cloned the cDNA sequence and full-length DNA sequence of the cinnamyl CoA reductase gene (CCR) involved in lignin biosynthesis from elephant grass, an energy plant, and analyzed its sequence characteristics. A 1316bp cDNA sequence of elephant grass CCR containing coding region and 3 'untranslated region and a 6133bp DNA sequence containing 5 exons and 4 introns were obtained. The cinnamyl-CoA reductase gene was successfully cloned from elephant grass. Various bioinformatics data obtained provide certain theoretical reference value for further research on this enzyme and better utilization of object grass [7].

1.2 Research progress of plant growth hormone signal transduction pathways

Hormones play a role in regulating plant growth and development, drought stress, nutrient absorption and stress resistance, so in-depth study of hormone signal transduction pathways is of great significance for understanding plant growth regulation mechanisms. Auxin signal transduction can be realized in two ways: one is through stimulating non-transcriptional level signals in the cytoplasm; The other is in the nucleus, which regulates the transcriptional expression of auxin response genes by stimulating signals in the nucleus [8]. Auxin promotes cell differentiation and organ primordium formation in the peripheral region of stem apex meristem through

polar transport [9]. Auxin promotes Cell expansion of hypocotyl cells as one of the typical auxin functions [10].

Zhou Peng et al. studied how DELLA proteins in the signal transduction pathway participate in the regulation of plant growth and development through cross-action. In the hormone signaling pathway, DELLA proteins, as an important regulatory factor, simultaneously play a role in the synthesis and signal transduction of various hormones, which is also one of the important molecular regulation of hormone cross-interaction. Through the analysis of model organisms such as Arabidopsis thaliana and rice, DELLA protein was found to participate in auxin signal transduction. Auxin signal transduction process mainly includes signal recognition, gene regulation and expression, and plant response to signal. In addition to the classical AUXIN SIGNALING pathway, indole-3-acetic acid (IAA) mediated TRANSPORT INHIBITOR RESPONSE1/ Auxin signaling F-BOX (TIR1/AFB) protein binds to Aux/IAA transcriptional regulators to activate the expression of downstream related genes. Scientists have also found some non-classical signal transduction pathways, such as ARF3 (ETTIN/ ETT) atypical auxin transduction pathway, which is also a very important signal transduction pathway [11]. Zhang Cunli et al. believe that although ethylene, a gaseous hormone, is simple, it plays an important role in the regulation of plant growth and development, and is closely related to seed germination, flowering and fruit, etc. Through years of research, scientists have described an approximately linear ethylene signal transduction pathway, and analyzed Arabidopsis thaliana as a model plant. It is concluded that the upstream of this pathway is composed of five ethylene receptors encoded by a multi-gene family of ETR1, ETR2, ERS1, ERS2 and EIN4, which bind to the protein kinase CTR1. Ethylene works with hormones such as cell growth hormone and cytokinin as well as environmental factors such as light. For example, the cross-action between ethylene and auxin plays an important role in plant growth and development [12]. This provides favorable support for us to explore the signal transduction pathway of plant growth hormone.

1.3 Research progress of some key enzyme genes of plant growth hormone signaling pathway

Combining biochemical, molecular and genetic biological tools, important regulators of auxin signaling pathways have been identified. Such as Auxin receptor protein TIR1/AFB (Transport Inhibitor Response1/Auxin Signaling F-Box), Auxin Binding protein ABP1 (Auxin-binding Protein1), Aux/IAA Auixn/ Auole-3-acetic acid (AuixN/Auxin-3-acetic acid) and Auxin Response Factor ARF (ARF).

1.3.1 Auxin Input Vector (AUX1)

The allogenic expression of auxin delivery vector AUX/LAX family can promote auxin input. AUX1 is the first cloned auxin input vector gene, which can encode a large number of pseudo-auxin input vector proteins. AUX1 is expressed in phloem, vascular column, root cap and apical epidermal cells. AUX1 belongs to the amino acid/auxin permeable family, located in the plasma membrane and widely present in plants. Aux1 is a homotropic transport of auxin protons and has high homology with the amino acid sequence of permease family proteins. Aux1 may be a part of the polyamine cationic transport family of common complex acids. Li pointed out that there are three types of auxin transporters found on cell membranes, AUX1/LAX (auxin resistant1/like aux1), ABCB/PGP (P-glycoprotein) and PIN (pinformed). They are responsible for the transport of auxin inside and outside cells, and AUX1/LAX input vectors help auxin enter cells. In 1996, Benntee et al cloned AUX1 gene in Arabidopsis Thaliana, and aux1 mutants showed severe rhizotropism. It is shown that AUX1 plays an important role in promoting the movement of auxin from gravity sensing to gravity response site, and the degree of root bending toward gravity mainly depends on the auxin concentration gradient formed by the input carrier, which is ultimately caused by the differential extension of epidermal cells [13].

1.3.2 Auxin Transport Inhibitor Response Protein 1 (TIR1)

Bai Huaju et al. proposed that since 1997, when Ruegger et al. screened a kind of Arabidopsis mutant with inhibition of auxin transport, TIR1 gene was isolated from this mutant, and its product was called transport inhibitor response protein 1, that is, TIR1. However, later studies believed that TIR1 could not play a role in auxin transport. Two research groups, Estelle and Leyser, separately proved in 2005 that TIR1 is the long-sought auxin receptor [14]. TIR1 is a soluble protein in the cytoplasm, belonging to a class of F-box proteins. It is an important part of the SCFTIR1 complex and plays an important role in the specific binding to target proteins. It has a set of leucine-rich repetitive sequence LRR domain. TIR1 is associated with the Ubiquitination protein degradation pathway. In addition, TIR1 contains a short spacer region of several amino acid residues, and a C-terminal fragment of about 70 amino acid residues. Further studies showed that auxin receptor TIR1 was involved in the degradation of Aux/IAA protein after binding with auxin, thereby regulating the transcription of downstream auxin response genes and participating in auxin signal transduction, which was regulated by auxin concentration dependence.

1.3.3 Auxin/indole-3-acetic acid (AUX/IAA)

Aux/IAA proteins, one of the transcription factors involved in auxin signaling, do not contain DNA-binding motifs, but are recruited to genomic regions by ARF proteins, which together with ARF proteins physically interact through a shared C-terminal domain [15]. Aux/IAA proteins usually contain one or two ethylene-responsive element binding factor-associated repressor, EAR) or an EAR-like inhibitor motif (domain1 (domain1, D1)), a central region necessary for TIR1/ AFB interaction and degradation (degron), or domain2 (domain2, D2) and a C-terminal PB1(Phox and Bem 1) protein-protein interaction domain that mediates homologous and heterodimerization. Rosa points out that most Aux/IAA proteins have four conserved amino acid sequence regions, Domain I,II, III, and IV, but some lack one

or more of these elements, and that Aux/IAA proteins have a rapidly induced role in auxin response because they are encoded by the Aux/IAA gene. The expression of some Aux/IAA protein genes is also induced by auxin, and the effects of Aux/IAA mutant were analyzed. The mutant plants showed resistance to auxin, enlargement of cotyledon and lateral root length at seedling stage. Experimental results such as the decrease in the number of lateral roots appear, which have an important indicator role in exploring the function of Aux/IAA protein [16].

1.3.4 Auxin Response Factor (ARF)

ARF proteins bind to a cis-regulatory sequence called an auxin response element (AuxRE), the core of which contains a TGTC motif that recruits ARF proteins. By analyzing the model plant Arabidopsis, structural analysis and protein chip data showed that different Arabidopsis ARF(ARF1, -3 and -5) preferentially bind to the larger TGTCGG motif by analyzing Arabidopsis as a model plant. The N-terminal of ARFs has a DNA domain (DBD), which binds to AuxREs TGTCNC-type Auxres, activating or inhibiting downstream gene transcription, but DBD cannot determine its regulatory characteristics, and the amino acid composition of the ARFs intermediate region (MR) affects its ability to activate transcription [15]. Gao Mengying et al. analyzed 23 ARF members of Arabidopsis Thaliana, and it is likely that ARF5, ARF6, ARF7, ARF8 and ARF19 are mainly responsible for transcriptional activation of auxin responses. These ARFs responsible for transcriptional activation are constitutionally bound to auxin response elements in their downstream gene promoter regions; however, in the absence of auxin, indole-3-acetic acid-inducing (AUX/ IAA) proteins bind to these ARFs and inhibit their binding to downstream gene promoters. As a result, the transcription of auxin response genes is weakened [17].

1.4 Research purpose and significance

Elephant grass is a new type of energy plant and animal husbandry grass, and its growth and development characteristics have attracted much attention. In order to

improve the planting efficiency of elephant grass and develop animal husbandry and industry in China, the growth hormone transduction pathway of object grass has been studied. This study intends to obtain all gene families of elephant grass growth hormone transduction pathway through bioinformatics analysis, and clarify the transmission process of elephant grass growth hormone signal and its key molecular mechanism through chromosome localization analysis, protein parameter analysis, whole genome replication analysis, phylogenetic tree analysis, expression analysis, protein interaction analysis and gene structure analysis. This will provide a theoretical basis for the regulation of plant growth and development. Through bioinformatics analysis of the regulatory factors of grass, key factors and regulatory networks related to growth hormone signal transduction pathways of elephant grass are obtained, which will provide an important reference for subsequent research on the mechanism of plant growth regulation and provide a deeper understanding and understanding of the biological functions of growth hormone and the regulatory mechanisms of plant growth and development. It is helpful to reveal the commonness and difference of growth hormone signal transduction pathways in elephant grass, which is of great significance to further explore the regulation of plant growth and development, and provide theoretical support for theoretical research in related fields.

CHAPTER 2
OBJECT, PURPOSE, AND METHODS OF THE STUDY

This study intends to obtain all gene families of elephant grass growth hormone transduction pathway through bioinformatics analysis, and clarify the transmission process of elephant grass growth hormone signal and its key molecular mechanism through chromosome localization analysis, protein parameter analysis, whole genome replication analysis, phylogenetic tree analysis, expression analysis, protein interaction analysis and gene structure analysis.

The object of the study growth hormone signal transduction pathway in elephant grass.

The subject of the study elephant grass.

2.1 Plant growth hormone signal transduction pathway gene acquisition

The auxin gene of purple elephant grass was obtained through literature review. After obtaining the auxin pathway genes of purple elephant grass, BlastP tool of TBtools software was used to find the auxin pathway genes of elephant grass through comparison with the genome data of elephant grass. Identification conditions: identity>80 p<1e-9.

2.2 Protein parameters of target grass cell auxin gene were calculated

The obtained 20 Protein sequences of the elephant straw cell growth hormone gene were sorted into txt documents, and then the Protein parameter calculation tool of TBtools was used to import the above documents to obtain the protein sequence length, molecular weight, isoelectric point and other attribute values. Export it as a table.

2.3 Genomic localization and genome-wide replication analysis of elephant grass cell auxin gene

The ID of 57 elephant grass genes that need to be studied was sorted into a new txt document, and the 57 genes were renamed to another new document in turn. The LINK files of 57 genes in elephant grass gene bank were sorted out. Identify tandem duplicate genes and set appropriate RGB colors against txt documents. The Gene Location Visualize from GTF/GFF tool of TBtools software is used to import the above sorted files successively, display the auxin signal transduction pathway genes on chromosomes, and derive the localization analysis diagram.

Using the One Step MCScanX function of TBtools, the auxin signal transduction pathway gene family was analyzed collinearly according to the genome sequence file and genome annotation file of elephant grass, and the whole genome copy of related genes was obtained.

2.4 Selection pressure analysis and positive selection analysis were performed for the whole genome replicators of the auxin gene of target grass cells

Selection pressure analysis can reveal the adaptive changes and functional evolution of genes or proteins in the evolutionary process by comparing the differences in selection pressure between different species, different genes or proteins. In selective pressure analysis, common methods include comparing the ratio (Ka/Ks) of a sequence's non-synonymous mutation rate (Ka, a mutation that causes an amino acid change) to its synonymous mutation rate (Ks, a mutation that does not change an amino acid). If the Ka/Ks ratio is significantly greater than 1, it indicates that these genes may be affected by positive selection. If the Ka/Ks ratio is close to 1, it indicates that these genes may be affected by neutral evolution. If the Ka/Ks ratio is significantly less than 1, it indicates that these genes may be affected by negative selection.

In a positive selection analysis, the ratio of non-synonymous mutation rates (Ka, mutations that cause amino acid changes) to synonymous mutation rates (Ks, mutations that do not change amino acids) between two or more genes or species is compared (Ka/Ks). If the Ka/Ks ratio is significantly greater than 1, it indicates that these genes may have been affected by positive selection, that is, beneficial mutations occurred during evolution and were retained by natural selection.

2.5 Phylogenetic tree analysis was carried out for cell somatic genes

In order to fully understand the genetic relationship and biological function of the somatoxin signal transduction pathway of elephant grass cells, multiple sequence alignment analysis was performed on the identified elephant grass somatoxin genes, and the phylogenetic tree was constructed. Download the sequence file of elephant grass auxin gene, find out the protein sequences of 57 auxin signal transduction pathway genes that need to be studied, and organize them into fasta files. MEGA X tool was used to construct phylogenetic Tree with Maximum Likelihood method for 57 protein sequences of target grass, and Interactive Tree Of Life (iTOL) tool was used to beautify the evolutionary tree.

2.6 The expression of auxin gene was analyzed

Transcriptome data were used to obtain the expression levels of auxin signal transduction pathway genes in different tissues (roots, stems and leaves) during different development stages. Then, TBtools HeatMap was used to draw gene expression heat maps, and the expression levels were normalized using log2 (TPM+1).

2.7 Protein interaction analysis was performed on the auxin gene

Due to the close relationship between elephant grass and millet, String-db (URL: http://www.bioconductor.org/packages/release/bioc/html/STRINGdb.html) website download millet protein sequence and protein interaction network. The

PPI Predict tool of TBtools was used to analyze the protein interaction of auxin protein in the target grass cells. The result was also illustrated by Cytoscape tool to map the protein interaction network, so as to facilitate the analysis of the interaction between genes.

2.8 Carry out gene structure view of auxin gene

Gene Structure View (Gene Structure View) is an advanced analytical tool for visualizing gene organization structure and annotation information, which can help us better understand gene organization structure and function, determine gene transcription start site, splicing variation, promoter and terminator location, etc. The original ID of elephant grass was used to construct phylogenetic tree and obtain nwk format file. Then, the Gene Structure View (Advanced) tool of TBtools was used to construct the gene structure view, which could compare the structural differences of different genes and analyze the evolutionary relationship and functional evolution of genes.

CHAPTER 3
EXPERIMENTAL PART

3.1 Search for elephant grass cell growth hormone gene

Auxin signal transduction pathway of elephant straw mainly involved in AUX1, TIR1 (Transport Inhibitor Response1), AUX/IAA (Auxin/ Indole-3-acid), and ARF (Auxin Response) Factor), SAUR (small auxin up RNA), G3H, etc. Through bioinformatics analysis, we obtained 57 auxin signaling pathway genes in 6 genomes of elephant grass, and 76 auxin pathway genes in purple elephant grass. The AUX1 gene family has 16 genes, the AUX/IAA gene family has 4 genes, the TIR1 gene family has 10 genes, the ARF gene family has 8 genes, the SAUR gene family has 1 gene, and the G3H gene family has 18 genes (Tab. 3.1).

Table 3.1 – **The number of 6 gene families in different species**

gene family	Cp	Cg	Si	Os	elephant grass
AUX1	10	5	4	5	16
TIR1	12	5	5	5	10
AUX/IAA	6	1	2	3	4
ARF	6	3	3	3	8
SAUR	16	5	7	5	1
G3H	29	12	11	9	18

3.2 Calculation of protein parameters of auxin pathway genes in target grass cells

According to the Protein parameter calculation tool of TBtools, protein molecular weight, isoelectric point, instability coefficient, fat solubility index and hydrophilic index can be obtained (Tab. 3.2).

Table 3.2 – **Computational analysis of protein parameters**

ID	Gene	Molecular Weight	Theoretical pI	Theoretical pI	Aliphatic Index	Grand Average of Hydropathicity
maker-chrA1-augustus-gene-1652.104-mRNA-1	AUX1.1	15254.7	7.69	20.12	98.98	0.543
maker-chrA4-augustus-gene-158.74-mRNA-1	AUX1.2	58586.24	9.77	41.09	91.17	0.279
maker-chrA5-snap-gene-164.117-mRNA-1	AUX1.3	58258.57	9.71	38.35	95.76	0.222
maker-chrA5-snap-gene-430.86-mRNA-1	AUX1.4	49773.01	10.75	55.84	85.2	-0.197
maker-chrA6-snap-gene-118.111-mRNA-1	AUX1.5	65559.94	10.44	46.11	87.2	-0.239
maker-chrB1-augustus-gene-372.103-mRNA-1	AUX1.6	26184.46	10.42	50.17	106.16	0.013
maker-chrB2-augustus-gene-196.81-mRNA-1	AUX1.7	34609.05	8.71	55.15	93.31	-0.121
maker-chrB5-snap-gene-1212.88-mRNA-1	AUX1.8	55649.79	11.76	68.31	82.2	-0.468
maker-chrB5-snap-gene-1215.88-mRNA-1	AUX1.9	56450.78	11.54	65.37	85.28	-0.366
maker-chrB6-augustus-gene-86.86-mRNA-1	AUX1.10	110232.5	9.34	61.56	76.65	-0.363
augustus_masked-chrB6-processed-gene-646.1-mRNA-1	AUX1.11	47082.96	9.56	35.84	85.48	-0.024
maker-chrB6-snap-gene-2006.119-mRNA-1	AUX1.12	33769.7	10.27	78.87	75.34	-0.46
maker-chrB6-snap-gene-2006.120-mRNA-1	AUX1.13	26473.51	9.84	56.41	85.84	-0.364
augustus_masked-chrB7-processed-gene-219.28-mRNA-1	AUX1.14	33971.11	9.08	56.8	86.26	-0.261
maker-chrB7-augustus-gene-1088.78-mRNA-1	AUX1.15	29664.72	11.3	55.54	64.34	-0.538
maker-ScaffoldUN-augustus-gene-364.73-mRNA-1	AUX1.16	56747.55	8.95	34.58	91.17	0.35
maker-chrA1-snap-gene-144.87-mRNA-1	TIR1.1	64837.9	7.1	49.7	94.34	0.056

ID	Gene	Molecular Weight	Theoretical pI	Theoretical pI	Aliphatic Index	Grand Average of Hydropathicity
maker-chrA1-augustus-gene-930.87-mRNA-1	TIR1.2	62769.57	8.27	45.56	96.88	0.114
maker-chrA3-snap-gene-141.118-mRNA-1	TIR1.3	62718	5.05	57.31	83.26	-0.155
snap_masked-chrA3-processed-gene-760.67-mRNA-1	TIR1.4	64139.51	8.12	49.62	94.54	-0.036
maker-chrA6-snap-gene-800.92-mRNA-1	TIR1.5	85141.17	6.54	68.46	57.3	-0.61
maker-chrB1-snap-gene-153.109-mRNA-1	TIR1.6	64927.04	7.1	47.74	93.68	0.057
snap_masked-chrB3-processed-gene-491.55-mRNA-1	TIR1.7	63968.19	6.79	49.12	94.56	-0.025
maker-chrB4-snap-gene-1107.98-mRNA-1	TIR1.8	72777.79	5.38	51.42	85.22	-0.121
maker-chrB6-snap-gene-1158.67-mRNA-1	TIR1.9	66246.1	6.57	48.31	90.82	0.09
maker-chrB6-snap-gene-319.102-mRNA-1	TIR1.10	87008.48	8.57	72.54	59.91	-0.557
maker-chrA3-augustus-gene-200.97-mRNA-1	AUX/IAA.1	42107.56	6.09	48.52	76.76	-0.361
maker-chrA6-augustus-gene-799.89-mRNA-1	AUX/IAA.2	21814.11	9.39	64.32	78.73	-0.355
maker-chrB4-snap-gene-1164.86-mRNA-1	AUX/IAA.3	43392.9	5.9	44.4	74.86	-0.382
maker-chrB6-augustus-gene-320.84-mRNA-1	AUX/IAA.4	14722.17	9.07	46.2	85.98	-0.375
maker-chrA1-augustus-gene-287.93-mRNA-1	ARF.1	107245.66	8.63	55.07	70.17	-0.429
maker-chrA3-snap-gene-547.66-mRNA-1	ARF.2	37639.76	8.73	69.48	76.13	-0.444
maker-chrA3-augustus-gene-687.100-mRNA-1	ARF.3	101909.92	9.28	61.99	71.81	-0.482
maker-chrA6-augustus-gene-1093.64-mRNA-1	ARF.4	76806.86	5.55	50.57	73.66	-0.455
maker-chrB3-augustus-gene-381.101-mRNA-1	ARF.5	81230.99	5.86	63.65	70.29	-0.424

ID	Gene	Molecular Weight	Theoretical pI	Theoretical pI	Aliphatic Index	Grand Average of Hydropathicity
maker-chrB4-augustus-gene-807.86-mRNA-1	ARF.6	72541.62	5.61	63.12	71.16	-0.552
maker-chrB4-augustus-gene-833.76-mRNA-1	ARF.7	22115.05	5.33	41.71	72.78	-0.37
maker-chrB6-augustus-gene-8.74-mRNA-1	ARF.8	60254.83	6.02	44.56	70.72	-0.49
maker-chrA3-snap-gene-334.74-mRNA-1	Ppu.SAUR	42412.8	8.94	53.25	75.6	-0.392
maker-chrA1-augustus-gene-301.114-mRNA-1	C3H.1	66438.34	5.23	46.37	84.06	-0.097
maker-chrA1-snap-gene-301.126-mRNA-1	C3H.2	53916.17	5.56	42.73	87.53	-0.033
augustus_masked-chrA1-processed-gene-307.24-mRNA-1	C3H.3	49547.95	5.48	40.32	81.44	-0.139
maker-chrA1-snap-gene-307.93-mRNA-1	C3H.4	71281.16	6.42	38.71	87.54	-0.12
maker-chrA1-augustus-gene-1717.116-mRNA-1	C3H.5	65379.97	9.12	60.59	82.67	-0.233
maker-chrA4-augustus-gene-52.91-mRNA-1	C3H.6	27102.94	6.11	53.6	87.95	-0.006
maker-chrA4-snap-gene-52.100-mRNA-1	C3H.7	32431.15	9.5	52.11	89.97	-0.15
maker-chrA5-augustus-gene-8.101-mRNA-1	C3H.8	67985.57	5.63	51.37	84.97	-0.132
maker-chrA6-snap-gene-190.124-mRNA-1	C3H.9	73971.45	10.77	63.03	77.63	-0.504
maker-chrA6-augustus-gene-210.92-mRNA-1	C3H.10	20174.98	5.42	72.07	87.54	-0.097
maker-chrA7-augustus-gene-821.116-mRNA-1	C3H.11	66417.59	8.91	59.26	72.15	-0.288
maker-chrB1-snap-gene-306.98-mRNA-1	C3H.12	67292.07	6.08	44.8	88.75	-0.04
maker-chrB1-augustus-gene-1941.101-mRNA-1	C3H.13	67720.05	9.53	50.51	75.83	-0.307
maker-chrB1-augustus-gene-1941.102-mRNA-1	C3H.14	68319.15	6.61	45.93	87.9	0

ID	Gene	Molecular Weight	Theoretical pI	Theoretical pI	Aliphatic Index	Grand Average of Hydropathicity
maker-chrB5-augustus-gene-918.100-mRNA-1	C3H.15	67944.54	5.71	50.77	86.19	-0.128
maker-chrB6-augustus-gene-1924.99-mRNA-1	C3H.16	74565.13	8.15	50.44	84.09	-0.216
maker-chrB6-snap-gene-714.94-mRNA-1	C3H.17	37607.16	8.33	43.73	85.23	-0.08
augustus_masked-chrB7-processed-gene-1264.3-mRNA-1	C3H.18	112148.76	8.9	48.38	75.53	-0.273

Molecular Weight refers to the relative molecular mass of a molecule, which is an important parameter to describe the size and mass of a molecule. Theoretical pI is important for understanding the properties of protein solubility, electrophoretic separation, and interactions. Instability Index is an index used to evaluate the stability of protein sequences. The higher the instability coefficient, the more unstable the protein, which may be more prone to degradation or deactivation. The highest instability coefficient is AUX1.12, the lowest instability coefficient is AUX1.1. Aliphatic Index is an index used to evaluate the hydrophilicity and hydrophobicity of a protein sequence. The higher the lipid solubility index, the more hydrophobic the protein is and the more likely it is to interact with non-polar fatty acids. The highest lipid solubility index is AUX1.6 and the lowest is TIR1.5. The Grand Average of Hydropathicity (GRAVY) is an index used to evaluate the hydrophilicity and hydrophobicity of a protein's amino acid sequence. The hydrophilic index can be calculated from the amino acid composition of proteins and their chemical properties, and is used to predict the solubility and folding state of the protein. The higher the value of the hydrophilic index, the more hydrophilic the protein is and the more it likes to interact with water. It can be seen from the table that the highest is AUX1.1 and the lowest is TIR1.5.

3.3 Genomic localization and genome-wide replication analysis of elephant grass cell auxin gene

According to the localization analysis of 57 genes in elephant grass, the distribution map of 6 gene families in elephant grass chromosomes was drawn (Fig.3.1).

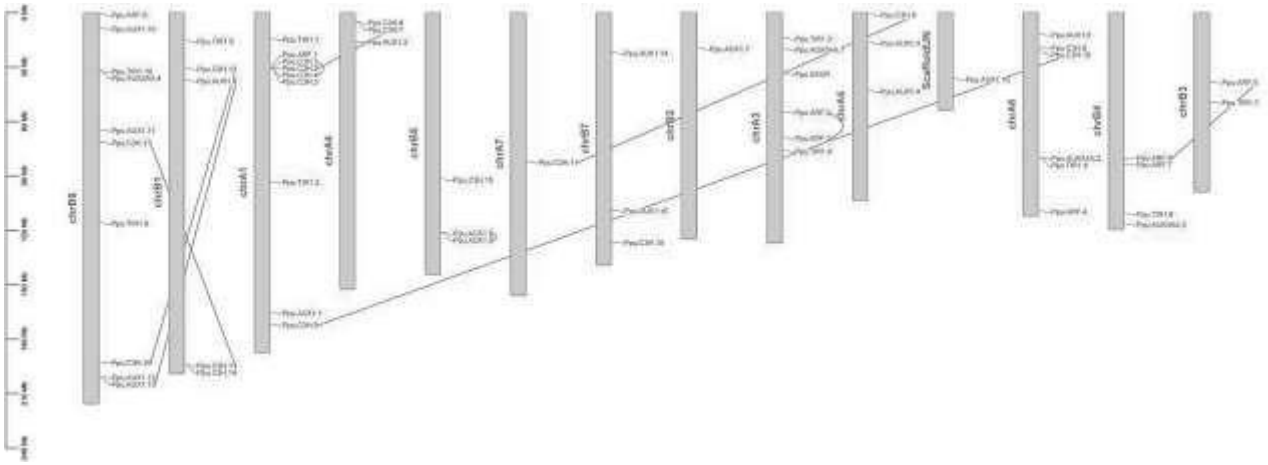

Figure 3.1 – Localization of 6 family genes on chromosomes

The 6 gene families of elephant grass are distributed on 14 chromosomes of elephant grass. Among them, the B6 chromosome has the most gene distribution, followed by the A1 chromosome, and the chromosome with the least gene distribution has only one gene. AUX1 family genes are mainly distributed in A1, A4, A5, A6, B1, B2, B5, B6, B7 and ScaffoldUN. The TIR1 family genes were mainly distributed in A1, A3, A6, B1, B3, B4 and B6. AUX/IAA genes were mainly distributed in A3, A6, B4 and B6. The ARF family genes were mainly distributed in A1, A3, A6, B3, B4 and B6. SAUR family genes are mainly distributed in A3. The G3H gene is mainly distributed in A1, A4, A5, A6, A7, B1, B5, B6 and B7. Although all auxin signaling pathway genes are distributed in the whole genome, the distribution of different genes on chromosomes is also specific. In addition, B6 of chromosome Ppu) TIR1.10 / Ppu) AUX/IAA. 4, Ppu. AUX1.12 / Ppu AUX1.13, B1 chromosome Ppu. C3H. 13 / Ppu C3H. 14, A1 on chromosome Ppu C3H. 1 / Ppu. C3H. 2, Ppu. C3H .3/Ppu.C3H.4, Ppu.C3H.6/Ppu.C3H.7 on chromosome A4, and Ppu.AUX/IAA.2/Ppu.TIR1.5 on chromosome A6 are tandem repeats. Ppu.AUX1.6

and Ppu.AUX1.13, Ppu.AUX1.8 and Ppu.AUX1.9, Ppu.ARF.2 and Ppu.ARF.3, Ppu.ARF.5 and Ppu.ARF.6, Ppu.C3H.1 and Ppu.C3H.4, Ppu.C3H.2 and Ppu.C 3H.6, Ppu.C3H.5 and Ppu.C3H.9, Ppu.C3H.8 and Ppu.C3H.11,

Ppu.C3H.12 and Ppu.C3H.16, Ppu.C3H.13 and Ppu.C3H.17 are collinear gene pairs, and whole genome replication gene pairs.

3.4 Selection pressure analysis and positive selection analysis were performed for the whole genome replicators of the auxin gene of target grass cells

By comparing the differences in selection pressure between different genes (Tab. 3.3), the Ka/Ks ratio of all genomes is significantly less than 1, indicating that all genes may be affected by negative selection. No genes had a Ka/Ks ratio significantly greater than 1, indicating that these genes were not affected by positive selection, that is, did not undergo beneficial mutations during evolution and were retained by natural selection.

Table 3.3 – **Collinear gene pair selection pressure analysis and positive selection analysis results**

Seq1	Seq2	Ka/Ks	selection pressure analysis
maker-chrB1-augustus-gene-372.103-mRNA-1	maker-chrB6-snap-gene-2006.120-mRNA-1	0.926952927164779	negative selection
maker-chrB5-snap-gene-1212.88-mRNA-1	maker-chrB5-snap-gene-1215.88-mRNA-1	0.8172246250868003	negative selection
maker-chrA3-snap-gene-547.66-mRNA-1	maker-chrA3-augustus-gene-687.100-mRNA-1	0.2570725823301566	negative selection
maker-chrB3-augustus-gene-381.101-mRNA-1	maker-chrB4-augustus-gene-807.86-mRNA-1	0.18014737443462783	negative selection
maker-chrA1-augustus-gene-301.114-mRNA-1	maker-chrA1-snap-gene-307.93-mRNA-1	0.5184769778877577	negative selection
maker-chrA1-snap-gene-301.126-mRNA-1	maker-chrA4-augustus-gene-52.91-mRNA-1	0.5732430699983854	negative selection
maker-chrA1-augustus-gene-1717.116-mRNA-1	maker-chrA6-snap-gene-190.124-mRNA-1	0.5793896769464856	negative selection
maker-chrA5-augustus-gene-8.101-mRNA-1	maker-chrA7-augustus-gene-821.116-mRNA-1	0.4587466713626822	negative selection

Seq1	Seq2	Ka/Ks	selection pressure analysis
maker-chrB1-snap-gene-306.98-mRNA-1	maker-chrB6-augustus-gene-1924.99-mRNA-1	0.338440731 7633852	negative selection
maker-chrB1-augustus-gene-1941.101-mRNA-1	maker-chrB6-snap-gene-714.94-mRNA-1	0.551565001 1489674	negative selection

3.5 The phylogenetic tree analysis of auxin gene was carried out

In order to fully clarify the relationship between genes in each family of elephant grass, multiple sequence comparison analysis was performed on 6 identified family genes. After the phylogenetic Tree Of 57 protein sequences of the target grass was constructed with Maximum Likelihood method, the evolutionary tree was prettified by the Interactive Tree Of Life (iTOL) tool, and the phylogenetic analysis of 57 protein sequences of the elephant grass was obtained (Fig. 3.2).

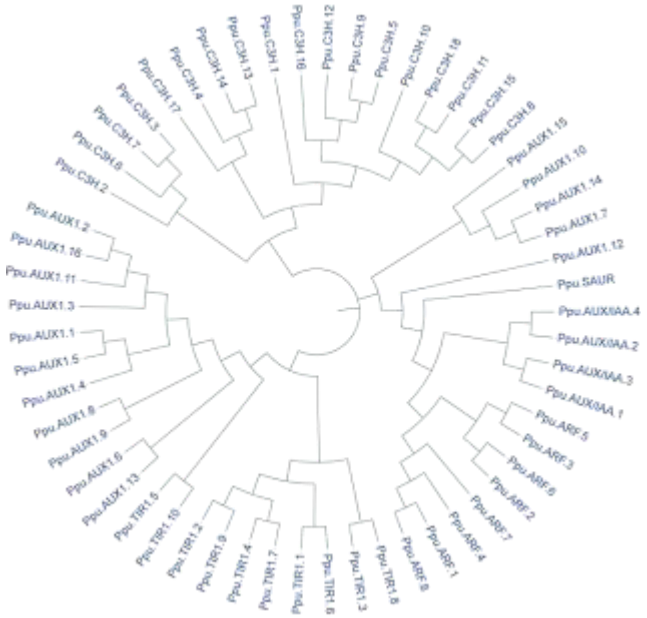

Figure 3.2 – Phylogenetic analysis of 20 protein sequences of elephant grass

It can be seen from Figure 2 that some AUX1 and TIR1 gene families come from the same branch, and some AUX1, AUX/IAA, SUAR and ARF gene families come from the same branch, which shows that the relatives are closer.

3.6 The expression of auxin gene was analyzed

Based on the genomic information of elephant grass, the HeatMap tool of TBtools was used to draw the expression heat map of auxin family genes of elephant grass (Fig. 3.3).

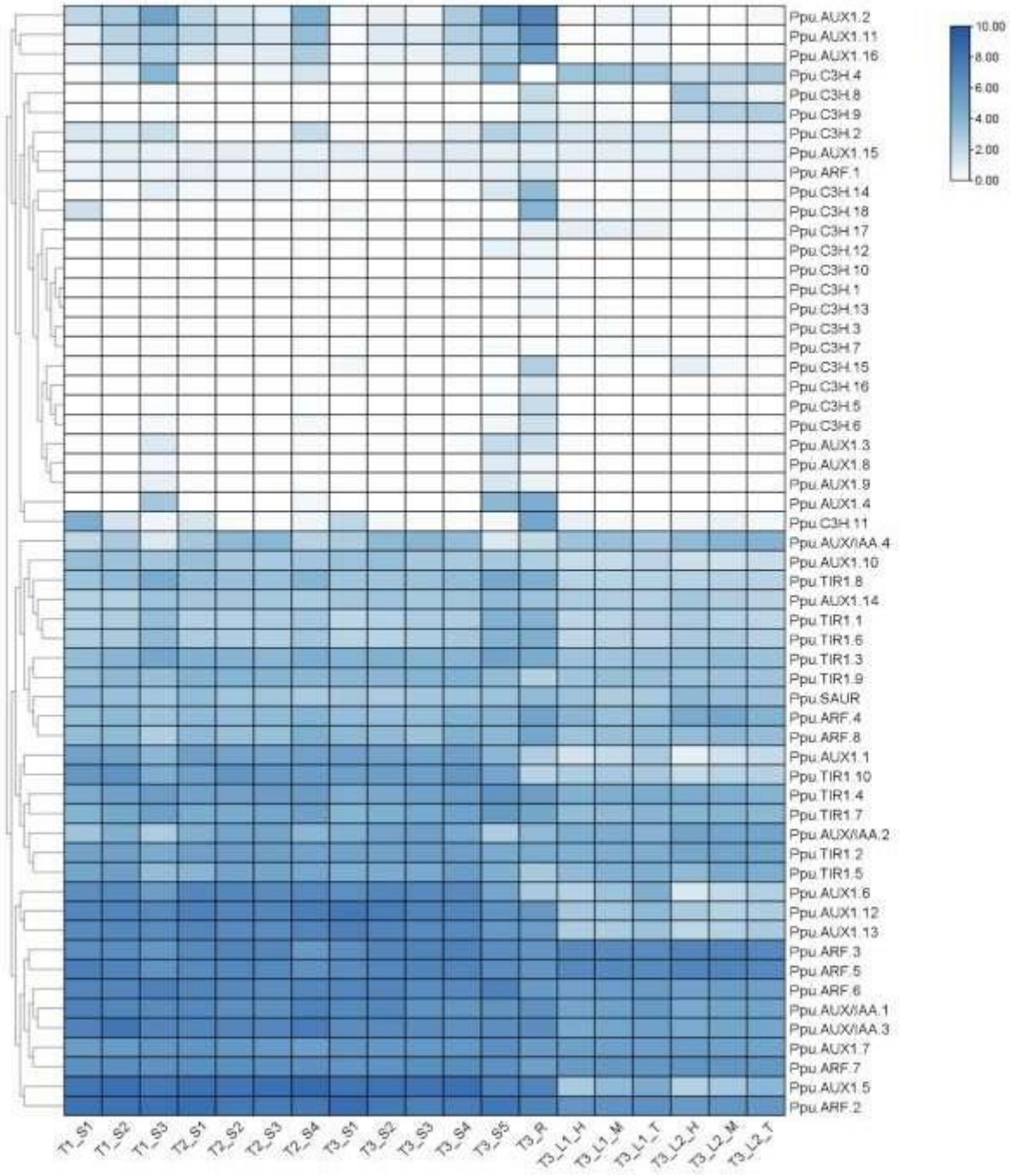

Figure 3.3 – Analysis of elephant grass gene expression in various tissues

The results showed that ARF.2, AUX1.5, ARF7, AUX1.7, AUX/1AA.3, AUX/1AA.1, ARF.6, ARF.5, ARF.3, AUX1.13, AUX1.12, AUX1.2 and so on were highly expressed in root. C3H.4, C3H.3 and C3H.7 were not expressed in the root, and other genes were expressed in the root, but the expression size was different. The expression of C3H family genes in root was lower than that of other five family genes. In different periods of stem, the expression of the same gene is also different, among which the most obvious differences are AUX1.2, AUX1.11, AUX1.16 and C3H.11, which can change from strong expression to weak expression, that is, from dark to light expression, and the expression levels of the first three genes are similar in different periods. The expressions of ARF2, AUX1.5, ARF7, AUX1.7, AUX/1AA.3, AUX/1AA.1, ARF.6, ARF.5, ARF.3, AUX1.13, AUX1.12 and AUX1.6 in the stem were large. At different stages of leaves, the expressions of ARF2, AUX1.5, ARF7, AUX1.7, AUX/1AA.3, AUX/1AA.1, ARF.6, ARF.5 and ARF.3 were large. Except that C3H.7 and C3H.3 were less expressed in different parts or different developmental stages, all the other genes were expressed in different developmental stages or different developmental parts of plants, indicating that they participated in the development process of a part of elephant grass. Only a small amount of C3H family genes were detected in different tissues, and C3H.5, C3H.6 and C3H.16 were only expressed in the roots, indicating that the gene family played a small role in the regulation of the growth and development of elephant grass. The other family genes were expressed in different developmental stages or different developmental parts of the roots and leaves, indicating that they were involved in the whole development process of the roots and leaves of elephant grass, and played an important role in the regulation of the growth and development of elephant grass.

3.7 Protein interaction analysis was performed on the auxin gene

By using the PPI function of TBtools, the protein relationship of the gene family of auxin signal transduction pathway in target grass cells was further predicted, as shown in Fig. 3.4.

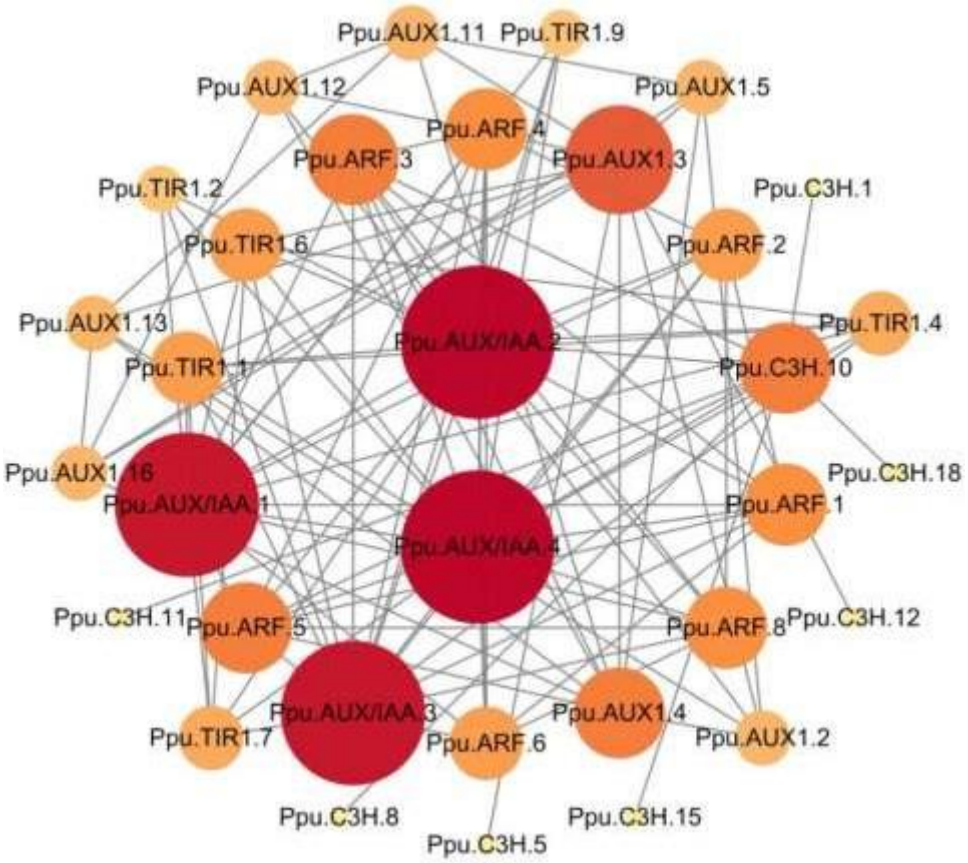

Figure 3.4 – Protein interaction between genes of elephant grass families

It can be seen from the protein interaction network result diagram that there are 33 nodes. There are corresponding interactions between any protein node and other protein nodes, among which AUX/IAA family is the core of the elephant grass gene family protein network, indicating that it plays an important role in the auxin signal transduction pathway.

3.8 Discussion

Elephant grass is a new type of energy plant with high photosynthesis and biomass, while auxin is a kind of plant hormone that promotes plant growth, callus formation and rooting. This study analyzed the genes involved in the auxin signal transduction pathway of elephant grass cells. There are mainly 6 gene families involved in the signal transduction pathway. acetic acid includes AUX1, TIR1 (Transport Inhibitor Response1), AUX/IAA (Auxin/indole-3-acetic acid), ARF (Auxin Response Factor), and SAUR (small) auxin up RNA), G3H. By comparing with purple elephant grass, it was found that the copy number of elephant grass and purple elephant grass was not different, indicating that the relationship between the two species was indeed close. It was found that the highest instability coefficient was AUX1.12 and the lowest instability coefficient was AUX1.1. The highest lipid solubility index was AUX1.6 and the lowest was TIR1.5. The highest hydrophilic index is AUX1.1 and the lowest is TIR1.5.

Chromosome localization analysis showed that 3 gene families of elephant grass were distributed on 14 chromosomes of elephant grass. Among them, the B6 chromosome has the most gene distribution, followed by the A1 chromosome, and the chromosome with the least gene distribution has only one gene. Seven tandem repeat genes and 10 genome-wide replication gene pairs were found. The density of the two ends of the gene distribution on each chromosome is greater than the density in the middle, suggesting that these genes may be more easily cloned at the top or bottom of the chromosome [18]. Through the selection pressure analysis and positive selection analysis of the whole genome replication gene pairs, it was found that no Ka/Ks ratio of elephant grass cell auxin genes was significantly greater than 1, indicating that these genes were not affected by positive selection, but were affected by negative selection, that is, no beneficial mutations occurred in the evolution process and were preserved by natural selection.

Phylogenetic analysis of auxin signal transduction pathway genes was carried out using the coding sequence of elephant grass. The results showed that all the

identified gene families came from different branches. At the same time, it was found that genes in the same gene family came from two different branches. For example, the AUX family was divided into two parts, and a part of the AUX1 and TIR1 gene families came from the same branch. Some of the AUX1, AUX/IAA, SUAR and ARF gene families come from the same branch. The expression analysis results showed that ARF.2, AUX1.5, ARF7, AUX1.7, AUX/1AA.3, AUX/1AA.1, ARF.6, ARF.5, ARF.3, AUX1.13, AUX1.12, AUX1.2 and so on were highly expressed in root. The expressions of ARF2, AUX1.5, ARF7, AUX1.7, AUX/1AA.3, AUX/1AA.1, ARF.6, ARF.5, ARF.3, AUX1.13, AUX1.12 and AUX1.6 are relatively large in the stem, and in different periods of leaves, The expressions of ARF2, AUX1.5, ARF7, AUX1.7, AUX/1AA.3, AUX/1AA.1, ARF.6, ARF.5 and ARF.3 were large.

CONCLUSIONS

This study mainly revealed the high copy number and transcription level of the somatostatin signal transduction pathway family genes in elephant grass cells, and the main results were as follows:

(1) In this study, 57 pathway genes of 6 gene families were identified in the cytokinin signaling pathway of elephant grass, distributed on 14 chromosomes.

(2) AUX1.2, AUX1.3, AUX1.4, AUX1.8, AUX1.9, AUX1.11, AUX1.16 are only expressed in part of the roots and stems, and are almost completely not expressed in all stages of the leaves; The remaining AUX1 family genes are expressed in all stages of the rhizomes and leaves, AUX1.5 and AUX1.6 are highly expressed in the stems, and AUX1.7 is highly expressed in all stages of the rhizomes and leaves.

(3) The TIR1 family was expressed at all stages of the roots and leaves, and the expression levels of TIR1.1, TIR1.3, TIR1.6, TIR1.8 and TIR1.9 were less in the stems and leaves at all stages, while the expression levels of TIR1.10 were less in the leaves, and the expression levels of TIR1.5, TIR1.9 and TIR1.10 were less in the roots.

(4) Although the number of genes in AUX/IAA family and SAUR family is small, they are expressed in all stages of the roots and leaves, while AUX/IAA.2 and AUX/IAA.4 are less expressed in some stages of the roots and stems.

(5) ARF.1 was only slightly expressed in roots, and other ARF family genes were highly expressed in all stages of roots and leaves.

(6) The expression level of C3H family was less or even not expressed at all stages of the roots, most genes were slightly expressed in the roots, and C3H.3, C3H.4 and C3H.7 were not expressed in the roots. A small amount of C3H.2, C3H.4, C3H.8, C3H.9 were expressed in some stages of leaves, and a small amount of C3H.2, C3H.4, C3H.11, C3H.14, C3H.18 were expressed in different stages of stems.

The elephant grass genome can provide theoretical support for studying the diversity, speciation and evolution of this family, and provide important resources for understanding important economic traits and adaptation mechanisms. This also provides new resources for the development of other species with significant economic, ecological and research value.

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