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 **Bioinformatic analysis of *Cenchrus purpureus* genes for cytokinin signalling**

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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KYIV NATIONAL UNIVERSITY OF TECHNOLOGIES AND DESIGN

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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

_____
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ASSIGNMENTS
FOR THE QUALIFICATION THESIS
Wang Fujie

1. Thesis topic **Bioinformatic analysis of *Cenchrus purpureus* genes for cytokinin signalling**
Scientific supervisor Iryna Voloshyna, Ph.D., As. 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

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EXECUTION SCHEDULE

№	The name of the stages of the qualification thesis	Terms of performance of stage	Note on performance
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 _____Wang FUJIE

Scientific supervisor _____Iryna VOLOSHYNA

SUMMARY

Wang Fujie. Bioinformatic analysis of *Cenchrus purpureus* genes for cytokinin signalling – Manuscript.

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

Elephant grass is an important pasture. It is a new type of energy plant, which is native to tropical and subtropical areas of Asia, Africa and America. It is a kind of high yield and forage grass, also can be used as feed for animals eat, widely used in agricultural production and industrial application. To reveal the development process of the genetic basis of cytokinin signal transduction, we through bioinformatics method to explore and analyze the elephant grass cytokinin signal transduction pathways of major gene family. This study using BlastP tools appraisal get elephant grass cytokinins three genes in signal transduction pathways of 20 members of the family; Protein characteristics analysis found that protein molecular weight between 8103-139430, isoelectric point between 5-10, unstable coefficient between 34-68, fat soluble index between 52-96 and hydrophilic index between 0.96 to 0.05; Gene structure analysis showed that the number of exons in different members of different families varied. Through the genetic analysis of chromosome positioning, found that elephant grass cytokinin signal transduction pathway 3 gene families, the three distribution on elephant grass 10 chromosomes gene families, in B6 gene on chromosome distribution; most Phylogenetic analysis through phylogenetic trees showed that the same gene families clustered together, which revealed the evolutionary relationship of organisms and better understood the similarities and differences between biological species. The expression levels of CRE1 family genes and B-ARR family genes were higher than those of AHP family genes in roots, while AHP family genes were mostly expressed in leaves. Protein interaction analysis revealed that the interaction between proteins was related to each other to understand the structure and function of the protein network. Among them, CRE1.8 was the main core gene of the elephant grass gene family protein network. This study provides an

important reference for further understanding the growth and development mechanism of elephant grass, and lays a foundation for related genetic improvement and growth regulation research in the future. Elephant grass has been listed as a new energy plant in the 20th century, with important edible value, economic value and ecological value. By obtaining the cytokinin signal transduction pathway genes of purple elephant grass, we searched for the cytokinin signal transduction pathway genes of purple elephant grass, and analyzed the data of the studied genes by bioinformatics methods, so as to explore the important genes for the growth and development of purple elephant grass. By studying the elephant grass cytokinin signal transduction pathway, can have a thorough understanding of plant cytokinin signal transduction mechanism, revealing the molecular mechanism of plant growth and development. To explore the mechanism of plant adaptation to the environment, provide theoretical basis for the plant ecological adaptation and optimization, can provide theoretical support for plant breeding and production, to cultivate high yield, art, and provide a scientific basis for quality of crop varieties.

Key words **:** *Elephant grass; cytokinin signaling pathway; chromosomal localization; expression analysis; phylogenetic tree analysis.*

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INTRODUCTION

Elephant grass is an important pasture. It is a new type of energy plant, which is native to tropical and subtropical areas of Asia, Africa and America. It is a kind of high yield and forage grass, also can be used as feed for animals eat, widely used in agricultural production and industrial application. To reveal the development process of the genetic basis of cytokinin signal transduction, we through bioinformatics method to explore and analyze the elephant grass cytokinin signal transduction pathways of major gene family. This study using BlastP tools appraisal get elephant grass cytokinins three genes in signal transduction pathways of 20 members of the family; Protein characteristics analysis found that protein molecular weight between 8103-139430, isoelectric point between 5-10, unstable coefficient between 34-68, fat soluble index between 52-96 and hydrophilic index between 0.96 to 0.05; Gene structure analysis showed that the number of exons in different members of different families varied. Through the genetic analysis of chromosome positioning, found that elephant grass cytokinin signal transduction pathway 3 gene families, the three distribution on elephant grass 10 chromosomes gene families, in B6 gene on chromosome distribution; most Phylogenetic analysis through phylogenetic trees showed that the same gene families clustered together, which revealed the evolutionary relationship of organisms and better understood the similarities and differences between biological species. The expression levels of CRE1 family genes and B-ARR family genes were higher than those of AHP family genes in roots, while AHP family genes were mostly expressed in leaves. Protein interaction analysis revealed that the interaction between proteins was related to each other to understand the structure and function of the protein network. Among them, CRE1.8 was the main core gene of the elephant grass gene family protein network. This study provides an important reference for further understanding the growth and development mechanism of elephant grass, and lays a foundation for related genetic improvement and growth regulation research in the future.

Purpose of the study – elephant grass has been listed as a new energy plant in the 20th century, with important edible value, economic value and ecological value. By obtaining the cytokinin signal transduction pathway genes of purple elephant grass, we searched for the cytokinin signal transduction pathway genes of purple elephant grass, and analyzed the data of the studied genes by bioinformatics methods, so as to explore the important genes for the growth and development of purple elephant grass. By studying the signal transduction pathway of elephant grass cytokinin, we can understand the mechanism of plant cytokinin signal transduction and reveal the molecular mechanism of plant growth and development. Exploring the adaptation mechanism of plants to the environment can provide theoretical basis for ecological adaptation and optimization of plants, provide theoretical support for plant breeding and production, and provide scientific basis for cultivating high-yield, stress-resistant and high-quality crop varieties.

Object of study – Key genes of ccytokinin signal transduction pathway in elephant grass: cytokinin receptor 1 (CRE1); cytokinin receptor 1 (AHP); B-type Arabidopsis response regulator (B-ARR)

Subject of study – Pennisetum purpureum Schumach

CHAPTER 1
LITERATURE REVIEW

1.1 Overview of elephant grass research

1.1.1 Brief Introduction of elephant grass

Pennisetum purpureum Schumach (Pennisetum purpureum Schumach) is a large herb of the gramineae and millet family that grows perennial in clumps and often has underground stems. Elephant grass is a common perennial herb. It is widely distributed in tropical, subtropical and temperate regions around the world, and is a highly adaptable and vigorous grass species. Elephant grassland is a kind of perennial warm-season herbages commonly planted in tropical and subtropical areas. It has the characteristics of freshness, juiciness, high yield and good palatability[1] . Elephant grass is native to Africa and then introduced and cultivated in India and Myanmar. At present, it has been successfully introduced and cultivated in Jiangxi, Sichuan and Guangdong [2] . Elephant grass often has underground stems. Culms erect, up to 4 m in height; Leaf tongue short, leaf blade linear, flat, hard. Panicle; Main shaft densely pubescent, spikelet lanceolate, subsessile, veins inconspicuous; Anther apex ciliate; Style base united. Leaf blade tubular, wall thick.

1.1.2 Research progress of elephant grass

Elephant grass has strong adaptability to drought, salt and cold stress, and its underground stems and roots have strong growth and nutrition abilities, making it an important lawn and forage plant. At the same time, a variety of bioactive components contained in elephant grass, such as flavonoids, alkaloids, polysaccharides, etc., have a variety of pharmacological effects such as anti-oxidation, anti-inflammation, antibacterial, hypoglycemic and so on. Therefore, elephant grass has a wide application prospect in the fields of medicine and health care. Elephant grass plays an important role in the protection and restoration of grassland ecosystem, which can improve soil quality and grassland productivity, so it plays an important role in the sustainable development of grassland ecosystem.

In recent years, with the development of genomics technology, researchers at home and abroad have begun to conduct genomic studies on elephant grass, in order to better understand the biological characteristics and stress adaptation mechanism of elephant grass.

At present, there are studies on hierarchical clustering methods based on principal components in energy production using biomass. The genotypes in the active germplasm bank of elephant grass were used to generate half-sibling families, and at the same time, the cultivation of high-yield genotypes with the goal of energy production was also determined[3] . Zhu Jie[4] et al. used SSR molecular marker technology to analyze the genetic variation of Guimu No. 1 hybrid elephant grass and 7 test materials that could naturally overwintering, and found that it had rich genetic polymorphism. Rajasekaran et al. found that cytokinin levels were lower in elephant grass embryogenic calli compared with those in non-embryonic calli [5] . Wu Juanzi et al[6] cloned CpCCoAOMT2 gene from xianggrass and conducted preliminary bioinformatics analysis, and found that CpCCoAOMT2 protein was closest to CpcCoAOMT2 protein in gramineous plants, while it was closest to gymnosperms and ferns, and the most distant relationship was dicot plants.

1.2 Overview of cytokinin signal transduction pathways

cytokinin (CTK) is a class of plant hormones that promote cell division. Its main function is to induce shoot formation and promote cell growth, generally produced in plant roots, and to promote the differentiation and growth of a variety of tissues. Cytokinins and auxins act synergistically on plant growth and development. CTK plays an active role in cell division, and participates in the regulation of cell growth, differentiation and other related physiological activities. CTK is mainly distributed in sites of active cell division, including root tips, shoot tips, immature seeds, germinating seeds, and the inside of growing fruits. CTK is essential for the induction of embryogenic callus in plants and can improve the induction rate of embryogenic callus. However, the concentration of cytokinin

cannot be too high, and it must be used in conjunction with auxin [7] . Cell division depends on CLV3 and AHKs signal regulation pathways, thereby further affecting the expression of STM and IPT7, and achieving the regulation of plant meristem[8] . Cytokinins are important plant hormones that regulate plant growth and development and participate in cell division, differentiation and morphogenesis.

The cytokinin signal transduction pathway plays a key role in many organisms. The signaling system for cytokinins is based on a dual signaling system. After binding to cytokinin, cytokinin receptors undergo autophosphorylation and transfer phosphate groups to phosphotransfer proteins in the cytoplasm through transmembrane transport. The phosphorylated proteins enter the nucleus and transfer phosphate groups to response regulators (ARRs) to induce gene expression, thereby regulating plant growth and development. Signaling of the classical plant hormone cytokinin (CKs) is based on interactions between proteins that constitute a multistep phosphate transfer system (MSP) : catalytic receptor-sensor histidine kinases (HKs), phosphate ester transmitters (HPts), and transcription factor-response regulators (RRs)[9] . It has been demonstrated that cytokinin signaling may be mediated by a two-component system similar to bacteria and fungi, which transacts external signals to downstream effectors and regulators through the phosphate esters of the plasma membrane anchored receptors[10] . The cytokinin signal transduction pathway is a hot research area. Researchers at home and abroad are exploring its molecular mechanism and physiological function, which provides an important reference for further revealing the regulatory mechanism of plant growth and development.

1.3 Key enzymes of cytokinin signal transduction pathway

1.3.1 CRE1 (cytokinin receptor 1)

cytokinin receptor kinase 1 (CRE1) is a protein on the plant cell membrane. It is an important component of cytokinin signal transduction and a receptor protein that plays a key role in the cytokinin signal transduction pathway. CRE1 can sense cytokinin signals in plant cells, trigger a series of signal transduction responses in

the cells, and regulate cell division and growth and development by activating downstream signal transduction pathways. When cytokinin binds to CER1, it activates the kinase activity of CER1, which in turn initiates downstream signaling. The CHASE region of CRE1 is the binding site of CTK, and CTK binds CRE1 in the form of a ligand[11] .Activated CER1 can interact with other signaling components, such as activation of the inositol polyphosphate kinase pathway to regulate cell growth and division. In addition, CER1 also interacts with transcription factors, thereby affecting the transcription and expression of cytokine-responsive genes. The study of cytokinin receptor 1 is important to gain insight into the mechanism of the cytokinin signal transduction pathway.

Recent studies have shown that, in addition to kinase activity, the cytokinin receptors CRE1/AHK4/WOL also possess phosphatase activity. This is characterized by the transfer of phosphate groups from phosphorylated AHPs to aspartic acid in CER1. This suggests that cytokine-mediated phosphate group delivery is a bidirectional and reversible process[12] . CRE1 was shown to bind cytokinin in a yeast cell-based experiment, thus providing direct evidence for this notion that the membrane-anchored protein is a true cytokinin receptor [13] . At the same time, studies have shown that Arabidopsis CRE1 encodes a histidine kinase, which contains two C-terminal reaction regulatory regions, one histidine kinase region and two transmembrane regions [14] .

1.3.2 Arabidopsis histidine phosphotransfer proteins (AHP)

Arabidopsis histidine phosphotransfer proteins (AHPs) are a class of proteins in plant cells and represent an important family of signal transduction proteins in Arabidopsis. They participate in the histidine phosphate transfer reaction during cell signaling in plant cells. AHP protein is an intracellular phosphotransferase that can accept the phosphorylation signal from cytokinin receptor kinase on the plant cell membrane and transfer it to a series of downstream signal transduction molecules, thereby regulating plant growth and development. There are 11 gene families of AHP in Arabidopsis. The amino acid sequences of each family member are very similar, but their expression patterns and physiological functions are

different. These family members possess phosphotransferase activity and play a role in transmitting and regulating signals in the histidine kinase signal transduction pathway. AHP functions to regulate phosphate transfer between receptor kinases and response regulators. When the conserved histidine site on AHP receives the phosphate group from the CTK receptor HK, the phosphate group is transported from the cytoplasm to the nucleus, and binds to the response regulator RR in the nucleus, thereby activating the expression of downstream genes to complete the signal transmission[15] [16] .

AHP proteins are encoded by a series of genes, and histidine phosphate transporters in Arabidopsis are composed of six members, among which AHP1-AHP5 are functional histidine phosphate transporters, including conserved His phosphorylation sites[17] . The AHP proteins encoded by these genes have different tissue expression patterns and functional properties, and they play different roles at different stages of plant growth and development. AHP proteins play an important role in the phosphate transfer reaction in plant cells. They can regulate biological processes such as cell division, cell expansion, plant growth and development. Therefore, AHP proteins have important applications in plant molecular genetics and biotechnology.

1.3.3 B-ARR, B-type Arabidopsis response regulator

ARR stands for "B-type Arabidopsis response regulator". B-ARR is a specific name for a single B-type response regulator. B-arr is a specific member of the BRR family, which is a type B response regulator. As a member of the BRR family, B-ARR is specifically involved in the regulation of cytokinin signal transduction and affects plant growth and development and other physiological processes. B-ARR is a family of response regulators responsible for cytokinin signaling in Arabidopsis plants. It is a transcription factor-like protein in cells and belongs to the family of response regulators. They are involved in regulating the transmission and biological response of cytokinin signaling through cytokinin receptors and cytokine-activated kinase cascades. The proteins of the b-type ARR transcription factors contain a conserved N-terminal receiving domain, an intermediate DNA-

binding domain, and a C-terminal transcriptional activation domain [19] [21] .When the cytokinin receptor is activated, the kinase delivers the phosphate group to the B-ARR via phosphorylation, thereby activating the B-ARR. Activated B-ARR further regulates transcription of downstream genes, as well as responses to other cytokinin signaling responses. Transcription of class B ARRs is not induced by CTK. As transcription factors, class B ARRs can directly regulate the expression of class A ARRs gene after their activity is activated by cytokinin [23] .

Specifically, B-ARR regulates the expression of cytokine-responsive genes by binding to DNA, thereby affecting cell division, growth and development, and other physiological processes in plants. Some studies have also shown that there are 12 B-type ARRs encoded in Arabidopsis, among which ARR21 and ARR13 are closely related. The over-expression of ARR21 gene and the constitutive expression of ARR21 after the deletion of DDK domain seriously hinder the growth and development of seedlings [20] . They play an important role in regulating root and stem development, leaf unfolding, and floral organ formation.

1.3.4 A-ARR, A-type Arabidopsis response regulators

A-ARR stands for "A-type Arabidopsis response regulators". ARR refers to a class of response regulators. ARR stands for "Arabidopsis Response Regulator" and refers to specific types of response regulators, of which A-ARR is one type, specific to A-type response regulators. A-ARR mainly participates in the regulation of plant cytokinin signal transduction pathway, activates or inhibits the activity of transcription factors, regulates gene expression, and affects plant development and physiological processes. Current studies have shown that part of the function of A-type ARR is to reduce the sensitivity of plants to cytokinin and negatively regulate the signaling pathway of cytokinin [18] .

The a-arr gene encodes proteins that share common features of response regulators, including an export domain, A receiving domain, and a DNA-binding domain.

In the cytokinin signaling pathway, briefly, activated cytokinin receptor kinases activate A-ARR via phosphorylation, leading to changes in transcription

factor activity and ultimately regulating plant growth and development. The most important characteristics of class A ARRS are that they can be induced by exogenous cytokinin treatment and act as negative regulators in the cytokinin signal transduction pathway [22] .

A-ARR plays an important role in the cytokinin signaling pathway in Arabidopsis. As receptors for cytokinin signaling, they regulate physiological processes such as plant cell division, growth, and senescence by participating in cytokinin sensing and signaling. A-ARR is involved in regulating the expression and activity of genes such as transcription factors, kinases and phosphatases in the cytokinin signaling pathway, thereby controlling downstream cell growth and differentiation.

1.4 Purpose and significance of the study

Elephant grass has been widely used in plant molecular biology, genetics, physiology and biotechnology due to its short life cycle, small genome, abundant genetic variation and easy genetic transformation.

The analysis and research of the signal transduction pathway of elephant grass cytokinin is to obtain the purple elephant grass cytokinin pathway genes, and then use the BlastP tool to find the elephant grass cytokinin pathway genes. Through the localization analysis of the cytokinin gene on the genome, the whole genome duplication analysis and the positive selection analysis, the selection pressure analysis of the whole genome duplication gene pair was performed, and then the phylogenetic tree analysis, expression analysis, and protein interaction analysis of the cytokinin genes were performed. Finally, Qpcr was performed to verify the results, and the mitogen signal transduction pathway of grass was analyzed, and the genes that play important roles in the growth and development of grass were finally mined.

By obtaining the cytokinin signal transduction pathway genes of purple elephant grass, we searched for the cytokinin signal transduction pathway genes of purple elephant grass, and analyzed the data of the studied genes by bioinformatics

methods, so as to explore the important genes for the growth and development of purple elephant grass. By studying the signal transduction pathway of elephant grass cytokinin, we can understand the mechanism of plant cytokinin signal transduction and reveal the molecular mechanism of plant growth and development. Exploring the adaptation mechanism of plants to the environment can provide theoretical basis for ecological adaptation and optimization of plants, provide theoretical support for plant breeding and production, and provide scientific basis for cultivating high-yield, stress-resistant and high-quality crop varieties.

Conclusions to chapter 1

In this chapter, the current status of cytokinin signal transduction pathway in grass was discussed, and the research progress of elephant grass and several key genes of cytokinin signal transduction pathway were introduced.

1. Elephant grass is widely distributed in tropical, subtropical and temperate regions all over the world. It is a kind of grass species with strong adaptability and strong vitality. Elephant grass contains a variety of bioactive components with anti-oxidation, anti-inflammatory, antibacterial, hypoglycemic and other pharmacological effects, so it has a wide application prospect in medicine, health care and other fields. Elephant grass plays an important role in the sustainable development of grassland ecosystem because it can improve soil quality and grassland productivity.

2. Cytokinins promote cell division. Its main function is to induce bud formation and promote cell growth, and to promote the differentiation and growth of a variety of tissues. CTK plays an active role in cell division, and also participates in the regulation of cell growth, differentiation and other related physiological activities.

3. Cytokinin receptor kinase 1 It is an essential component of cytokinin signaling and plays a key role in the cytokinin signaling pathway. CRE1 senses cytokinin signals in plant cells and regulates cell division and growth and development by activating downstream signal transduction pathways.

4. Arabidopsis histidine phosphotransfer proteins (AHPs) are an important family of signal transduction proteins in Arabidopsis. They are one of the intracellular phosphotransferases involved in the histidine phosphate transfer reaction during cell signal transduction in plant cells. It is able to accept phosphorylation signals from cytokinin receptor kinases on the plant cell membrane and transfer them to a series of downstream signal transduction molecules, thereby regulating plant growth and development.

5. B-ARR is a family of response regulators responsible for cytokinin signaling in Arabidopsis plants. The proteins of the b-type ARR transcription factors contain a conserved N-terminal receiving domain, an intermediate DNA-binding domain, and a C-terminal transcriptional activation domain. When the cytokinin receptor is activated, the kinase delivers the phosphate group to the B-ARR via phosphorylation, thereby activating the B-ARR. Activated B-ARR further regulates transcription of downstream genes, as well as responses to other cytokinin signaling responses. CTK was unable to induce transcription of class B ARRs. Class B ARRs can act as transcription factors, and their activity is regulated by cytokinin activation, thus directly affecting the expression of class A ARRs genes. They are involved in regulating the transmission and biological response of cytokinin signaling through cytokinin receptors and cytokine-activated kinase cascades.

6. A-ARR mainly participates in the regulation of plant cytokinin signal transduction pathway, activates or inhibits the activity of transcription factors, regulates gene expression, and affects plant development and physiological processes. The present study shows that the A-type ARR protein has A partial function to attenuate the sensitivity of plants to cytokinins and is responsible for regulating the signaling pathway of cytokinins.

CHAPTER 2.
OBJECT, PURPOSE AND METHODS OF THE STUDY

2.1 Search for the elephant grass cytokinin gene

Through literature review, the purple elephant grass cytokinin gene was obtained. After obtaining the purple elephant grass cytokinin pathway genes, the BlastP tool of TBtools software and elephant grass genome data were used to find the elephant grass cytokinin pathway genes by comparison. Identification condition: identity>80 p<1e-9.

2.2 Protein parameters were calculated for the cytokinin gene

The obtained 20 Protein sequences of the elephant germ cell kinin gene were sorted into txt files, and then the above documents were imported by the Protein parameter calculation tool of TBtools to obtain the sequence length, molecular weight, isoelectric point and other property values of the protein. Export it as a table.

2.3 Localization analysis and whole genome duplication analysis of the elephant grass cytokinin gene on the genome

The 20 elephant grass gene ids to be studied were sorted into the new txt document, and the 20 genes were renamed to another new document in turn. LINK files of 20 genes were sorted out in elephant grass gene Bank; The tandem repeat genes were identified and the appropriate RGB color controls were set to the txt document. The Gene Location Visualize from GTF/GFF tool of TBtools software was used to import the files in turn, display the genes of cytokinin signal transduction pathway on the chromosome, and export the localization analysis map.

2.4 Phylogenetic tree analysis of cytokinin genes was performed

In order to fully clarify the genetic relationship and biological functions of the genes in the signal transduction pathway of elephant grass cytokinin, multiple sequence alignment analysis was performed on the identified elephant grass cytokinin genes, and a phylogenetic tree was constructed.The protein sequences of

20 cytokinin signal transduction pathway genes needed to be studied were identified and sorted into fasta files. The phylogenetic Tree Of the 20 protein sequences was constructed using the Maximum Likelihood method using the MEGA-X tool, and the phylogenetic tree was beautificated using the Interactive Tree Of Life (iTOL) tool.

2.5 Cytokinin gene expression analysis was performed

The transcriptome data were used to obtain the expression levels of genes in the mitogen signal transduction pathway in different tissues (roots, stems, leaves) at different developmental stages. Then the HeatMap tool of TBtools was used to draw the gene expression heat map, and the expression levels were normalized by log2 (TPM+1).

2.6 Protein interaction analysis of the cytokinin gene was performed

Due to the close genetic relationship between elephant grass and millet, by String-db(website:http://www.bioconductor.org/packages/release/bioc/html/STRINGdb.html) website download millet protein sequence and protein interaction network. Then, the protein-protein interaction analysis was performed using the PPI Predict tool of TBtools, and the results were used to draw and beautize the protein-protein interaction network map by Cytoscape tool to facilitate the analysis of the interaction between genes.

2.7 Gene structure view of the cytokinin gene

Gene Structure View is an advanced analysis tool for visualizing the organization and annotation information of genes. It can help us better understand the organization and function of genes and determine the position of transcription start sites, splicing variants, promoters and terminators of genes. The original ID of elephant grass was used to construct a phylogenetic tree and obtain a file in nwk format. Then, the Gene Structure View (Advanced) 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.

Conclusions to chapter 2

To explore the characteristics of mitogen signal transduction pathway genes, the protein parameters of these genes were calculated, gene localization analysis on the genome, phylogenetic tree analysis, expression analysis, protein interaction analysis and gene structure view were performed.

CHAPTER 3
EXPERIMENTAL PART

3.1 Search for the elephant grass cytokinin gene

CRE1 (cytokinin receptor 1), AHP (Arabidopsis histidine phosphotransfer proteins), B-ARR (B-type) are the main genes involved in the signal transduction pathway of mitogen in elephant grass cells Arabidopsis response regulators). Through bioinformatics analysis, we obtained 20 cytokinin signal transduction pathway genes in the three genome of Xiangya sinensis, including 11 genes in the CRE1 gene family, 4 genes in the AHP gene family, and 5 genes in the B-ARR gene family. Elephant grass cytokinin pathway genes (20) and purple elephant grass (18) were not significantly different. It was higher than that of Pensetifolia (9), millet (8) and rice (8) (Table 3-1). The CRE1, AHP, and B-ARR gene families were strongly amplified in elephant grass and purple elephant grass compared to other species.

Table 3-1 Number of three gene families in different species

Gene family (horizontal) \ species (vertical)	CRE1	AHP	B-ARR
Pennisetum purpureum Schumach	11	4	5
Purple Aura Herb	10	4	4
Grain of corn	4	2	2
rice	4	2	2
Pennisetum	5	2	2

3.2 Protein parameters were calculated for the genes involved in the cytokinin pathway

According to the Protein parameter calculation tool of TBtools, the molecular weight, isoelectric point, instability coefficient, lipid solubility index and hydrophilic index of the protein can be obtained (Table 3-2). 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. The isoelectric point (Theoretical pI) is very important for understanding the solubility, electrophoretic separation and interaction properties of proteins. Instability Index is used to evaluate the stability of protein sequence. A higher instability coefficient indicates that the protein is more unstable and may be more susceptible to degradation or inactivation. The highest instability coefficient was AHP.3, and the lowest instability coefficient was CRE1.9. The Aliphatic Index is a measure used to assess the hydrophilicity and hydrophobicity of a protein sequence. A higher fat-solubility index indicates that the protein is more hydrophobic and prefers to interact with nonpolar fatty acids. The highest fat solubility index was B-ARR.5, and the lowest was CRE1.5. Grand Average of Hydropathicity (GRAVY) is a metric used to evaluate the hydrophilicity and hydrophobicity of a protein amino acid sequence. The hydrophilicity index can be calculated from the amino acid composition of proteins and their chemical properties and used to predict the solubility and folding state of proteins. A higher value of the hydrophilicity index indicates that the protein is more hydrophilic and prefers to interact with water. It can be seen from the table that the hydrophilic index of protein amino acids is all negative, among which the highest is B-ARR.5 and the lowest is CRE1.5.

Table 3-2

Protein parameter calculation and analysis

ID	Elephant grass gene	Molecular Weight	Theoretical pI	Instability Index	Aliphatic Index	Grand Average of Hydropathicity
maker-chrA4-snap-gene-782.77-mRNA-1	AHP.1	22061.71	5.33	48.73	70.25	-0.407
maker-chrA6-snap-gene-230.79-mRNA-1	AHP.2	25063.89	9.25	61.61	81.49	-0.274
maker-chrB1-snap-gene-596.71-mRNA-1	AHP.3	33770.65	6.7	67.76	81.35	-0.117
maker-chrB6-augustus-gene-1867.66-mRNA-1	AHP.4	17151.39	6.59	59.31	60.86	-0.597
augustus_masked-chrA2-processed-gene-1172.14-mRNA-1	B-ARR.1	93039.38	7.12	47.72	82.39	-0.283
maker-chrA6-snap-gene-60.114-mRNA-1	B-ARR.2	70768.4	5.05	46.69	83.33	-0.356
maker-chrB4-augustus-gene-203.84-mRNA-1	B-ARR.3	69112.17	6.02	42.46	77.21	-0.552
augustus_masked-chrB6-processed-gene-2063.23-mRNA-1	B-ARR.4	64537.41	5.04	46.45	84.05	-0.383

maker-chrB6-snap-gene-2064.81-mRNA-1	B-ARR.5	92628.53	6.16	39.95	95.51	-0.052
maker-chrA2-snap-gene-5.122-mRNA-1	CRE1.1	8103.22	6.55	59.76	78.77	-0.166
maker-chrA2-snap-gene-429.88-mRNA-1	CRE1.2	132455.02	6.27	44.58	92.45	-0.091
maker-chrA3-snap-gene-113.90-mRNA-1	CRE1.3	100581.14	5.85	37.4	85.99	-0.23
maker-chrA3-snap-gene-115.82-mRNA-1	CRE1.4	139429.96	9.16	42.9	83.85	-0.245
maker-chrA5-snap-gene-142.112-mRNA-1	CRE1.5	23402.52	8.71	67.4	52.25	-0.952
maker-chrA6-snap-gene-39.86-mRNA-1	CRE1.6	83189.5	8.27	43.74	89.26	-0.146
maker-chrB2-snap-gene-1042.93-mRNA-1	CRE1.7	44833.73	8.04	43.33	92.07	-0.1
maker-chrB2-snap-gene-1165.83-mRNA-1	CRE1.8	56356.77	7.23	42.08	93.94	-0.271
snap_masked-chrB4-processed-gene-1084.89-mRNA-1	CRE1.9	97269.13	5.66	34.85	85.04	-0.201
maker-chrB6-augustus-gene-	CRE1.10	85213.01	8.68	43.11	90.87	-0.127

2086.102-mRNA-1						
maker-ScaffoldUN-snap-gene-139.109-mRNA-1	CRE1.11	17669.36	5.56	43.58	85.85	-0.125

3.3 Localization analysis and whole genome replication analysis of the elephant grass cytokinin gene on the genome

According to the localization analysis of 20 genes in elephant grass, the distribution map of 3 gene families on elephant grass chromosome was drawn (Figure 3-1 below). The three elephant grass gene families are distributed on 10 chromosomes of elephant grass. Among them, the most genes were found on chromosome B6, followed by chromosome A6 and chromosome A2, and only one gene was found on the chromosome with the least gene distribution. CRE1 family genes were mainly distributed in A2, A3, A5, A6, B2, B4, B6 and ScaffoldUN. The B-ARR family genes were mainly distributed in A2, A6, B4 and B6. The AHP family genes were mainly distributed in A4, A6, B1 and B6. Although all mitogen signaling pathway genes are distributed throughout the genome, the distribution of different genes on chromosomes is also specific. Genome-wide replication analysis revealed no collinearity.

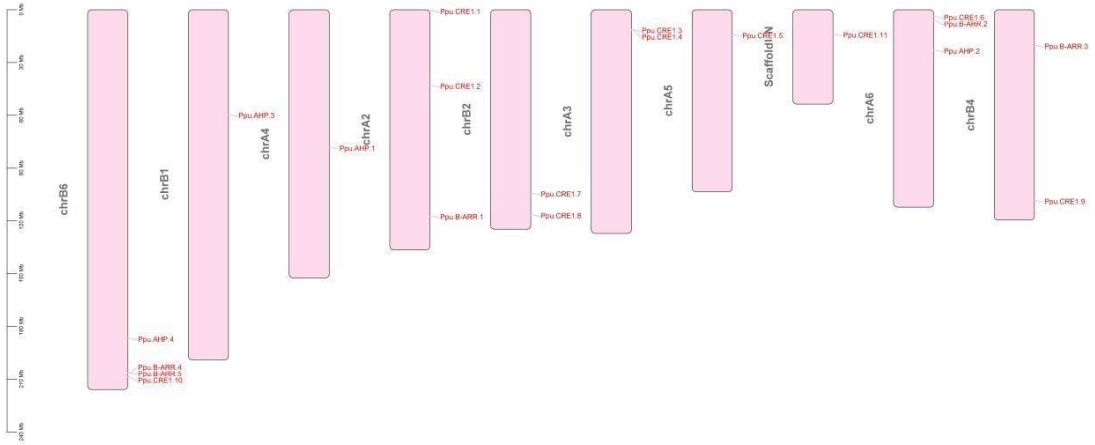

Figure 3-1 Localization of family genes on chromosomes

3.4 Phylogenetic tree analysis of cytokinin genes was performed

In order to fully clarify the genetic relationship between the genes of elephant grass families, multiple sequence alignment analysis was performed on the identified three genes of elephant grass families, using the software MEGA-X, The phylogenetic Tree Of 18 protein sequences was constructed by Maximum Likelihood method and then beautised by iTOL (Interactive Tree Of Life) tool. Phylogenetic analysis of 20 protein sequences of elephant grass (Figure 3-2) and protein sequence system analysis of three family genes of elephant grass and purple elephant grass (Figure 3-3) were obtained. Figure 3-2 shows that the two gene families of B-ARR and AHP are from the same branch, indicating that they are more closely related. The genes of the CRE1 family were divided into two branches, and the CRE1.1 gene was not in the same branch as the other family genes. Except for the CRE1.1 gene, CRE1.8 gene belonged to a different branch from other genes. Figure 3-3 shows that elephant grass and purple elephant grass gene families mostly come from the same branch, which further confirms that elephant grass and purple elephant grass have very close genetic relationship. The evolution of elephant grass and purple elephant grass family genes can be divided into two branches. CRE1.10 of Chinese elephant grass and Chinese elephant grass came from the same gene branch, and they came from the same gene branch with Chinese elephant grass CRE1.9 and Chinese elephant grass CRE1.6, which came from the same gene branch with the single Chinese elephant grass CRE1.8. The remaining elephant grass and purple elephant grass family genes are from another branch. However, the AHP and B-ARR family genes of elephant grass and purple elephant grass evolved from the same branch. Among the AHP family genes, the AHp-2 gene of elephant grass evolved earlier. AHP.3 and AHP.1 in elephant grass and AHP.1 and AHP.2 in purple elephant grass were derived from the same family evolutionary gene branch. In the evolutionary branch of B-ARR family genes, the b-arR.1 gene of elephant grass evolved earlier. B-ARR.3 of elephant grass and B-ARR.2 of purple elephant grass were from the same evolutionary branch. B-ARR.5 of elephant grass and B-ARR.4 of purple elephant grass also came from the same

evolutionary branch. This clade was not from the same clade as the other two B-ARR.3 and B-ARR.2 from the same clade, and the evolutionary relationship between these two clades was far away compared with the others. The B-ARR.3 and the B-ARR.2 of the purple elephant grass were from the same evolutionary branch.

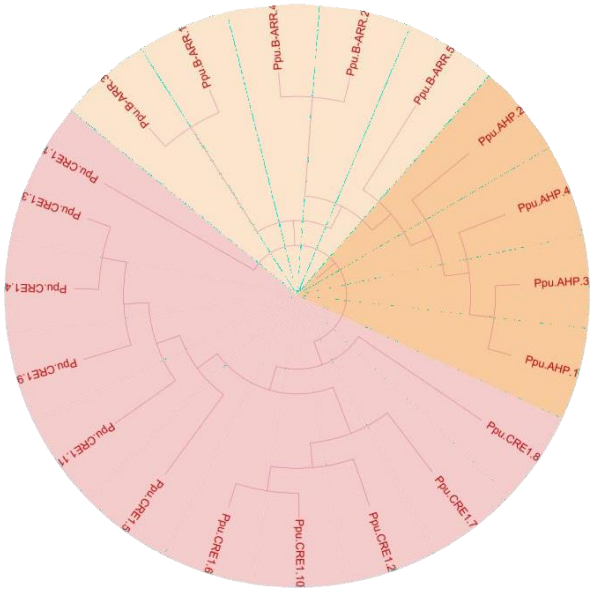

Figure 3-2 Phylogenetic analysis of 20 protein sequences in elephant grass

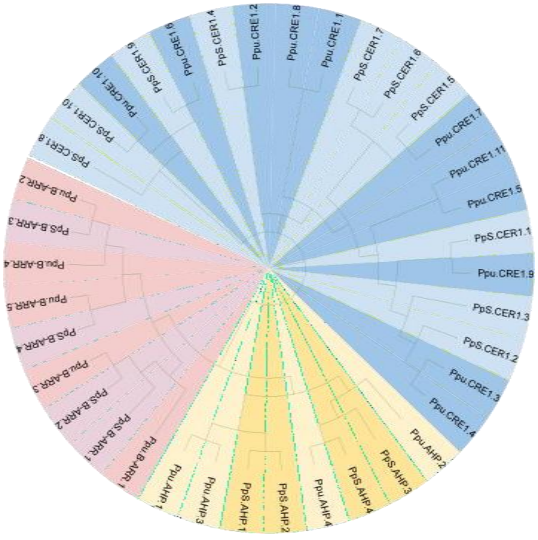

Figure 3-3 Systematic analysis of protein sequences of three family genes of elephant grass and purple elephant grass

3.5 Expression analysis of the cytokinin gene was performed

Based on the genome information of elephant grass, the HeatMap tool of software TBtools was used to draw the expression heat map of elephant grass family genes (Figure 3-4) [24] [25] . The results showed that the genes CRE1.3, CRE1.4, B-ARR.1, CRE1.9, CRE1.11, B-ARR.3, and B-ARR.5 were highly expressed in the root. However, B-ARR.2 was not expressed in roots. The remaining genes were all expressed in the roots, but the expression levels were different. The expression levels of CRE1 family genes and B-ARR family genes were higher than AHP family genes in roots. The expression of the same gene was also different in different stages of stem, and the most obvious difference was CRE1.6 and CRE1.10, which could change from strong expression to weak expression, that is, from dark to light color, and the expression levels of the two genes in different stages were similar. At different stages of leaves, the expression levels of most genes were small, but the expression levels of AHP.2 and AHP.4 were relatively large and similar. The expression of CRE1.2 was significantly different in different parts of the leaf at different stages. CRE1.3, CRE1.4, B-ARR.1 and B-ARR.3 were more expressed in roots than in different parts of stems and leaves and at different stages. CRE1.2 and AHP.2 and AHP.4 were more expressed in the leaves than in the other parts. However, the expression of AHP.1, CRE1.8, B-ARR.2, AHP.3, CRE1.1, CRE1.5 and CRE1.7 in stems were higher than those in other parts. Except for B-ARR.2 gene and CRE1.2 gene, all the other genes were expressed in different developmental stages or different developmental sites of rhizome and leaf, indicating that they were involved in the whole development process of rhizome and leaf of elephant grass and played an important role in the development of elephant grass.

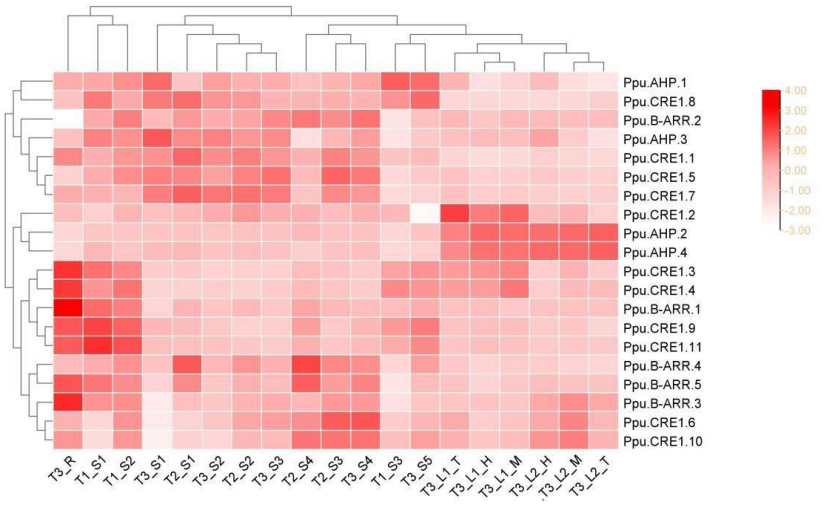

Figure 3-4 Expression analysis of elephant grass genes in each tissue

3.6 Protein interaction analysis of cytokinin genes was performed

Through the PPI function in the TBtools tool, the protein relationship of the gene family of the cytokinin signal transduction pathway was further predicted, as detailed in Figs. 3-5. There are 19 nodes in the protein-protein interaction network. There were corresponding interactions between any protein node and other protein nodes, among which PpuCRE1.8 was the core of the elephant grass gene family protein network, indicating that it plays an important role in the regulation of plant photosynthesis.

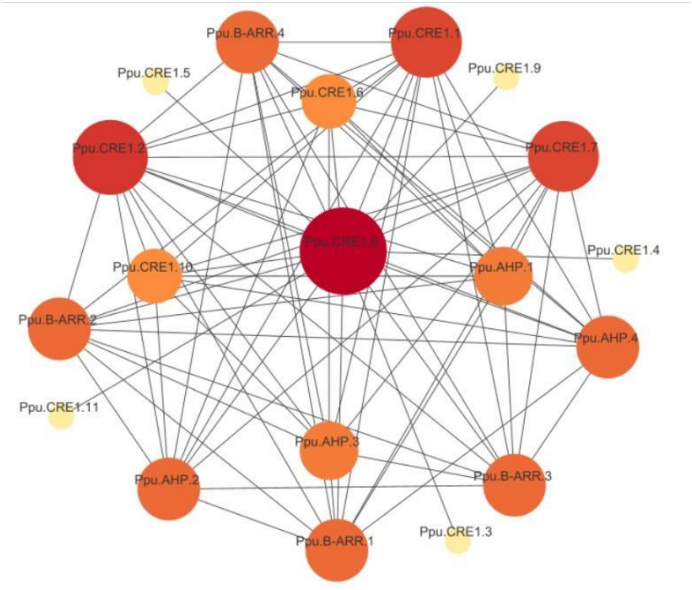

Figure 3-5 Protein interactions among the genes of the elephant grass family

3.7 Gene structure view of the cytokinin gene

The Gene Structure View (Figure 3-6) was constructed using the Gene Structure View (Advanced) tool of TBtools, which usually represents different parts of a gene in the form of lines or boxes, such as exons (CDS), introns (Utrs), etc. The figure shows that there are few exons of CRE1.11 and CRE1.1 genes in the CRE1.1 family genes. However, CRE1.4, CRE1.9, CRE1.2, CRE1.6 and CRE1.10 genes had fewer introns. However, CRE1.1 gene contains few introns and few exons. In the B-ARR family, there were no introns at both ends of b-arR.1. B-ARR.3 and B-ARR.4 have introns at both ends. B-ARR.2 has an intron on only one end. In AHP families, both exons and introns were less distributed.

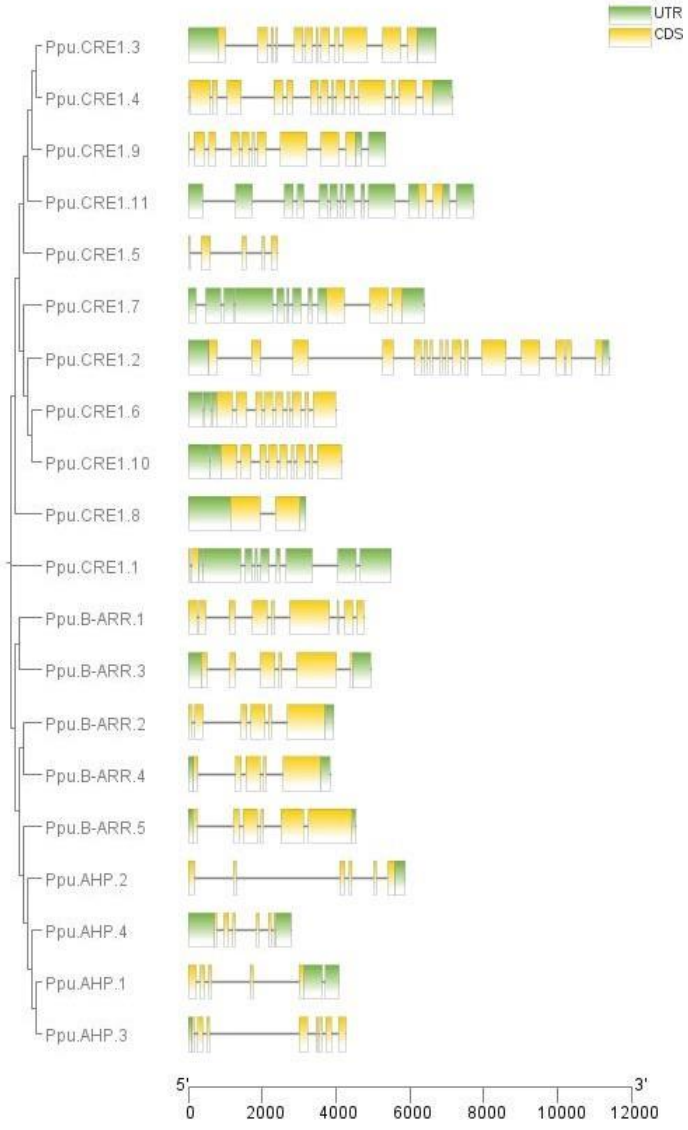

Figure 3-6 View of gene structure

3.8 Discussion

Elephant grass is a new energy plant with high photosynthetic and high biomass, and cytokinins are plant hormones that promote cell division, induce bud formation, and promote its growth. In this study, we analyzed the genes involved in the mitogen signal transduction pathway. There are four main gene families involved in the signal transduction pathway, and the first three gene families, including CRE1, AHP and B-ARR, were mainly introduced. By comparing with purple elephant grass, it was found that there was little difference in copy number between elephant grass (20) and purple elephant grass (18), indicating that the two species were indeed closely related. The protein parameters of the grass cytokinin pathway genes were calculated, and we found that the AHP.3 gene was the most unstable, while the CRE1.9 gene was the most stable. CRE1.5 was the most hydrophilic gene, and B-ARR.5 was the least hydrophilic gene. The highest hydrophilicity of the protein amino acid sequence was B-ARR.5, and the lowest was CRE1.5.

Chromosome localization analysis showed that the three gene families of elephant grass were distributed on every chromosome of elephant grass. Among them, the most genes were found on chromosome B6, followed by chromosome A6 and chromosome A2, and only one gene was found on the chromosome with the least gene distribution. The density at both ends of the gene distribution on each chromosome is greater than the density in the middle. This result suggests that these genes may be more likely to be cloned at the top or bottom of the chromosome [26] .

A phylogenetic analysis of cytokinin signal transduction pathway genes was performed using the coding sequences of elephant grass. The results showed that all identified gene families were from different clades. It was also found that the genes in the same gene family were from two different branches, such as CRE1.1 and the other genes in the family were from different branches. However, B-ARR is from the same branch as AHP. Elephant grass and purple elephant grass gene families mostly come from the same branch. The results of expression analysis

showed that CRE1 family genes, such as CRE1.3, CRE1.4, CRE1.9 and CRE1.11, were highly expressed in roots. Some studies have shown that CRE1 mainly develops in roots [27]. The expression of the same gene was also different at different stages of stem. The most significant difference was CRE1.6 and CRE1.10, and the expression levels of these two genes were similar at different stages. The expression levels of most genes were small at different leaf stages, but AHP.2 and AHP.4 showed large and similar expression levels. AHP family genes were less expressed in roots. CRE1.2, AHP.2, and AHP.4 were all less expressed in stems. However, the expression of B-ARR family genes was low in roots.

Conclusions to chapter 3

1. 20 members of 3 gene families in the mitogen signal transduction pathway were identified by BlastP tool;

2. Protein characteristics analysis showed that the molecular weight of the protein was between 8103 and 139430, the isoelectric point was between 5-10, the instability coefficient was between 34 and 68, the lipid solubility index was between 52 and 96, and the hydrophilic index was between -0.96 and -0.05.

3. Gene structure analysis found that the number of exons in different members of different families was different.

4. Through chromosome localization analysis of genes, it was found that three gene families of elephant grass cytokinin signal transduction pathway were distributed on 10 chromosomes of elephant grass, and the most genes were distributed on B6 chromosome.

5. Phylogenetic analysis through phylogenetic trees showed that the same gene families clustered together to better understand the similarities and differences between biological species;

6. The expression levels of each gene in different tissues were different by expression analysis. Among them, the expression levels of CRE1 family genes and B-ARR family genes in roots were higher than those of AHP family genes, while AHP family genes were mostly expressed in leaves.

7. Protein interaction analysis revealed that the interaction between proteins was interrelated, and CRE1.8 was the main core gene of the elephant grass gene family protein network.

CONCLUSIONS

This study mainly revealed the high copy number and transcript level of the family of genes in the elephant grass cytokinin signal transduction pathway. The main results are as follows:

(1) A total of 20 pathway genes from 3 gene families were identified in the cytokinin signal transduction pathway of elephant grass, distributed on 10 chromosomes.

(2) The most unstable gene in elephant grass was AHP.3, while CRE1.9 was the most stable. CRE1.5 was the most hydrophilic gene, and B-ARR.5 was the least hydrophilic gene. B-ARR.5 was the highest hydrophilicity of the protein amino acid sequence, and the lowest was CRE1.5.

(3) The expression of CRE1 family genes and B-ARR family genes was higher than that of AHP family genes in roots. CRE1.3, CRE1.4, B-ARR.3 and B-ARR.4 showed the highest expression. However, B-ARR.2 was not expressed in roots.

(4) The expression levels of AHP.1, CRE1.8, B-ARR.2, AHP.3, CRE1.1, CRE1.5 and CRE1.7 in stems were higher than those in roots and leaves.

(5) The expression levels of CRE1.2, AHP.2 and AHP.4 in leaves were higher than those in roots and stems.

(6) CRE1.8 is the core of the elephant grass gene family protein network, indicating its important role in the regulation of plant photosynthesis.

Elephant grass genomes can provide a system for studying diversity, speciation, and evolution in this family, and an important resource for understanding economically important traits and adaptation mechanisms. This also provides new resources for the exploitation of other species of significant economic, ecological, and research value.

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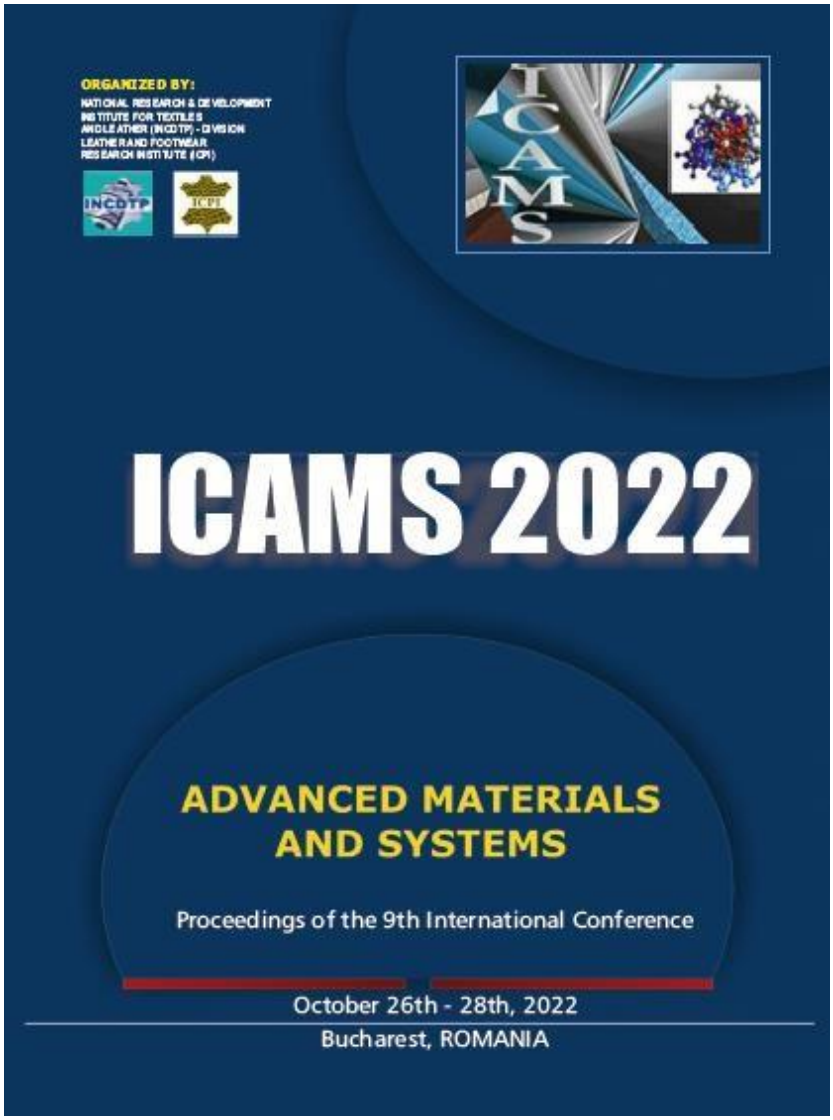
APPENDIX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NANOPIGMENTS FOR LEATHER FINISHING COATINGS

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The work is focused on obtaining nanopigments by adsorption of anionic dyes on positively charged montmorillonite. The effect of sequential modification of aqueous dispersions of montmorillonite with cationic and anionic compounds on the structural and charge characteristics of mineral dispersions was studied. The effect of chemical dispersion of agglomerates of aqueous montmorillonite dispersions after adding carbonate solutions was shown. The treatment of dispersions of original montmorillonite with sodium carbonate provides maximum dispersion of mineral aggregates by penetrating into the interstructural space of aluminosilicate packets, moving them apart and separating them. It was found that the modification of montmorillonite dispersed by sodium carbonate by adding basic chromium sulfate is accompanied by a change in the surface chemistry of the mineral and structural transformations. Structural changes are manifested by the formation of a developed structure of cationic montmorillonite. The cationic surface charge of montmorillonite and high specific surface of montmorillonite are important factors for ensuring effective adsorption of anionic dyes on the surface of the mineral. The efficiency of adsorption of anionic dyes on cationic montmorillonite is investigated. It was shown that the adsorption of dyes depended on the pH of the medium. The scheme of obtaining nanopigments, which were characterized by good covering power, saturated and intense colour was proposed.

Keywords: montmorillonite, pigment, leather finishing coating

INTRODUCTION

Traditional leather finishing involves applying a covering composition to the surface of leather. The finishing coating provides protection of leather from external atmospheric and mechanical impacts (Covington, 2009).

The type of leather coating depends on the content of pigments and can be (Covington, 2017; Zhuravsky *et al.*, 1996; Kasyan, 2019): aniline – a transparent coating without the use of pigments; semi-aniline – characterized by a small content of pigments to provide, mainly, a shade of color; and pigmented – with a significant content of pigments for complete coverage of the surface of leather with a colored covering layer.

Pigments provide color and covering power to the finishing coating (Winter *et al.*, 2017). Organic or inorganic pigments are used in the finishing coating of leathers. Covering compositions with organic pigments provide leather with shine, bright and intense color, but have low light fastness and heat resistance. Inorganic pigments create a high-quality coating with good light fastness and water resistance, but are characterized by a high tendency to sedimentation and are limited in color and brightness (Winter *et al.*, 2017; Osgood, 1990).

The ability of the coating to form a uniform coating stable composition with required thickness depends on the properties of the pigment, the origin of their surface, and the size of the particles.

The use of nanopigments provide improved physical and mechanical indexes of the leather finishing coating (Bondaryeva and Mokrousova, 2020; Bondaryeva *et al.*, 2021).

The aim of the work was to describe the scientific basis of patterns of anionic dyes adsorption on positively charged montmorillonite to obtain nanopigments for leather finishing coatings.

https://doi.org/10.24264/icams-2022.I.4

EXPERIMENTAL

Materials

Bentonite clay from the Cherkassky deposit (Ukraine), after thorough purification and washing was used as a basis for obtaining nanopigments. The main mineral was montmorillonite, the content was 85±3 %. The value of the exchange capacity was 72 mg-eq/100 g of clay. Humidity – 27 ± 3 %.

The sodium carbonate, basic chromium sulfate (III) and anionic dyes were used to modify dispersions of montmorillonite.

Methods

The nanopigments were obtained by sequential treatment of aqueous montmorillonite dispersions (100 g/l) with sodium carbonate, basic chromium sulfate and anionic dyes.

Firstly, 6,0% of sodium carbonate from weight of dry montmorillonite was used, and then the cationic form of montmorillonite was obtained by modifying the dispersion of Na^+-montmorillonite with chromium compound. For this purpose, the basic chromium sulfate was used – $Cr_2(SO_4)_3(OH)_{0.2b}$, chromium oxide (III) content was 25.6 %. A solution of basic chromium sulfate in the amount of 10.0% Cr_2O_3 (by weight of the montmorillonite) was added to the dispersion of Na^+-montmorillonite (MMT–Na^+). Mixing was continued until a homogeneous mass of gray colour was obtained. The pH value of the modified dispersion of cationic montmorillonite (MMT-Cr^{3+}) was 4.5-5.2.

The nanopigments were prepared by gradually mixing the cationic form of montmorillonite with the anionic dyes. Mixing was performed using a mechanical mixer (30–40 min, 40–45°C) to obtain time-stable dispersions in the form of nanopigments of saturated deep colour. The consumption of anionic dyes in a ratio of 1:1 according to the mineral component. The nanopigments were obtained as the colored modified dispersions of montmorillonite.

A laser-correlation spectrometer "ZetaSizer-3" (Malvern Instrument, USA) with a Multi Computing Correlator type 7032 CE was used to study the dispersion of mineral systems.

The adsorption of dyes from aqueous solutions on the cationic form of montmorillonite was determined by measuring the light transmittance of dye solutions of different concentrations.

The electrokinetic potential was determined by microelectrophoresis.

RESULTS AND DISCUSSION

In montmorillonite modification, molecules of polar liquids (for example, sodium carbonate) can freely penetrate into the interpackets space of montmorillonite, push them apart and increase the distance between packets. As a result, montmorillonite particles disperse spontaneously in water, their number per unit volume increases significantly, and the number of direct contacts for further interactions increases.

It is shown that treatment of dispersions of native montmorillonite with sodium carbonate provides maximum dispersion of mineral aggregates by penetrating into the interstructural space of aluminosilicate packets, moving them apart and separating them.

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