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 **Analysis of drought resistance of different wheat and cloning of drought resistance genes**

First (Bachelor's) level of higher education
Specialty 162 "Biotechnology and Bioengineering"
Educational and professional program "Biotechnology"

Completed: student of group BEBT-20
Xiaoran ZHANG

Scientific supervisor
Tetiana SHCHERBATIUK, Dr. Sc., Prof.

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

Kyiv 2024

KYIV NATIONAL UNIVERSITY OF TECHNOLOGIES AND DESIGN

Faculty: Chemical and Biopharmaceutical Technologies
Department: Biotechnology, Leather and Fur
First (Bachelor's) level of higher education
Specialty: 162 Biotechnology and Bioengineering
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
Xiaoran Zhang

1. Thesis topic **Analysis of drought resistance of different wheat and cloning of drought resistance genes**
scientific supervisor Tetiana Shcherbatiuk, Dr. Sc., 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	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 ______________Xiaoran ZHANG

Scientific supervisor _____________Tetiana SHCHERBATIUK

SUMMARY

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

As one of the most important crops in the world, wheat plays an indispensable role in our lives. However, the growth process of wheat is affected by drought and other adverse environments. Therefore, it has become an urgent issue to study the drought resistance mechanism of wheat. The aim of this experiment is to analyze and study the drought resistance mechanism of wheat, to find out the genes related to drought resistance of wheat among them, and to carry out molecular experiments to clone the drought resistance genes.The experimental methodology is as follows: Firstly, several different varieties of wheat were analyzed for drought resistance, and they were subjected to water fasting treatment, observed the phenotypic characteristics of different varieties of wheat and selected the two plants with the largest differences to be subjected to PEG simulated drought treatment, and then the phenotypes were observed and the leaves and roots of wheat were taken to be subjected to RNA-seq analysis, and genes that were significantly differentially expressed in both roots and leaves were selected to be subjected to GO and KEGG analyses. The genes related to drought stress that were significantly up-regulated in both leaves and roots were selected as candidate genes and verified by RT-PCR. According to the results of real-time fluorescence quantitative PCR (RT-PCR) analysis, the expression levels of the target genes in wheat leaves increased significantly under drought stress conditions, revealing that the genes may play an important role in drought tolerance in wheat. These genes were amplified by PCR and subjected to gel electrophoresis, and we will continue to verify the function of these genes at a later stage. In this experiment, we dug deeper into the mechanism of drought resistance in wheat and searched for drought-related genes to be cloned.GO analysis showed that the differentially expressed genes were mainly water deficit response, cold stress response, etc.; KEGG analysis showed that they were mainly enriched to phytohormone signaling pathway, and finally,we successfully cloned CS5A02G369800, CS7D02G549900, CS6A02G350500, CS7A02G422500, and provided valuable genetic resources for drought-resistant breeding of wheat. The cloning and functional analysis of the genes provide important clues for the in-depth understanding of the drought-resistant mechanism of wheat, and provide favorable information for the genetic improvement and drought tolerance of wheat.

Key words：Wheat; Gene cloning, drought stress, RT-PCR technolog

TABLE OF CONTENTS

INTRODUCTION 8

CHAPTER 1 CHAPTER 10

1.1 Wheat review 10

1.2 Plant response to drought stress 11

1.2.1 Physiological mechanisms of plants in response to drought stress 13

1.2.2 Evaluation criteria for drought tolerance of wheat 14

1.3 Function of gene cloning and its role in drought resistance 15

1.4 Research progress at home and abroad 15

1.5 Purpose and significance of the study 17

1.5.1 Effects of drought stress on plant growth 17

1.5.2 Study expectation and experimental significance 20

1.6 Future research outlook on wheat 22

Conclusions to chapter 1 23

CHAPTER 2 OBJECT, PURPOSE AND METHODS OF THE STUDY... 24

2.1 Experimental materials 24

2.2 Experimental method 25

2.2.1 Planting method of plant conditions 25

2.2.2 Analysis and processing of RNA-seq data 26

2.2.3 synthesis of cDNA 27

2.2.4 Design of the primers for RT-PCR 28

2.2.5 fluorescence quantitative PCR 28

2.2.6 Gene cloning 29

2.3 Drought stress treatment..30
2.4 Shooting method...31
Conclusions to chapter 2 31
CHAPTER 3 EXPERIMENTAL PART..33
3.1 Results of phenotyping of drought resistance in wheat plants......................33
3.2 PEG simulated drought experiment 34
3.3 Results of RNA-seq analysis of wheat leaves...35
3.4 Expression studies of genes related to drought resistance 38
3.5 Gel electrophoresis results 40
Conclusions to chapter 3 41
CONCLUSIONS...43
REFERENCE DOCUMENTATION 45

INTRODUCTION

As the most important crop in China, wheat is also of great significance in the world, and has always been highly valued by the world. Its cultivation has also become an important choice and inevitable path to solve many problems in the world. Wheat has different degrees of water demand in different growth stages, so drought will affect wheat in different stages of wheat growth. However, in recent years, with the influence of climate and other environmental changes, the drought phenomenon is becoming more and more common, and drought stress has become an important factor restricting the growth and yield of wheat.

Notably, gene cloning provides a new path for us to study the progress of drought resistance in wheat. Gene cloning is an important technology applied in the field of biotechnology. Is the process of artificial replication of a specific gene sequence. Drought resistance genes play an important role in the plant facing drought conditions, wheat drought resistance gene cloning is the cloning technology to obtain the gene sequence related to wheat drought resistance. By cloning and studying these genes, an in-depth understanding of their regulation mechanism can enhance the survival ability of plants under drought conditions, and apply them in the cultivation process of wheat plants, which can significantly improve the yield of wheat.

In this study, RT-PCR and gene cloning were used to clone genes related to drought resistance in wheat, analyze the expression and activity of these genes at seedling and jointing stage, explore their physiological mechanisms in the drought resistance, and study their regulatory network. The specific contents are as follows:

The relevance of the topic is look for wheat drought resistance genes, explore the mechanism of these genes and clone these genes.

The purpose of the study is the Explore the drought resistance mechanism of wheat and cultivate better wheat varieties.

The objectives of the study is Explore the mechanism of wheat drought resistance and find drought-related genes for cloning.

The object of the study is Wheat C6878 and wheat T103.

The subject of the study is study the differences between wheat C6878 and wheat T103, and drought resistance genes were found for cloning.

Research methods is Cloning technique by PCR technique.

The scientific novelty is Exploring the drought resistance mechanism of wheat.

The practical significance of the results obtained is Explore the mechanism of wheat drought resistance and find drought-related genes for cloning.

CHAPTER 1
CHAPTER

1.1 Wheat review

Wheat (*Triticum aestivum*) is a widespread crop grown around the world, which is an important grass plant and one of the most important food crops in the world. It has been widely cultivated, feeding people for thousands of years. It is one of the cornerstones of human agricultural civilization, providing a major food source for billions of people around the world, and also playing an important role in improving the modern economy. Exploring the growth conditions of wheat and how to cultivate better wheat varieties has become a common research topic of the people for nearly a hundred years. Here is a detailed overview of the wheat:

The root system of wheat is a slender beard root, which is an annual herb. The specific morphology of the stem is upright, usually between the height of 0.5 meters to 1.5 meters, but the height of different wheat varieties is not unique, there are height and short, due to different varieties and different growth conditions will lead to the difference of size. Among them, the dwarf Japanese varieties are more common and more extensive in daily planting. Its advantages of good lodging resistance make them widely praised in modern agriculture and obtain a higher status. At the same time, many varieties can grow to a higher height, showing a height of more than 1.5, but due to its easy lodging characteristics, its planting is not extensive. Wheat has parallel veins, leaves are generally linear, length of about 20cm, width is about 2cm about, smooth leaves, leaf sheath often with small thorns. The wheat is made of spikelets, each with dozens to hundreds of small flowers. These florets have stamens and pistil and usually present as unisexual flowers, but occasionally.

Wheat is one of the important research subjects, and its genome sequence has been fully interpreted, which provides the experimental basis for the genetic improvement and breeding of wheat, and is conducive to the operation of the next research step. The application of modern biotechnology makes wheat disease resistance, stress resistance and other traits can be improved through gene editing, transgenic and other methods to improve the yield and quality, bring us more convenient concessions, to adapt to different environmental conditions and market demand.

In general, wheat as one of the main food crops in the world, the survival and development of human plays a very important role, because of the role of wheat, so we should pay attention to the present and future wheat research, determined to give full play to the role of wheat, mining its function, improve its varieties, constantly adapt to the requirements of society and science and technology, constantly the next step of experimental operation. With the development of science and technology and the change of human needs, the research and breeding of wheat will continue to remain active. It is believed that in the near future, wheat will not only provide better food resources for human beings, but also bring unexpected functions in other fields.

1.2 Plant response to drought stress

Drought, as an abiotic stress factor, seriously restricts the development of agricultural production in the world. According to statistics, there are more than 60 countries in the arid and semi-arid regions, accounting for about 43% of the land area. Since the 21st century, with the destruction of ecological balance and global climate warming, the serious impact of drought on agriculture has gradually become the focus of people's attention. Drought has become the main factor limiting the crop yield in China and even the world. China's arid and semi-arid arable land area accounts for about 52.5% of the land area, of which the semi-arid areas account for 21.7% and the arid areas account for 30.8%. China's main grain production areas, north and northwest China are just in China's serious water shortage. Under natural conditions, due to the dry climate, severe evaporation, less annual precipitation and other reasons, the growth and development of plants are seriously affected, and it becomes more and more urgent to understand the drought resistance mechanism of plants. As a temperate plant, wheat has significant requirements for temperature and other conditions. The suitable temperature for wheat cultivation is 15℃ to 25℃, and too high or too low temperature is not conducive to wheat cultivation, among which the influence of temperature before sowing and flowering period is particularly significant on the growth and development of wheat. Drought is one of the most serious climate disasters in the growth process of wheat. It has a very significant impact on wheat, which will seriously affect the growth and yield of wheat. Therefore, when plants respond to drought stress, through a series of physiological and biochemical reactions, it is used to adapt to the emergence of drought and other adverse environments.

Drought can easily lead to physiological metabolic disorders in the growth process of wheat, which affects its stress resistance, increases the risk of plants by diseases and insect pests, and is not conducive to the growth of wheat. Its impact on wheat is not only limited to the simple yield decline, but also may cause a chain effect, which will have a serious negative impact on the growth, development and quality of wheat, and then affect food production and human life. Therefore, drought and other adverse environment has a great adverse impact on the growth and development of wheat.

1.2.1 Physiological mechanisms of plants in response to drought stress

Drought has a great impact on plants. In order to reduce water loss, plants can improve their drought resistance by wilting leaves, reducing the number of leaves, and falling leaves.

The components of biological membranes mainly include lipids, proteins, and a small amount of carbohydrates, of which lipids are one of its important components. Membrane lipid fluidity plays a key role in membrane function, while the degree of unsaturated fatty acids in membranes plays an important role in regulating membrane fluidity. Free radicals destroy the dynamic balance of membrane unsaturated fatty acids and saturated fatty acids through the peroxidation of membrane unsaturated fatty acids, resulting in less unsaturated fatty acids and more saturated fatty acids, so that the membrane forms a rigid state.

In addition, plants can alternatively regulate hormone signaling pathways, such as abscisic acid (ABA) and ethylene, to regulate growth, development and stress response. Episisic acid (ABA) is the main hormone synthesized in roots and transported to leaves when plants suffer from drought stress, which improves the plant tolerance to drought stress by controlling stomatal closure and inhibiting plant growth. Ethylene is also an important plant hormone, which has many effects on plant growth and development, including important contributions to improving drought resistance under drought stress. Under drought conditions, plants produce more reactive oxygen radicals, causing oxidative damage, while ethylene can modulate the activity of antioxidant enzymes to help plants cope with oxidative stress. Ethylene can also directly affect yield by increasing embryo and grain abortion.

A series of mechanisms produced by plants after drought stress jointly maintain the growth and development of wheat, and help wheat to resist a series of adverse environment interference, which is conducive to the growth and development of wheat.

1.2.2 Evaluation criteria for drought tolerance of wheat

As one of the most important food crops in the world, wheat is also facing the common problems with other crops —— Drought, soil drought, water shortage and hot climate are still a problem that China's agriculture needs to face for a long time. Therefore, in order to solve the climate problems in the growth and development of wheat, in order to realize the further development of China's agricultural economy, realize the well-off level of the national people and complete the goal of people's overall prosperity, it is crucial to evaluate and screen the drought resistance of wheat. This requires us to master the standards and methods for the evaluation of wheat drought tolerance in order to provide guidance for further experimental research on wheat drought resistance and breeding.

Drought tolerance of wheat can be assessed by yield. We can compare wheat drought resistance by comparing yield changes under drought conditions. Generally speaking, the drought resistance of wheat production is poor, and the slightly better drought resistance is correspondingly stronger. Observe the quality of wheat seeds under drought stress is also a standard to judge the strength of drought resistance, such as by observing the quality of grain or by measuring the protein content of wheat. Meanwhile, the growth rate of wheat is also an important criterion for assessing drought tolerance in wheat. Under drought conditions, wheat will show a clear trend of growth retardation and even wilting trend. So we can determine the drought resistance of wheat by observing the growth of the plants. In addition, wheat can also determine the ability to absorb water under drought conditions by its root condition, such as root length, root surface area, and root biomass, to determine its drought resistance.

1.3 Function of gene cloning and its role in drought resistance

Gene cloning plays a very important role in drought resistance in plants. As common in animals and plants and microbial bZIP transcription factors, according to the survey data, existing scholars success in wheat and drought related transcription factors cloning, and through the cloning gene into series experiments in tobacco, the results show that the tobacco drought resistance is further enhanced. In 2013, the field authority scientists through the successful use of wheat cloned new transcription factor gene, after the introduction into tobacco to obtain transgenic tobacco plants, experimenter the plant under adversity, the results show that transgenic plants than ordinary plants, in the seed germination rate, survival rate and plant internal water content have significantly improved. In addition, many scientists have successfully proved through a series of experiments that by cloning transcription factor genes in wheat, it will effectively improve the drought resistance of wheat.

In conclusion, the role of gene cloning technology covers many fields. It not only plays an important role in the fields of basic scientific research, genetic engineering and biomedicine, but also has great potential in improving plant drought resistance, providing new ideas and ways to solve the global drought problem.、

1.4 Research progress at home and abroad

The world's first transgenic plant was born in 1983, Vasil through genetic gun will β -gluconidase gene success into wheat, makes the world's first transgenic wheat, China also successfully developed in 1997 breeding of transgenic wheat plants, which makes the further development.

Initially, wheat needed to be genetically transformed with suspended cells and protoplasts, but with the development of science and technology, there are many kinds of explants to be selected in the genetic transformation process. At present, researchers have cloned dozens of functional genes related to drought resistance from different species, and made breakthrough progress in improving plant drought resistance through genetic engineering technology. According to the mode of action of drought resistance genes, drought resistance genes can be divided into two categories: the first is functional protein genes, whose coding products play a direct protective role in plant drought resistance; the second is regulatory protein genes, whose coding products play a regulatory role in the process of early signal transduction and gene expression. These two classes of genes play different roles in different growth stages in improving drought resistance **Помилка! Джерело посилання не знайдено.**.

As one of the most important agricultural products in the world, wheat has made an extremely important contribution to solving the problem of food and clothing and promoting the sustainable development of economy. Therefore, it is urgent for the researchers to study the adverse factors hindering the growth of wheat and improve the yield of wheat. Elumalai et al. introduced the HVA 1 gene of barley into wheat, followed by Patnaik et al. Guo Beihai et al. used the gene gun method to transfer the mountain spinach betaine aldehyde dehydrogenase gene into the wheat, and obtained the transgenic wheat with drought-resistant and salt resistance. Pellegrineschi et al. transformed Arabidopsis DREB1A into wheat, and the seedling drought treatment experiment found that the drought tolerance of transgenic wheat was significantly improved. Gao Shiqing et al. used the gene gun method to transform soybean Gm DREB transcription factors into wheat, and found that the drought tolerance of transgenic wheat was significantly improved under PEG simulated drought conditions. Wang Junwei used gene gun method to transform DREBIB and CBFI genes into wheat varieties. Drought resistance simulation test showed that 10 lines were significantly improved in three physiological indexes, chlorophyll content, proline content and photosynthetic rate, compared with the control, and the drought resistance of transgenic plants was improved. Du Lipu et al. introduced TPS of trehalose synthase gene into wheat, and PEG simulated drought treatment. The results showed that the drought resistance of TPS gene wheat plants was improved. Gao et al. introduced cotton Gh DREB gene into wheat by gene gun, and functional analysis showed that the chlorophyll and soluble sugar content of transgenic wheat leaves was improved under drought conditions compared with control plants, indicating that the drought resistance ability of Gh DREB transgenic wheat was improved. Saad et al. introduced rice SNAC 1 gene into wheat and conducted drought treatment. The test results showed that compared with wild type, the water and chlorophyll content of transgenic wheat leaves under drought stress increased, and the growth amount of wheat plants also increased, indicating that the drought resistance performance of transgenic wheat was improved **Помилка! Джерело посилання не знайдено.**.

All these research results show that the cultivation of drought-resistant wheat has reached a new height, which is conducive to the wheat drought resistance research to reach a higher level. At the same time, some agricultural scientific research institutions and universities in China are also actively carrying out relevant research, and strive to provide new solutions for wheat drought resistance improvement and wheat drought-resistant breeding. Make the very important contribution to the further development of agricultural economy.

1.5 Purpose and significance of the study

1.5.1 Effects of drought stress on plant growth

Drought refers to the climatic phenomenon in which precipitation is scarce or evaporation is greater than precipitation in an region for a long time. Wheat and other crops are extremely vulnerable to the interference of adverse conditions such as drought, and these adverse factors seriously restrict the growth and development of wheat, and have a huge impact on the yield and quality of the final wheat. Studies have shown that drought is crucial for the growth and development of wheat at various periods of its growth **Помилка! Джерело посилання не знайдено.**.

Drought, as an adverse environmental factor, has a huge effect on wheat growth. The impact of this adverse environment is not temporary, but long-term, and it has irreversible negative effects in all periods and growth stages of wheat. Drought reduces the germination rate of wheat seeds, which seriously affects the yield of wheat and has a negative impact on China's economy. Secondly, the wheat under long-term drought conditions will develop slower than the normal growth of wheat, and the wheat under drought conditions will also have short plants, leaf atrophy, plant wilting and other conditions, which will seriously affect the growth and development of small seedlings. Drought also has a huge impact on wheat during flowering and fruiting. Wheat under drought stress will have a lower flowering rate, thus reducing the wheat yield.

Drought stress with different duration and times also has serious effects on wheat yield formation. Most studies show that long drought stress can lead to crop stress damage, even if the late recharge is not conducive to its recovery. However, under the condition of short duration of drought stress, the compensation mechanism of crops is not induced, and the effect of water saving and increasing yield is not significant. The study on wheat shows that about 10 days is a good stress duration, but the difference between different varieties is significant **Помилка! Джерело посилання не знайдено.**.

In conclusion, drought will adversely affect the whole growth season of wheat. At the same time, the drought conditions of different degrees will also have different effects on wheat. Therefore, breeding with excellent drought resistance gene of wheat is an important task.

Drought will cause the water content in the soil to decline, leading to the severe water shortage of crops, so that the crops do not have sufficient water supply to complete various physiological activities. Under drought stress, plants close their stomata to reduce water evaporation, but this also causes less carbon dioxide absorption and affects photosynthesis in the plants. At the same time, under drought conditions, the concentration of sodium ions in the soil will increase accordingly with the decrease of water content, which is very easy to cause the accumulation of sodium ions in plants, thus inhibiting the growth and development of plants. Drought also leads to a large number of capillary heel and side root shedding, reducing the water absorption function of roots, forming a further vicious cycle, leading to a large number of plants wilting or even dehydration and death, seriously affecting the yield of crops, which is not conducive to the sustainable economic development.

If the soil is under drought conditions for a long time, the soil will cause serious barren transformation and salinization, which will not only affect the current growth of wheat, but also may affect the long-term growth of wheat and even lead to the abnormal growth of other crops. Drought stress also impacts on soil nitrate. Nitrogen in soil is not only the main nutrient element for the growth and development of crops, but also the mineral element that absorbs most from the soil by using the root system. Related studies showed that drought stress affects nitrate content in soil. Different degree of drought stress on different soil nitrate content is different, under the condition of severe stress, the soil surface content of nitrate nitrogen is significantly higher than the middle and lower, and late irrigation will lead to a lot of nitrate nitrogen washed into the deep soil, and the greater the degree of drought stress, shower the more serious pollution of groundwater. Schuuret al. and Pandey et al. found that drought stress decreased the nitrate content in farmland soil. Drought stress will not only change the growth environment of crops, but also affect the absorption and utilization of soil nitrate nitrogen**Помилка! Джерело посилання не знайдено.**, and also affect the nitration of soil.

The intensity, duration, and soil type of drought will have a significant impact on the soil organic carbon content. The decomposition of organic carbon depends on the activity of microorganisms, and under drought conditions, microorganisms do not have sufficient water supply to conduct metabolism and decompose organic matter, so the decomposition rate of organic carbon slows down. Moreover, under drought stress, soil erosion increases, which further leads to the loss of organic matter in the soil, especially during the drought before heavy rains. Compared with drought stress, the organic carbon content of soil under wet conditions increased significantly. Xiao **Помилка! Джерело посилання не знайдено.** et al showed that drought stress had little effect on soil organic carbon content. Sanaullah et al. showed that the organic carbon content in alfalfa soil decreased under drought stress. The results of **Помилка! Джерело посилання не знайдено.**also showed that dry stress leads to the decrease of soil organic carbon content in wheat farmland. The results show that drought stress on different and crop soil organic carbon content is not the same, this is mainly due to the soil organic carbon content by climate, vegetation, soil properties and management measures and other physical factors, biological factors and human factors, and there is the interaction between various factors.

1.5.2 Study expectation and experimental significance

As one of the most important food crops in the world, wheat has made an important contribution to solving the problem of food and clothing for people around the world. But due to global climate change, the frequency and intensity of drought have increased dramatically. Most of the main wheat producing areas in the world are distributed in arid and semi-arid areas, and the main wheat producing areas in the north of Qinling Mountains and Huaihe River in China, with different degrees of drought. Wheat is very sensitive to drought stress, and the yield loss is up to 58% -92% after experiencing drought stress during the key growth stages of its life cycle, such as the heading period and grain filling period. Therefore, it is of great significance to study the cloning of wheat genes. This paper aims to explore the cloning of drought-resistant genes in wheat. The mechanism of wheat drought resistance gene was revealed under drought stress. Through this study, new genetic resources and theoretical support for subsequent wheat drought breeding.

It is believed that the study of wheat drought resistance mechanism, the search for drought resistance genes and cloning will bring many aspects of convenience to the near future. It is believed that in the future, the drought resistance mechanism of wheat can be further explored, so as to find relevant genes, design drought resistance strategies more specifically, and cultivate wheat more suitable for growing in drought conditions**Помилка! Джерело посилання не знайдено.**, so as to better cope with the impact of drought and other adverse environment on wheat plants. More effectively deal with wheat adverse reactions in drought, such as growth retardation, wheat plant wilting, wheat production and other problems.

At the same time, the study of wheat drought resistance genes can also provide precondition for wheat other good genes, for us to further reveals the mechanism of wheat drought resistance, and for the future found wheat cold resistance, land salinization, insect resistance genes provide reference, further gene cloning and through transgenic technology to cultivate more excellent wheat varieties. In this study, we first analyzed the drought resistance of several different wheat varieties, treated them with water break, observed the phenotype characteristics of different wheat varieties and selected the two strains for PEG simulated drought treatment, then observed the phenotype and took the leaves and roots of wheat for RNA-seq analysis, from which genes related to drought resistance were obtained 16.

1.6 Future research outlook on wheat

Cloning of wheat drought genes is an important research field17, its future research direction may further analyze the function mechanism of drought genes, is conducive to the future further study of the mechanism of known drought genes in wheat, including its participation in the signal conduction pathway, regulation of gene network, etc., to reveal the deeper details of drought mechanism, provide a theoretical basis for subsequent genetic engineering improvement. At the same time, it is conducive to the discovery of new drought resistance related genes, through high-throughput technologies, excavate the undiscovered drought resistance related genes in wheat, as well as new regulators interacting with known genes, and enrich the drought resistance gene resources. Functional verification and genetic engineering improvement can be carried out. Gene editing technologies such as CRISPR / Cas 9 can be used to accurately verify the identified drought resistance genes, and multi-gene superposition or regulatory network can be constructed to improve the drought resistance of wheat. It is beneficial to realize the comprehensive study of salinity tolerance and drought tolerance. The growth environment of wheat is often accompanied by the double pressure of salinity and drought. In the future studies, we can jointly mine and utilize the genes of salinity resistance and drought resistance to realize the comprehensive adaptation of wheat to compound stress. At the same time, we can transfer the identified drought-resistant genes to different wheat varieties, investigate their performance in different genetic backgrounds, and select the drought-resistant wheat varieties suitable for different ecological environment and cultivation conditions 20.

The further development of these research directions is expected to provide more theoretical support and practical guidance for wheat drought-resistant breeding 20, and promote the improvement of wheat yield and quality to adapt to the increasingly severe climate change and production environmental pressure.

Conclusions to chapter 1

1. As one of the most important crops in the world, wheat plays an indispensable role in our lives. However, wheat is highly susceptible to adverse environments such as drought during its growth. Therefore, it has become an urgent problem to study the drought resistance mechanism of wheat.
2. Drought has a great impact on plants, and in order to reduce water loss, plants can improve their drought resistance by wilting leaves, reducing the number of leaves, and losing leaves. At the same time, membrane structure and drought resistance are also closely related. In addition, plants can also regulate hormone signaling pathways, such as abscisic acid (ABA) and ethylene, to regulate growth and development and stress response.
3. Research progress at home and abroad shows that significant progress has been made in the study of plant drought resistance.

CHAPTER 2
OBJECT, PURPOSE AND METHODS OF THE STUDY

2.1 Experimental materials

The main experimental materials used in this study included multiple wheat varieties, including wheat C6878, wheat T1305, wheat T103, and wheat T101. Based on wheat above, to verify whether the identified target genes were also significantly upregulated under drought stress conditions. All lines were cultured under standard growth conditions in the laboratory and ensured the consistency of the culture conditions to ensure the reliability of the experimental data.

In order to ensure the normal growth of wheat, ensure the healthy development of wheat, we also ensure sufficient sunshine, and at the same time, ensure the temperature suitable for the growth of wheat in the greenhouse, ensure the supply of fertilizer, and equipment nutrient soil to ensure the healthy growth of wheat.

Various physiological and biochemical reagents were also prepared in this study. These reagents mainly include kits provided by Beijing Polymer Biotechnology Co., Ltd., and kits provided by Nanjing Nuoweizan Biotechnology Co., Ltd., for reverse transcription, RT-PCR and other experimental processes. The equipment in the experiment includes greenhouse temperature control equipment, plant growth box, refrigerator, electronic balance, ultra-clean bench, vortex oscillometer, centrifuge, electrophoresis instrument, etc., to ensure the reliability of the results.

2.2 Experimental method

2.2.1 Planting method of plant conditions

Using this laboratory, we used a systematic wheat culture and treatment process. First of all, in order to ensure the healthy growth and development of wheat, we provide nutrient soil for the growth of wheat and ensure the fertilizer conditions. To meet the climatic conditions required for wheat growth, because wheat is very sensitive to climate changes, so the greenhouse temperature is controlled between 15℃ and 25℃, to ensure the growth conditions of wheat. At the same time, the soil is deeply ploughed and fertilized regularly to ensure fertile soil and good drainage. Soil PH values were controlled between 6.0 and 7.5. During the growth of wheat, ensure the supply of light and water, etc. Give adequate light conditions to promote photosynthesis and nutrient uptake, and give adequate water supply.

Because wheat drought resistance was studied in this experiment, the drought conditions in wheat cultivation were strictly controlled. For plants requiring drought stress, water was cut off and 18% PEG simulated drought conditions, and the treatment time of drought stress was determined.

Several different wheat plants were analyzed for drought resistance and water cut off. The water cut treatment of wheat is an experimental operation under drought conditions to explore the phenotypic changes of wheat under drought conditions and a series of internal physiological indicators, and to find more drought-resistant wheat varieties. In order to ensure the reliability of the experiment, the growth status of wheat seedlings and the culture environment should be consistent. The moisture content of the drought-treated wheat was strictly controlled to reduce the water content in the soil. The time of water cut-off treatment can usually last for several days to several weeks, which should be set according to the growth trend and apparent state of wheat. Attention should be paid to maintaining appropriate environmental conditions during water cut treatment to ensure the supply of suitable temperature and light conditions. At the same time, during the period of water cut off treatment, photos were recorded regularly to facilitate the real-time observation of wheat conditions.

2.2.2 Analysis and processing of RNA-seq data

RNA-seq is a technique used to determine the sequence and quantity of RNA in cells. Here are the general steps for RNA sequencing:

The mRNA was extracted from the selected wheat drought-resistant samples, and the quality and concentration of the selected RNA were further determined and evaluated.

The mRNA was fragmented and the cDNA library was synthesized and sequenced on a large scale.

The appropriate RNA sequencing platform is selected, and the constructed libraries will be sequenced to obtain the corresponding data.

The raw data obtained from sequencing was analyzed by the steps of quality control, sequence comparison, and expression level analysis to obtain the expression level and structural information of the RNA. Further analysis of the RNA data obtained from the analysis, including pathway enrichment analysis and differential gene expression analysis, with the aim to reveal its biological functions and regulatory mechanisms.

When performing the analysis, we can screen for the desired genes in many ways. For example, through the differential analysis of gene expression, the most obvious change of drought resistance genes can be selected for the next step.

2.2.3 Synthesis of cDNA

In the experiment, the reaction solution should be configured according to the proportion listed in the following table, and the configuration should be performed on ice.

1.Genomic DNA reactions were removed, as shown in Table 2.1

Table 2.1-Configuration of reaction fluid

reagent	application amount
5×gDNA Eraser Buffer	2.0 μ L
gDNA Eraser	1.0 μ L
Total RNA	1.0 μ L
RNase Free dH2O	Up to 10 μ L

2.The reverse transcription reaction

As follows, the whole reaction needs to be conducted on ice, first configured according to the number of reactions + 2, and then distributed to each reaction tube. See Table 2-2

Table 2.2-Configuration of reaction fluid

reagent	Application amount
Reaction solution for step 1	10.0μl
PrimeScript RT Enzyme Mix 1	1.0 μ l
RT Primer Mix＋4	1.0 μ l
5×PrimeScript Buffer 2（for Real Time)	4.0 μ l
RNase Free dH2O	4.0 μ l
Total	20.0 μ l

37℃ 15min

85℃ 5sec

4℃

2.2.4 Design of the primers for RT-PCR

RT-PCR primer design can be assisted by some online software or tools: some commonly used primer design software include Primer3, NCBI Primer-BLAST, OligoAnalyzer, etc. Before primer design, the target gene is determined to obtain the sequence of the gene. The target genes and gene sequences identified in this experiment were obtained from the gene database (such as NCBI).

In this experiment, the NCBI Primer-BLAST primer design software was used to design the primers. After the primer design, the designed primers can be evaluated using the tools provided by the primer design software or other online tools, such as OligoAnalyzer.

2.2.5 fluorescence quantitative PCR

Arrange the mixture according to Table 2-3:

Table 2.3-Configuration of reaction fluid

2 × ChamQ Universal SYBR qPCR Master Mix	10
Primer1（10 μ L)	0.4μL
Primer2(10 μ L)	0.4μL
Template DNA/cDNA	2 μ L
ddH2O	To 20.0 μ L

The Real Time PCR reaction was performed

The two-step PCR reaction procedure was performed as follows:

Stage 1: Predenaturation Stage2:PCR reaction

Reps:1 Reps:40

95℃ 30s 95℃ 5s

60℃ 30s

2.2.6 Gene cloning

Gene cloning generally includes the following steps:

1. Design of the primers

The primers applied in this experiment are shown in Table 2-4The primers used in this experiment are as follows:

Table 2.4-Primer sequences

Primer name	Primer sequences
CS6A02G350500-RF	GCCGCCCATCAATACTCTGT
CS6A02G350500-RR	GATGCCCTTCTTCCTCCTCC
CS5A02G369800-RF	ATGGAGTACCAGGGGCAGAC
CS5A02G369800-RR	CACCAGCAGACCACTGGAAT
CS7D02G549900-RF	CACCAGCAGACCACTGGAAT
CS7D02G549900-RR	GTCCAGGCAGCTTGTCCTT
CS7A02G422500-RF	CCCAGGGATTCCCCTCAGTA
CS7A02G422500-RR	ATGTCGAGATCGATGGGCAC
CS1B02G145800-RF	CCACAACTAGCACCGAGAGG
CS1B02G145800-RR	GCCGCCCATCAATACTCTGT

2. PCR amplification of the target gene

Configure the reaction solution according to the instructions of the PCR kit and mix the mixed reagents evenly. The correct primers, including the forward and backward primers, were selected and added to the reaction solution to ensure that the reagents in each reaction system were mixed evenly while avoiding contamination. According to the instructions of the selected PCR instrument and kit, the PCR procedure was designed, including the reaction temperature and time parameters. It was placed in a PCR apparatus for DNA amplification and successful amplification by analysis of the agarose gel electrophoresis.

3. Agarose gel electrophoresis

Take a clean conical flask, add agarose and TAE buffer, put in the microwave oven on high heat to no flocculent precipitate, add nucleic acid dye and pour into the mold. After the glue solidifies for 30 minutes, pull out the comb teeth and carefully put them into the electrophoresis tank. Carefully add the sample and DNA marker to the hole with a pipette. Do not contact the glue as far as possible. Close the electrophoresis slot cover and open the electrophoresis apparatus switch. Note that when using the pipette, even if the gun head is replaced, black is the cathode, red is the anode, and determine that the Yin and Yang pole wire is connected correctly.

2.3 Drought stress treatment

Drought stress is a state of plants under drought conditions, mainly manifested as a phenotypic feature of plant wilting due to insufficient water supply. Drought is a typical environmental pressure in the growth process of crops, which will adversely affect the growth and development of plants and seriously affect the yield of crops. In this experiment, drought stress treatment was required. Through drought stress treatment, it is better to observe the difference in drought resistance of different varieties of wheat, and more convenient to locate the genes related to drought resistance in wheat. The drought stress treatment in this experiment is to simulate the coping mechanism of plants under drought conditions and observe their phenotypic changes. Mainly through water cut treatment and PEG simulated drought treatment. Water cut treatment is the drying process of wheat by gradually reducing the moisture content after wheat irrigation; PEG simulated drought treatment by watering 18% PEG-6000 for one day to simulate the drought conditions. After completing drought stress, statistical analysis of the experimental results is needed to assess the ability of wheat to respond to drought. The treated leaves and roots were then RNA extracted and processed for RNA sequencing.

Through these steps, wheat drought stress can be simulated in the laboratory, investigating wheat growth, physiological and molecular response mechanisms, and potential drought-resistant adaptation strategies.

2.4 Shooting method

In the course of the experiment, in order to detect the changes of soil seedlings, the shooting of wheat needs to follow certain shooting methods. During the shooting process, a black cloth should be placed behind the wheat to arrange several different varieties of wheat, and take photos in the next few days to record the results of the experiment.

Conclusions to chapter 2

1. Firstly, the drought resistance of several different wheat varieties was analyzed, the water cut treatment was carried out, the phenotypic characteristics of different wheat varieties were observed, and the two plants with the greatest differences were selected for PEG simulated drought treatment, then the phenotype was observed and the leaves and roots of wheat were analyzed by RNA-seq, and the genes that were significantly differentially expressed in roots and leaves were selected for GO analysis and KEGG analysis, and finally the

genes related to drought stress that were significantly up-regulated in both leaves and roots were selected as candidate genes. and RT-PCR validation.

2. According to the results of real-time quantitative PCR (RT-PCR), the expression level of the target gene in wheat leaves increased significantly under drought stress, suggesting that this gene may play an important role in drought resistance of wheat. These genes were then amplified by PCR and gel electrophoresis, and the functional verification of these genes was continued at a later stage.

3. Finally, it was successfully cloned genes associated with drought resistance.

CHAPTER 3
EXPERIMENTAL PART

3.1 Results of phenotyping of drought resistance in wheat plants

As the world's most widely planted and cultivated crop in the highest total production, wheat is highly susceptible to drought and other adverse conditions during its growth and development process. Therefore, it is urgent to explore the physiological mechanism of wheat drought resistance. This experiment is to find the genes related to drought resistance in wheat and clone them. In the process of finding wheat drought resistance genes, we need to treat drought stress in different varieties of wheat.

Wheat C6878, wheat T1305, wheat T103 and wheat T101 were cut off, and the results are shown in Figure 3.1:

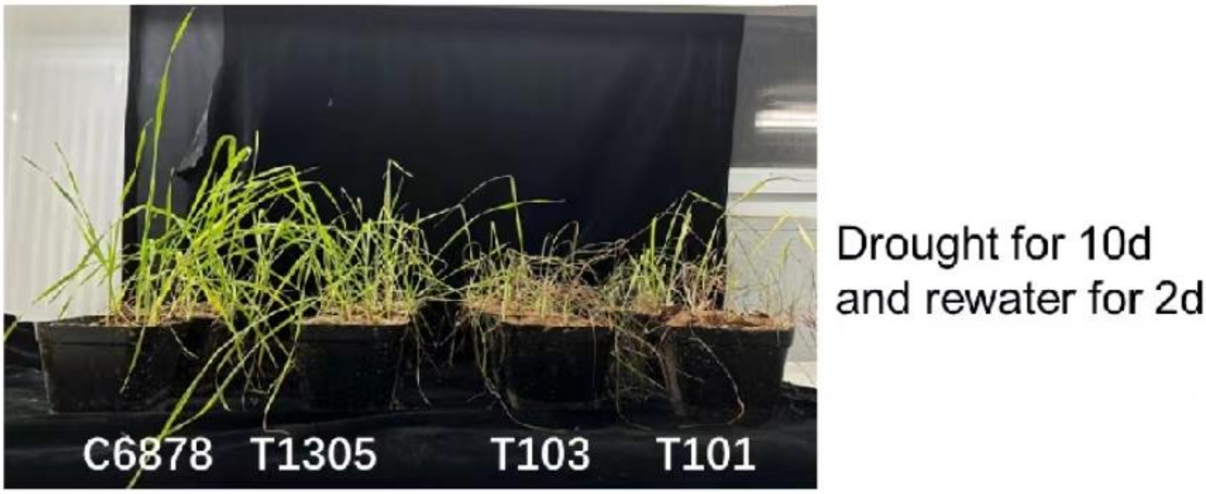

Figure 3.1-Wheat water cut treatment

According to Fig, wheat is greatly affected by drought stress, with the biggest difference between wheat C6878 and T103.

Drought conditions have a significant negative impact on plant growth, resulting in wheat growth inhibition, manifested by reduced plant height growth, reduced leaf area, and reduced biomass accumulation in above and below ground parts. Analytically available, drought stress causes stomatal closure closed in wheat leaves under drought stress to reduce water transpiration. This reduces the stomatal conductance of the plant and reduces water loss, but also limits CO2 entry, affecting photosynthesis and growth. Drought stress, which causes chlorophyll content to decline in wheat leaves, is probably due to accelerated leaf aging and blocked photosynthesis.

3.2 PEG simulated drought experiment

PEG (polyethylene glycol) is a commonly used laboratory reagent that can be used to mimic drought stress conditions. In this experiment, PEG was required to simulate drought treatment, and the treatment results are shown in Figure 3.2:

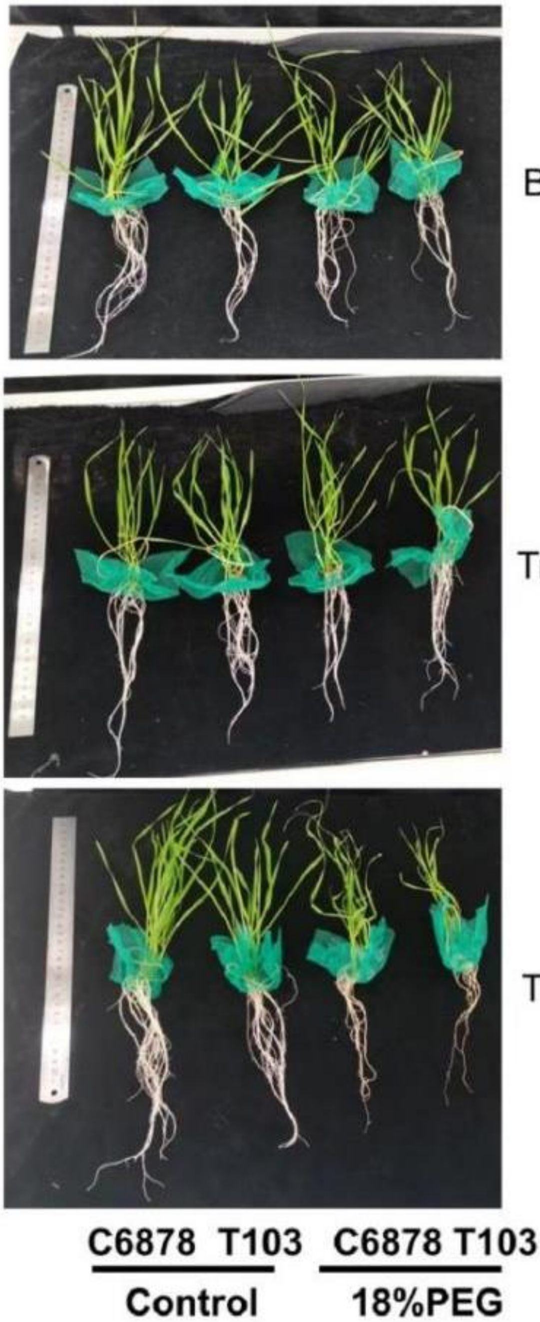

Figure 3.2-PEG simulated drought-treated wheat

3.3 Results of RNA-seq analysis of wheat leaves

RNA-seq analysis of leaves and roots after PEG simulated drought treatment, and the preliminary analysis results are shown in Figure 3.3:

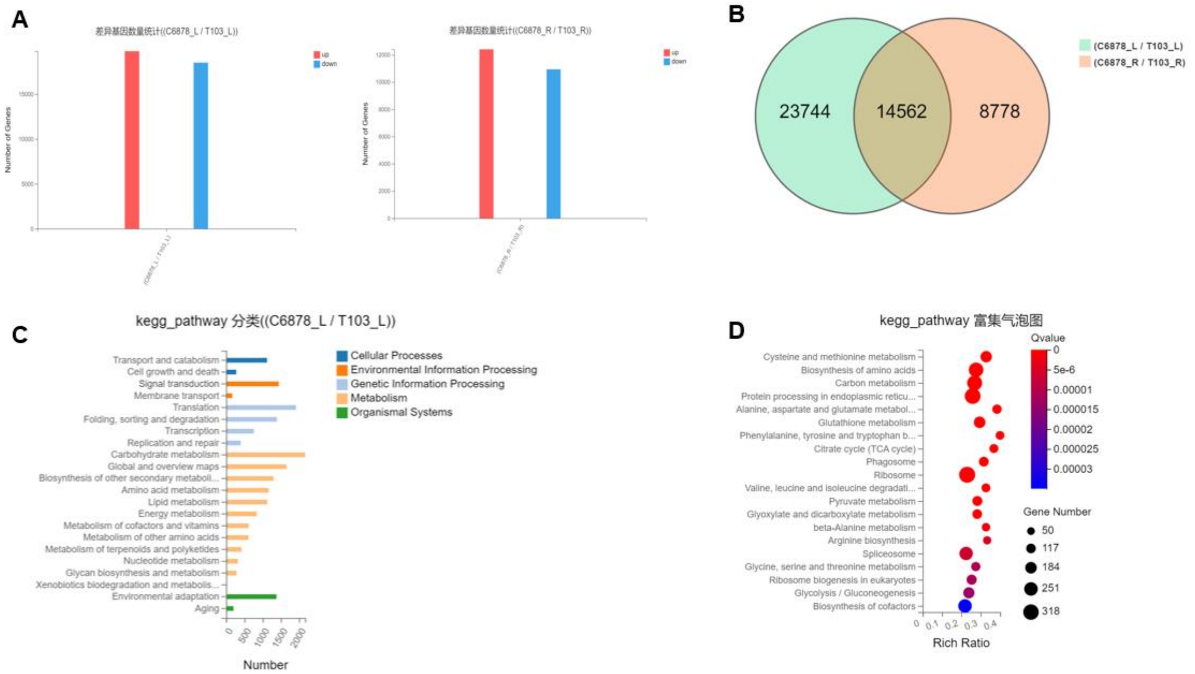

Figure 3.3 -RNA-seq analysis

Through analysis, the number of genes was 47084,38,306 in wheat T103 and C6878 and C6878, and 23,340 differential genes identified in wheat C 6878. KEGG analysis yielded the following genes related to drought stress, verified by RT-PCR, and some of them were selected for cloning.

Table 3-1 and Table 3-2 are the selected genes related to wheat drought resistance. According to these selected required genes for the next experimental analysis:

Table 3.4-Functional analysis of the genes involved in drought resistance

Gene ID	Type	log2 (C6878_L / T103_L)	log2 (C6878_R / T103_R)	Qvalue (C6878_L/T103_L)	Qvalue (C6878_R/ T103_R)	GO_P Term
TraesCS7D02G549900	mRNA	8.967061597	7.794978736	2.91E-50	2.88E-37	GO:0009415：response to water
TraesCS7A0	mRNA	2.20799093	5.250678724	5.88E-	1.05E-71	GO:0009415：response to

2G560000				165		water
TraesCS6A02G350500	mRNA	15.61473049	7.51688356	1.79E-51	1.00E-62	GO:0009414：response to water deprivation；GO:0009631：cold acclimation；GO:0009737：response to abscisic acid
TraesCS5A02G369800	mRNA	3.918191148	5.8187534	0	1.45E-222	GO:0009414：response to water deprivation GO:0009631：cold acclimation；GO:0009737：response to abscisic acid

Table 3.5-Functional analysis of the genes involved in drought resistance

Gene ID	Type	log2 (C6878_L / T103_L)	log2 (C6878_R / T103_R)	Qvalue (C6878_L/ T103_L)	Qvalue (C6878_R/ T103_R)	kegg_pathway_term
TraesCS7A02G422500	mRNA	5.521213579	8.485487044	1.09E-88	1.36E-22	MAPK signaling pathway - plant
TraesCS1B02G145800	mRNA	4.429524481	6.191881402	6.96E-175	1.70E-59	MAPK signaling pathway - plant+++04075：Plant hormone signal transduction

Drought resistance genes were selected and primed, as shown in Table 3.6:

Table 3.6-Primer sequence

Primer name	Primer sequence(5’→3’）
CS7A02G422500-RF	CCCAGGGATTCCCCTCAGTA
CS7A02G422500-RR	ATGTCGAGATCGATGGGCAC

CS1B02G145800-RF	CCACAACTAGCACCGAGAGG
CS1B02G145800-RR	GCCGCCCATCAATACTCTGT
CS6A02G350500-RF	GCCGCCCATCAATACTCTGT
CS6A02G350500-RR	GATGCCCTTCTTCCTCCTCC
CS5A02G369800-RF	ATGGAGTACCAGGGGCAGAC
CS5A02G369800-RR	TCCCTGGTCGGCTGGAG
CS7D02G549900-RF	CACCAGCAGACCACTGGAAT
CS7D02G549900-RR	GTCCAGGCAGCTTGTCCTT
CS7A02G560000-RF	CGTCATGGAAAGCATCACGG
CS7A02G560000-RR	TCATTCCAGTGGTGTGCTGG
B-ACTIN-F	GACCGTATGAGCAAGGAGAT
β-ACTIN-R	CAATCGCTGGACCTGACTC

3.4 Expression studies of genes related to drought resistance

To investigate the relative expression of wheat drought resistance genes in roots and leaves in different varieties of wheat, we used real-time PCR to determine the relative expression of drought resistance related genes in two wheat varieties — wheat C6878 and wheat T103.

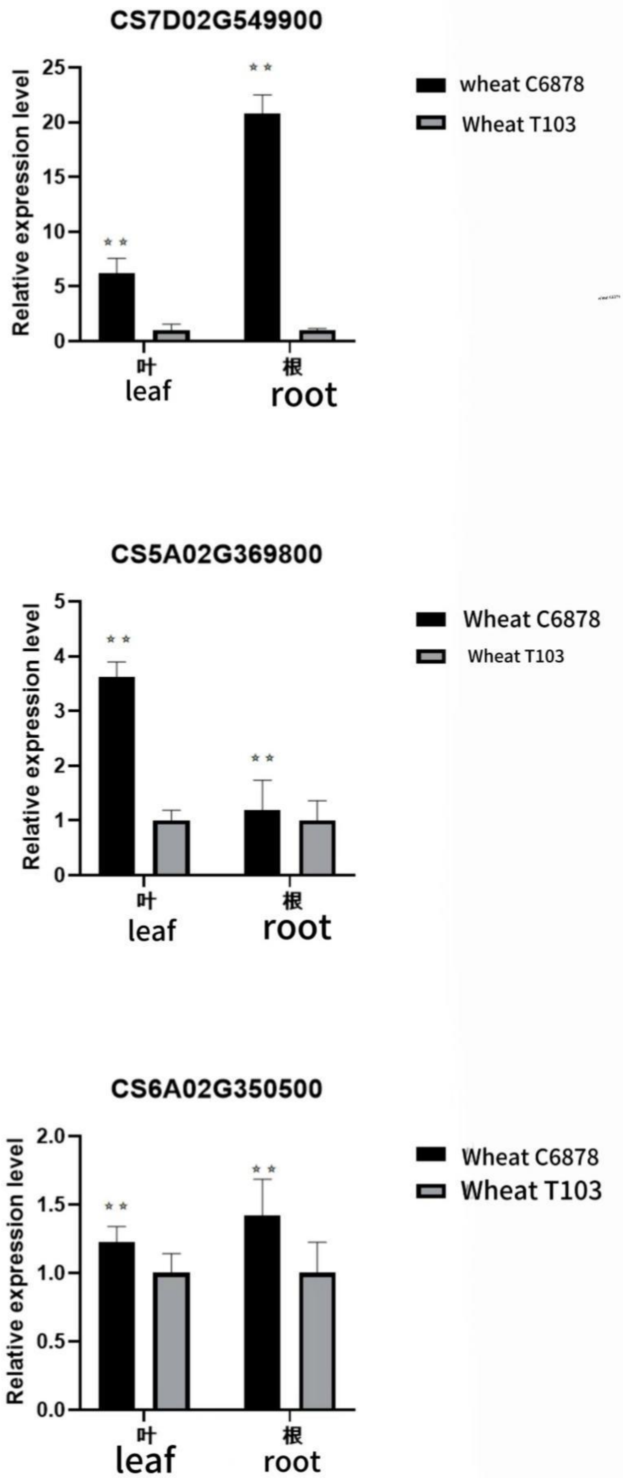

Figure 3.7-Relative expression of some genes involved in drought resistance in wheat

From the figure, the relative expression of the above drought resistance genes was significantly higher in the day of wheat 6878 than in the day of wheat 103. The trend of gene content in one day in wheat 6878 was overall higher than the trend of drought resistance in wheat 103. This verifies that the above genes do play a role in the drought resistance mechanism in wheat.

3.5 Gel electrophoresis results

The following results were obtained by analytical agarose gel electrophoresis imaging:

Figure 3.8-Results of the agarose gel electrophoresis

From left to right genes are CS5A02G369800, CS7D02G549900, CS6A02G350500 and CS7A02G422500. Gene lengths were 667bp, 709bp, 1209bp, and 1881bp. In line with the above results and analysis, the successful amplification of the target gene can be verified.

Conclusions to chapter 3

1. We successfully cloned the genes related to drought resistance by PCR DNA amplification technology and performed agarose gel electrophoresis detection.

2. We have successfully cloned the drought resistance gene, which provides reference significance for future wheat genetics and breeding, and is conducive to the cultivation of excellent wheat varieties.

CONCLUSIONS

As the most important crop in China, wheat is also of great significance in the world, and has always been highly valued by the world. Its cultivation has also become an important choice and inevitable path to solve many problems in the world. Wheat has different degrees of water demand in different growth stages, so drought will affect wheat in different stages of wheat growth. However, in recent years, with the influence of climate and other environmental changes, the drought phenomenon is becoming more and more common, and drought stress has become an important factor restricting the growth and yield of wheat.

Drought stress is a stress state in which wheat growth is obviously hindered due to water shortage in the environment. Drought stress is not only an important factor affecting the growth of crops in China, but also an important factor affecting the growth of global crops. Especially in arid areas, drought on crops becomes an important condition restricting plant growth, and is a major challenge and threat to the growth and development of global crops. Therefore, in order to promote the growth of wheat plants, improve wheat yield, improve the drought resistance of wheat is urgent.

In this experiment, through water cut treatment and PEG simulated drought treatment, we could find wheat growth wilting, which proved that the inverse environment such as drought did adversely affect wheat growth and development. In this experiment, the changes of wheat under drought conditions showed growth stagnation, wheat stopped or slowed growth, saving energy and water supply necessary physiological activities. At the same time, the leaf wilting phenomenon, the leaf becomes smaller and thinner, is also to save water and energy. The impact of drought on wheat is also reflected in the reduction of wheat production. To sum up, wheat will show a series of physiological and morphological changes in a series of adverse environments such as drought, threatening its survival and growth, and seriously affecting the wheat yield.

The GO term of differentially expressed genes was mainly water shortage and cold stress; KEGG was enriched in phytohormone signaling pathway, and CS5A02G369800, CS7D02G549900, CS6A02G350500, CS7A02G422500 provided gene resources for wheat drought breeding in the later stage.

Gene cloning provides a new path for us to study the progress of drought resistance in wheat. Gene cloning is an important technology applied in the field of biotechnology, which is the process of artificial copying of a specific gene sequence. The impact of drought on crops is great, which requires us to find the genes related to drought resistance in wheat, and wheat drought resistance gene cloning is the cloning technology to obtain the gene sequence related to wheat drought resistance. Through the cloning and research of these genes, a deep understanding of their regulatory mechanism, so as to enhance the survival ability of plants under drought conditions. If they are used in the cultivation process of wheat plants, the yield of wheat will be significantly increased and the further development of agricultural economy will be promoted.

REFERENCE DOCUMENTATION

1. Zhang Hongmei. (2014). PhD in proteomics on drought stress response and LEA protein in wheat (dissertation, Northwest A & F University). doctor https://kns.cnki.net/kcms2/article/abstract?v=LGlPPz-4LgG_BZyENDPPwXCsXc0FnuEvkLbc5y-vcLW-oPDFpAoqwSvU8x_3zJM-FBzppAnVm0fJM8unI9OoAa-OFF16W3bV7cOUi9Kqzh16XCyWPRWBP_5-oe5jaPKRm6-HqvFocEmzj5XwY01dbQ==uniplatform=NZKPTlanguage=CHS

2. Dong Li, Chen Jiaqi, Fan Yue & Wang Ni. (2019). Progress in genetic engineering for wheat drought resistance. Jiangxi agriculture (10),102.doi:10.19394/j.cnki.issn1674-4179.2019.10.087.

3. Qin Na. (2015). PhD Research on maize C_4 PEPC gene wheat response to drought stress (dissertation, Nanjing Agricultural University). doctor https://kns.cnki.net/kcms2/article/abstract?v=LGlPPz-4LgEnDXJaX_OcVxanu9lKcDqLdFR3up2zI7RI0eQJs_r0NkSK-E9RYz2TH_UZDKb2QI3zXLV1myUU9AI-goaP0-fzS-11UPA3UutDK1xJeIhb19hkRLrchUrcHmIRdk5AlBvbAmL2S3T0Hw==uniplatform=NZKPTlanguage=CHS

4. Aotegen Baiyin, Yang Xueju, Zhang Shuhua, Zhao Yong, Ju Xiaoying & Lang Minglin. (2017). Progress in genetic engineering for wheat drought resistance. Genomics and Applied Biology (02), 826-833.doi:10.13417/j.gab.036.000826.

5. Chen Xinyu. (2023). Growth and development of wheat roots and their response mechanism to nitrogen and drought (dissertation, Yangzhou University). doctor https://link.cnki.net/doi/10.27441/d.cnki.gyzdu.2023.000054doi:10.27441/d.cnki.gyzdu.2023.000054.

6. Wang Jun. (2022). Ph. D., simulation study on wheat yield and carbon and ni trogen evolution in soil water under drought stress (dissertation, Gansu Agric ultural University). doctor https://link.cnki.net/doi/10.27025/d.cnki.ggsnu.2022.000070doi:10.27025/d.cnki.ggsnu.2022.000070.

7. Yang Qiliang, Zhou Bing, Liu Xiaogang, Qi Yafeng, Jia Weibing & Yang Jurui. (2013). Effect of deficient irrigation and nitrogen application on nitronitrogen distribution and water utilization in the root area. Journal of Agricultural Engineering (04), 142-150.

8. Yin Yanzhe. (2013). The influence of rehydration on the transport of water ni trogen under the condition of multi-pit irrigation (thesis, Taiyuan University o f Technology). Master https://kns.cnki.net/kcms2/article/abstract?v=LGlPPz-4LgGfsMQcg2QRvbhcrmA_wBKmTflsU0kn7pgDO2avA-_jWLOPGlKJq1BvjSg7BWeDG0RfV-EHJkf1-x7yo5ki4SW6HFOlH1PupYznOJHALYn2_x9Fea0VNZgUdvKxlWtsitJBXc1wvVLfUQ==uniplatform=NZKPTlanguage=CHS

9. Gao Lina, Chen Suying, Zhang Xiying, Sun Hongyong, Wang Yanmei & Sha o Liwei. (2009). Effect of winter wheat field coverage on soil temperature an d fertility process in the North China Plain. Agricultural research in arid area s (01), 107-113.

10. Liu Hongbin, Zhang Yungui, Li Zhihong, Zhang Weili, Lin Bao. (2005). Stud y on nitronitrogen pollution in deep groundwater in plain agricultural area of Beijing. Soil Journal (03), 411-418.

11. Xiao Lie, Liu Guobin, Li Peng & Xue. (2017). Effects of short-term CO 2 c oncentration and drought stress on carbon and nitrogen and microbial rhizosp here effects in L. chinensis soil. Journal of Applied Ecology (10), 3251-3259. .doi:10.13287/j.1001-9332.201710.009.

12. Xue Ran. (2017). Research on soil nutrient and microbial responses to differe nt water supply patterns in the rhizosphere of early growth wheat (dissertation

, Lanzhou University). doctor https://kns.cnki.net/kcms2/article/abstract?v=LGlPPz-4LgGms0ENWPqi3OJZ-7o3Ge2ZBybGAW8hR2Z9z9HmHtqNwPF78TasAPCN_QJ3vvxAeOFf0OZfdMq7e7wdO2zBlpXzID8G9vzHm7s8eGXrCobgTHvMwW49w1AWZOxWbdSIMVZzc_NHwbBKAQ==uniplatform=NZKPTlanguage=CHS

13. Zhang Hao, Zhang Rongtao, Wu Wei Wei, Sun Xuming & Yue Hong. (2024). Community structure and function of endophytic bacterial communities in different drought-resistant wheat varieties. Journal of Wheat Crop (03), 399-406

14. Zhang Yuedong. (2023). Screening and analysis of wild European plum drought resistance genes based on adversity group study (dissertation, Inner Mongolia Agricultural University). Master https://link.cnki.net/doi/10.27229/d.cnki.gnmnu.2023.001335

15. Zhou Quan. (2023). 244 masters of drought resistance evaluation and identification of spring wheat germplasm resources (dissertation, Northwest A & F University). Master https://link.cnki.net/doi/10.27409/d.cnki.gxbnu.2023.002439

16. Li Shan. (2022). Identification of key genes for drought resistance in foxtail millet based on transcriptome and co-expression network analysis (master's dissertation, Shanxi Agricultural University).https://kns.cnki.net/kcms2/article/abstract?v=8dkf_uZKVx238q3eXeHqHJF0L_XLc0UdMO2muXpPEO-Te6GBCNpQSuQbcPWyJUZDE5PF7L_3LptvwhZGxp7imyncTSrmt26dQmBvIqz4AB_dfI3beZsvbPmLOy7B-LpA&uniplatform=NZKPT&language=CHS

17. Wang Ke. (2023). Vin-1C6 gene transformation and AATI gene cloning (dissertation, Inner Mongolia Agricultural University). Master https://link.cnki.net/doi/10.27229/d.cnki.gnmnu.2023.001550doi:10.27229/d.cnki.gnmnu.2023.001550.

18. Wu Jiayi. (2023). Primary Study on Cloning and function of neuropeptide F receptor gene of P. xylostella (dissertation, Jiangsu University). Master https://link.cnki.net/doi/10.27170/d.cnki.gjsuu.2023.001849doi:10.27170/d.cnki.gjsuu.2023.001849.

19. Wu Yanji Ji. (2023). Cloning and identification of wrinkle leaf mutation gene in Chinese cabbage (dissertation, Shenyang Agricultural University). docto rhttps://link.cnki.net/doi/10.27327/d.cnki.gshnu.2023.000009doi:10.27327/d.cnki.gshnu.2023.000009.

20. Xie Armon. (2023). The NF-Y family gene of wheat TaNF-YA 1 mediated the function of plants against osmotic stress (dissertation, Agricultural University, Hebei). Master https://link.cnki.net/doi/10.27109/d.cnki.ghbnu.2023.000208doi:10.27109/d.cnki.ghbnu.2023.000208.