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PLANT-BREEDING VALUE OF WHEAT IS DEPENDING ON THE FUNCTIONAL FEATURES OF POWDERY MILDEW PHYTOPATHOGENS

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ANNOTATION

Tao Ye. Plant-breeding value of wheat is depending on the functional features of powdery mildew phytopathogens. – The qualifying scientific work based on the manuscript.

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Wheat is an archaeophyte crop with a cultivated area of more than 200 million hectares and a total harvest of almost 800 million tons. The bulk of the people on the globe practice wheat farming and eating. In the absence of reserves to increase planted areas, the primary direction of constant wheat consumption is to enhance yields and increase the percentage of crops suited for the production of ecologically friendly meals. *Blumeria graminis* f. sp. *tritici* (Bgt), which infects wheat worldwide, is the source of the deadly wheat powdery mildew. Creating highly resistant cultivars is one of the most crucial and cost-effective ways to prevent the loss of wheat due to Bgt. Realization of this direction is possible due to the development and introduction of varieties and the discovery of powdery mildew resistance genes and resistance regulation genes, which are essential for the breeding of new varieties with disease resistance. It needs the formation of a theoretical basis for inheritance features and genetic control of qualitative selection traits.

The dissertation work provides a theoretical foundation and practical solution to the issues of creating source material of winter wheat with resistance to powdery mildew for breeding purposes and establishing the molecular and genetic basis of plant resistance to powdery mildew. The urgent task of modern science is to expand theoretical research and solve practical problems in order to create varieties with a controlled level of powdery mildew.

Therefore, the authors identified the adult disease resistance of powdery mildew in 86 new wheat lines. The experiment was conducted at the Wheat Test Base in Henan Province in 2020–2022. The test materials were from 45 relevant breeding units in China. There are 86 new wheat lines. The test carried out powdery mildew resistance identification. And analyzing the source of disease resistance genes. In the 2020

resistance test for powdery mildew, only 11.7% of the 86 new wheat lines identified performed well. There is no line of disease resistance in the adult stage that is immune or near-immunized. There are four lines that are highly resistant to disease during the adult stage. There are two lines with moderate resistance to disease in the adult stage. There are four lines that are moderately susceptible. They accounted for 4.7%, 2.3%, and 4.7% of the total identified materials, respectively, and the overall resistance was poor.

We use pedigree analysis and parental resistance to disease to derive disease resistance genes for varieties that are resistant to disease. It is speculated that the genetic sources of some powdery mildew-resistant varieties may be Aikang 58, Jimai 22, Yumai 34, Zhengmai 366, Liangxing 66, Zhoumai 16, Zhoumai 18, Yumai 47 and Xiaoyan 926A.

In the early stages of this research, the yeast two hybrid (Y2H) cDNA library was constructed using the excellent common wheat variety Bainong 64, which has been widely promoted in production and has completed genome sequencing. Based on Y2H, *Pm46* was used as a bait protein to screen the Y2H system cDNA library, and the function of the obtained interacting protein genes, which may be related to the resistance of disease, was preliminary proved. As a result, *TaGDSL*, a gene that plays a positive regulatory role in wheat powdery mildew resistance, was cloned and characterized.

Cloning of the *Pm46* gene: the *Pm46* gene was cloned from the cDNA of Bainong 64, with a total length of 1545 bp and encoding 514 amino acids.

Screening the interaction protein of *Pm46* by Y2H: In-Fusion technology was used to introduce the CDS of *Pm46* into the yeast double hybrid bait vector pGBKT7 to construct the pGBKT7-Pm46 bait vector. The growth of yeast has no toxic effect or self-activation effect, indicating that the full length of *Pm46* can be used as a bait to screen the Y2H system libraries; in this study, 5 proteins interacting with *Pm46* that may be related to disease regulation were screened by Y2H.

Function verification of the interacted protein genes: the gene silencing system induced by barley stripe mosaic virus (BSMV-VIGS) was used to silence 5 of the screened interacted protein genes, which may be related to the resistance to disease in

the common wheat of Bainong 207, respectively. The results show that *TaGDSL* plays an active regulatory role in the powdery mildew resistance pathway.

The over-expression vector of the *TaGDSL* gene and the RNAi interference vector were constructed, and the vectors were transferred into the common wheat Bainong 207 by the Agrobacterium infection method. The stable knockdown expression of *TaGDSL* plants was built by *Agrobacterium tumefacien*s transformation. The results also showed that wheat was more susceptible to disease when the *TaGDSL* gene was silenced, and the result is consistent with the VIGS assays. The germination and mycelia growth rates of powdery mildew on the leaves of positive transgenic plants were much faster than those of wild-type wheat leaves.

The silencing (RNAi) *TaGDSL* plants had enhanced powdery mildew resistance. Functional identification of the *TaGDSL* gene from wheat by the long-growth period assay proved that silencing of the *TaGDSL* gene could resist powdery mildew in wheat plants. After inoculation with powdery mildew, the yield of wheat crops – the straw biomass, grain, number of spikelets, and 1000-grain weight of the RNAi lines were significantly higher than those of the WT lines at maturation. These results support the idea that *TaGDSL* is an active regulator of powdery mildew resistance. Our findings demonstrate that *TaGDSL* silencing is potentially useful since it can help generate genetically modified genotype materials with powdery mildew resistance in wheat.

Key words: wheat, powdery mildew, varieties, lesion plants, genotype, genes, resistance, agronomic valuable traits, yield, breeding value.

АНОТАЦІЯ

Тао Ye. Селекційна цінність пшениці залежно від функціональних особливостей фітопатогенів борошнистої роси. – Кваліфікаційна наукова праця на правах рукопису.

Дисертаційна робота на здобуття наукового ступеня доктора філософії за спеціальністю 201 «Агрономія». – Сумський національний аграрний університет Міністерства освіти і науки України, м. Суми, 2023 р.

Пшениця – це культура-археофіт, посівна площа якої сягає понад 200 мільйонів гектарів, а валовий збір складає близько 800 мільйонів тонн. Вирощування і споживання пшениці поширені у більшості народів світу. При відсутності резервів для розширення посівних площ основним напрямом для стабільного споживання пшениці є підвищення врожайності, а також збільшення частки культури щодо придатності для виробництва екологічно чистих продуктів харчування. Борошниста роса пшениці, спричинена *Blumeria graminis* F. sp. tritici (Bgt), є руйнівним захворюванням в усьому світі. Одним з найважливіших екологічно чистих та економічних методів зменшення втрат пшениці, спричинених Bgt, є виведення високостійких сортів. Реалізація цього напряму можлива завдяки розробці та упровадженню у виробництво сортів з відомими генами стійкості до борошнистої роси. Необхідні також гени регуляції стійкості для виведення нових сортів з резистентністю до хвороби. Це вимагає теоретичного дослідження успадкування і генетичного контролю важливих селекційних ознак.

Дисертаційна робота спрямована на теоретичне обґрунтування та практичне вирішення питань створення вихідного матеріалу пшениці озимої зі стійкістю до борошнистої роси для селекційних цілей та вивчення молекулярно-генетичних основ резистентності рослин до борошнистої роси. Поглиблення теоретичних досліджень і вирішення практичних завдань з метою створення сортів з контрольованим рівнем борошнистої роси є актуальним завданням сучасної науки.

Таким чином, автори виявили стійкість дорослих рослин до борошнистої роси у 86 нових ліній пшениці. Експеримент проводився на випробувальній базі з вирощування пшениці в провінції Хенань в 2020-2022 роках. Тестовані селекційні матеріали були отримані з 45 відповідних селекційних підрозділів Китаю, де були створені 86 нових ліній пшениці. В ході тесту була проведена оцінка стійкості до борошнистої роси, а також аналіз джерел генів резистентності до хвороби. При тестуванні резистентності до борошнистої роси в 2020 році з 86 нових сортів пшениці тільки 11,7 % показали хороші результати. На дорослій стадії розвитку рослин не виявлені лінії зі стійкістю до хвороб, яка була б на рівні імунної або

близької до імунізації. Високу стійкість до хвороби на стадії дорослих рослин мали 4 лінії. Ще 2 лінії на цій стадії характеризувалися помірною стійкістю до хвороби. Помірну сприйнятливість мали 4 лінії. Вони становили 4,7 %, 2,3 % та 4,7 % від загальної кількості оцінених зразків відповідно, а вцілому стійкість була низькою.

Нами використаний аналіз родоводу та дані резистентності батьківських форм до хвороб, щоб виявити гени стійкості у досліджуваних сортів. Вірогідно, що генетичними джерелами деяких стійких до борошнистої роси сортів можуть бути Aikang 58, Jimai 22, Yumai 34, Zhengmai 366, Liangxing 66, Zhoumai 16, Zhoumai 18, Yumai 47 та Xiaoyan 926A.

На ранній стадії цього дослідження була сконструйовано колекцію клонів ДНК (кДНК) двогібридних дріжджів (yeast two hybrid - Y2H) з використанням чудового сорту звичайної пшениці Bainong 64, який широко застосовувався у виробництві, а також завершено секвенування геному. Для скринінгу бібліотеки кДНК системи Y2H був використаний як білок-приманка ген Pm46, створений на двохгібридній заквасці. Попередньо було доведено, що стійкість до хвороб може бути пов'язана з функцією отриманих взаємодіючих білкових генів. В результаті цього було клоновано та охарактеризовано TaGDSL, ген, який відіграє позитивну регуляторну роль у стійкості пшениці до борошнистої роси.

Клонування гена Pm46 виконано за участі кДНК сорту Bainong 64 загальною довжиною 1545 пар нуклеотидів, які кодували 514 амінокислот.

За допомогою Y2H виконано скринінг взаємодії білка Pm46. Для конструювання вектора-приманки pGBKT7-Pm46 була використана технологія In-Fusion для введення кодуючої послідовності (CDS) Pm46 в дріжджовий подвійний гібридний вектор-приманку pGBKT7. Зростання дріжджів не мало токсичної дії і не призводило до самоактивації. Це вказує на те, що повна довжина нуклеотидів Pm46 може бути використана як приманка для скринінгу системних бібліотек Y2H. У цьому дослідженні з допомогою Y2H були обстежені 5 білків, що взаємодіють з Pm46 і, можливо, пов'язані з регуляцією захворювань рослин.

Здійснено перевірку функціонування взаємодіючих білкових генів. Для пригнічення п’яти з відібраних взаємодіючих білкових генів, які можуть бути

пов'язані зі стійкістю до хвороб у звичайної пшениці сорту Bainong 207, відповідно була використана система інгібування генів, індукована вірусом смугастої мозаїки ячменю (BSMV-VIGS). Результати показують, що TaGDSL відіграє активну регуляторну роль у механізмі розвитку борошнистої роси.

Були сконструйовані вектор надмірної експресії гена TaGDSL та вектор РНК-інтерференції (RNAi), які методом зараження Agrobacterium перенесено в звичайну пшеницю Bainong 207. Шляхом трансформації Agrobacterium tumefaciens була створена стабільна нокдаун-експресія мічених рослин. Отримані результати підтвердили, що пшениця була більш сприйнятливою до хвороб, коли ген TaGDSL був “вимкнений". Цей результат узгоджується також з аналізами VIGS. Швидкість проростання та росту міцелію борошнистої роси на листках позитивних трансгенних рослин була набагато вищою, ніж у листя пшениці дикого типу (WT).

Підвищену стійкість до борошнистої роси мали рослини з вектором надмірної експресії селективного “мовчання” (RNAi) гена TaGDSL. В результаті аналізу тривалого періоду росту в рослин пшениці показана функціональна ідентифікація гена TaGDSL, в результаті чого “вимкнений" ген TaGDSL може протистояти борошнистій росі. Після інокуляції борошнистою росою урожайність посіву пшениці – біомаса соломи, зерна, кількість колосків і маса 1000 насінин у ліній RNAi були значно вищими, ніж у ліній WT при дозріванні. Цими результатами підтверджуються, що TaGDSL є активним регулятором стійкості до борошнистої роси. Наші висновки демонструють, що “вимкнений" TaGDSL є потенційно корисним, оскільки може допомагати у створенні генетично модифікованих генотипічних матеріалів з резистентністю до борошнистої роси пшениці.

Ключові слова: пшениця, борошниста роса, сорти, ураження рослин, генотип, гени, резистентність, агрономічні цінні ознаки, урожайність, селекційна цінність.

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ABBREVIATIONS

Pm46 – Powdery mildew 46;

GDSL – Gdsl-type (GDSL) lipase;

Bgt – *Blumeria graminis* f. sp. *Tritici;*

RNAi – RNA interference;

VIGS – Virus-induced gene silencing;

qRT-PCR – Real-time quantitative Polymerase Chain Reaction;

cDNA – complementary DNA;

bp – Base pair;

Gus – β-glucuronidase;

Kan^+ – Kanamycin Solution;

Amp^+ – Ampicillin;

Rif^+ – Rifampin;

LB – Luria-Bertani;

Escherichia coli – *E.coli;*

CDS – Coding sequence;

hpi – Hours post-inoculation;

BSMV – Barley stripe mosaic virus;

PDS – Phytoene desaturase;

H_2O_2 – Hydrogen peroxide;

OE – Overexpression.

INTRODUCTION

Relevance of the topic. Wheat belongs to Triticum of the gramineae family. According to the number of chromosomes, the triticum can be divided into diploid, tetraploid, hexaploid and octaploid triticum. The common wheat is hexaploid wheat. Common wheat (*Triticum aestivum* L) is one of the most important food crops in the world. Wheat provides about a fifth of our food calories and protein. According to FAO data, from 2014 to 2016, the global average wheat planting area was 220 million hm^2, the yield per unit area was 3,323 kg/hm^2, and the total output was 738 million tons. China's annual wheat output accounts for about 17 percent of the global total, making it the world's largest wheat producer. China's wheat planting area is widely distributed throughout the country. In recent years, the planting area has stabilized at about 24 million hm^2, ranking first in the world. Wheat powdery mildew caused by *Blumeria graminis* F. sp. *tritici* is one of the increasingly serious fungal diseases in wheat production in China and other countries. Powdery mildew is a kind of living parasitic fungi, which has the characteristics of short life cycle, easy long-distance transmission of spores and strong sexual recombination ability. After powdery mildew infection, the wheat plant is easy to lay down, the leaf is dry, the death rate is fast, seriously affects the normal growth and development of wheat.

Actuality of theme. When the yield of powdery mildew resistant wheat reached a certain height, the quality of wheat became an increasingly concerned problem. In the actual production process, wheat quality is easily affected by many factors, such as disease, insect pest, drought and so on. Powdery mildew of wheat, as one of the serious fungal diseases, has been increasingly rampant in recent years. Therefore, how to control powdery mildew of wheat economically, safely and effectively has become an urgent problem to be solved in wheat production.

Connection of work with scientific programs, plans, themes. The research was carried out in accordance with the thematic plans of research works of the National Natural Science Foundation of China in the framework of the topic

«Function of Tamlo-B1 interacting protein TaANKs of powdery mildew gene in wheat» (Nos. 31901538, 202001-202212).

The purpose and objectives of the study. The aim of the research was to increase the efficiency of winter wheat by creating a source material with a controlled level of resistance to powdery mildew in the crop. The goal was to solve the following tasks:

1. To study the gene pool of domestic varieties of winter wheat on the basis of resistance to resistance to powdery mildew.

2. To estimate the relationship of the sign of resistance to powdery mildew with a set of economically valuable traits.

3. To determine the role of individual genes in the control of processes and mechanisms of resistance to powdery mildew in winter wheat plants.

Object of study. Regularities of manifestation of economic, morphological and genetic traits in winter wheat varieties and establishment of breeding value on their basis.

Subject of study. Breeding value and biological features of the source material of winter wheat by the main characteristics and ability to powdery mildew resistance.

Research methods. The common Chinese wheat variety, Bainong 64, carrying the *Pm46* gene, has been shown to play an important role in powdery mildew resistance and was collected and preserved by Henan Institute of Science and Technology (HIST) in Xinxiang, China. Bainong 207 was developed and maintained by HIST. Penong 64 and Bainong 207 were used for gene expression analysis, respectively. Leaves of three different individuals from the same treatment were collected and mixed at each time point. Bainong 207 is also used for BSMV-VIGS, RNAi, and OE testing. The tissue expression of *TaGDSL* in roots, stems and leaves of Bainong 207 was detected at the adult stage. Leaf samples were collected after 1h, 12h, 24h, 48h and 36h of treatment. Bainong 207 was used for expression analysis and was grown in a climate chamber of 23°C/18°C with a 14-h light / 10-h dark cycle

and 70% relative humidity.

The scientific novelty lies in solving an important scientific problem of creating and evaluating the source material of winter wheat with a resistance to powdery mildew.

For the first time. Discovering new resistance genes and analyzing their molecular mechanism is the key to control powdery mildew. The purpose of this study was to clone the *TaGDSL* gene from wheat, explore its role in powdery mildew resistance by using modern molecular biological methods, and analyze its function, so as to provide theoretical basis for the creation of wheat powdery mildew resistance varieties.

It was improved. the scheme of evaluation of selection material of winter wheat on the indicator of resistance to powdery mildew.

The issue of improving the quality of the crop by controlling certain breeding traits has been further developed.

The practical significance of the results. Based on the results of the research, a working collection of winter wheat samples with resisting to powdery mildew was transferred to the laboratory of selection and seed production of Henan Grain Crop Genome Editing Engineering Technology Research Center of China; Materials of study are included in educational programs on disciplines of educational level of Bachelor of Agronomy at Henan Institute of Science and Technology. We identified a gene, *TaGDSL*, that plays an important role in the resistance to powdery mildew. This gene is induced by powdery mildew and plays an important role in wheat resistance to powdery mildew. The gene has been patented in China.

The personal contribution of the applicant is to plan and conduct research, summarize scientific data of references (literature) on the topic of the dissertation, analysis of experimental data, formation of conclusions and recommendations for plant breeding, preparation and writing of scientific papers. Scientific articles have been published both independently and in co-authorship.

Approbation of dissertation results. The results of the research were published and discussed at "The 6th International Symposium on Genomics and Crop Genetic Improvement - Molecular Breeding" conference, in particular at "Proceedings of the International Scientific and Practical Conference «Honcharivski Chytannya»" international ones. The main items, research results and conclusions of the work during 2019-2022 were presented and discussed at the meetings of the Department of Agrotechnology and Soil Science of Sumy National Agrarian University.

Publications. The main items of the dissertation are covered in Вісник Сумського національного аграрного університету Серія «Агрономія і біологія» publications, including "Research status and prospect of genes related with resistance to powdery mildew of wheat", "Cloning and Bioinformatics Analysis of Wheat Powdery Mildew Resistance Related Gene *TaGDSL*" and "accessions from the 4th WWSRRN for resistance to powdery mildew in the north-eastern forest steppe of Ukraine" articles in Plant Genetic Resources Scientific Journal. "Research progress on identification of wheat main diseases and insect pests, powdery mildew and pathogen", "Mechanisms of detoxification tolerance to heavy metals in wheat" article is published in Sciences of Europe. The articles included in the database Scopus (Web of Science): "Genome-wide identification and characterization of the Lateral Organ Boundaries Domain (LBD) gene family in polyploid wheat and related species"; "Genome-Wide Analysis of Serine Hydroxymethyltransferase Genes in Triticeae Species Reveals That TaSHMT3A-1 Regulates Fusarium Head Blight Resistance in Wheat".

The structure and scope of the dissertation. The dissertation structure contains an annotation, a list of symbols, introduction, five chapters, conclusions, proposals for breeding practice, a list of references, appendixes.

CHAPTER 1

MECHANISMS OF PLANT RESISTANCE TO POWDERY MILDEW (LITERATURE REVIEW)

Wheat (*Triticum aestivum* L), 2n=6x=42, AABBDD is Gramineae, Triticeae, Triticum, and is one of the earliest cultivated plants the world. It originated from the Middle East near the Mediterranean Sea and was introduced into China later. It is reported that wheat cultivation in China has a history of at least four or five thousand years [1]. At present, wheat is also the second largest crop after rice, which is cultivated all over the world. Wheat production and output rank the first in the world, with 43 countries and more than one third of the population taking it as the staple food [2]. Wheat has high nutritional value and can provide about one-fifth of the calories and protein of human needs [3]. As the world population continues to increase, wheat will become more and more critical. In addition, wheat is the most important food for trade and international aid. According to UN COMTRADE, the world's wheat export in 2016 amounted to 148 million tons. China is the world's largest wheat production and consumption of the country, annual production accounts for about one-fifth of the global total, the world's largest output [4]. China's agricultural production level is constantly improving, but pests and diseases are still an essential factor to limit agricultural production. Powdery mildew is one of the wheat diseases with the most extensive range and a great influence on yield. Wheat powdery mildew is by living nutrition obligate parasitic fungi of wheat powdery mildew caused a worldwide disease, can often result in 13%-34% of yield loss, on the pathogenesis of heading and filling stage, a severe loss will generate 50% of output, in extreme infected cases can lead to dry leaves, and even plant death [5]. In the past 40 years, wheat powdery mildew has spread rapidly from local areas in the southwest and southeast coastal regions to almost all wheat areas in China due to the improvement of wheat production conditions and the variation of pathogen virulence structure, causing considerable losses to China's grain production [6]. The most economical and effective method to control wheat powdery mildew is to

cultivate resistant varieties. The discovery of resistance genes and resistance control genes is significant for the breeding of new wheat resistant varieties.

1.1. Research progress of wheat powdery mildew

Research progress of powdery mildew

Erysiphales are a kind of very important bio-trophic pathogens, mainly parasitizing the aboveground parts of cruciferous plants and causing harm to a variety of crops [7]. Since the first scientific name of Powdery mildew was first identified by Linnaeus, they have played an important role in the history of plant pathology for over 280 years [8]. The morphological characteristics of white, powdery surface mycelium, conidia and dark brown tea spore (Obturator) make Powdery mildew fungus one of the most famous and easily recognized plant pathogens [9]. Powdery mildew fungi often develop in a relatively humid environment, and their high water content of conidia, compatibility with host and sensitivity to chemical fungicides make them significantly different from other plant pathogens [10]. In the field, powdery mildew will become prevalent in a relatively short time after landing on the host surface, and can be identified and managed by culture of different crops, chemistry, biology, and host resistance [11]. Powdery mildew can cause disease in many angiosperms. In fact, most of them harm dicotyledons, except for *Erysiphe graminis* (synonym) and *Blumeria graminis* (synonym), which cause disease in monocotyledons. The conidia and conidia of powdery mildew are white and powdery, and the diseased plant can be identified from a distance [12]. The most obvious characteristic of Powdery mildew is that it has two different growth stages. The first stage is asexual reproduction or conidia stage, which forms a large number of white conidia on the diseased host plants. These conidia are easily transmitted by air flow and become the source of secondary inoculation. The second is the stage of sexual reproduction, in which sexual spores are formed in the later asexual stage. The spores in the ascus are called perithecia cleistothecia, chasmothecia in the mature stage. These fruitoids are cephalic, small, globular at first, and yellowish brown, dark brown, brown, or black. The obturator shell has distinct appendages, which are filiform and branching, bulbous at the base and curved or spiky at the apex. The

appendages are of great value to the classification and identification of this genus. Ascus are sac-like structures, mostly oval in shape, and contain 2 to 8 ascospores at maturity, depending on the fungus species [13, 14].

Harm and distribution of powdery mildew in wheat

Wheat is susceptible to a variety of diseases throughout its life. These diseases are widely distributed and highly adaptable, which pose a significant threat to wheat yield [15]. Illnesses caused by fungal pathogens alone reduce wheat yield by 15 to 20 percent per year [16]. Generating billions of dollars in damage to the global economy [17]. After powdery mildew infection, wheat plants are prone to lodging. Their leaves dry and die quickly, which seriously affects the average growth and development of wheat [17, 18]. Powdery Mildew caused by Powdery wheat mildew can cause severe yield loss and grain quality deterioration in a short time [18]. Wheat powdery mildew reduced winter wheat by 13% and spring wheat by 20% [19, 20, 21]. According to statistics, wheat powdery mildew is distributed from 60° N to 44° N and can occur in many wheat-growing areas all year round. The crop yield loss in Russia, Brazil, and China is as high as 35%, 62%, and 40%, respectively. Since 2004, wheat powdery mildew has occurred over 6 million hectares every year in China. Studies have shown that it is a crucial disease mainly occurring on the leaves. In severe cases, the stalk, leaf sheath, and ear of wheat will also be infected, and even the leaves will dry up, and the whole plant will die. The pathogen of wheat powdery mildew is the obligate parasite of living nutrition, causing only parasitism on living wheat [22]. Wheat powdery mildew can occur in all wheat growth stages and continuously threaten wheat growth [23]. When humidity is above 70%, air temperature is 15-20℃, nitrogen fertilizer is excessive, and the wheat planting density is high, wheat plants are green and weak, and white powder disease is likely to occur. In a dry land, with insufficient water, fertilizer, or lodging in the wheat field, the disease resistance of wheat will be weakened, powdery mildew often will be more serious [24].

But belongs to ascomycetes subphylum fungi, conidia are elliptic, the obturator shell of pathogenic bacteria is black spherical, containing 9-30 ascus. Ascospore is

round to elliptic. The ascospore shell is usually formed in the late wheat growth stage and can release ascospore after maturity (Fig.1.1). Powdery mildew is widely distributed, with rapid toxicity variation and complex and changeable physiological species of pathogenic bacteria [25, 26].

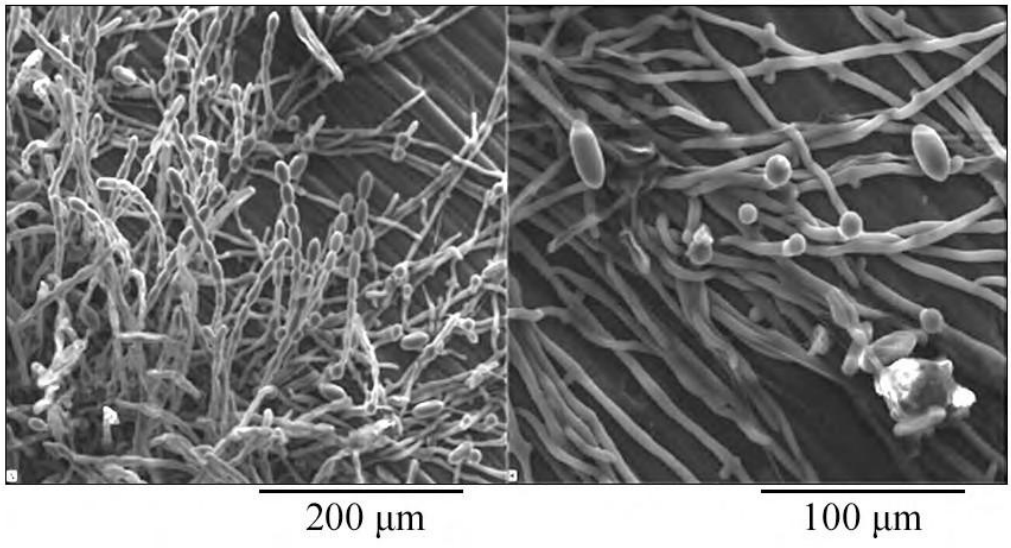

Figure 1.1. The scanning electron micrograph of *B. graminis* [25].

Growth cycle of wheat Powdery mildew

Wheat powdery mildew infected wheat in a suitable environment and began to reproduce. The invasion process is as follows: first, A single conidium is blown onto the leaf, and about 1 hour later, the primary bud tube appears at one end of the obsidian (Fig.1.2). The primary bud helps identify the host surface cells, attach them tightly to the leaf surface, and extract water from the host surface. Over the next few hours, a second bud tube grows from the other end of the spore, elongates toward the leaf surface, and forms an enlarged structure at its back called an aptamina, which attaches to the epidermis. After about 12h, powdery mildew penetrates the cell wall of the host cell by invading the nail. Through the interaction between powdery mildew and host cells, about 50-70% of the spores can successfully penetrate the cell wall, depending on the environmental conditions, the host cells, and the spores themselves (with the most significant impact). The spores that successfully penetrate the cell wall, powdery mildew bacteria will form haustorium after 24 hours, which is a particular infection

structure with finger-like protrusions. Houston can invade the plasma membrane of the host, also known as the organ in which the pathogen takes nutrients. After the successful establishment of the haustorium, airborne hyphae began to grow in the epidermal cells of the leaves and gradually infected other cells nearby, then formed more haustorium, and finally formed the colony of white hyphae net. After about 4-5 days of growth, hyphae will form short upright sporophytes with 5-10conidia chains, and yet, a large number of mature conidia will be released to start the next infection cycle. This clonal propagation is the primary propagation mode of powdery mildew when the conditions are suitable [27]. However, in winter, powdery mildew had sexual reproduction and existed in the closed capsule form on the leaves of the aged host [28]. When released from the ascus, the ascospore behaves like conidia and begins a new round of infection [30].

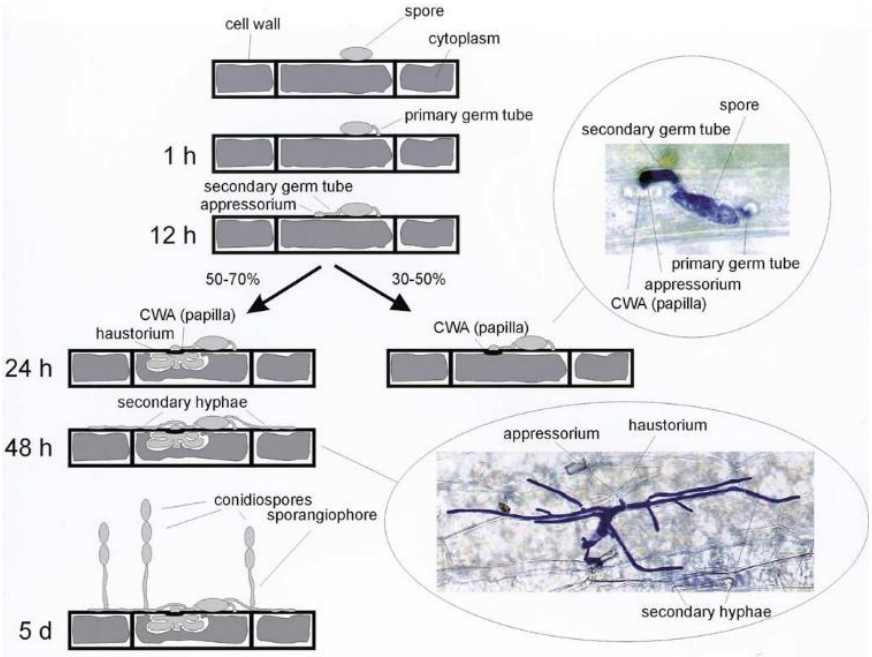

Figure 1.2. Schematic Diagram of powdery mildew bacteria development [24] and scheme of the asexual life cycle of the powdery mildew fungus [24].

Research progress of powdery mildew resistance genes in wheat

In 1930, an Australian scholar reported for the first time that there was an anti-powdery mildew gene in wheat Thew, and it was dominant, thus revealing a wave

of genetic research on wheat powdery mildew. The first powdery mildew gene was named *Pm1* in 1950 and was located on the 7AL chromosome of wheat. So far, more than 90 powdery mildew resistance genes and their alleles, called PM1-65, have been identified [30]. About half of these powdery mildew resistance genes are derived from normal wheat. Also, about one-third of the related species derived from wheat include one-grain wheat, emem-grain wheat, rough-goatgrass, and Timofee-wili wheat. The remainder is derived from haretodes and haretodes rye [31]. Now, most of the resistance genes have lost resistance to powdery mildew or are very weak [32, 33]. Only a small number of genes or alleles remain resistant to powdery mildew [34]. In the main wheat-growing areas, *Pm8* resistance was lost [35, 36]. The resistance of *Pm2* and *Pm4b* was also gradually lost in the Yellow and Huai wheat region [37]. At present, only *Pm1c, Pm12, Pm21, Pm24*, and *Pm35* genes still have a strong resistance to powdery mildew, among which *Pm21* is a rare broad-spectrum resistance gene [38]. Moreover, some disease resistance genes have been applied in wheat breeding, such as Nannong 9918 carrying powdery mildew resistance gene *Pm21*, Liangxing *99* carrying powdery mildew resistance gene *Pm52*, Bannong AK58 moving powdery mildew resistance gene *Pm8*, etc. [39], and achieve more significant economic benefits. Researchers study of wheat powdery mildew in the past was mainly focused on positioning and cloning of disease resistance gene mining. The current research results show that the resistance genes and powdery mildew in the evolution process, the resistance of the resistance genes out quickly, as the disease has progressed, this situation will be more and more can't meet the needs of wheat genetic improvement, is an urgent need to explore new ways of wheat powdery mildew resistance breeding to improve wheat lasting broad-spectrum resistance to powdery mildew.

Research progress of pathogen identification

The ability to identify organisms that cause specific crop diseases is the cornerstone of crop disease prevention and understanding and control With the premise.

Traditional methods of identifying fungal plant pathogens rely on interpretation of visual symptoms and/or isolation of pathogens, Culture and laboratory identification.

The accuracy and reliability of these methods largely depend on the experience and skill of the diagnostic staff [40]. New methods such as immunological methods, DNA/RNA probe techniques and PCR amplification techniques are becoming more and more popular Is applied to the diagnosis of plant pathogens. The greatest advantage of these techniques over traditional diagnostic methods may be height Specificity. They can distinguish between different fungal species and also identify single species [41]. To study and understand the biology, population structure and dynamics of plant pathogenic fungi, host/pathogen interaction New opportunities are provided by gene flow in pathogen populations and inoculum movement. Along with fungal genomics and genes As more functional information becomes available, the range of applications for molecular diagnostics will expand [42]. PCR-based methods are very sensitive and can detect trace amounts of pathogen DNA, which is used to study systemic infections Or early detection of disease before symptoms. Compared with culture method, PCR method is faster and often in sampling The results can be obtained within 1 to 2 days. Are more reliable than visual symptom recognition because they do not rely on distinguishing between symptoms of the disease Nuance required skills [43].

The combination of morphological and molecular identification will greatly accelerate and improve the pathogen The identification speed and accuracy of bacteria provide technical support for disease prevention and monitoring in forage production.

1.2. Advances in plant disease resistance

Molecular mechanism of plant disease resistance

Plants in the natural environment in the growth and development of the whole process will be subject to various pathogenic micro-organisms invasion, and pathogenic microorganisms mainly include pathogenic fungi, bacteria and viruses, etc. For a long time in the co-evolution of plants and pathogens, various defense systems have gradually evolved to inhibit the destruction of pathogens. When pathogenic bacteria invade the plant, a series of signals can be generated immediately in the plant body and transmitted to activate the plant's defense system to resist the invasion of pathogenic bacteria. Plant defense system mainly includes two immune defense line; the first line of

defense is the body's immune response (Pathogen - Associated Molecular Patterns, PAMPs, PAMP - Triggered Immunity, PTI), the process is Triggered by the Pathogen Associated Molecular Patterns, when Pathogen invasion to the surface of a plant, grows on the plant cell membrane on the surface of the pattern recognition receptors can identify the Pathogen Associated Molecular Patterns PAMP, through the signal transduction, triggering an immune system response. The second line of defense is effector-triggered (ETI). Effect factors trigger this process. When pathogenic bacteria invade the surface of plants, plant disease-resistant genes secrete Effector factors that can recognize pathogenic bacteria and trigger immune system response mediated by Effector factors [44].

The path of plant resistance to pathogen invasion is a very complex network. Signaling molecules play an essential role in this network. Still, the same signaling molecule can be produced in different response pathways, and the same pathogen can also stimulate other signaling molecules [45, 46]. When a pathogen enters a plant, the inside body of the plant can produce a series of signal molecules immediately, carry on transmission, excite plant oneself defense system then, make plant has the ability to resist pathogen thereby. Many signaling molecules play a role in stimulating and regulating plants' defense systems, including Ca^{2+}, salicylic acid (SA), jasmonic acid (JA), ethylene (ET), and reactive oxygen species (ROS). Ca^{2+} can not only maintain the osmotic pressure of plants but also participate in regulating the signal transduction pathway of pathogenic bacteria in plants. It is an essential second messenger molecule of plant cells. Pathogenic bacteria induce. Ca^{2+} crosses the cytoplasmic membrane, resulting in intracellular Ca^{2+} As the concentration increases, the osmotic pressure increases, and the corresponding protein kinases are activated [47]. Salicylic acid can activate the production of some related proteases, thus making plants resistant to disease. Experiments have shown that salicylic acid content will accumulate in large quantities after plants are invaded by pathogenic bacteria [48]. Also, salicylic acid in the process of plant resistance to pathogen invasion and H_2O_2 is closely related, and H_2O_2 can determine the host disease resistance response in plants. Simultaneously, salicylic acid may inhibit catalase activity [49]. Jasmonic acid and ethylene are ubiquitous in plants.

They are not only the growth regulators in plants but also the signal molecules in plants in response to the invasion of pathogens. Studies have shown that salicylic acid, jasmonic acid, and ethylene have a close cross-connection in response to pathogenic bacteria's invasion [50].

In the long process of plant-pathogen co-antagonistic evolution, plants evolved a cellular dual innate immune system to resist the invasion of Pathogens such as Bacteria, Fungi and Virus. They are Basal immunity and R-gene-based disease resistance [51]. The former is also known as PTI (PAMPs-triggered immunity). Pattern recognition receptors (PRRs) detect and recognize danger signals caused by external invasion. One of these pathogens is called pathogen-associated molecular patterns (PAMPs), The host source is called damage-associated Molecular Patterns (DAMPs) [52], and these two modes hinder the replication of pathogens and the occurrence of diseases. Recognize effector factors by R protein (Resistance protein) and induce severe Hypersensitive response (HR). The phenomenon that causes Programmed cell death (PCD) at the infection site is resistance-gene immunity. Avirulence effector (Avr) -induced immunity (ETI) [53]. It has been found that R protein contains 90% Nucleotide binding (LEucine-rich repeat receptors) [54]. The corresponding pathogenic pathogens - plants - resistance in long perfect synergy into the period, the dynamic change trend available four stage model to generalize the "Z" word. Stage 1: Plant cell transmembrane receptor PRRs can detect PAMPs and stimulate PTI to resist the spread of pathogens. Stage 2: The pathogen invades host cells by secreting Effector to evade PRRs recognition, and at the same time enhances pathogen toxicity and causes the susceptibility induced by Effector factors (triggered susceptibility, ETS); Stage 3: Intracellular R protein in plant cells induces ETI through specific recognition of effector factors, and the infected parts of plant cells limit the range of infection due to severe HR response. Stage 4: Under the long-term plant-pathogen interaction and natural selection, pathogens evolve new reactive factors to evade specific detection and screening of R proteins, and plants subsequently evolve new R proteins to trigger ETI again [55]. It can be seen from the above that accurate recognition of diverse pathogens by plant immune receptors is the prerequisite for stimulating plant resistance [56].

Traditional microbiology and plant pathology studies mainly explore plant disease resistance by analyzing the physiological and biochemical manifestations of plant disease resistance. In recent years, with the generation of molecular biology and bioinformatics and rapid development in order to further study the theory of resistance to broaden the way of combining structural biology from the level of protein molecules parsing cellular immune mechanism, enrich the study of plant immune signaling pathways abundance, for proven the pathogen recognition mechanism of the immune receptor opens a new road, It has greatly promoted the scientific theory and technology of plant disease resistance.

Research progress on physiological and biochemical reactions of crop diseases

As an important basis for developing disease-resistance breeding strategies, research on disease-resistance mechanism is not only the basis for disease-resistance breeding, but also can provide new ideas and directions for disease-resistance breeding [57]. In the long process of co-evolution of plants and pathogens, plants have established a complex and accurate defense system to resist all kinds of foreign invasion, the invasion and expansion of pathogens on the surface and in the body of plants are restrained to a certain extent, that is, the resistance of plants to pathogens. Almost as important content of plant cells, water molecules involved in the whole process of plant life cycle, in a plant pathogen injury when plants start all defensive measures, through the metabolism and accumulate defense related substances, as in the metabolic process of reactants and reaction products, water molecules in maintaining cell inside and outside osmotic pressure, It plays an irreplaceable role in ion transport and other processes [58]. Wang Chun et al. [59] studied the interaction between Powdery mildew and Arabidopsis, and found that after 28 days of powdery mildew infection, Arabidopsis plants maintained lower leaf osmotic potential by maintaining higher tissue water content [60].

Inoculated powdery mildew in *Poa pratensis* L. and found that the relative water content in plants decreased after inoculation, so the relative water content could be used as an indicator of powdery mildew resistance. After pathogen invasion, the plant

somatic membrane is damaged and the permeability changes, and the relative conductivity can be used as an embodiment of the degree of membrane peroxidation. The higher the conductivity is the higher the degree of membrane peroxidation is, and the fine cell membrane is seriously damaged [61, 62] studied the effects of different trace element deficiency on powdery mildew occurrence in cucumber (*Cucumis sativus* L.) and found that the electrical conductivity of copper deficient plants was significantly higher than that of normal plants after 1 day of inoculation, but decreased significantly at the later stage of disease. Studies have shown that stress leads to serious disorder in plant physiology, biochemistry and metabolism, resulting in massive accumulation of toxic and harmful substances. Under stress, plants can protect themselves by activating and synthesizing Water soluble protein (WSP), WSC, Pro and other osmotic regulatory substances [63]. Plants will also use antioxidant enzymes (such as SOD, POD, CAT, etc.) to resist the damage to their metabolism caused by the outbreak of reactive oxygen species caused by pathogen invasion. MDA is one of the main physiological indexes to detect the degree of lipid peroxidation in plant tissues. [64]. There have been a lot of studies on the physiological basis of plant resistance to pathogen invasion. Studies have shown that immune and highly resistant *Lagerstroemia indica* L. varieties enhance disease resistance by increasing SOD activity, while MDA content changes in immune and resistant varieties are consistent. Wang et al. [65] found that endophytic fungi 2T1 and 3T1 treatment of Cinnamomum oleifora increased the activities of POD and CAT and MDA content in plants. Wang et al. [65] reached a similar conclusion in their study of Meloidogyne incognita infecting *Nicotiana tabacum* L. with differential resistance. [66] measured PAL, POD activity and WSP by treating Malus domestica anti-tebutazol mutant UV-TS1-10 and *Botryosphaerira dothidea* sensitive strain TS1 with pesticides It was found that pesticide treatment increased the activity of enzymes and the content of WSP in the mutant. It was studied the effects of continuous cropping on resistance of cotton (*Gossypium hirsutum* L.) to *Fusarium oxysporum* F. sp. *vasinfectum* [67]. Continuous cropping weakened the reaction of antioxidant enzymes in cotton flowers, reduced the intensity of antioxidant enzymes alleviating membrane peroxidation, and greatly weakened the resistance of cotton to disease. By studying the mechanism of

Bacillus subtilis inhibiting *Phytophthora parasitica* var. *nicotianae*, [68] found that the content of β-1, 3-glucan in the cell wall of the former strain decreased, and the integrity of cell membrane was destroyed, resulting in the growth of mycelia blocked or malformed.

Shang et al. [69] reported that *Pasteuria penetrans*, which harasses root-knot nematodes, somewhat inhibited host plant infestation by reducing the rise and fall of protective enzyme activity in the host. Wang et al. [70] found that POD activity in resistant sugarcane (*Saccharum officinarum*) was enhanced when *Sporisorium scitamineum* was infected, predicting that POD was involved in the early disease resistance process of sugarcane. The research results of Li et al. [71] showed that the contents of Pro, MDA and CAT activity in sick *Vigna unguiculata* Walp roots were higher than those in healthy roots after infection with Meloidogyne incognita. POD and SOD activities were lower than those of healthy roots. Cheng [72] reported WSP of cabbage (*Brassica pekinensis* Rupr.) and cabbage (*Brassica oleracea* L.) infected by *Plasmodiophora brassicae* Woron Compared with control and susceptible varieties, pathogen infection increased WSC content, MDA content first and then decreased, SOD and POD activities also increased after inoculation. Li [73] showed that POD, SOD, PAL, MDA and WSP could be used as representative physiological indicators to identify the response of *Lycium barbarum* L. to infection by *F.* oxysporum. Tian et al. [74] studied the clustering of 21 *Momordica charantia* L. germplasm resources and the physiological and biochemical responses of different powdery mildew resistance materials, and found that the clustering analysis was consistent with the field resistance identification results. The researchers believed that the contents of Chl and WSC and the activities of POD and PPO could be used to identify the early infection of powdery mildew. Chen [75] determined *Astragalus propinquus* after infection by *F. oxysporum*, *F. sonali* and *F.acuminatum.* The activity of SOD, CAT and POD in *Astragalus membranaceus* showed no significant difference with the infection of different pathogens.

Research progress on plant structural resistance

In the complex process of pathogen - host plant interaction, pathogen stimulates the host plant to produce morphological and structural changes, forming physical barriers and preventing pathogen invasion. As an early place for plants to wrestle with pathogens, the protective effect of pathogenic bacteria on the surface of plants should not be underestimated. A large amount of wax and cuticle covering epidermal cells are the main components of these structures. In preventing the invasion of pathogen, the composition of epidermal cells, the structure of fine cells, the size and position of various cells, the morphology of stomata, water pores and dermal pores, and the existence of thick wall cells all play their respective roles. The tissues and organs such as leaves, fruits and branches secrete water droplets on the wax surface to gather into a waterproof layer to resist the adsorption, infection, germination or proliferation of pathogenic bacteria. In order for pathogens to invade the host, they need to complete adhesion and germination to produce mycelium step. Waxes and leaf pilates largely controlled leaf surface humidity. As early as 1931, Johnstone discovered that the leaves of certain apple species with waxy powder can rapidly expel water droplets to reduce the success rate of *Venturia inaequalis* infection and improve disease resistance. It was noted that plant varieties with a waxy layer in the field generally suffered less disease than those without a waxy layer, mainly because it was difficult for infection droplets to gather on the surface. Chen [76] and Yao [77]. obtained the same conclusion by studying the relationship between sheath blight and wax content and disease resistance of rice (*Oryza sativa* L.) respectively. Some researchers hold different views. Wang [78] believes that wax has poor wetting ability and is not easy to adhere to raindrops and form water films. In addition, pathogen invasion of host plants requires a wet surface, and the presence of a large amount of wax is not conducive to pathogen spores invasion and growth and development. The thicker the wax layer on the surface of the plant, the harder it is for pathogens to invade. The cuticle is involved in the defense process of pathogen invasion from early to middle and late [79, 80]. The thickness and hardness of the outer wall of epidermal cells are also important components of host plant structural disease resistance. In addition to the above mentioned structures, the xylem, vascular bundle and sclerenchyma of the host plant also play an important role in preventing the

pathogen from spreading further [81]. In addition, the density, structure and opening and closing rules of water pores and stomata are also related to disease resistance. Some studies have found that stomatal density and size determine the infection efficiency of pathogenic fungi spores. Plant transpiration rate determines the degree and efficiency of pathogen invasion and germination by influencing foliar humidity [82]. Stomata, as the main place for gas and water exchange in plants, plays an important physiological role in their growth and development, and is also the main channel for pathogen to enter plants. In conclusion, in recent years, many scholars have studied the change characteristics of stomata microstructure and ultrastructure, and investigated the process of stomata regulating plant growth and development, so as to clarify the mechanism of plant structural resistance to disease. The relationship between stomata and disease resistance varies from crop to crop and disease [83, 84, 85]. Therefore, whether stomata can be used as a physiological indicator of plant resistance to disease needs to be determined according to specific research. The interaction between host and pathogen is a complicated process [86]. The morphological and structural differences of hosts also show the diversity and richness of defense against pathogenic bacteria. Although the plant structure resistance related research started sooner than other aspects, but because of imperfect research means And can't answer for a long time of difficult and dispute and plant disease workers not fully appreciated in subjective prejudice plant form of state structure on the disease resistance of the importance of reason for a long time not making much progress the study. The disease resistance and its mechanism of plant morphology are more complex, involving physiological and biochemical factors. Therefore, we believe that with the rapid development of electron microscopy, physiological and biochemical technologies, molecular biology, genomics, metabolomics and proteomics, as well as some interdisciplinary or marginal disciplines, the role of plant morphological structure in disease resistance will attract more attention from researchers and further research in this field will surely be further developed. In order to solve the complex relationship between plant and pathogen, but also to provide reference and theoretical support for disease resistance breeding.

Research progress in the response of plant endogenous hormones to disease

resistance

A large number of studies have shown that endogenous hormones participate in and regulate multiple life activities in plants [87]. Endogenous hormones in plants mainly include Abscisic acid (ABA), Ethylene (ET), inole-3-acetic acid (IAA), Cytokinin acid (IAA). CTK) and Gibberellic acid (GA), and each type of hormone has a regulatory effect on plant growth and development within a certain concentration range [88]. Plant endogenous hormones can be involved in resisting the infection of pathogenic bacteria, inducing the defense response of the host, avoiding or reducing the harm caused by pathogenic bacteria, and are closely related to disease resistance [89]. Xu [90] pointed out that in the interaction between rice and *Burkholderia glumae,* which causes ear disease, the infection of this pathogen is involved in inducing changes in a series of endogenous hormones, and such induction effect is more significant in resistant varieties than susceptible materials. Zhong et al. [91] found that the infection of Potato virus Y (PVY) to tobacco plants resulted in a significant reduction of IAA content in the plant, and the decrease of susceptible varieties was greater than that of resistant varieties. Under the action of *Cercospora sojina,* the increase of IAA content in soybean leaves was conducive to the enhancement of resistance to disease, and the decrease of IAA and GA content in susceptible varieties [92].

The research results of Gao et al. [93] showed that IAA was involved in the resistance process of maize *(Zea mays* L.) to head smut (pathogen: Black fungus of millet shaft, *Sphacelotheca destruens*). Luo et al. [94] found that CTK not only promoted cell division and enlargement, but also significantly promoted plant growth, but also played a positive role in plant resistance to pathogen invasion. Liu et al. [95] found that GA plays a role in regulating plant height, tillering and other traits of crops, and it also participates in the healing process of plants by inducing the expression of disease-resistance genes. ABA is involved in many processes of crop growth and development, mainly regulating the aging and programmed death of cells. Cellular immunity is achieved by inducing the expression of programmed cell death related genes in plants. As the effect factors of plant response to stress, SA and JA are also

important signal molecules in the signal transduction pathway of plant disease resistance. It is generally believed that SA positively regulates plant defense against pathogens [96]. As a broad spectrum physiological effect factor and an important signal molecule in the pathway of disease resistance signal transduction, JA plays a very important role in the process of plant defense against pathogens [97]. The endogenous hormone content of host plants will change correspondingly after pathogen infection. In this study, the physiological response mechanism of wheat powdery mildew induced by endogenous hormone was explained by measuring the variation rule of endogenous hormone content in wheat leaves with different resistance after artificial inoculation of powdery mildew. It provides theoretical support for the prediction of early onset of powdery mildew resistance, epidemic monitoring and resistance breeding of wheat.

Research progress of genes related to plant susceptibility

Plants for pathogen resistance and disease, now most of the scholars in the fields of disease research, few people engaged in disease research, the study of disease genes is very few, but the plant disease resistance and disease of plants and pathogen interaction are equally important [98, 99].

The concept of susceptibility factors was first proposed by British scholars Schulze and Vogel in 2000 [100]. Subsequently, in 2002, Eckardt [101] made a further discussion on the concept of susceptible genes, regarding them as essential factors for the successful invasion, growth, development, and reproduction of pathogenic bacteria. Hy trialability gene, which can facilitate pathogen infection and facilitate affinity in plants, is currently defined as a susceptibility gene [101]. In the interaction between host and pathogen, infection genes assist pathogen invasion through the following three aspects to increase the degree of plant infection. First: When pathogens invade the host, susceptibility genes can help the host identify the pathogens and their affinity. For example, the host specialization toxin (HST) is capable of producing specialization in plants due to the interaction between the susceptibility gene and the virulent gene product of the pathogen [102]. Secondly, susceptibility genes can encode negative regulators with immune signals. For example, the CPR1/CPR30 gene in Arabidopsis

can encode and translate F-box protein and has the ability to regulate the accumulation of SNC1 protein negatively. When the CPR1/CPR30 gene is mutated, the plant's disease resistance ability is significantly improved [103]. Third: in pathogen and host mutual affinity, after invading the host, the disease gene can assist the growth and reproduction of pathogenic bacteria, for the metabolism and structure of pathogenic bacteria to provide the necessary nutrients. For example, in *Arabidopsis thaliana*, pepper, tomato, and lettuce and other higher plants widely exist A kind of disease genes, the host of the translation initiation eIF4G and poly real viruses effect VPg, polymerase NIb and PABP (poly real A binding protein) translation initiation complex formation, combining the RNA virus m 5 'end cap structure, help complete the translation and viral RNA synthesis of viral proteins, when they had mutations, can improve the host resistance to the virus [104]. Many experimental results showed that when the susceptible genes were mutated or lost, the resistance of plants to pathogen invasion was greatly enhanced. Finally, the invasion ability of pathogens was weakened.

The first gene was discovered by Vogel, an American researcher, an essential gene in *Arabidopsis thaliana* infection. Vogel named the gene *PMR6*. Deleting the *PMR6* gene resulted in a mutation that showed high resistance to powdery mildew [105]. Subsequently, the disease susceptibility genes were cloned on many crops. After the $OsSSI_2$ gene was silenced in rice, the plant resistance to blast and leaf Bsr was significantly improved. After the mutation of the *BSR-D1* gene, a large amount of hydrogen peroxide would accumulate in the cells, thus improving the disease resistance of rice [106]. After the interference of the *GHWRKY106-1* gene with RNA interference technology in cotton, the expression of PRs, a protein related to disease course, would be significantly enhanced in cotton, thus improving the disease resistance of cotton [107]. When the *HVBI-1* gene was overexpressed in barley, the disease resistance of barley would be weakened, while when the *HVBI-1* gene was silenced, the disease resistance of barley would be enhanced [108]. When the *TaS3* and *Blufensin1* genes were silted, wheat resistance to powdery mildew and stripe rust was significantly enhanced [109]. At present, the cloned plant susceptibility genes mainly include transcription factors, enzymes, transmembrane proteins, and other types [110].

1.3. Research progress of *Pm46* gene

Gene discovery and research status

Wheat powdery mildew resistance inheritance is diverse, which is controlled by both main effect quality genes and quantitative traits of micro-effect polygenes. Since waterhouse, an Australian scholar, first reported in 1930 that the wheat variety Thew carried a dominant powdery mildew resistance gene, scientists have identified several genes in wheat and related genera resistant to powdery mildew. These resistance genes are mainly dominant, and only a few are recessive [111]. International designation for the powdery mildew resistance gene in wheat is Pm. To solve the damage of wheat powdery mildew, breeders transferred the powdery mildew resistance genes into wheat to cultivate the disease-resistant varieties. For example, a new variety of wheat resistant to powdery mildew [112] can be obtained by transferring a rye chromosome with the *Pm8* gene into normal wheat [112, 113]. Some anti-powdery mildew genes also have a polygenic effect. For example, the resistance gene *Lr34* was obtained using the mapping cloning method, which encodes a transferase subfamily protein at an ATP binding site and can also be used for rust and powdery mildew [114]. Adult resistant genes *Pm39* and *Pm46* have also been proved to be "one-cause-multipotent" and resistant to wheat rust and powdery mildew [115].

In 1979, Canadian scientists Dyck and Samborski found a leaf rust resistance gene at the adult stage from Pakistani wheat. Later, they introduced the resistance gene into wheat variety Thatcher by successive backcross and obtained a resistant strain RL6077 (Thatcher*6/PI 250413) [116]. Later, it was found that RL6077 was also resistant to stripe rust and stem rust [117, 118]. In 2009, Lagudah et al. confirmed that there was no *Lr34* gene in RL6077 using molecular markers and speculated that RL6077 contained a new multi-disease resistance gene [119]. Hiebert et al., by observing the chromosome pairing behavior, refuted the previous views on the translocation of the *Lr34* gene on 7DS to other stains [120]. Further, genome-wide SSR molecular markers were used to analyze the osmotic chromosome fragments from donor wheat PI 250413 in RL6077, and it was found that 5 polymorphic SSR molecular markers (Xcfd71, Xbarc98, Xcfd23,

Xwmc457, and Xwmc48) were associated with the leaf rust resistance genes in Thatcher/RL6077 and RL6058/RL6077 populations. Then linkage analysis using a third isolated population from RL6077 showed that the 4DL SSR marker Xcfd71 was closely linked to the resistance gene. The new gene in RL6077 was officially named *Lr67* because no rust-resistant genes have been reported on the 4DL. Herera-foessel et al. located stripe rust resistance genes *Yr46* and *Lr67* in RL6077 to the same region of the 4DL chromosome. Subsequently, Herera-Foessel et al. also found that the Lr67/Yr46 site could provide stem rust and powdery mildew resistance and presented the symptoms of tip necrosis, so it was named as the polypotent site: Lr67/Yr46/Sr55/Pm46/Ltn3. [121]. The full length of the predicted Pm46 resistant protein gene consists of 1545 bases encoding 514 amino acids, contains 12 predicted transmembrane helices and is most similar to the STP13 family of H+ / monosaccharide co-transporters, which promotes hexose cross-membrane transport. Their corresponding pleiotropic or tight chain gene, named *Sr55*, *Pm46*, and *Ltn3*, can be used to provide a broad spectrum of durable wheat resistance [122]. In terms of geographical distribution, the *Pm46* gene was found mainly in local varieties in The Punjab of India and was rarely carried in other regions. There are few reports of the *Pm46* gene in Wheat varieties in China. Wang Zhiwei et al. used molecular marker CSTM4_67G to detect 42 wheat varieties and higher generations grown in Yunnan province and found that Yunmai 75, Yun15D4-15, Yimai 1, Yimai 3, Fengmai 32, and Fengmai 35 contained dual-resistant adult rust-resistant gene Lr67/Yr46/Sr55, accounting for 14.29% of the tested materials [123]. Both barley and wild barley themselves carry the *Lr67* lineal homologous gene (*HvSTP13*), but neither has the G144R mutation-specific for the disease-resistant allele. Milne et al. introduced G144R variation into *HvSTP13* and obtained stable transgenic barley lines. Disease identification showed that transgenic barley showed leaf rust resistance at the seedling stage and plant stage, suggesting that the *Lr67* gene mediates conservative disease resistance in barley and wheat [124].

Disease resistance mechanism of Pm46 gene

The research of John - Moore and Sybil Herera-Foessel et al. showed that Lr67res protein might reduce hexose transport by forming an inactive heterodimer protein complex that produces a dominant-negative interference mechanism [116]. This is consistent with dominant or semi-dominant resistance phenotypes given by *Pm46* genes and with phenotypic susceptibility due to deletion of this locus. Dimer-mediated dominant negative interference with transporter activity has been found in other plant sugar transport families [116]. The partial resistance of Lr67res protein to different vivisection pathogens in wheat and barley may be due to the host cells' resistance to extracellular hexose detection, thus increasing the ratio of hexose/sucrose in extracellular hexose. This, in turn, induces a sugar-mediated signal response, creating an environment that is more hostile to the growth of the pathogen. The inhibition of hexose detection by Lr67res is similar to the invertase activity response induced by ubiquitous plant pathogens invading cell walls, which will change the extracellular hexose/sucrose ratio and cause hexose-mediated defense response [107].

Sugars contribute to various physiological processes and act as substrates and signaling molecules in plant defense responses [125]. Activation of sucrose transport by some bacterial virulence protein-coding genes promotes host susceptibility, whereas eliminating these genes induces host resistance [126]. It remains determined whether the *Pm46* gene is also detrimental to host resistance to inanimate nutritive pathogens in field-grown crops. Nevertheless, as a valuable tool for developing broad-spectrum resistance in crops, the *Pm46* gene provides a favorable breeding strategy for combining different forms of broad-spectrum resistance.

1.4. Advances in GDSL gene research

Research progress of lipase gene as a class of hydrolyser that can hydrolyze a variety of substrates such as sulfur esters, arylesters, phospholipids and amino acids [127], Gdsl-type (GDSL) lipase (Lipase, EC 3.1.1.3) differs from other lipase types with GxSxG conserved amino acid sequences due to its specific structure -- the protein N 'terminal has GDSL conserved amino acid sequences [128].

GDSL lipase is a hydrolyzer, which can hydrolysate a variety of substrates such as thiolates, aryl esters, phospholipids, and amino acids. GDSL lipase has a unique structural characteristic with *GDSL* conserved amino acid sequence at the N' end of the protein, different from other lipase types with GxSxG conserved sequence. Upton and Buckley [129] first identified the conserved domain and named it (PFAM PF00657). Subsequent studies have found that this type of lipase is widely present in prokaryotes and eukaryotes. With the development of more plant genome sequencing and bioinformatics, GDSL lipase is found to be a large gene family. At present, GDSL lipase is widely known to be involved in the average growth and development of plants, organ morphogenesis, secondary metabolism, stress, and other physiological activities, and plays an important role in the lipid metabolism of oil crop seeds [130]. However, systematic understanding of the structure, classification, evolution, expression, and function of the family's genes is lacking.

Gene structure and species of GDSL lipase family

Many studies have reported the functions and effects of GDSL hydrolase/ lipase in crop growth and development, plant organ morphogenesis, secondary maturation, stress response and oil metabolism of oil crop seeds [131, 127]. As one of the 11 largest gene families [131], the plant *GDSL* hydrolase family is reported to have 108 and 114 members in arabidopsis and rice, respectively [132]. The *GDSL* gene family was found in corn, cabbage or *Brassica napus*, *Selaginella moellendorffi*i, poplar and *Alfalfa truncatula*, *Physcomitrella patens*, *Chlamydomonas reinhardtii*, grapes, *Phaeodactylum tricornutum* and *Sorghum bicolour* and other species have 50-150 members in the genome of vegetables, fruits, trees and food crops [127, 133, 134].

Plant GDSL lipase is A large gene family. Volokita et al. [134] compared and analyzed the amino acid sequences of 604 *GDSL* genes encoded by different land plants and found that plant *GDSL* gene family members formed three large subfamilies (subfamily A, B and C) on the phylogenetic tree. Each clade contains *GDSL* genes from a different plant. Most *GDSL* family genes in plants consist of 5 exons and 4 introns [134]. It analyzed the gene structure of hundreds of *GDSL* genes in different plants and

found that 6 introns were highly conserved, and the distribution characteristics of these 6 introns were significantly different in the three subfamilies: Intron 1 and 6 were conserved and unchanged in the three subfamilies, and existed in most *GDSL* genes [134]. Intron 5 is conserved in subfamilies A and B, while introns 2, 3, and 4 are unique to subfamilies A, B, and C, respectively. Members of the *GDSL* gene families are distributed on all chromosomes of plants, but the distribution is not uniform [134]. Some *GDSL* genes were distributed in clusters on chromosomes. Taking the Arabidopsis genome as an example, there were two or more *GDSL* genes in clusters or tandem arrangement on 12 chromosomal loci. Some genes are duplicated in other parts of the same chromosome or on different chromosomes, resulting in multiple copies [134, 135]. Comparative analysis of *GDSL* gene families including bryophyte, picea sitchensis, poplar, arabidopsis, grape, rice and sorghum shows that at least 11 genes are duplicated before the evolutionary divergence of bryophyte and embryophyte. Others occurred after the evolutionary bifurcation of bryophytes and embryotes, and before the evolutionary bifurcation of gymnosperms and angiosperms, thus further expanding the number of *GDSL* gene families in embryotes [135]. In addition, the gene degeneration mechanism is also a major force driving the evolution of *GDSL* family.

GDSL is involved in growth development and stress response

Pathogens can induce the expression of *GDSL* genes in some plants, hormones such as salicylic acid, ethylene, jasmonic acid, and abiotic stress factors, indicating that they may be involved in plant resistance and stress response [135]. It reported the news of salicylic acid inducing arabidopsis *GDSL* lipases *GDSL1* disease-resistant activity, *GDSL1* mutant plants of saprophytic fungi spore canola raw chain grid (*Alternaria brassicicola*) is more sensitive than the wild type, the recombinant expression GDSL1 protein with lipase activity, integrity. It can directly damage the fungal spores and inhibit its germination. Besides, the lipase can also induce the plant to produce resistance to the fungus system [136]. Further studies found that excessive expression of GDSL1 in plants can enhance resistance to various pathogenic fungi and bacteria, and *GDSL1* induces phylogenetic resistance of plants through ethene-mediated signaling

pathways [137]. Similarly, the expression of *GDSL2* in *Arabidopsis thaliana* can be caused by salicylic acid, jasmonic acid, and ethylene, and has an inhibitory effect on fungal spore germination. However, *GDSL2* may mediate plant disease resistance by down-regulating auxin signaling pathways [138]. It conducted a similar study on the *CaGDSL1* homolog of pepper and found that its expression was induced by salicylic acid, jasmonic acid, ethylene, bacterial infection, high salt, drought, injury and other stress factors [139]. Unexpectedly, capsicum plants with down-regulated *CaGDSL1* expression have increased background resistance to *Xanthomonas campestris* Pv. It can be seen that, as a large plant gene family, lipases have diverse functions. Different lipases in the same species can differentiate into different functions, and the functions of homologous lipases in other species may also be different [139].

It has also been reported that members of this gene family are significantly increased in expression when induced by hormones, chemicals, environmental stress and pathogen infection. For example, *BrSIL1* and *CaGDSL1* esterase genes in *Brassica rapa* and *Capsicum annuum*, which depend on SA resistance signaling pathway, are induced to be expressed under pathogen infection. Members of this gene family can be found to be closely related to plant disease resistance [139, 140]. Abundant studies have been conducted on the involvement of this gene family hydrolase in plant cuticle formation [141], mediating CTK and GA signaling channels, and regulating seed germination and disease resistance [142, 143, 144, 145] proved that some endogenous hormones can also induce the expression of genes in this family, thus participating in the process of plant disease resistance and plant stress. Oh et al. [137] found that salicylic acid can induce the expression of GDSL1 recombinon, and this hydrolase can improve the defense ability of plants by directly destroying the integrity of fungal spores and inducing disease resistance of plant system. Some scholars have carried out relevant studies on the over-expression of genes in this family and its positive or negative regulation by endogenous hormones to improve disease resistance [139, 146].

Related research on GDSL gene

GDSL genes in both microorganisms and plants have important roles and potential applications. Plant *GDSL* genes can resist fungal pathogens and stress, and participate in the growth and development of plants and lipid metabolism, so it has great application potential in agriculture. It can be bred by some molecular biological techniques to cultivate crops with good quality and strong stress resistance. *GDSL* genes has a wide range of substrate specificity and multiple hydrolysis functions, and plant lipase is cheaper and easier to obtain than microbial lipase, which may have great application value in food, cosmetics, pharmaceuticals and other fields in the future. The study of *GDSL* genes started late, and the characteristics and functions of this family of genes have not been fully studied. In particular, there are still many problems to be discovered and studied in the study of *GDSL* gene in plants [146].

Although *GDSL* genes have been cloned from many plants, their studies are not in-depth. For example, the tertiary structure and crystal structure of *GDSL* gene still need to be further explored. Therefore, it is necessary to carry out detailed studies on the substrate binding mechanism, catalytic mechanism and flexibility of active sites [146].

In conclusion, as a huge and complex plant gene family, *GDSL* genes are extensively and deeply involved in the complex process of plant defense against pathogens. Through analysis, this experiment found that *GDSL* genes were differentially expressed in artificially inoculated wheat with differential resistance. In this study, the wheat lipase gene *GDSL* screened by yeast double hybrid was cloned and bioinformatics analysis was conducted to construct the expression vector and genetic transformation of *GDSL* gene, in order to lay a foundation for the expression pattern, functional identification and wheat molecular breeding [139].

1.5. VIGS and RNAi technology

Technical principle of VIGS

VIGS is a technique that uses recombinant virus specificity reduces endogenous gene activity, based on post-transcriptional gene silencing (PTGS) [147]. Usually VIGS viral vector can be combined with the host plants of the target gene, using PTGS as a

natural antiviral defense line to fight the virus proliferation, genetic transformation mediated by agrobacterium infect plants, inserted into the part of the viral genome, its RNA degradation mechanism and the way of RNA interference are very similar, both in the virus genome to add multiple cloning sites, to their target genes into the host plant. VIGS vector inoculation in plants is usually obtained through agrobacterium tumefaciens infection by integrating t-DNA containing the viral genome into the host genome of at least one cell for standard transcription translation. This leads to the production of double-stranded RNA (dsRNA) from the viral ssRNA template, and Dicer proteins cut this viral dsRNA into short interfering RNA (siRNAs) duplicates, approximately 21-24 nucleotides in length. These siRNAs, in turn, are incorporated as single-stranded RNA molecules into RISC (RNA-induced silencing complex), which screens and destroys RNA complementary to siRNA [148, 149, 150]. In the particular case of VIGS, the viral RNA and target gene mRNA were cleaved. The virus-derived silencing signals are further amplified and spread systematically throughout the plant (Fig. 1.3). It is assumed that siRNAs of about 21nt length mediate short-range transport, while RNA-dependent RNA polymerase 6 (RDR6) requires long-range transport, possibly amplifying the silencing signal. The systematic propagation of silencing signals occurs regardless of the successful movement of virus particles in the plant. When VIGS was applied to susceptible plants, the target gene mRNA of host plants was degraded in most plants [151].

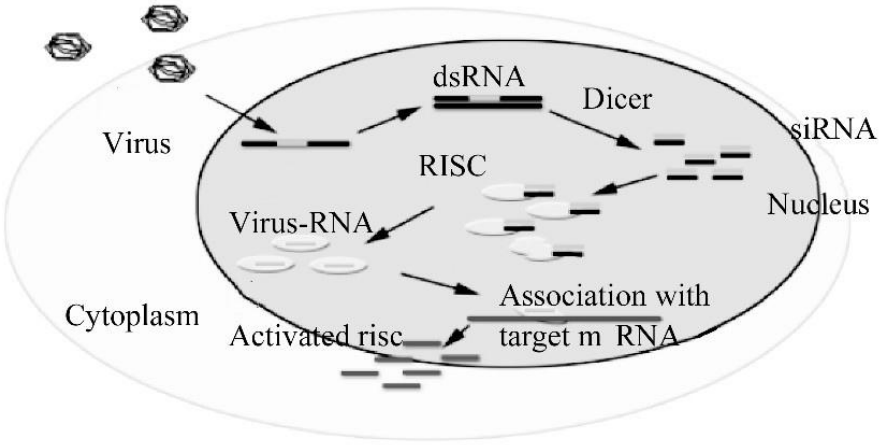

Figure 1.3. The Molecular Mechanism of VIGS Technology [152].

Application of VIGS technology

Large-scale sequencing of functional genomics in non-model plants provides primary data for studying the structural evolution of genomes or the history of repeated events in plant lineages. These new data have also contributed significantly to gene discovery and pave the way for further understanding of gene function evolution, plant-pathogen interactions, biosynthetic and developmental pathways [147]. However, genetic tools are mainly limited to model plants such as *Arabidopsis thaliana*, rice (*Oryza sativum*), or tobacco (*Nicotiana tabacum*), and therefore methods for analyzing gene function in other non-model plants are minimal [152, 153, 154]. It is particularly challenging for most plants to establish a repeatable stable genetic transformation program. As a result, VIGS, which can silence specific genes, is a powerful technique that has been successfully used in a variety of species. As the tools of functional genomics are increasingly used in plant species such as *Zea mays*, *Hordeum vulgare*, and wheat, it is tough to analyze gene function by conventional methods. VIGS technology enables the rapid study of gene function. Since almost all VIGS vectors originally used in dicotyledonous plants were derived from viruses that originally host Solanaceae, some VIGS vectors were successfully extended to other solanaceae plants (especially tomatoes, bell peppers, and petunias) [155]. Tobacco rattle virus (TRV) has a high susceptibility to a wide range of hosts and mild post-infection symptoms and is

preferred as a VIGS resource for dicotyledons. More recently, VIGS are effective against rosaceae plants such as arabidopsis, peas, and cassava [156]. TRV's experimental host range has now been extended to several species of buttercup. Recently, a new VIGS vector system was developed from apple latent spherical virus (ALSV), which can also be used in a variety of higher dicotyledonous plants, including night plants, arabidopsis and legumes. Monocotyledons such as barley, rice, wheat, and maize are also susceptible to TRV [157]. VIGS has become an essential reverse genetic tool for revealing the gene function of species that have difficulty achieving stable genetic transformation or achieving transformation.

Principles of RNAi technology

As a gene knockout technique, RNA interference (RNAi) has been widely used to analyze the gene functions of various organisms. It is a post-transcriptional gene silencing phenomenon induced by double-stranded RNA [158, 159]. Because of its high specificity and effectiveness, it has become a useful tool for gene function analysis. Detailed molecular mechanism of RNAi as shown: first of all, long dsRNA by the Ribonuclease III (RNase III) family, and was cut into 21 nucleotides in length. When each siRNA is disbanded, one of the two strands is preferentially incorporated into the RNA-induced silencing complex (RISC). The antisense strand of siRNA was hybridized with the mRNA as a guide, and RISC cleaved the mRNA near the center (Fig. 1.4) [160].

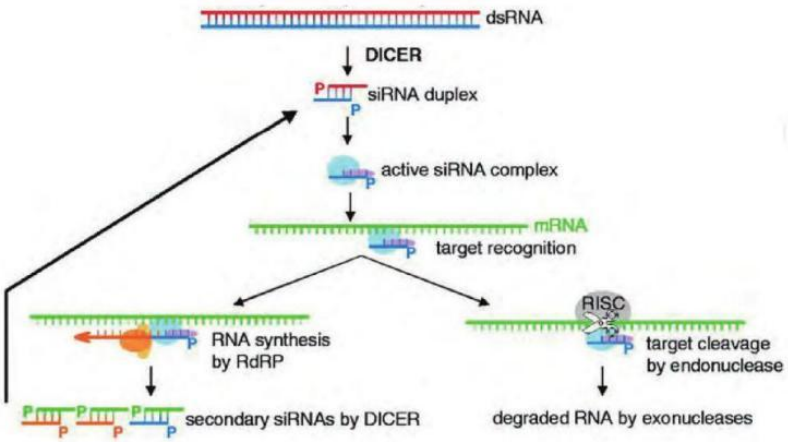

Figure 1.4. The Mechanism of RNAi [160].

Application of RNAi technology

As a highly significant gene suppression technology, RNAi technology has been widely used in crop genetic improvements, such as disease resistance, quality improvement, and abiotic stress tolerance. Fire demonstrated for the first time that RNAi could be used for pest control by injecting C. elegans with bacteria that express dsRNA targeting [161]. Mao et al. [160] designed specific dsRNA according to CYP6AE14, the cytopigment gene of corn earworm, and introduced it into cotton, and obtained transgenic plants with apparent insect resistance to corn earworm [162], which further confirmed the feasibility of using RNAi technology to cultivate insect-resistant crops. At the same time, Bt insecticidal protein Cry3Bb1 and DvSnf7 insecticidal resistant corn have been commercially cultivated [163]. Yang Xiang dong et al. [164] constructed a P3 gene RNAi vector of soybean Mosaic virus SC-3 strains and introduced it into cultivated soybean varieties, and found that transgenic soybean plants had good resistance to multiple soybean Mosaic virus strains such as SC-3, SC7, SC15, SC18 and SMV-R and watermelon Mosaic virus under field conditions, and the resistance traits could be stably inherited. Zhong Xiao Fang et al. [162] introduced The RNA interference fragment of HG-RPS-23 gene into soybeans and obtained a new transgenic soybean material that could significantly improve the resistance to the physiological subspecies of soybean cystodes 3 [91]. By blocking the expression of ACC oxidase, ethylene formation in tomatoes could be significantly reduced and shelf life could be extended, while synthesis of fruit softening substances such as mannosidase and acetylhexanase could be inhibited, which could increase the shelf life of tomatoes [165]. At present, RNAi transgenic crops are mainly completed by agrobacterium-mediated method, which has the advantages of simple operation and low cost. Agrobacterium contains Ti plasmids and Ri plasmids, and a section of transferred DNA (T-DNA) is attached to the plasmids. After agrobacterium enters the cells, it can integrate this section of t-dna into the genome of the infected plant and inherit it stably [166, 167]. At present, RNAi technology in plant research is widely used in many fields, such as disease resistance, insect resistance, quality improvement and breeding, abiotic stress such as drought, salinity, cold tolerance in the areas of study were made certain

progress, in the study of crops at various stages of crop growth and development, biological and abiotic stress response has extensive application prospect.

Agrobacterium-mediated genetic transformation

Plant transformation has become an important means of integrating foreign genes into plant genome, and genetic transformation can change plant traits. Although many plant transformation technologies are available, obtaining single copy transgenic events and associated costs remains a significant challenge [168]. Agrobacterium-mediated transformation has many advantages and is still the preferred method for genetic transformation. Stable plant transformation is usually mediated by *Agrobacterium tumefaciens*. Agrobacterium is a plant pathogen, which can cause the formation of crown galls in the tissues infected by agrobacterium, and it is easy to infect the injured parts of dicotyledons [168]. Agrobacterium contains Ti and Ri plasmids, and there is a transferrable T-DNA on the plasmids. After agrobacterium enters the cell, the T-DNA can be integrated into the genome of the infected plant and can be stably inherited [169, 170] The researchers first incorporated the transformed gene into the T-DNA of the Ti plasmid, and then transferred the gene into plant cells through agrobacterium-mediated transformation. The general steps of *Agrobacterium tumefaciens* transformation can be divided into several stages: construction of expression vectors of target genes, transformation of expression vectors into *Agrobacterium tumefaciens*, infection of plants to be transformed by *Agrobacterium tumefaciens*, screening of positive plants and identification of positive plants [170]. At present, agrobacterium transformation has been widely applied in many plants, mainly dicotyledons, such as *Arabidopsis*, tomato, soybean, etc. With the continuous maturation and development of this technology, agrobacterium-mediated genetic transformation can gradually be used to transform monocotyledons, such as rice, maize, wheat, etc [169].

1.6. Breeding strategies and possible schemes for resistance to powdery mildew winter wheat varieties

Wheat is one of the food crops with the largest planting area, maximum yield and highest nutritional value in the world. Wheat has been struggling under the stress of

adversity during its growth and development. Both biological stress and abiotic stress have great influence on wheat growth. Lower stress levels are manifested by slower growth and reduced resistance to disease. Severe stress levels can lead to significant reductions in wheat yields or even failure to harvest. Powdery mildew, a fungal disease caused by powdery mildew, is common in red wheat production and has been recorded in major wheat cultivation areas in the world. In the past, powdery mildew was more common in temperate, wet and rainy areas. In recent years, due to the blind increase of planting density and nitrogen fertilizer application amount, coupled with the long-term single planting mode, powdery mildew has become increasingly serious in wheat production [80]. Although powdery mildew can be controlled with fungicides, screening and breeding resistant varieties is the most economical, safe and effective method for powdery mildew control [81]. Breeding resistant varieties is the most economical and effective way to control powdery mildew of wheat. However, there are some limitations in the practical production of powdery mildew resistance genes into resistant varieties. With the deterioration of the disease year by year, this situation will be less and less able to meet the needs of wheat genetic improvement. In order to improve wheat powdery mildew resistance, it is urgent to explore a new way of wheat powdery mildew resistance breeding [17].

Conclusions to chapter 1

There are practically no powdery mildew resistant wheat varieties in production but varieties with this characteristic are extremely necessary. It is desirable that such varieties combine resistance to powdery mildew with such valuable trait as high yield capacity.

Conversional breeding is a time-consuming and lengthy process. However, these methods provide the initial material with the desired characteristics and the development of new varieties of winter wheat with powder mildew resistance. The achievements of traditional breeding in creating varieties with powder mildew resistance are convincing.

Biotechnology methods are relevant and promising as well. These methods

significantly speed up the selection process.

A promising approach in modern breeding is the combination of molecular genetic developments with traditional breeding methods.

CHAPTER 2

CONDITIONS, MATERIALS AND METHODS OF RESEARCH

The research was conducted in accordance with the thematic plans of research works of Henan Institute of Science and Technology in the National Natural Science Foundation of China (Grant Number 31872129, 2018-2023). The research, which was the base of dissertation consisted, of two experiments:

1. Study of the collection and obtaining the source material of winter wheat with high ability to powdery mildew resistance. The experiment was performed on the Planting base of Henan Institute of Science and Technology (2020-2022);

2. The discovery and functional analysis of the *TaGDL* gene (2019-2022). This experiment was performed in China (Henan Institute of Science and Technology (Xinxiang, China).

2.1. Experiment 1. Study of the collection and obtaining of initial material of winter wheat with high ability to powdery mildew resistance

Powdery mildew is an important disease in wheat production. Breeding and planting resistant varieties is the most economical way to control powdery mildew. It is an effective and environmentally friendly approach. The research on powdery mildew resistance of newly developed wheat lines can provide scientific basis for variety certification and layout. In this study, the powdery mildew resistance was identified in 86 new wheat lines recruited to participate in regional trials, in order to provide a basis for further research and utilization of these wheat lines.

1.1.1. Test materials

The experimental materials were 86 new wheat samples, susceptible control Xiaoyan 22 and auxiliary control Xinong 979, which participated in the regional trial from 2020 to 2021. Stripe rust strains used for disease resistance identification were mixed strains of stripe 32 and 33. Detailed information is shown in Appendix Table 1.

Xiaoyan 22

Xiaoyan 22 is a mild spring and medium ripe wheat variety with compact plant type, plant height of 88cm, general lodging resistance, good fruit bearing, general cold resistance, and heavy dry tip of flag leaf.

Xinong 979

The seedlings of Xinong 979 are slightly creeping, dark green leaf color, narrow and long flag leaf, compact plant type, good plant type structure, hard stem, good elasticity, plant height 75-78cm. Ear type medium to large, ear layer is more homogeneous, ear nearly rectangular, medium and size is more uniform.

2.1.2. Test design

The experiment was conducted in the wheat experimental demonstration station of Henan University of Science and Technology. Each new wheat strain was sown in 2 rows, 1 meter in length, 0.25 meters in row spacing, 0.10 meters in plant spacing, 0.5 meters in aisle width between row rows, and 1 meter in protective rows around the experimental area. One row of control cultivar was sown between every 10 materials. The experiment was seeded in early October 2020, and white powder was inoculated in mid-March of the following year. The strain was a mixed strain of powdery fungus, which was provided by the College of Life Science and Technology, Henan University of Science and Technology. The powdery mildew resistance of the new wheat lines was identified when the control varieties were sufficiently diseased. Field cultivation, water and fertilizer management, topdressing, weeding and pest control were determined according to the local field production practice.

2.1.3. Identification method of powdery mildew resistance in adult wheat

Wheat powdery mildew was identified by artificial inoculation in the adult stage of the field (Fig. 2.1), and the disease was recorded in the heading stage and milk ripe stage of wheat. The grade of the disease was 0-9, in which grade 0 was Immunity, grade 1-2 was High resistance. Grade 3-4 was Medium resistance, grade 5-6 was Medium susceptible, grade 7-8 was High susceptible, and grade 9 was

Extremely susceptible (Fig. 2.2).

(a) (b)

Figure 2.1. Field performance of wheat powdery mildew in adult stage: (a) - Susceptible; (b) - Disease resistance [171].

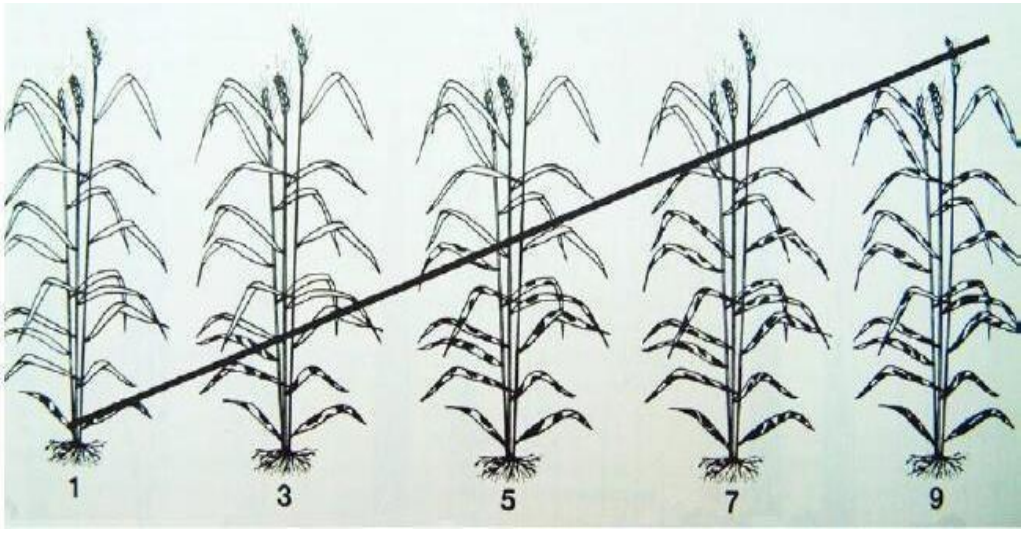

Figure 2.2. Diagram of rating scale of wheat powdery mildew evaluation at the adult stage. 1: highly resistant; 3: moderate resistant; 5: moderate susceptible; 7-9: highly susceptible [171].

2.2. Experiment 2. The discovery and functional analysis of the *TaSFT2L* gene

2.2.1. Plant material

The wheat (*Triticum aestivum* L.) cultivar Bainong207 was used for gene transformation and functional analyses of the *TaGDSL* gene.

2.2.2. Strains and vector

On $pGADT_7$, $pGBKT_7$, plant silencing expression vector pTCK303 and plant overexpression vector pCAMBIA1301 were provided by http://www.miaolingbio.com/. PMD-19T vector was purchased from TaKaRa Biotechnology Co., LTD. *Escherichia coli* (*E.coli* DH5α) and Agrobacterium GV3101 strains were provided and preserved by Henan Grain Crop Genome Editing Engineering Technology Research Center.

2.2.3. Enzymes and main reagents

DNA Gel recovery kit and plasmid small amount extraction kit were purchased from TIANGEN Company. TaKaRa Mini BEST Plant RNA Extraction Kit, PrimeScript™ II 1ST Strand cDNA Synthesis Kit, Rt-qpcr Kit (TB Green® Premix Ex Taq™ II (Tli RNaseH Plus), T4 ligase, In-Fusion® HD Cloning Kit SeamLess clonal enzyme, Restriction endonuclease, PrimeSTAR® GXL DNA Polymerase high-fidelity enzyme, GoldY2H competent cell, 2 * Tap Mastcr Mix, yeast transformation kit, were purchased from TaKaRa biotechnology Co., LTD; DL 2000^+ and DL 5000 DNA markers were purchased from Bao Bioengineering (Dalian) Co., LTD.

2.2.4. Instruments and equipment

(1) Illumination incubator;

(2) ultra-low temperature (-80℃) refrigerator;

(3) Gradient PCR instrument;

(4) ultramicro spectrophotometer;

(5) Ultra-clean workbench;

(6) Desktop high speed refrigerated centrifuge;

(7) Constant temperature incubator;

(8) 7300 real-time fluorescence quantitative PCR instrument;

(9) Confocal laser microscope;

(10) Varioskan Flash multifunctional microplate reader;

(11) SH220N Graphite Furnace Digestion Instrument, Haineng Instrument Co., LTD. (Jinan);

(12) Optima 2100 DV Inductively Coupled Plasma Emission Spectrometer, PE, USA;

(13) Bio-Best 140E UV-Vis Gel Imaging analysis System, SIM, USA;

(14) P25T/Horizon 11.14 Gel electrophoresis system, Biometra;

(15) Intelligent artificial climate greenhouse;

(16) autoclave pot;

(17) Ultra-clean workbench;

And pipETte, miniature palm centrifuge, water bath, constant temperature shaking table, drying box, fume hood and other experimental equipment.

Reagent and medium preparation

(1) EDTA: Inhibits metalloproteolytic enzyme, and is generally prepared into 0.5 M liquid for reserve. 186.1g ethylenediamine tetraacetic acid disodium dihydrate ($EDTA{\cdot}2H_2O$) was added to 800 mL water, stirred vigorously with magnetic stirrers, adjusted the pH value to 8.0, and then constant volume to 1 L, which was autoclaved and used for sterilization. EDTA solution needs to be completely dissolved when the pH value of NaOH is adjusted to close to 8.0.

(2) 1 M Tris-HCl (pH 8.5) (1L) weighed 121.1g Tris base, added ddH_2O to about 800 mL, stirred evenly, adjusted pH value to 8.5 with 1 m HCl, constant volume to 1L, sterilized for later use.

(3) CTAB extraction buffer: 1 mM Tris-HCl (pH 8.0), 25 mM $EDTA{\cdot}2\ H_2O$, 0.25 mm NaCl, 0.5%SDS. To prepare 200 mL solution for example, 4.8456 g Tris, 2.922 g $NaCl_2$, 1.861 g $EDTA{\cdot}2H_2O$, 1g SDS, HCl adjusted pH 8.0, constant volume to 200 mL should be added.

(4) Preparation of onion subcellular localization expression buffer: 150 mM acetosyringone (AS) and 10mM $MgCl_2$ were prepared, and the ratio of 20 mM $MgCl_2$/ 150 mM AS was mixed.

(5) LB medium: weigh 10 g sodium chloride, 10 g peptone, 5 g yeast extract, add deionized water and constant volume to 1000 mL, autoclave at 121 ℃ for 15 min, then cool for later use. To prepare solid medium, add 15g/ L agar powder, adjust pH to 7.5, then sterilize the medium, add antibiotics with the corresponding concentration, pour them into a disposable pETri dish, seal them with aseptic sealing film, and store at 4℃ for later use.

(6) GUS staining solution: 50 mM phosphate buffer (pH 7.2), 0.1% Triton X-100, 2 mM potassium ferricyanide, 2 mM potassium ferricyanide, 10 mM EDTA, 2 mM X-glue.

2.2.5. Methods of research

Extraction of Wheat DNA

(1) Taking the tender leaves, put them into a mortar and grind them into powder with a grinding rod after being pre-cooled by liquid nitrogen, and add 650 μL preheated CTAB solution;

(2) Put EP tube with CTAB and sample into 65℃ water bath for about 20-30 min;

(3) 600 μL of chloroform: isoamyl alcohol (24:1) was added, mixed, placed on ice for 10 min, centrifuged at 12000 rpm for 10 min, sucked 400 μL of supernatant, and loaded into a new EP tube;

(4) The same volume of isopropyl alcohol was added, mixed and placed in the refrigerator (-20 ℃), frozen for 40 min, 12000 rpm, centrifuged for 10 min, discarded supernatant;

(5) To add 75% ethanol for 200 μL washing 2-3 times, and then pour on sterile filter paper to dry upside down;

(6) ddH_2O (20-40 μL) was added to dissolve and used as the target fragment of DNA template amplification.

Extraction of RNA from wheat

(1) RNA Extraction

① The young and tender plant tissues were frozen by liquid nitrogen and then placed in a mortar, quickly ground into powder in liquid nitrogen. 100 mg was taken in a 1.5 mL centrifuge tube, and RNA was extracted immediately to avoid the effect of RNase.

② The sample, which was ground into powder, was added to a 1.5 mL centrifuge tube containing the lysate (500 μL Buffer RL, 10μL 50×DTT Solution) without RNA enzyme. The palmate-shaped vortiometer was used to vortex for 30 min until there was no obvious precipitation in the lysate.

③ The lysate was centrifuged at 4 ℃ at 10 000 rpm for 10-15 min. Carefully transfer the upper aqueous phase to a new 1.5 mL RNase Free Tube centrifuge, add 1/2 volume of liquid anhydrous ethanol, and siphon evenly using a pipette.

④ Immediately transfer all the mixture (including precipitation) into the adsorption column; Centrifugation was performed at 12 000 rpm for 1min, and the filtrate was discarded.

⑤ 500 μL Buffer RWA protein removal solution was added to the adsorption column, followed by 12 000 rpm low-temperature centrifugation of 30 s-60 s, and filtrate was discarded.

⑥ Adding 600 μL Buffer RWB (confirm that anhydrous ethanol has been added) along the wall of the adsorption column helps to wash the salt adhered to the wall completely. Centrifuge at 12 000 rpm at 4℃ for 30 s, then discard the filtrate.

⑦ The adsorption column was put back and centrifuged at 10 000 rpm for 2 min at low temperature.

(2) DNase digestion

① Using DNaseI to digest DNA in RNA, the reaction system is as follows:

10 x DNase1 Buffer 5 mL

Recombinant DNase I 4 mL

RNase Free to 50μL

② To add 50 μL DNase I reaction solution to the center of the adsorption membrane, and let stand at room temperature for 15-20 min.

③ 350 μL Buffer RWB was added along the wall of the adsorption column, centrifuged at 12000 rpm for 30 s at 4℃, and the filtrate was discarded.

(3) RNA purification

① 600 μL Buffer RWB was added along the wall of the adsorption column again, centrifuged at 12 000 rpm for 30s at 4℃, and the filtrate was discarded.

② The adsorption column was placed on the collecting tube and centrifuged at 12 000 rpm for 2 min at 4 ℃ to remove the remaining ethanol from the membrane.

③ The column was placed into a new 1.5 mL RNase Free Collection Tube, and 50μL RNase Free water or 0.1% DEPC was added to the center of the membrane to elute RNA. The column was placed at room temperature for 5 min, and centrifuged at 12 000rpm at 4 ℃ for 2 min.

④ The first eluent was added back to the adsorption column, stood at room temperature for 5 min, and centrifuged at 12 000 rpm for 2 min. After elution, the RNA was labeled and stored in the refrigerator immediately.

⑤ After extraction, 2 μL RNA was extracted for RNA integrity detection by 1% agarose gel electrophoresis, and another 1μL was used to determine the A 260 and A 280 values of RNA concentration and purity by nucleic acid protein analyzer.

Synthesis of the first strand of cDNA

The first Strand of cDNA was synthesized according to TaKaRa's PrimeScript™ II 1ST Strand cDNA Synthesis Kit.

(1) Add the following components to 0.2 mL centrifuge tube:

RNA	1μg
Oligo dT Primer (50 μM)	1μL
RNase Free ddH_2O	10μL

(2) Then put the centrifuge tube into the PCR instrument and react at 65 ℃ for 10 min.

(3) Put the centrifuge tube on ice for 5min, add the following mixture 10μL:

5×PrimeScript II Buffer	5μl
4 μl RNase Inhibitor (40 u/μl)	0.5μL
PrimeScript II RTase (200 u/μl)	1 μL
RNase Free ddH_2O	10 μL

(4) Lightly shake and mix, react at 42 ℃ for 60 min, denaturate at 70 ℃ for 15min, and store at -20℃ for later use.

PCR amplification of the target fragment

(1) High fidelity enzyme amplification system

① The reaction system is as follows (50μL)：

5×PrimeSTAR GXL buffer	10μl
2.5 mmol/L dNTPs	4μl
10 mmol/L forward primer	2μl

10 mmol/L reverse primer	2μl
cDNA as template	5μl
PrimeSTAR GXL DNA polymerase	1μl
RNase Free ddH_2O	50μl

② After adding the sample, mix evenly and avoid bubbles and slight centrifugation;

③ PCR reaction: predenaturation 94 °C for 5 min; Denaturation at 94 °C for 30 s, 58 °C for 1.5 min, 32 cycles; It was extended 50 μL at 72 °C for 7 min and stored at 4 °C.

④ The 8 μL PCR product was detected by electrophoresis.

(2) Common 2*ES Tap enzyme amplification system

① The reaction system is as follows:

10 mmol/L forward primer	2μL
10 mmol/L reverse primer	2μL
cDNA template	4μL
2*Ex Taq enzyme	25μL
RNase Free ddH2O	50μL

② After adding the sample, mix evenly and avoid bubbles and slight centrifugation;

③ PCR reaction: pre-denaturation at 94 °C for 5 min; Denaturation was 95 °C for 30 s, xx°C for 30 s (annealing temperature was based on primer temperature ±5°C), extension at 72°C for xx min (depending on fragment size and enzyme amplification efficiency), with 25-35 cycles; 72 °C for 10 min, stored at 4 °C;

④ Appropriate PCR products were taken and detected by electrophoresis.

Agarose nucleic acid electrophoresis

(1) Size of the DNA fragment (for example, 0.35 g agarose and 35 mL electrophoresis buffer were required for 1% concentration). After weighing, the gel was included in the triangular flask, and the corresponding electrophoresis buffer was added, and the gel was shaken slowly and evenly.

(2) So that it melts evenly. Then, after it cools slightly, Nucleic acid dye (EB dye) is added and poured into the glue-making plate after shaking well, and an appropriate comb is placed on the shelf to be solidified.

(3) After complete condensation at room temperature, pull out the comb, place the agar gel in a clean electrophoresis tank and slowly pour the appropriate amount of TAE buffer;

(4) Switch on the power supply, generally 100-120 V voltage, electrophoresis 15-30 min;

(5) After electrophoresis, turn off the power, observe the electrophoresis band and its position on the gel imager, and take photos to record.

Purification and recovery of PCR products and digestion products

(1) All PCR products were sampled for electrophoresis. After electrophoresis, the target band was cut off and weighed into a clean 1.5mL centrifuge tube.

(2) To add 1-3 times the gel weight volume of sol solution, 55℃ water bath or metal bath, during which gently turn the centrifugal tube up and down until the glue block is completely dissolved, take cooling to room temperature for the next step; (for fragments smaller than 300bp, 1/2 volume of isopropyl alcohol of sol solution should be added);

(3) The centrifugal adsorption column was set in the collection tube, and the above rubber solution was added to the adsorption column. After standing for 2 min at room temperature, it was centrifuged at 12 000 rpm for 1 min at high speed. The waste liquid in the collection tube was poured out, and the adsorption column was put into the

collection tube.

(4) To add 600 μL rinse solution, stand at room temperature for 2-5 min, centrifuge at 12 000 rpm for 1min, pour out the waste liquid in the collection tube. Repeat cleaning once;

(5) The adsorption column was put into the collection tube and continued to be idled at 12 000 rpm for 2 min to remove the residual bleaching liquid on the adsorption column. Put the adsorption column on the absorbent paper and place it for 3-5 min. After drying thoroughly, proceed to the next step;

(6) Puting the adsorption column into a clean centrifugal tube, drop 30-50 μL elution buffer in the center of the column (65-70℃ preheating effect is better), place for 2-3 min, centrifuge at 12000 rpm for 2min, repeat this step for better effect.

(7) To take 2 μL of the recovered product for electrophoresis detection or concentration measurement for the next step of connection.

Enzyme digestion of vector plasmid

(1) The concentration of the sample to be cut by the enzyme was determined before digestion, and appropriate restriction endonuclease and buffer were selected according to digestion requirements. The digestion reaction was carried out according to digestion efficiency, and the system was as follows:

(1) Add the following ingredients to 0.2mL PCR tube (100 μL system):

Target segment/vector	10-20μl
10×restriction enzyme Buffer	10μl
restriction enzyme 1	4μl
restriction enzyme 2	4μl
RNase Free ddH_2O	100μl

(2) After mixing, place the mixture at the required temperature for 1-3h;

(3) Agarose electrophoresis was used to identify the digestion results and the

undigested plasmids were used as the control. If the product was digested by PCR, it could be directly recycled; if the product was digested by plasmid, it might need to be recycled after electrophoresis.

Construction of fragment and clone vectors

(1) T4 ligase method and System (10μ L)

① Add the following reagents to a 0.2mL centrifuge tube:

Target fragment	6-7μL
Vector after enzyme digestion	1-2μL
T4 DNA ligase buffer	1μL
T4 DNA ligase	1μL
ddH_2O	10μL

The amount of gene fragment and vector used for connection shall be added according to the principle of mole number 1:1.

② Mix carefully, slightly centrifugal;

③The fragment and vector were constructed overnight at 16℃.

(2) In-fusion clonal reaction linkage system:

① Add the following reagents to a 0.2 mL centrifuge tube:

Target fragment	xμL
Vector after enzyme digestion	xμL
The In-fusion	1μL
Ligase buffer	2μL
ddH_2O	10μL

② Mix carefully, centrifuge slightly, connect at 50 ℃ for 30 min, and ice bath for 5 min. The connected samples can be directly used for transformation or stored in a 4 ℃ refrigerator for short-term storage.

③ Note that regardless of the fragment size, the higher cloning efficiency can be achieved by adding 50-200 ng vector and insert fragment respectively. If the PCR fragment is less than 0.5 kb, the fragment addition amount is less than 50 ng to achieve the highest cloning efficiency. The recommended molar ratio of insert fragment to vector is 2:1.

Preparation of competent Escherichia coli

(1) *E. coli* DH5α single colony with good activity was selected and inoculated in 5mL LB liquid medium, and placed in 37℃ shaker for overnight culture;

(2) The 100 μL overnight culture liquid was transferred to 100 mL LB liquid medium, and the OD_A600 value was determined to reach 0.5-0.6 (about 3-4 hours).

(3) The culture was transferred to a 50 mL round-bottom centrifuge tube in the ultra-clean workbench for ice bath for 10 min, then centrifuged at 4000rpm/ min for 10-15 min at low temperature, and placed on ice.

(4) After discarding the supernatant, the precipitated thalli were suspended with 25 mL precooled 0.1m $CaCl_2$ and ice bath for 30 min.

(5) Centrifugation at 4 ℃ at 4 000 rpm/min for 10 min;

(6) Discard the supernatant, add 1.5 mL (30% glycerol, 0.1M $CaCl_2$) suspension, shake gently, mix thoroughly, and put the felt state into 1.5 mL PCR tube, and immediately store in the refrigerator at -80℃;

(7) Two tubes of competent cells were taken to verify the competent state activity, and 1μL sterile ddH_2O and 1μL plasmid were added into sterile plate respectively for transformation. After transformation, the quality of competent state was observed after overnight culture. There should be no colony growth on the plate containing ddH_2O, and the effectiveness of the receptive state was evaluated according to the colony number on the plate containing plasmid transformation.

Plasmid DNA transformed Escherichia coli

(1) To add all the junction products into 50 μL DH5α large intestine competent

cells, mix them gently, and take an ice bath for 30 min;

(2) Using 42 ℃ water bath for 90 s, placed on ice for 5 min;

(3) 600μL LB medium was added, mixed upside down, and incubated at 180 rpm at 37 ℃ for 45 min-1 h.

(4) Cells were collected by centrifugation and coated on LB plate containing corresponding antibiotics.

(5) Colonies were identified by PCR after inverted culture at 37℃ for 12-16h.

PCR identification of monoclonal plaque

(1) The larger monoclonal plaque was selected and placed in the PE tube of 800μL corresponding resistance culture liquid. After propagation at the suitable temperature for 6-8 hours, PCR amplification was performed as template to identify the inserted fragment. The reaction system was as follows:

Primer 1	0.5μl
Primer 2	0.5μl
template	1μl
2*Tap Mastcr Mix	5μl
ddH_2O	10μl

(2) After adding the sample, mix evenly and avoid bubbles and slight centrifugation;

(3) PCR reaction: same as above PCR reaction steps;

(4) Appropriate amount of PCR products were taken and detected by electrophoresis.

(5) The correctly identified monoclonal plaque was mixed into the corresponding resistant culture medium for propagation and extraction of plasmid.

Plasmid DNA extraction

(1) The correct monoclonal colonies were selected and identified, and 5 mL LB

medium (containing corresponding antibiotics) was added and shaken for 8-12h. Then take 1mL bacterial solution for preservation. The remaining bacterial solution were collected by centrifugation at 12000rpm and the supernatant was discarded.

(2) Adding 250 μL P1 containing RNase1 (1% glucose, 50mM/L EDTA pH8.0, 25 mM/ Tris-HCl pH8.0) into the centrifuge tube for full mixing and oscillation mixing. Then add 250 μL P2 (0.2 mol/L NaOH, 1% SDS), mix gently 6 to 8 times, make sure all the bacteria in the centrifuge tube contact with solution P2, the solution will be clear and bright. Then 350 μL P3 was added (60 mL potassium acetate, 11.5 mL glacial acetic acid, 28.5 mL H_2O per 100 mL solution), and mixed gently immediately. Flocculent precipitation may occur at this time, which does not affect the quality of plasmid.

(3) Centrifuged at 12 000 rpm for 10 min, the supernatant was absorbed and added to the adsorption column (balanced with binding buffer in advance). After centrifugation at 12 000 rpm for 1min, the waste liquid in the collection tube was poured out.

(4) Add 600 μL bleaching solution to the adsorption column, centrifuge it at 12 000 rpm for 60 s, and pour away the waste liquid in the collection tube. Repeat this step once; after that, the adsorption column was centrifuged at 12 000 rpm for 2 min to remove the residual ethanol as far as possible. Dry until alcohol-free.

(5) The adsorption column was removed and put into a sterilized 1.5 mL centrifuge tube, 90 μL eluent was added, placed at room temperature for 5min, centrifuged at 12 000 rpm for 2 min at high speed, repeat this step.

(6) taking 2 μL plasmid and use agarose gel electrophoresis to detect or measure the concentration.

Identification of recombinant plasmid by enzyme digestion

The extracted recombinant plasmid was identified by the corresponding endonuclide digestion. The digestion system was reduced to 20 μL. After 2-10h digestion, the effect of digestion was detected by electrophoresis and the plasmid recombinant results were analyzed.

Preparation of competent states of Agrobacterium tumefaciens

(1) A single colony of *Agrobacterium tumefaciens* was isolated and inoculated in 5 mL LB liquid medium (containing 50 mg/L rifampicin) for overnight shaking culture at 220 rpm at 28℃.

(2) 1 mL of the activated bacterial solution was taken for overnight culture and inoculated into 200 mL LB liquid medium containing the same antibiotics. The OD_{A600} value was about 0.5-0.8 after shaking culture at 220 rpm at 28 ℃ for 3-4 h.

(3) The bacteria solution was placed in a 50 mL round-bottom centrifuge tube and centrifuged at 3 000 g at 4℃ for 5 min to collect the bacteria.

(4) Discard the supernatant, fully suspend agrobacterium cells with 20 mM $CaCl_2$ cooled by 20 mL ice bath for 30 min, 3 000 g centrifugation at 4 ℃ for 5 min, and collect thalium;

(5) Discard the supernatant, add 1.5mL (30% glycerol, 20 mM $CaCl_2$) suspension and shake it gently to make it fully mixed and uniform, divide and divide the bacterial liquid into a 1.5 mL centrifuge tube that is exposed to cold, freeze storage at -80℃ or liquid nitrogen.

Agrobacterium mediated transformation

(1) The plasmid vector was added into 100 μL *Agrobacterium tumefaciens* cells (GV3101), and was absorbed, beaten and mixed, then ice bath for 10 min.

(2) Quick-freezing with liquid nitrogen for 5min.

(3) Quickly put in 37 ℃ water bath for 5 min, 5 min on ice.

(4) Add 800μL LB medium, mix upside down, and incubate at 180 rpm at 28℃ for 3h；

(5) Cells were collected centrifugally and coated on LB plates containing corresponding antibiotics；

(6) After 3 days of inverted culture at 28 ℃, colonies were identified by PCR.

Preparation of competent states of yeast

TaKaRa kit was purchased from Bogri Medical Technology (Beijing) Co., LTD.

(1) The bacteria solution was revived, and the preserved Y2HGold strain was taken out from the -80 ℃ refrigerator, streaked on YPDA solid medium, and placed in a constant temperature incubator at 30 ℃ for about 30 h;

(2) The mellow monoclonal colonies were selected, propagated into 2mL centrifuge tube, and incubated overnight at 200 r/min on a constant temperature shaker at 30℃.

(3) 50 mL of propagation solution, propagate the bacteria solution in the previous step into 100 mL conical flask at a ratio of 1:50, and incubate it on a constant temperature shaker at 30 ℃ for 200 r/min for 4-6 hours. After 4 hours, measure the value of OD600 every half an hour until the value of OD600=0.5.

(4) The cultured liquid was transferred to a 50 mL centrifuge tube, centrifuged at 3000*g for 10 min, and the supernatant was poured out.

(5) The thallus was suspended with 30 mL sterilized ultrapure water, centrifuged at 3000*g for 10 min, and the supernatant was poured out and repeated once.

(6) The bacteria were re-suspended with 1.5 mL LiAc/TE Master Mix and transferred to a 2 mL centrifuge tube, centrifuged at 12000 r/min for 30 s, and the supernatant was poured out.

(7) The bacteria were suspended with 600 μL LiAc/TE Master Mix to obtain competent yeast cells. The cells were divided into 1.5 mL centrifuge tubes, 100 μL for each tube, and placed on ice for use.

Note: LiAc/TE Master Mix configureuration system is as follows:

reagent	10 mL system
1 M LiAc	1.1 mL
10*TE	1.1 mL
ddH_2O	7.8 mL

2.2.6. Experimental design

Plant growth conditions

Common wheat varieties Bainong 207, Bainong 64 and Bainong Aikang 58 were provided by the College of Life Science and Technology of Henan University of Science and Technology. Banong AK58 of common wheat has excellent traits and has been sequenced. In order to facilitate the follow-up of high-quality reference sequences, the cDNA library constructed by Banong AK58 was selected in this study. General wheat Bainong 207 has excellent comprehensive traits, showing moderate resistance to powdery mildew at seedling stage and moderate resistance at adult stage. Therefore, this study selected Bainong 207 as the receptor material to verify the function of candidate genes in wheat powdery mildew, and it was convenient to obtain transgenic plants with excellent comprehensive traits.

Screening of yeast library

1. Bait vector construction

(1) Empty vector pGBKT$_7$ was extracted$_7$The vector pGBKT$_7$. Figure 2.3 shows the diagram.

(2) pGBKT$_7$ was digested with *EcoR* I and *Bam*H I double enzyme$_7$Empty vector, and recover linearized pGBKT$_7$.

(3) The cloned *Pm46* was connected to pGBKT$_7$ by seamLess connection technology$_7$.

(4) Transfer the connected vector into DH5α.

(5) The long transformants were selected for identification, and the bacterial solution identified correctly by PCR was sent for sequencing.

(6) The bacterial solution with correct sequencing was propagated and plasmid was extracted.

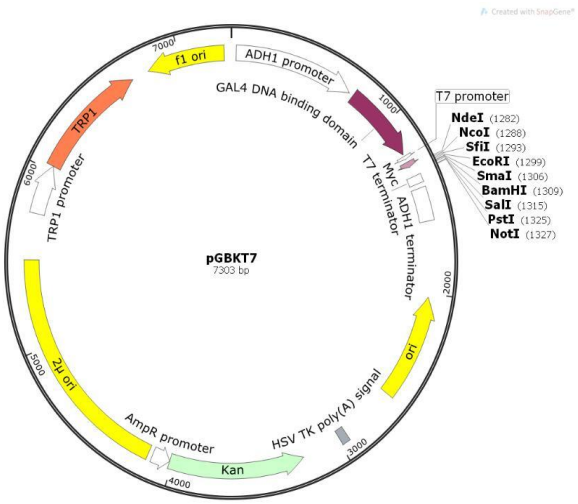

Fig. 2.3. PGBKT$_7$ Vector plasmid map [34].

2. Bait vector pGBKT$_7$-Pm46 transformed yeast Y2HGold.

(1) 1.5 μg pGBKT$_7$-Pm46 plasmid, 300 μL PEG/ LiAc master Mix, 100μL yeast competent cells were mixed and oscillated with whirlpool oscillator for 60 s.

(2) 42 ℃ water bath for 45 min, mixing up and down every 7-8 min.

(3) Centrifuge at 700 g for 5-10 min and pour off the supernatant.

(4) 100 μL 0.9% NaCl solution was added to resuspension, and SD-TRP plate was applied.

(5) Incubate at 30 ℃ in a constant temperature incubator for 3-5 days.

(6) The inverters were identified by PCR and sequenced.

Note: PEG/ LiAc Master Mix conFigureuration system is as follows:

reagent	15 mL system
1 M LiAc	1.5 mL
10*TE	1.5 mL
50 % PEG	12 mL

3. Transfer wheat cDNA library

(1) $pGBKT_7$ with correct identification is $pGBKT_7$-Pm46 bacterial solution, resuscitation and propagation. The bacteria solution was propagated to 50 mL at 1:50 and incubated at 200 r/min on a constant temperature shaker at 30 ℃ for 4-6 hours. After 4 hours, the value of OD600 was measured every half an hour until it reached OD600=0.5.

(2) Centrifuge at 700 g for 10 min, pour out the supernatant, and re-suspend the bacteria with 30 mL of sterilized ultra-pure water.

(3) Centrifuged at 700 g for 10 min, the supernatant was poured out, and the thalli were re-suspended with 1 mL LiAc/TE Master Mix, and transferred to a 1.5 mL centrifuge tube.

(4) Centrifuged at 700 g for 10 min, the supernatant was poured out, and the bacteria were re-suspended with 600 μL LiAc/TE Master Mix, that is, the sensible state $pGBKT_7$-Pm46, set on ice.

(5) 7.5 μg wheat cDNA library, 600μL sensitive $pGBKT_7$ - Pm46, 100 μL salmon extract, 2.5 mL PEG/ LiAc Master Mix.

(6) Use whirlpool oscillator to shock for 60 s.

(7) Take a water bath at 30 ℃ for 45 min, and mix it up and down every 15 min.

(8) Add 160 μL dimethyl sulfoxide, shake well, and bath at 42 ℃ for 20 min.

(9) Centrifuged at 200 g for 5 min, the supernatant was poured out and the thallus was re-suspended with 3 mL 2*YPDA.

(10) Culture at 180 r/min for 90 min on a 30 ℃ shaking table.

(11) Centrifuged at 700 g for 5 min, the supernatant was poured out, and the bacteria were suspended with 3.6 mL 0.9% NaCl solution, and the defect plate was coated.

4. Detection of decoy vector's self-activation ability

(1) The recombinant decoy vector $pGBKT_7$ was constructed$_7$-Pm46. For details, see 2.2.10.

(2) Self-activation detection of decoy vector was carried out according to yeast two-hybrid method.

5. Toxicity test of bait protein to yeast

PGBKT$_7$ constructed in PGBKT$_7$- *Pm46-B1 vector and* empty vector (yeast strain Y2HGold) was transformed into Y2HGold, and diluted at 1/10 and 1/100 on SD/ -TRP medium for 4-5 days, respectively.

Construction of yeast two-hybrid vector

Wheat cDNA was amplified using primer pGADT$_7$-GDSL F/R and primer PGBKT$_7$- Pm46 F/R, denoted as pGADT$_7$- GDSL and PGBKT$_7$- Pm46, respectively. The amplified products were detected by agarose gel electrophoresis and the results were consistent with the design results. pGADT$_7$ was connected to the pGADT$_7$ vector digested by *EcoR* I and *Xho* I (Fig. 2.4-A). Recorded as GDSL-PGADT$_7$ (pGADT$_7$ vector digestion system was conFigured), the recovered product pGBKT$_7$-Pm46 was connected to the pGBKT$_7$ vector digested by *Bam*H I and *EcoR* I enzymes (Fig. 2.4-B). It was denoted as Pm46-PGBKT$_7$ (pGBKT$_7$ vector digestion system was conFigured according to the system in 3.2.3). The GDSL-PGADT$_7$ and Pm46-PGBKT$_7$ linkage products were transformed into EScoli DH5α, and monoclonal PCR identification was randomLy selected, and the clones with target bands were sent to the company (Wuhan, Jin Kailui) for sequencing. DNAMAN software was used to analyze the sequencing results, and the correct clones were used to extract plasmids.

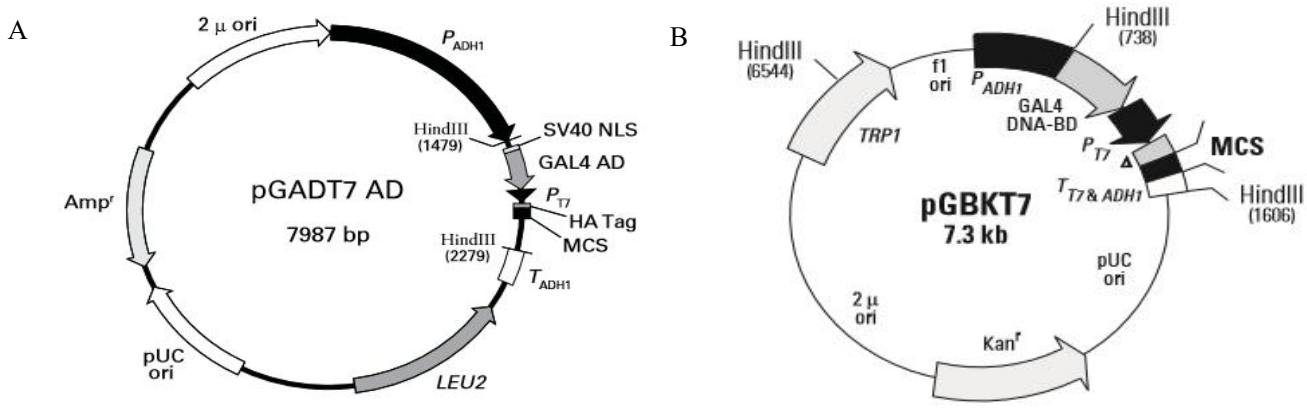

Figure 2.4. PGADT$_7$ vector map (A) and pGBKT$_7$ (B) vector map [34].

Interaction verification between TaGDSL gene and TaPm46 gene

Yeast transformation kit was used and GDSL-pGADT$_7$ and Pm46-pGBKT$_7$ vectors were transferred to yeast Y2H as experimental group. Vectors pGBKT$_7$ and pGADT$_7$, TaPm46-PGBKT$_7$ and pGADT$_7$, pGBKT$_7$ and TaGDSL-PGADT$_7$ were used as negative control, and vectors PGADT$_7$-T and PGBKT$_7$-53 were used as positive control. The transformed yeast was smeated on SD/ -Leu / -Trp, SD/ -His / -Leu / -Trp, SD/ -Ade / -His / -Leu / -Trp defective medium, and cultured in a 30 ℃ constant temperature incubator for 3-7 days. The growth of yeast was observed and photographed every day.

1. Construction and identification of bilayer fluorescent complementary expression vectors.

(1) Linearization of pXY106 plasmid vector by double digestion reaction. Meanwhile, the plasmid pXY104 was digested by (*Xba* I/ *Bam*H I) double enzyme digestion reaction. The specific steps are as follows: put the PCR tubules on ice and add reactants into the tubules: 1 ul *Xba* I restriction onuclease and *Bam*H I restriction onuclease, 2 μL Buffer(10×), 16 μL target fragment or plasmid, and then take a metal bath at 37 ℃ (plasmid for 30 min, gel recovery solution for 3 h). After the digestion, the undigested plasmid was taken as negative control and agarose gel electrophoresis was used to identify and test whether the digestion reaction was successful.

(2) In-fusion technology was used to construct pXY106-TaGDSL-NYFP and PXY104-TaPm46-CYFP fluorescence complementary expression vectors. The steps are as follows: Place the PCR tube on ice and add reactants to the PCR tube: The vector 2 μL, the target gene fragment 5 μL, the homologous recombinant ligase 1 μL and the Buffer 2 μL were gently absorbed and mixed with a pipetting device. After that, the vector was taken in a metal bath at 37 ℃ for 30 min, followed by an ice bath for 5 min. During the reaction, a timer was strictly used.

(3) the above build fluorescent complementary expression vector into cells of e. coli DH5a, will be transformed e. coli after coating on LB culture medium containing corresponding antibiotics plate, then 37 ℃ in the constant temperature incubator upside down for the night train, after being grown on the tablet of monoclonal colony, pick a

number of single colony and propagation.

(4) The selected single colonies were identified by bacterial liquid PCR reaction, and then the identified correct monoclonal colonies were sent to Wuhan Jinkarui Biological Engineering Co., LTD for sequencing. After that, the plasmid vector was extracted and sequenced correctly.

2. BIFC expression vector was transferred into agrobacterium tumefaciens competent cells.

(1) The competent cells of *Agrobacterium tumefaciens* were first taken out from -80 ℃, and then placed in a prepared ice box for thawing.

(2) Siphon 2ul fluorescent complementary expression vector into 100 μL of *Agrobacterium tumefaciens* competent cells with pipette, and gently suck and hit with pipette to mix evenly.

(3) After mixing, put it in an ice bath for 30 min in an ice box, and then put it in liquid nitrogen for 1 min (press the centrifugal tube with tweezers to ensure that it is immersed in liquid nitrogen). Take it out and put it in a 37 ℃ metal bath for 5 min. Strictly follow the timer during the reaction.

(4) In the sterilized ultra-clean workbench, the reaction products were added to 1 mL LB liquid medium (without antibiotics), sealed with plastic film, and cultured for 3 h at 220 rpm at 28 ℃ in a vibrating culture apparatus.

(5) Centrifuge the cultured bacterial liquid at 4300 rpm for 1 min, pour out most of the medium until the remaining 100 μL, mix the bottom cells with the remaining medium gently with a pipette, and then spread them on the LB solid medium plate with corresponding resistance with a coating rod. After the liquid on the surface was dried, the petri dish was covered and sealed with plastic wrap, and then cultured upside down in a constant temperature incubator at 28 ℃ for 2-3 days.

(6) After single colonies grew on LB medium plate, 3-5 single colonies were selected for propagation and culture for 8-12 h, and then PCR reaction was carried out

for identification.

3. Fluorescence detection of transient transformation of tobacco.

(1) The successfully transformed and identified positive agrobacterium solution was incubated overnight at 180 rpm at 28 ℃ in a constant temperature shaker for propagation.

(2) Centrifuge washing of the above proliferating bacteria solution to make agrobacterium cells no longer contain any antibiotics. The specific operation steps are as follows: Centrifuge for 5000r for 1 min, and pour off the supernatant. Pipette was used to add 1mL ddH_2O to it and gently suck it to make the germ cells suspended again. Centrifuge for 4200r for 1 min, and pour off the supernatant. Then 10 mL of injection medium prepared in advance was added, transferred to a conical flask and gently shaken, and cultured overnight at 190 rpm at 28 ℃ in an oscillating incubator.

(3) Preparation of injection medium. 50 mL infusion medium was formulated with 250 mg D-glucose, 5 mL MES mother solution, 5 μ L acetosyringone mother solution, 5 mL Na_3PO_4-12H_2O mother solution, and 50 mL sterile water. Mix thoroughly and store at -4℃.

(4) Spectrophotometer is used to measure the culture of bacteria liquid, if the OD600 is too high, add medium dilution, if the OD600 is too low, continue to culture, so that the OD600 of bacteria liquid between 0.5-0.8.

(5) Mix two pieces of bacterial liquid in a 1:1 volume and add it to a centrifugal tube, gently suck and mix, then let it stand for 3 h in a dark environment, so that the two vectors can fully interact with each other.

(6) Fully reacted agrobacterium solution was injected into tobacco planted 3-4 weeks in advance, and leaves with good growth were selected during injection. With disposable syringe, remove the needle) to learn the right amount of mixed bacteria liquid, with sterile gloves with left hand lightly to resist tobacco leaf positive, right hand gently to bacteria liquid in the syringe injection in tobacco leaf, from the back blades with marker stroke out the scope of the microbial proliferation, remember good bacteria

liquid injection and the label of date and the name of the gene expression vector. The injection should avoid the veins to ensure successful injection into the tobacco leaves.

(7) The injected tobacco was cultured in a greenhouse with appropriate temperature and light (light in the daytime and darkness at night) for 48 h, during which appropriate amount of water was poured every day.

(8) After 48 h culture, tissue cells of injected tobacco leaves were torn and observed by laser confocal microscope under yellow excitation light at 515 nm for light signal (yellow) in tobacco leaf cells.

The expression level of TaGDSL gene was analyzed by real-time fluorescence quantitative PCR (qRT-PCR)

(1) Expression patterns of *TaGDSL* gene in wheat under powdery mildew stress at different times.

Seedlings with the same growth potential were selected and treated with powdery mildew. After powdery mildew stress of 0 h, 3 h, 6 h, 12 h and 24 h, leaves were taken and treated with liquid nitrogen before being stored in a refrigerator at -80 ℃. According to the coding sequence of the cloned *TaGDSL* gene, primers for specific PCR were designed using Primer Premier 6.0 in the conserved region (Appendix Table 2). The expression level of *TaGDSL* gene was analyzed by qRT-PCR. The $2^{-\Delta\Delta CT}$ method was used to analyze the gene expression characteristics of *TaGDSL* gene at different time under powdery mildew stress in wheat.

(2) Tissue specific expression of *TaGDSL* gene in wheat.

Samples of young roots, young leaves, stems, leaf sheaths, stem nodes, stamens and pistils of normal wheat plants were taken, and immediately frozen in liquid nitrogen -80℃. RNA was extracted from the above tissue parts of wheat and reversely transcribed into cDNA. Gene expression characteristics of *TaGDSL* gene in different tissue parts of wheat were analyzed by qRT-PCR.

Barley stripe mosaic virus (BSMV) - VIGS inoculation and TaGDSL function analysis

Virus induced gene silencing (VIGS) is an effective tool for plant gene deletion

and primers (Appendix Table 2) were designed. Gene function research methods such as RNA interference (RNAi) or genome editing have the advantages of fast, simple, no inefficient and cumbersome genetic transformation process, avoiding genotype-specific effects among different genetic backgrounds, and timely targeting tissue-specific genes.

Amplification of cDNA fragments of phytoene desaturase gene (*TaPDS*) and *TaGDSL* was done by oligonucleotide primers with *Sma*1 sites that had been reverse-inserted into RNAγ of BSMV to establish BSMV cDNA clones: *TaPDS* and *BSMV: TaGDSL* for gene silencing. In vitro transcription assays as well as intermixture formulae of RNAβ, RNAα, RNAγ and RNAγ-derivative clones were performed as reported by Gunupuru et al. [172, 173, 174]. Then, for inoculation, 10 mL of the intermixture werr rubbed gently on the 2nd leaf surface of the two-leaf stage plants. At five days post inoculation, qRT-PCR was conducted to verify the transcript patterns of *TaPDS* and *TaGDSL*. Then, for 14 days, they were treated with powdery mildew. Maximum root lengths were determined prior to and after treatment. Roots and leaves of inoculated seedlings were separately harvested, rinsed using deionized water for physiological analyses and dried for 72 h at 80 °C to a constant weight. Treatments were repeated six times. The primers for this assay are shown in Appendix Table 2.

Construction of TaGDSL gene expression vector in wheat

pTCK303 vector contains two specific polyclonal sites (Fig. 2.5), which can be inserted into two reverse repeat sequences. After two reverse complementary sequences can be inserted between the two polyclonal sites, hairpin ring RNA molecule (dsRNA) can be transcribed and formed. DsRNA is digested into 21-23bp siRNA fragment. Driven by helicase and endonucase, siRNA specifically degrades mRNA, resulting in the silencing of endogenous target genes and thus inhibiting the expression of target genes. pTCK303 vector Figure is shown below.

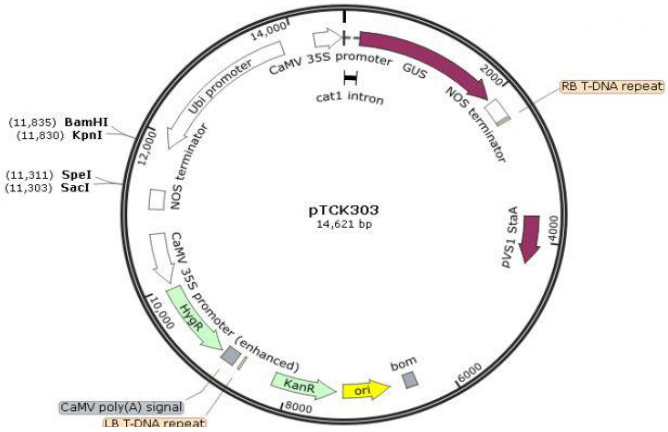

Figure 2.5. Plasmid map of pTCK303 expression vector

(1) Construction of RNA interference (RNAi) vector.

DNAMNAN 6.0 software was used to analyze the enzyme site sequence of TaGDSL gene, and primers (Appendix Table 2) were designed based on the polyclonal site of RNA interference vector pTCK303.

① The *TaGDSL* gene was amplified by Appendix Table 2 primers with *Spe*1 and *Sac*1 restriction endpoints. The same restriction endonuclease was used to cut the pTCK303 vector. *TaGDSL* gene was amplified and purified by gel electrophoresis. The recovered product of plant RNA interference vector *pTCK303-TaGDSL* and the recovered product of *TaGDSL* silencing gene *Spe*1 and *Sac*1 terminal were ligated and transformed into DH5α receptor cells, which were coated on LB solid medium containing kanamycin. Cultured at 37 ℃ for 12-16 h, positive clones were screened out.

② The *TaGDSL* gene with reverse fragments of *BamH* 1 and *Kpn* 1 was amplified by the same method, and the *pTCK303-TaGDSL*-RNAi plasmid was digested by *Bam*H I and *Kpn* I. The plasmid was purified and recovered by gel electrophoresis. The plasmids were identified by *BamH* 1 and *Kpn* 1 digestion. Fragment size was detected by 1.5% agarose gel electrophoresis. The correct vector plasmid verified by enzyme digestion was sequenced, and subsequent experiments were carried out after the sequencing was completely correct.

(2) Construction of overexpression (OE) vector.

After analyzing the restriction sites of *TaGDSL* gene sequence with DNAMNAN 6.0 software, primers (Appendix Table 2) with *Xba* 1 and *Sac* 1 restriction sites were

designed by combining the polyclonal sites of plant overexpression (OE) vector pCAMBIA1301 (Fig. 2.6). *TaGDSL* amplified by primers was linked to the recovered product of pCAMBIA1301 vector digested by the same enzyme. Enzyme digestion is carried out in the same way. The ligands were transformed into DH5α cells and coated with LB solid medium containing kanamycin. Cultured at 37℃ for 12-16 h, positive clones were screened for monoclonal identification. After monoclonal identification, plasmid was extracted by recombinant vector. The recombinant plasmid was identified by *Xba* 1 and *Sac* 1 double digestion. The empty vector pCAMBIA1301 was used as control. The vector was named pCAMBIA1301-TaGDSL.

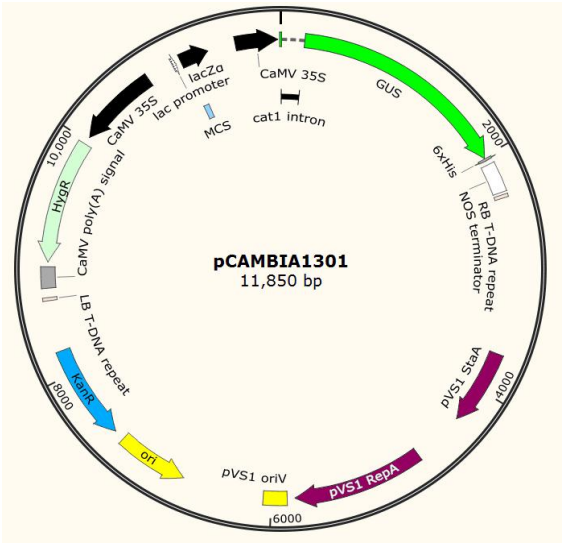

Figure. 2.6. Plasmid map of pCAMBIA1301 expression vector map [34].

(3) Plasmid DNA was transformed into *Agrobacterium* strains.

The successfully identified *pTCK303-TaGDSL* and *pCAMBIA1301-TaGDSL* plasmids as well as pTCK303 and pCAMBIA1301 empty plasmids were transformed into agrobacterium tumefaciens receptor cell GV3101. After transformation, the plasmids were coated on plate containing Kanamycin and rifampicin resistance. The monoclonal clones were identified. After successful identification, the positive strains were identified and preserved.

(4) *Agrobacterium*-mediated genetic transformation.

The constructed vector was transformed into the *Embryonic calli* of wheat Bainong207 with *A. tumefaciens* (strain EHA105)-mediated transformation according to

the method [175, 176]. Then sent to company (Wuhan GeneCreate Biological Engineering Co., Ltd.) for genetic transformation of wheat and transgenic wheat will be obtained.

(2) Screening T0 generation transgenic plants.

According to GUS (primer in Appendix Table 2) staining, the wheat positive T0 generation plants were preliminatively screened and screened at the molecular level. The CTAB method was used to extract and screen the DNA of wheat plants for subsequent identification. Using genomic DNA of T0 generation of positive plants as template, amplification primers were designed according to GUS reporter gene on the vector, and molecular level tests were performed to identify positive plants. 10 μL PCR products were taken and 1% agarose gel electrophoresis was performed. Untransformed plants and empty plasmids were used as controls to identify positive plants and label them.

The transgenic plants were cultured in an artificial climate chamber (12 h photoperiod at 55 Klux light intensity, 65% relative Humidity, temperature 20-25 °C). Culture to maturity, after maturity, harvesting and preservation of individual plants. Subsequent gene function analysis was conducted in T2 generation wheat.

Functional analysis of TaGDSL gene in transgenic wheat

(1) Bioinformatics analysis of *TaGDSL* gene.

BLASTP alignment was conducted through NCBI website, and the sequences with high similarity were downloaded. Neighbor-joining phylogenetic tree was constructed by combining the downloaded sequences with MEGA 7.0 pairs. According to the cloned *TaGDSL* gene sequence, the protein coding region (CDS) was identified and translated into protein using BioXM2.7. Use online tools ProtParam ExPASy site (https://web.expasy.org/protparam/) to analyze the basic physical and chemical properties of protein, including the theory of amino acid composition, relative molecular weight and pI value, etc.; Using ProSWEETale tools to analyze protein hydrophobicity (http://ca.expasy.org/tools/proSWEETale.htmL); Using TMpred tool (http://www.cbs.dtu.dk/services/TMHMM-2.0/) to predict transmembrane region and

across the membrane direction and signal peptide analysis.

(2) Transgenic plant treatment.

The effects of *TaGDSL* expression on powdery mildew in wheat grains and straw were studied by pot experiment. Transgenic plants and WT were transplanted into pots (10 cm × 24 cm × 14 cm) containing 500g soil. The seedlings were first cultured in a growing chamber (12 h photoperiod at 55 Klux light intensity, 65% relative humidity, temperature 20-25 °C) for 3 days, then transferred to natural light for growth until harvest, and then the parts, including the roots, leaves, nodes, husk, and grains, 80°C were dried for 3 days.

(3) *TaGDSL* gene expression analysis.

Quantitative primers for *TaGDSL* gene and specific primers (Appendix Table 2) for wheat reference gene were designed. In the same culture environment, when wheat sprouted into three leaves, cDNA of T2 generation positive plants was extracted, and untransformed plants were used as control. Fluorescence quantitative analysis was used to analyze the expression of *TaGDSL* gene in T2 generation plants. The differences of plant expression levels were analyzed, and the single plants with high silencing levels of *pTCK303-TaGDSL* gene and high expression levels of *pCAMBIA1301-TaGDSL* gene were selected for powdery mildew stress experiment.

(4) Identification of transgenic plants.

GUS (β-glucosidase) gene is not found in many plants, and its detection method is very simple, rapid, sensitive and stable, so GUS gene is widely used as a reporter gene in transgenic plants.

① Preparation method of GUS staining solution: X-gluc staining solution was 50X concentrated solution, and an appropriate amount of X-GluC and GUS Buffer were thoroughly mixed at a ratio of 1:50 to prepare GUS staining solution. The staining solution was best prepared for use now and can be stored at 4 ℃ for 3 days in a short time.

② GUS staining procedure: An appropriate amount of wheat leaves were cut into a

200 μL PCR tube, and 100 μL GUS staining solution was added to the PCR tube to completely immerse the wheat leaves. PCR tubes containing wheat leaves and GUS dye solution were placed in an incubator at 37 ℃ for 1 day. The GUS dye solution was extracted, and the same volume of 70% ethanol was added, and then placed in the incubator at 37 ℃ for 1 day. Until completely remove the chlorophyll in the leaves. GUS staining was observed.

(5) Powdery mildew identification and gene silencing efficiency analysis.

The leaves of wheat plants with virus phenotype were cut and inoculated into the middle and lower parts of wheat leaves. The remaining parts of wheat leaves were stored in a refrigerator at -80 ℃. RNA was extracted and gene silencing efficiency was analyzed. The wheat powdery mildew leaves used for inoculation were carefully placed on 6-BA plates, and fresh wheat powdery mildew spores were evenly shaken off the placed leaves, then the disease of wheat leaves was observed regularly. Two biological replicates were performed for each gene.

Statistic analysis of data

In this study, we used three biological replicates for each treatment in an experiment. Significant differences between treatments were compared by Duncan's multiple range tests ($P < 0.05$) using ANOVA in SPSS software (version 20.0; SPSS, Inc.,Chicago, IL, USA).

Conclusions to Chapter 2

1. Experiment 1 was carried out in conditions favorable for winter wheat growing. As initial material, In this study, the resistance to powdery mildew was identified in 86 samples of winter wheat that participated in regional tests from different breeding establishments, in order to provide a basis for further research and utilization of these materials.

2. In experiment 2, molecular biology approach was used to study the molecular mechanism of resistance to powdery mildew in winter wheat. *Gdsl-type* (*GDSL*) gene was functionally characterized in wheat. Method of gene discovery and functional validation in *TaGDSL* gene homologous cloning and expressions analysis was used. We

assessed the correlations between *TaGDSL* gene expressions in wheat and resistance to powdery mildew. It is necessary to describe the method of gene discovery and functional validation in order to confirm and understand the reliability of subsequent experiments.

CHAPTER 3

COLLECTION STUDY AND OBTAINING THE SOURCE MATERIAL OF WINTER WHEAT WITH HIGH ABILITY OF RESISTANCE TO POWDERY MILDEW

3.1. Identification results of powdery mildew resistance of wheat at adult stage

There were no immune or near-immune materials in the new wheat samples. 4 high-resistance materials (4.7%); Medium resistance material (2), accounting for 2.3%; 4 were medium sensitive materials, accounting for 4.7%; There were 78 highly sensitive materials, accounting for 88.3%. Overall resistance is not good (Fig. 3.1).

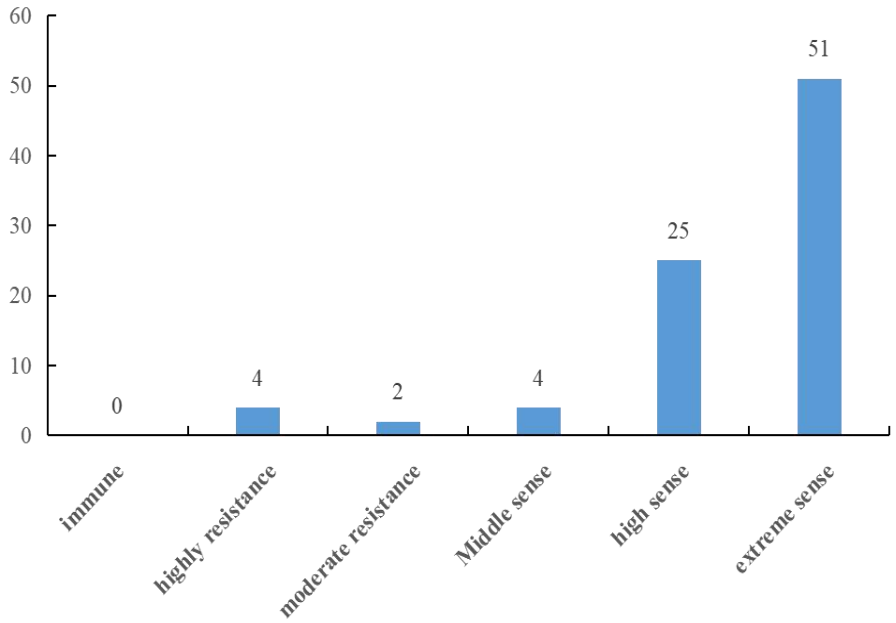

Figure 3.1. Identification results of resistance to wheat powdery mildew in 86 tested varieties.

After the spring of 2020, the climate conditions were suitable for the infection, propagation and prevalence of powdery mildew. The occurrence degree of powdery mildew in wheat was serious, and the control variety Xiaoyan 22 (CK1) and the auxiliary control variety Xinong 979 (CK2) showed high susceptibility to powdery mildew in the field. The overall resistance of 86 tested materials was poor, and there were no immune or near-immune new samples. Only four high-resistance materials, namely Baojingmai 166, Lingke 686, Xinong 837 and Huimai858, accounted for 4.7% of the total materials (Table 3.1.). There were two medium-resistant samples, Shandao 198 and Baojing 718, which accounted for 2.3% of the total test materials (Appendix Table 4). The moderately susceptible lines of mildew were Mengmai 101, Longmai 838, Xinong 943 and Sichun 519, accounting for 4.7% of the total (Appendix Table 4). The remaining 78 samples, including Kenmai 22 and Mengmai 59, showed high resistance in the field in the 2020 powdery mildew resistance test, accounting for 88.3% of the total material (Table 3.2.), and the overall resistance was not good (Appendix Table 3).

Table 3.1. Results of High-resistance Identification of Wheat Test Lines from 2020 to 2021.

Line	Powdery mildew	
	Reaction	Type
CK1	9	ES
CK2	9	ES
7	1	HR
14	1	HR
21	1	HR
43	1	HR

Not: *ES: Easy susceptible*
HR: High resistance

Table 3.2. Results of High-susceptible Identification of Wheat Test Lines

from 2020 to 2021.

Line	Powdery mildew		Line	Powdery mildew	
	Reaction	Type		Reaction	Type
CK1	9	ES	44	7	HS
CK2	9	ES	45	7	HS
2	7	HS	47	7	HS
6	7	HS	48	7	HS
12	7	HS	50	7	HS
13	7	HS	53	7	HS
15	7	HS	54	7	HS
16	7	HS	56	7	HS
17	7	HS	57	7	HS
18	7	HS	58	7	HS
19	7	HS	59	7	HS
20	7	HS	62	7	HS
22	7	HS	63	7	HS
23	7	HS	64	7	HS
24	7	HS	65	5	MS
25	7	HS	66	7	HS
26	7	HS	67	7	HS
31	7	HS	68	7	HS
32	7	HS	69	7	HS
33	7	HS	71	7	HS
35	7	HS	73	7	HS
36	7	HS	74	7	HS
37	7	HS	75	7	HS
38	7	HS	78	7	HS
39	7	HS	82	7	HS
40	7	HS	83	7	HS
41	7	HS	86	7	HS
42	7	HS	-----	-----	-----

Not: *ES: Easy susceptible*
HR: High susceptible

3.2. Powdery mildew resistance line pedigree and resistance performance

A total of 86 new wheat samples were selected to identify powdery mildew resistance in the adult stage. Through the identification results, the new samples with medium or above sensitivity were screened out, and the genealogy and the sources of resistance genes were analyzed (Table 3.3).

Table 3.3. Pedigree and resistance performance of new resistant lines

line	Pedigree	Infection Type
7	Jimai22/4520	HR
14	Yumai34/Xinong686	HR
21	Zengmai366/(Xinong9814/D9401)	HR
42	Xinong501/Liangxing66	HR
4	Xinong979/Zhoumai16	MR
8	BJN171/BJM172	MR
5	Zhoumai18/Mengxuan205-4	MS
84	Yumai47/Xiaoyan22	MS
65	Xiaoyan926A/Xiaoyan22	MS
61	Aikang58/Xumai1-97	MS

Not: *HR: High resistance*
MR: Medium resistance
MS: Medium susceptible

3.3. Gene source analysis of resistance to powdery mildew in wheat

Due to the 2020 wheat powdery mildew pandemic, among the 86 new wheat lines tested, only 4 samples showed high resistance, 2 samples showed moderate resistance, and 4 samples showed moderate sensitivity. The remaining 78 tested materials were all highly susceptible to powdery mildew, and the overall resistance was poor. Through the identification results, a new wheat strain with moderate or above sensitivity was screened, and its genealogy was analyzed to analyze its possible white powder resistance genes. The analysis results are as follows:

New wheat line	Type	Parents	Type	Parents	Possible source of resistance
Lingke686	HR	Xinong686	HS	Yumai34	Yumai34
Xinong627	HR	Xinong501	MS	Liangxing66	Liangxing66
Shandao198	MR	Xinong979	HS	Zhoumai16	Zhoumai16
Longmai838	MS	Xiaoyan22	HS	Yumai47	Yumai47
Xichun519	MS	Chunmai1-97	MS	AK58	AK58

3.4. Comprehensive evaluation results

Combined with the results of resistance to powdery mildew, a total of 9 new samples with moderate or higher resistance to powdery mildew were selected (Table 3.4). They are: Aikang58, Baojinging 166, Baojinging 718, Lingke 686, Xinong 837, Xinong 627, Sichun 519, Longmai 838 and Xinong 943. These materials can be used as excellent germplasm resistant to powdery mildew.

Table 3.4. Disease resistance results of new disease-resistant lines

Line	Powdery mildew	
	Reaction	Type
AK58	0	HR
Baojingmai166	2	MR
Baojingmai718	0	HR
Lingke686	1	HR
Xinong837	0	HR
Xinong627	1	HR
Xichun519	0	HR
Longmai838	2	MR
Xinong943	2	MR

Not: *HR: High resistance*
MR: Medium resistance

Conclusions to chapter 3

The results showed that only 11.7% of the 86 new wheat lines identified showed good resistance in the 2020 powdery mildew resistance test, among which 4 showed high resistance (Baojingmai 166, Lingke 686, Xinong 837, Huimai858), 2 showed moderate resistance and 4 showed moderate resistance. The overall resistance was not good. The origin of resistance genes of new wheat samples with excellent resistance to disease was analyzed by genealogy analysis. The results showed that the resistance genes of some of the new powdery mildew resistance lines might be derived from Aikang 58, Jimai 22, Yumai 34, Zhengmai 366, Liangxing 66, Zhoumai 16, Zhoumai 18, Yumai 47 and Xiaoyan 926A. The results of disease resistance identification and analysis of possible sources of disease resistance genes provide scientific basis for further research and application of these materials.

CHAPTER 4

THE DISCOVERY AND FUNCTIONAL ANALYSIS OF THE TaGDSL GENE

4.1. Screening of interacting proteins

4.1.1. *Pm46* gene cloning

A CDS sequence of Chinese spring was obtained by searching the genetic information of *Pm46* on NCBI. As a reference sequence, primers were designed at the 5 'and 3' ends.The full length of *Pm46* was amplified from the cDNA of Bainong 64.The amplified *Pm46* was then connected to the PMD19-T Vector and transferred into *E.coli* for identification and sequencing.The full length of cDNA of *Pm46* was 1545 bp, encoding 514 amino acids. Figure 4.1 shows the electrophoretic results of PCR.

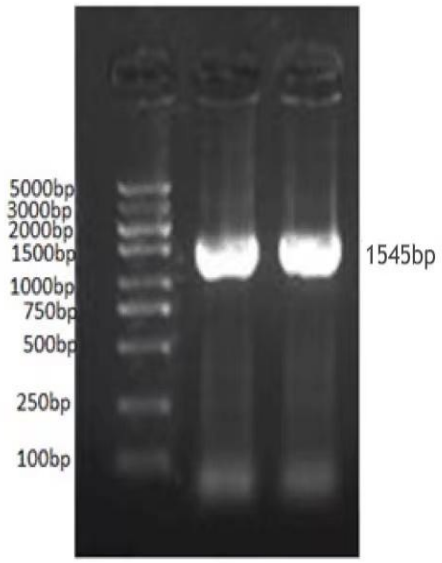

Figure 4.1. Electrophoresis of *TaPm46* amplification.

4.1.2. Bait pGBKT$_7$-TaPm46 vector construction and validation

In order to further verify the function of *TaPm46* gene and screen its interacting proteins, GXL enzyme was used to conduct PCR amplification with cDNA obtained from reverse transcription as template. PCR products were detected by 1.5 % agarose gel electrophoresis and about 1545 bp fragment was obtained (Fig.4.2a), which was

recovered to obtain the target gene p*GBKT7-Pm46*. After digestion, pGBKT$_7$ plasmid was recovered and connected with seamless clonase. After transformation into *E.coli DH5a*, monoclonal colonies were selected for identification. The bacterial solution with clear and bright bands consistent with the target size was selected and sent for sequencing.

The recombinant vector was verified by *EcoR* I and *Bam*H I double enzyme digestion. Electrophoresis was performed on 1 % agarogase gel and the results were observed. A 1545 bp band could be observed after enzyme digestion (Fig.4.2b), indicating that the recombinant bait vector *pGBKT7-Pm46* was successfully constructed, indicating that the direction and reading frame of *TaPm46* gene and the bait vector were correct. There was no base mutation in the gene coding region (Fig.4.2c).

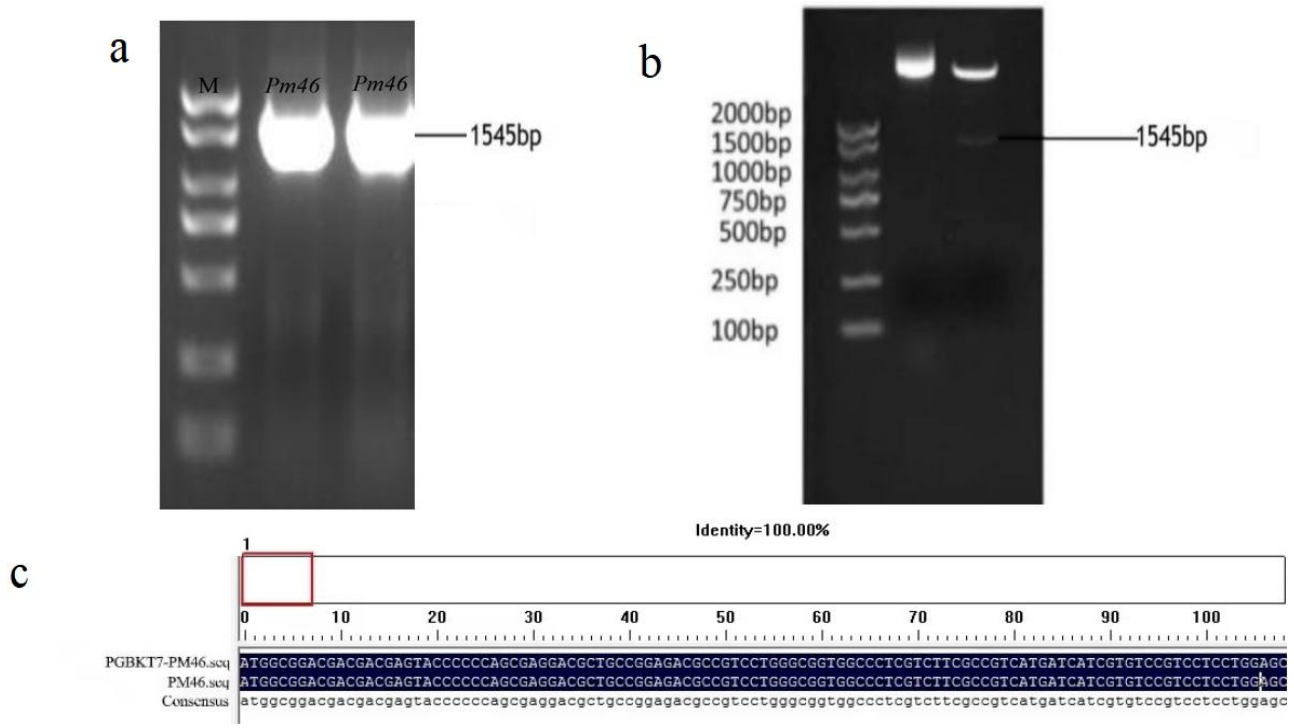

Figure 4.2. Electrophoresis of *TaPm46* amplification: (a) PCR electrophoresis of *pGBKT7-Pm46* vector transformed *E.coli* transformants; (b) Enzyme digestion verification of *pGBKT7-Pm46* gene; (c) Comparison of *pGBKT7-Pm46* gene sequences. M: DNA maker.

4.1.3. Bait vector self-activation detection

The pGBKT$_7$ vector contains the GAL4 DNA-binding domain (DNA-BD), which can transcribe the autosynthetic protein. Therefore, it is necessary to verify that the

fusion protein product expressed by the decoy vector inserted into the yeast two-hybrid system has no self-activation effect on the reporter gene and exclude false positives in the yeast two-hybrid system. Since pGBKT$_7$ contains the Trp synthesis gene, the Y2H Gold yeast strain transformed into pGBKT$_7$-Pm46 vector could only grow on SD / -Trp deficient medium. If the yeast grew on SD / -Trp /X-a-Gal /ABA deficient medium, it indicated that the gene had self-activation ability. The gene could not be used to study yeast two hybrid protein interaction.

The pGBKT$_7$-Pm46 was transformed into Y2HGold yeast to verify whether the decoy vector had self-activation ability. The results showed that bait vector (pGBKT$_7$-Pm46) could only grow normally on SD / -Trp deficient medium, but not on SD / -Trp /X-a-Gal /ABA deficient medium (Fig. 4.3). That is, the constructed decoy vector has no self-activation ability and can be used for subsequent experiments.

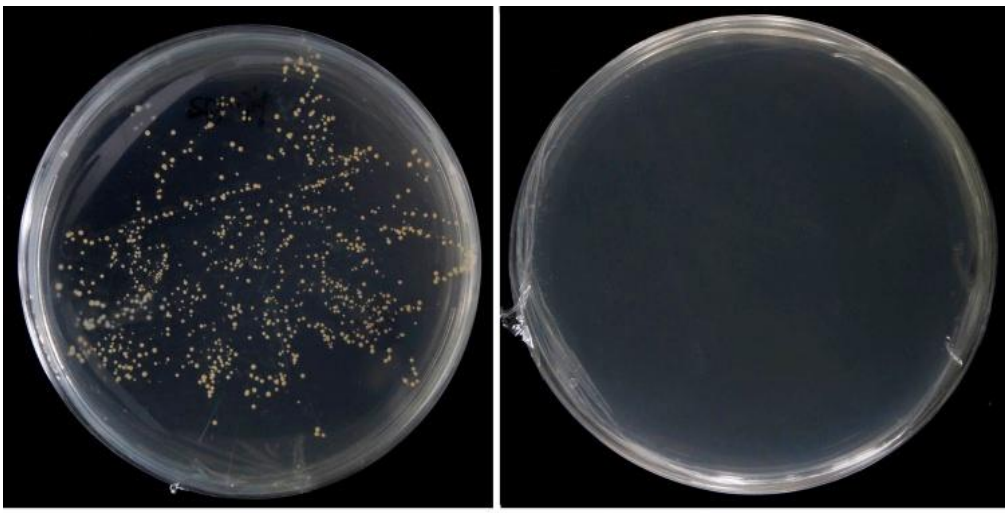

Figure 4.3. Schematic diagram of self-activation detection of bait vector.

4.1.4. Bait vector toxicity test

In the process of library screening, it is necessary to detect the toxicity of the constructed bait vector to prevent the yeast cells from being affected. If the toxicity is produced to the yeast cells, the yeast growth is poor at the late stage of yeast growth, and the concentration of yeast cannot meet the minimum requirements of library screening.

The Y2HGold yeast transformed by pGBKT$_7$-Pm46 vector and pGBKT$_7$ empty vector were diluted and cultured on SD / -Trp medium with 1/10 and 1/100, respectively, and colonies appeared 4-5 days later. The number of monoclones grown from pGBKT$_7$-Pm46 containing bait vector pairs was roughly the same as that from pGBKT$_7$, and both colonies were larger than 2 mm (Fig. 4.4), indicating that bait vector pGBKT$_7$-Pm46 had no toxic effect on yeast Y2HGold and could be used for screening yeast two-hybrid.

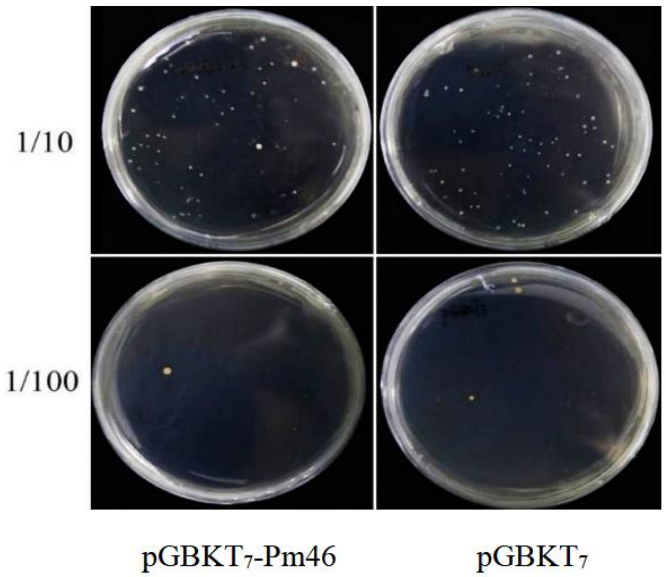

Figure 4.4. Toxicity detection of bait vector pGBKT$_7$-Pm46 to Y2HGold strain.

4.1.5. Yeast double hybrid screening interactive proteins

The prerequisite of yeast two-hybrid system to screen interacting proteins is to have a complete wheat cDNA library. Therefore, the abundance of wheat cDNA library was detected by PCR using pGADT$_7$ vector sequences as primers. The results are shown in Figure 4.5, indicating that the constructed wheat cDNA library can be used for the next step of interaction screening.

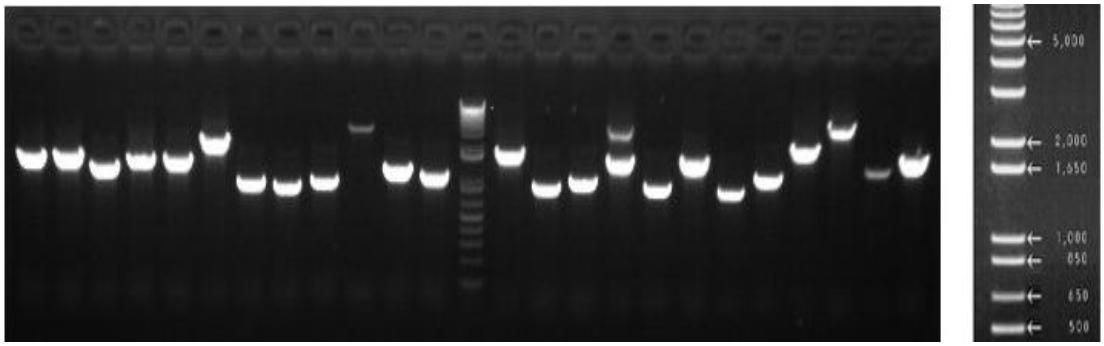

Figure 4.5. Schematic diagram of wheat cDNA library abundance detection.

Construct pGBKT$_7$-Pm46 was transferred into wheat cDNA library and cultured on SD / -Trp, SD / -Leu / -Trp, SD / -Ade / -His-Leu / -Trp defect plates for 3 days, as shown in Figure 4.6.

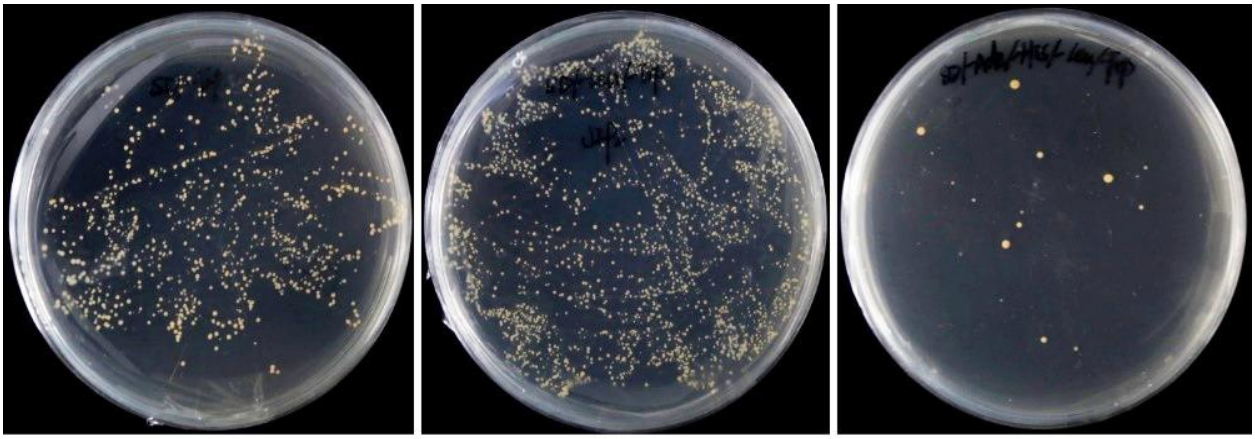

Figure 4.6. SD / -Trp, SD / -Leu / -Trp, SD / -Ade / -His-Leu / -Trp transformants.

Toxicity test and yeast self-activation test indicate that this system can be used to screen proteins interacting with *Pm4*6 gene. The transforter in Figure 4.7 is the product of the combination of bait protein and prey protein, and the sequence on pGADT$_7$ vector is used as primer to detect the transforter. PCR electrophoresis results are shown in Figure 4.7.

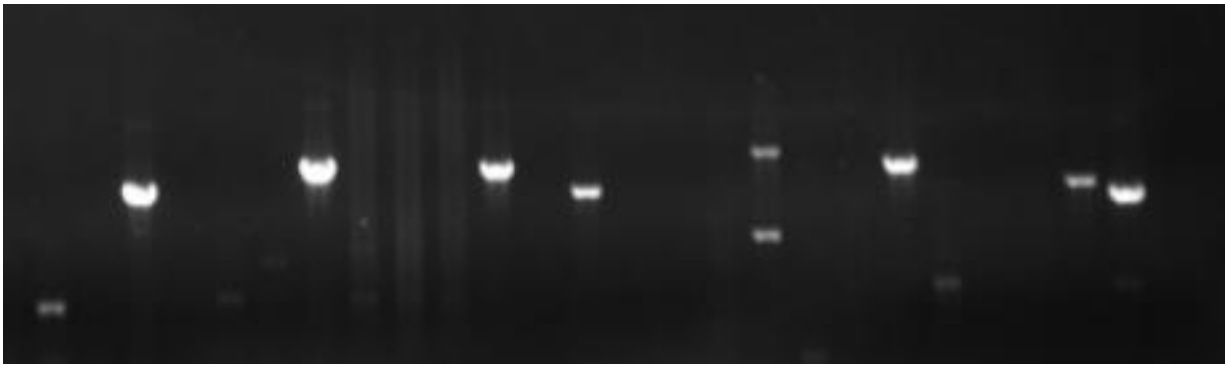

Figure 4.7. SD / -Trp, SD / -Leu / -Trp, SD / -Ade / -His-Leu / -Trp transformant identification electrophoresis.

Toxicity test and yeast self-activation test showed that the system could be used to screen proteins interacting with *pGBKT$_7$-Pm46* gene, indicating that these transformants were the combination products of bait protein and prey protein. The sequence on pGADT$_7$ vector was used as primers to detect transformants, and 12 yeast-positive clones were obtained (Table 4.1). The positive clone plasmid was extracted and transferred into *E.coli* for sequencing. A total of 4 genes were obtained after repeated cloning was removed by sequencing. NCBI was used to

analyze these genes and remove redundant sequences to obtain 4 different gene fragments that interact with *pGBKT7-Pm46* and may be related to plant immunity, which are as follows: Lipase Protein, ribosome Protein, cysteine transmembrane Protein and a chlorophyll-binding Protein named PIP (Pm46 Interaction Protein) 1-4 (Table 4.1).

Table 4.1. Some of the putative interaction clones by screening the Yeast Two Hybrid library using *TaPm46* as a bait

Each fragment	**Protein function prediction**
Interact fragment	**Predicted function**
GDSL	esterase/lipase
L18 mRNA	ribosomal protein
CYSTM1	Cysteine transmembrane protein
LHCP	Chloroplast binding protein

4.2. Basic Information Analysis of *GDSL* gene

4.2.1. Extraction of total RNA from wheat

The extraction quality of total RNA is the premise to determine the results of this experiment. Extracting high purity and complete total RNA is an essential guarantee for RT-PCR. After RNA was extracted from wheat leaves, 0.8% Agar gel electrophoresis was used to detect the total RNA quality, as shown in Figure 4.8. The results show that the extraction effect is good and the integrity is good. The OD 260/280 values detected by the UV spectrophotometer ranged from 1.7 to 2.0, indicating that the RNA samples obtained in this experiment had high purity, which could be used for subsequent reverse transcription experiments and amplification fragments to construct vectors.

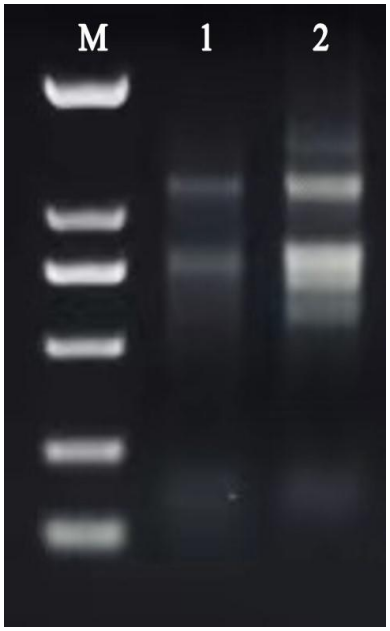

Figure 4.8. RNA detection by electrophoresis: M is standard 2000 + Marker; 1-2 are RNA samples.

4.2.2. Full-length cloning of wheat *TaGDSL* gene

According to the conserved sequence of wheat *TaGDSL* gene in NCBI and the full-length design of specific primers, the target gene was amplified by RT-PCR using wheat cDNA as a template. The amplified products were analyzed by 1.5% agarose gel electrophoresis.The results showed that the size of the amplified band was the same as that of the target fragment (Figure. 4.9). After the electrophoretic gel was cut, the target bands were recovered with an ordinary agarose DNA recovery kit. The recovered product is linked to pMD19-T and transformed into competent *E.coli.* cells. After monoclonal verification, the correct monoclines were sequenced. The monoclonal plasmid with correct sequencing results was propagated and recorded as pMD-19T-*TaGDSL* plasmid. Sequencing results showed that the entire length of the gene was 1269 bp, which was consistent with the sequence in the GenBank database.

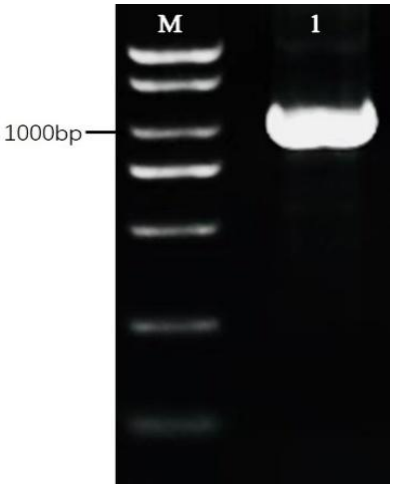

Figure 4.9. *TaGDSL* gene PCR amplification: M - standard 2000 + Marker; 1 - Amplified band.

4.2.3. Sequence analysis and bioinformatics analysis of wheat *TaGDSL* gene

In order to further explore the phylogenetic process of the *TaGDSL* gene and understand the genetic relationship of this gene, amino acid sequences of durum wheat and barley and other related proteins in the *TaGDSL* gene family were searched and downloaded from the NCBI database (Fig. 4.10). A phylogenetic tree was constructed by using MEGA7.0 (Fig. 4.11). In the figure, wheat *TaGDSL* was most closely related to durum wheat *TdGDSL* and barley *HvGDSL*.

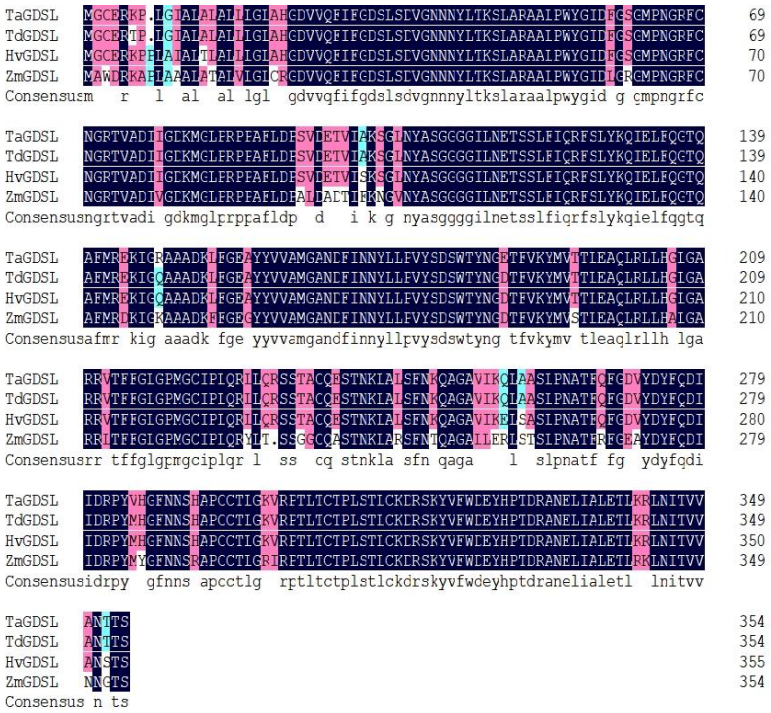

Figure 4.10. Homologous analysis of *GDSL* amino acid sequences: Ta - *Triticum aestivum*; Td - *Triticum Durum*; Hv - *Hordeum vulgare*; Zm - *Zea mays*.

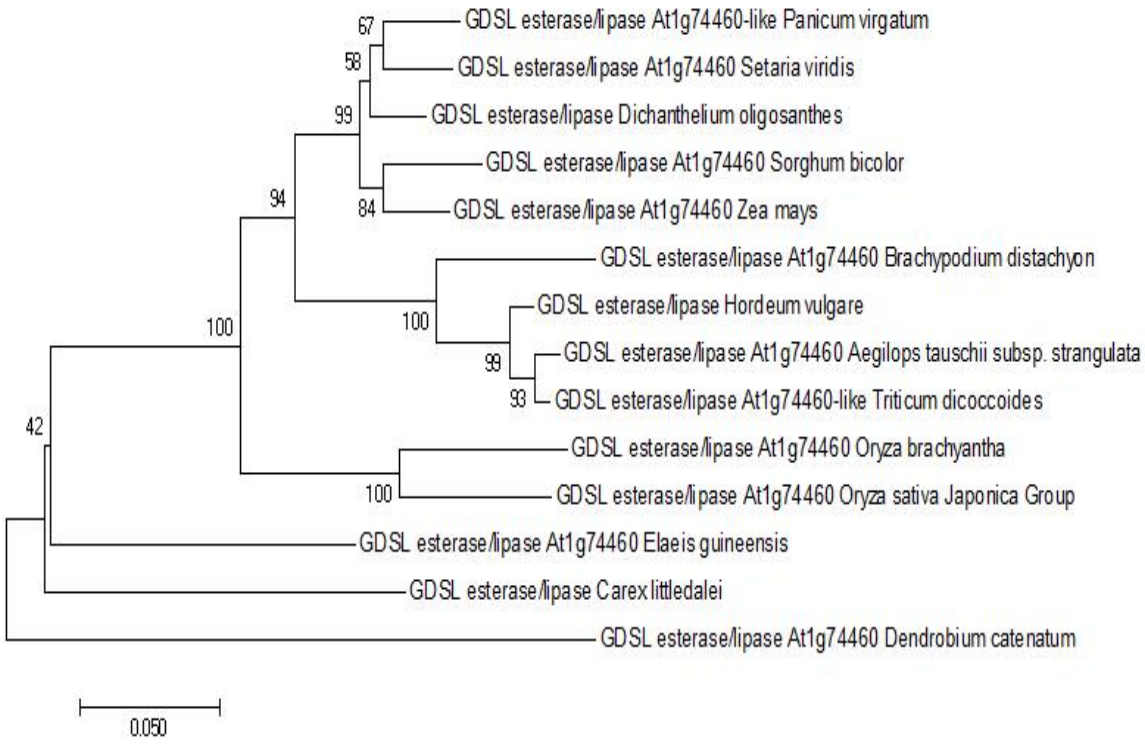

Figure 4.11. Phylogenetic tree analysis of *GDSL* genes in other species.

Protaparam analyzed the physical and chemical properties, and its molecular formula was $C_{3669}H_{6071}N_{1269}O_{1502}S_{422}$, its relative molecular weight was 38.99 kD, and the theoretical isoelectric point Pi was 8.19. The SINGALP 4.1 analysis showed that the sequence had a signal peptide. However, according to the online analysis of the TMHMM gene Server v.2.0, the *TaGDSL* protein has a transmembrane structure region (Fig.4.12). Using Extasy online website (http://web.expasy. org/cgi-bin/protscale/protscale.pl?1), the hydrophilicity/hydrophobicity of the amino acid sequence of this gene was analyzed (Fig.4.13). The hydrophobic and hydrophilic regions coded by *TaGDSL* appear alternately and evenly distributed. Therefore, we predicted that the *TaGDSL* protein was hydrophilic.

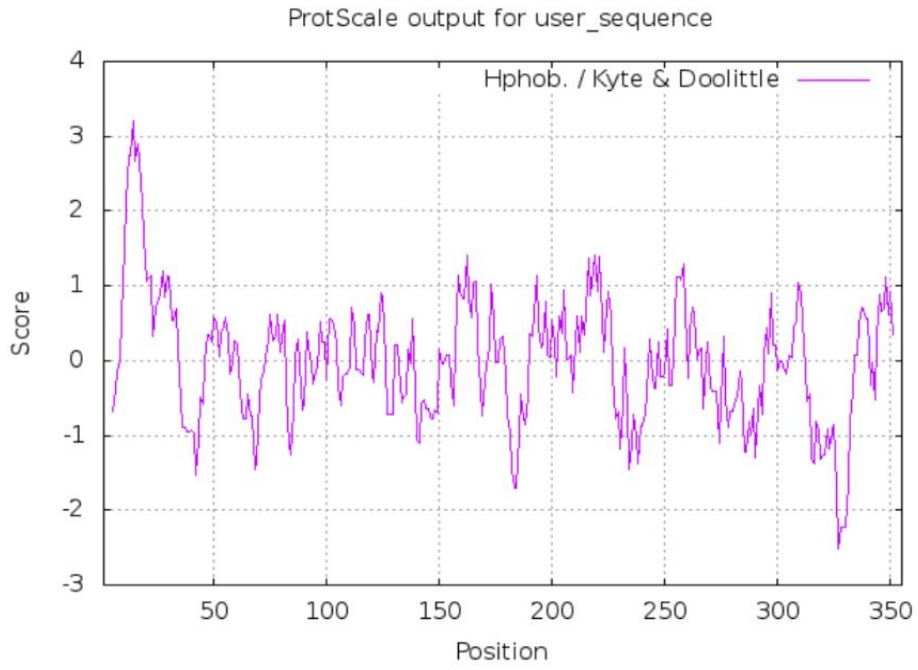

Figure 4.12. Hydrophilic and hydrophobic analysis of *TaGDSL* gene.

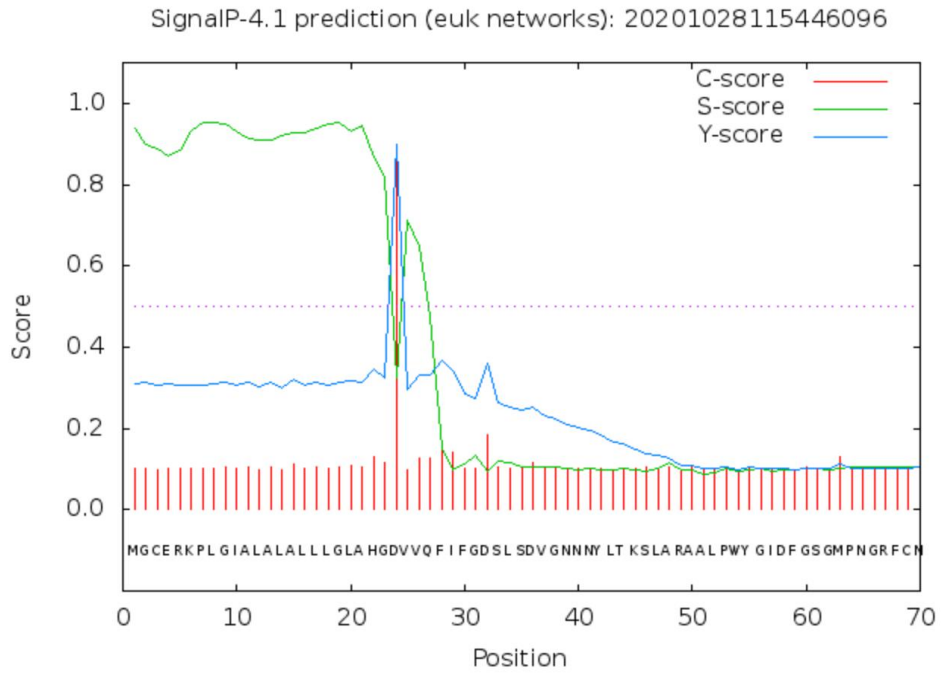

Figure 4.13. Transmembrane domain prediction of *TaGDSL* gene.

4.3.1. Construction of bi-molecular fluorescent complementary vector

The wheat cDNA was amplified by designing specific primers pGADT$_7$-GDSL F/R

and pGBKT$_7$-Pm46F /R. The primers are shown in Table Appendix 2. The full-length pGADT$_7$-GDSL and pGBKT$_7$-Pm46 genes containing the adaptor sequence connecting the pGADT$_7$ vector and pGBKT$_7$ vector were obtained. The amplification products of pGADT$_7$-TaGDSL and pGBKT$_7$-Pm46 were purified and recovered. The recovered pGADT$_7$-TaGDSL and pGBKT$_7$-Pm46 were ligated with PmD19-T and transformed into *E.coli*. The monoclones were selected for PCR positive verification and sent to the company for sequencing. The correctly sequenced pGADT$_7$-TaGDSL and pGBKT$_7$-Pm46 were ligated with pGADT$_7$ and pGBKT$_7$ vectors digested by *EcoR* I and *Xho* I, *Bam*H I and *EcoR* I, and then ligated with recombinase. After the ligation and transmutation of *E.coli DH5α*, the fragments of 1545 bp and 1269 bp could be detected by sequencing, which were consistent with the fragments of *TaPm46* and *TaGDSL*, indicating that the expression vector was successfully constructed.

For the construction of the bimolecular fluorescent complementary vector, *TaGDSL* gene and *TaPm46* gene were amplified by PCR, and the gene fragments were ligated into (*Xba* I / *Bam*H I) and (*Xba* I / *Bam* H I) respectively by in-fusion I) pXY106-TaGDSL-nYFP vector and pXY104-TaPm46-cYFP vector were constructed on double digested fluorescent vector pXY106 and pXY104. For the double enzyme digestion of the plasmid vector, the plasmid without enzyme digestion was used as the control, and 1.5% agarose gel was used for electrophoresis to test whether the vector was cut in the enzyme digestion reaction. The pXY104 plasmid control, pXY104 linearized vector, pXY106 plasmid control, pXY106 linearized vector, it can be seen that the speed of the linearized vector in electrophoresis is faster than that of the plasmid control, indicating that the enzyme digestion reaction successfully cut the vector. The results were consistent with the expected results (Fig. 4.14), and the next experiment could be carried out. The size of the TaGDSL gene fragment was about 750 bp, which was consistent with the expected results, indicating that the pXY106-TaGDSL-nYFP fluorescent complementary expression vector was successfully transferred into *E.coli* cells.

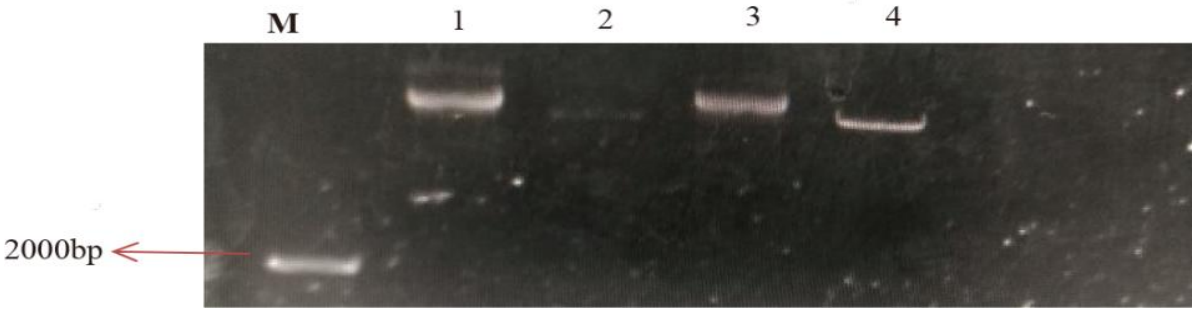

Figure 4.14. The speed of the linearized vector in electrophoresis: 1 - pXY104 plasmid control; 2 - pXY104 linearized vector; 3 - pXY106 plasmid control; 4 - pXY106 linearized vector.

The constructed fluorescent complementary expression vector was transformed into competent *E.coli* cells, and the transformed *E.coli* cells were used as the template for PCR amplification. The results of transformation were detected by electrophoresis on 1% agarose gel. The bands of DL 2000+ Marker were compared to determine whether *E.coli* contained the target gene expression vector. The electrophoresis results are shown in Figure 4.15 and Figure 4.16, in which the target vector is indeed contained in *E.coli* and the fragment size is consistent with the expected result, indicating that the experiment has successfully transferred the fluorescent complementary expression vector into *E.coli.* The positive ones were sent to Wuhan Jinbeginning Bioengineering Co., LTD for sequencing. The results are consistent with the reference sequence.

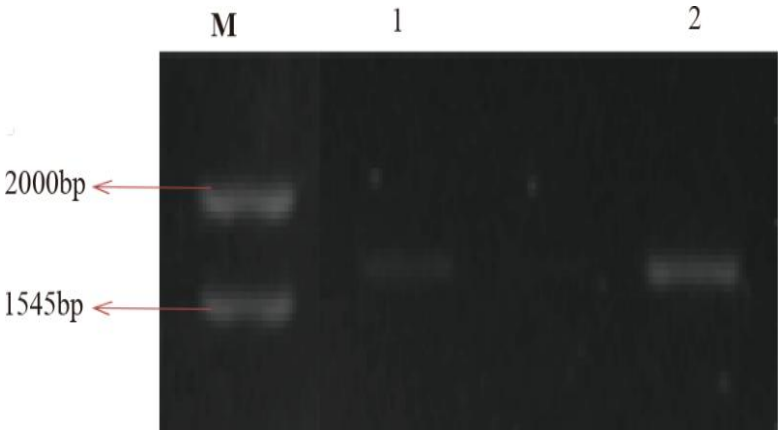

Figure 4.15. Identification of pXY104-TaPm46-cYFP by PCR: M - DL2000+ Marker; 1, 2 - length of destination fragment.

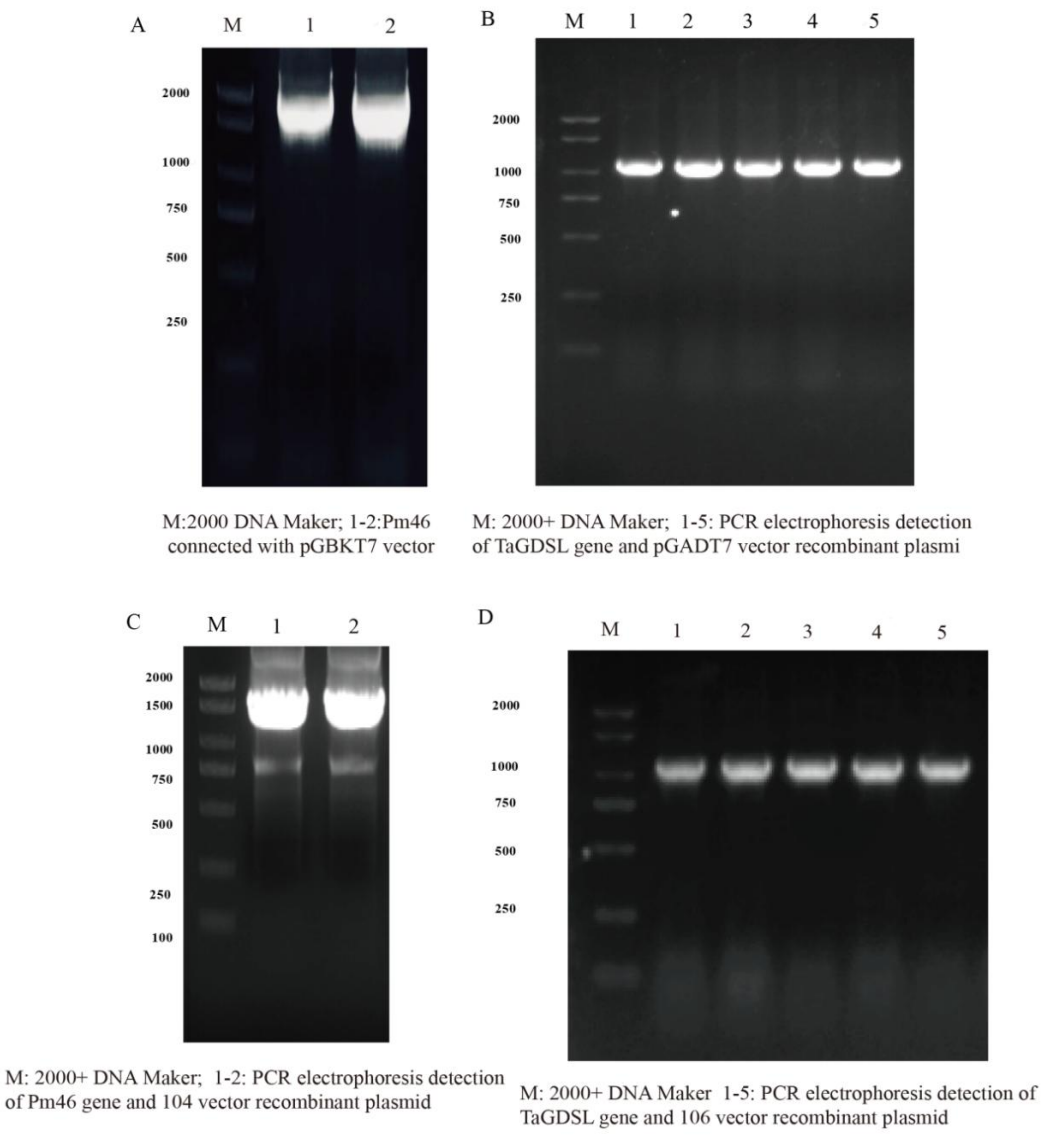

Figure 4.16. Identification of pXY106-TaGDSL-nYFP by PCR.

The results indicated that the pXY106-TaGDSL-nYFP vector and pXY104-TaPm46-cYFP expression vector were successfully constructed. The length of *TaPm4*6 gene fragment was about 1545 bp, which was consistent with the expected result, indicating that pXY104-TaPm46-cYFP fluorescent complementary expression vector was successfully transferred into *E.coli*.

4.3.2. The interaction between *TaGDSL* and *TaPm46* was verified by yeast two-hybrid

Transcriptional activators of specific genes in eukaryotes usually have two separable domains, namely DNA-binding domain (BD) and transcrip-tional

activation domian (AD). Both have their own functions in the domain and do not affect each other. However, an activator that is fully activated for a specific gene must contain both domains; otherwise, it cannot express the expression of genes specifically activated after BD region of different sources binds to AD. According to this principle, we constructed the TaGDSL-pGADT$_7$ and Pm46-pGBKT$_7$ yeast two-hybrid vectors to verify the interaction relationship between *TaGDSL* gene and *TaPm46* gene. We will co-transfer yeast Y2HGold of TaPm46-pGBKT$_7$ and syndrome TaGDSL-pGADT$_7$, TaPm46-PGBKT$_7$ and pGADT$_7$, pGBKT$_7$ and syndrome TaGDSL-PGADT$_7$, pGBKT$_7$ and pGADT$_7$, We found that only co-transposition TaPm46-pGBKT$_7$ and TaGDSL-pGADT$_7$ could grow on SD/-Leu/ -Trp, SD/-His/-Leu/ -Trp, SD/ -Ade /-His/-Leu/ -Trp deficient medium. Subsequently, the yeast co-transformed with TaPm46-pGBKT$_7$ and TaGDSL-pGADT$_7$ vectors were diluted 10-fold, 100-fold and 1000-fold to SD/ -Ade /-His/-Leu/ -Trp deficient medium containing X-α-gal and ABA, respectively. After 3-7 days of culture, the yeast was found to turn blue (Fig.4.17). Photos were taken and recorded. The results indicated that *TaGDSL* gene and *TaPm46* gene could interact in yeast.

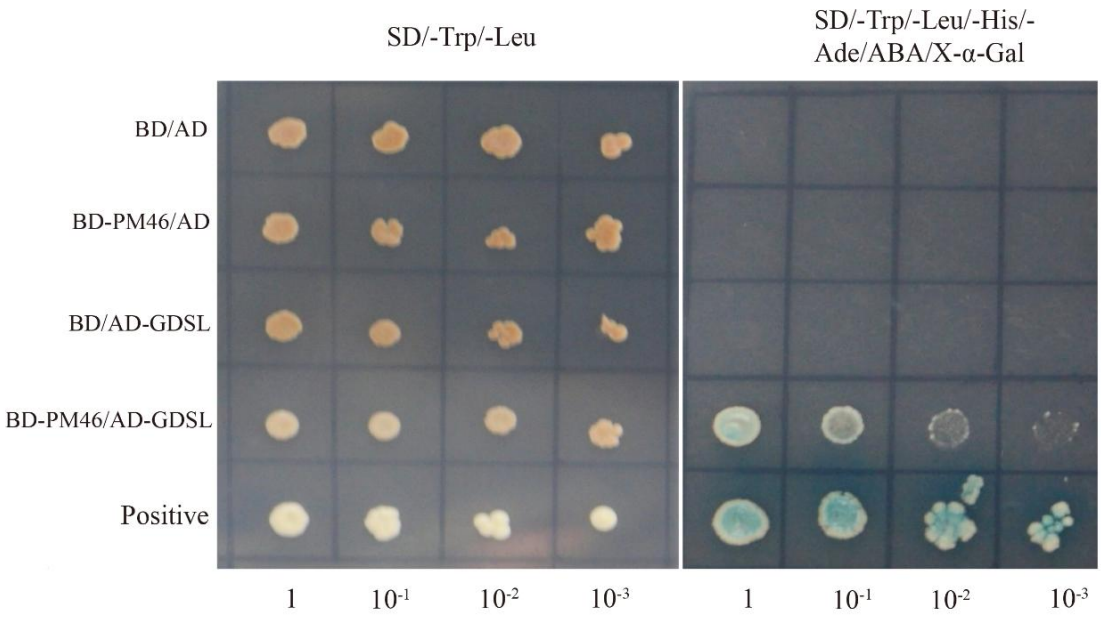

Figure 4.17. Analysis of yeast two-hybrid interaction between *TaPm46* and *TaGDSL* gene.

4.3.3. Bimolecular fluorescence complementation assay was used to verify the interaction

According to the results in 3.3.2, *TaGDSL* and *TaPm46* can interact in yeast. In order to verify whether *TaGDSL* and *TaPm46* can interact in plants, the interaction between *TaGDSL* and *TaPm46* in tobacco cells was investigated by using a bimolecular fluorescence complementation experiment.

In this study, *Agrobacterium tumefaciens* containing pXY106-*TaGDSL* and pXY104-TaPm46, pXY104 and pXY106 vectors were amplified. The combination of pXY106-TaGDSL and pXY104-TaPm46 was used as the experimental group, and the combination of pXY104 and pXY106 was used as the control group. After 48 h, the leaves of tobacco were observed under a confocal microscope. Yellow fluorescence phenomenon could be observed in the epidermal cells of tobacco (Fig. 4.18), indicating that TaGDSL interacts with TaPm46 in plant cells. When only Agrobacterium pXY104 and pXY106 are injected, No yellow fluorescence was observed.

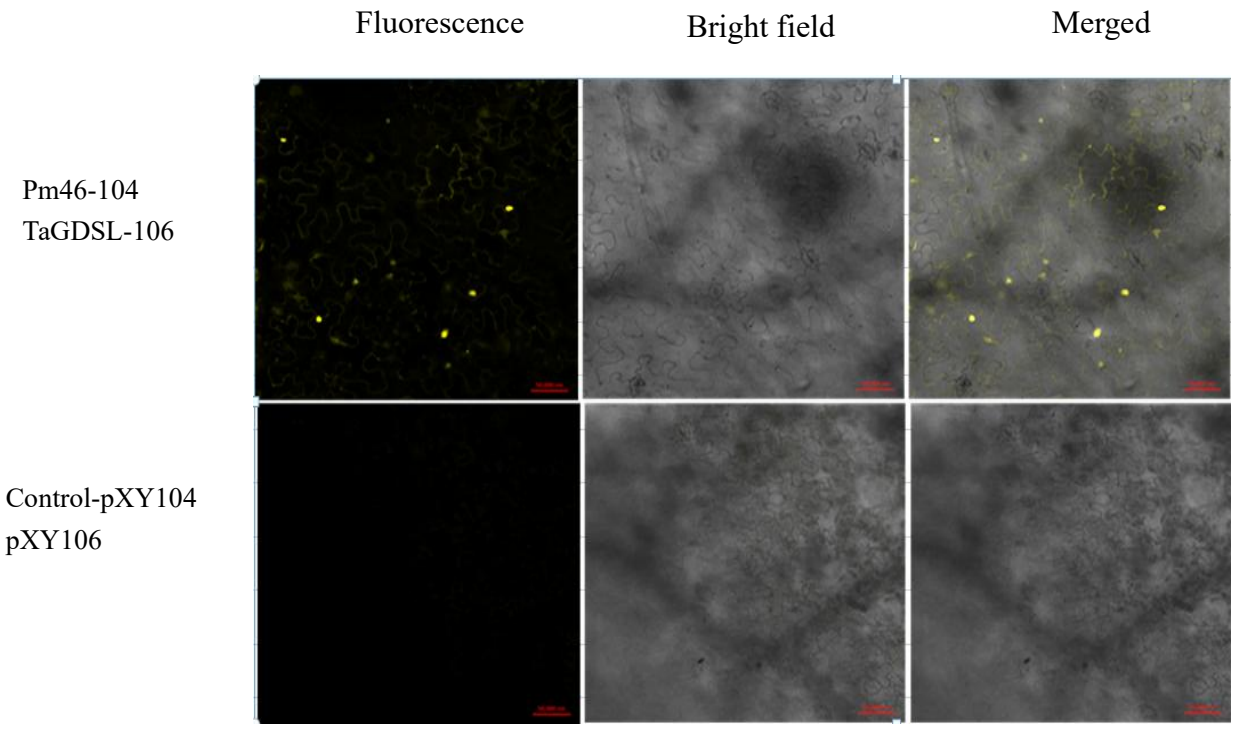

Figure 4.18. The interaction between *TaPm46* and *TaGDSL* detected by Bi-Fluorescent Complementation (Bars = 50μm).

4.4. *TaGDSL* gene function analysis

4.4.1. Barley stripe mosaic virus (BSMV)-virus induced gene silencing (VIGS) technology was used to screen the interacting protein genes

(1) Construction and detection of recombinant viral vector BSMV: *TaGDSL1*, BSMV: *TaGDSL2*, BSMV: *TaGDSL3*, BSMV: *TaGDSL4*. The silenced fragment of *TaGDSL1, TaGDSL2, TaGDSL3, TaGDSL4* genes was obtained by PCR amplification, with a length of about 250bp. Figure 4.19 shows the electrophoresis diagram of PCR amplification of the target fragment.

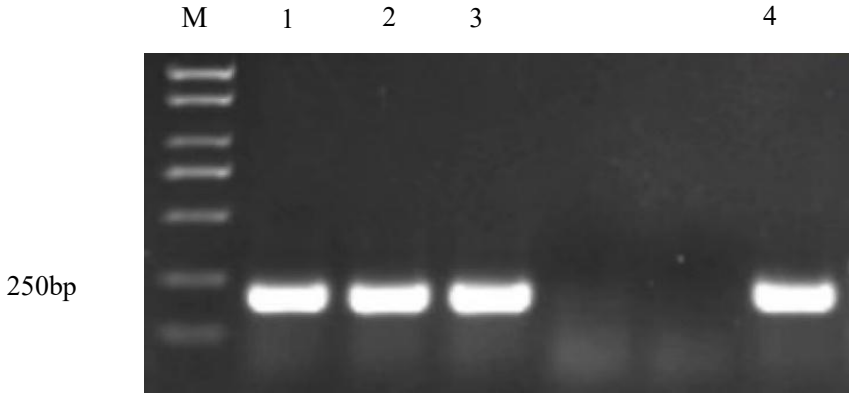

Figure 4.19. Identification of the genes silence fragment by PCR (M is 2000+ DNA Maker; lane 1, lane 2, lane 3 and lane 4 are amplified fragments of target genes *TaGDSL1, TaGDSL2, TaGDSL3, TaGDSL4,* respectively).

(2) Vector linearization and in vitro transcription.

The recombinant viral vectors BSMV: *TaGDSL1*, BSMV: *TaGDSL2*, BSMV: *TaGDSL3*, BSMV: *TaGDSL4*, α, β, γ, target genes and viral vector plasmids were linearized by corresponding enzys. Then the recombinant viral vectors BSMV: *TaGDSL1*, BSMV: me*TaGDSL2*, BSMV: *TaGDSL3*, BSMV: *TaGDSL4*, α, β, γ, γ-pDS and target genes were recovered and purified, and the linearized fragments of viral vector plasmid were detected respectively.

The in vitro transcription part is the key to the success of the whole BSMV-VIGS. The in vitro transcription RNA of BSMV: *TaGDSL1*, BSMV: *TaGDSL2*, BSMV: *TaGDSL3*, BSMV: *TaGDSL4*, α, β, γ, γ-*PDS* and the target gene fragment was obtained by in vitro transcription, as shown in Fig. 4.20. According to the figure, we have obtained a large amount of in vitro

transcription RNA with very clear bands and no obvious tailing phenomenon. All these indicated that the in vitro transcription was successful and could be carried out in the next experiment.

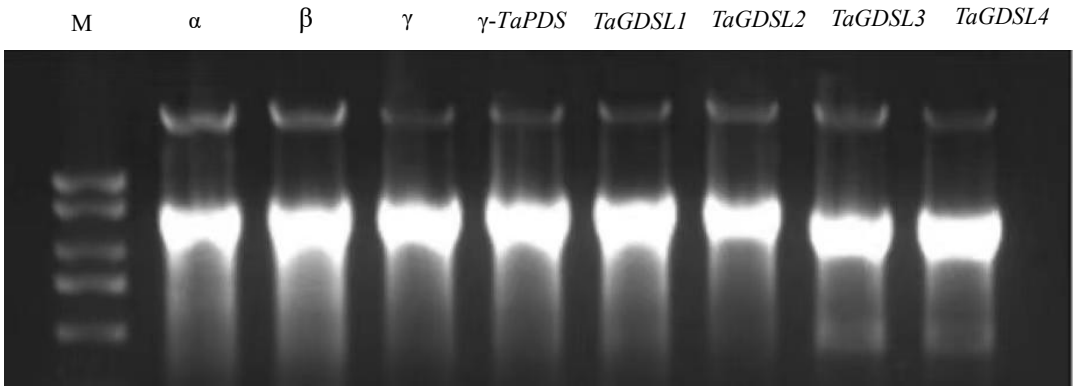

Figure 4.20. RNA transcription was verified by PCR. M is 2000+ DNA Maker, lane 2, lane 3, lane 4, lane 5, lane 6, lane7, lane 8 and lane 9 are amplified fragments of target genes α, β, γ, γ -*TaPDS*, BSMV: *TaGDSL1*, BSMV: *TaGDSL2*, BSMV: *TaGDSL3*, BSMV: *TaGDSL4* respectively.

(3) Detection of gene silencing system.

The BSMV: *TaPDS*-inoculated plants exhibited significant photo-bleaching (Fig.4.21). *TaPDS* transcription levels in BSMV: *TaPDS*-inoculated plants were inhibited by over 80%, relative to mock-inoculated plants, implying that the BSMV-VIGS system is appropriate for gene silencing studies of *TaGDSL*. The *TaPDS* shown in the figure 4.21 refers to the photobleaching that occurs when the virus carrying the PDS gene fragment is applied to the plant for about two weeks.

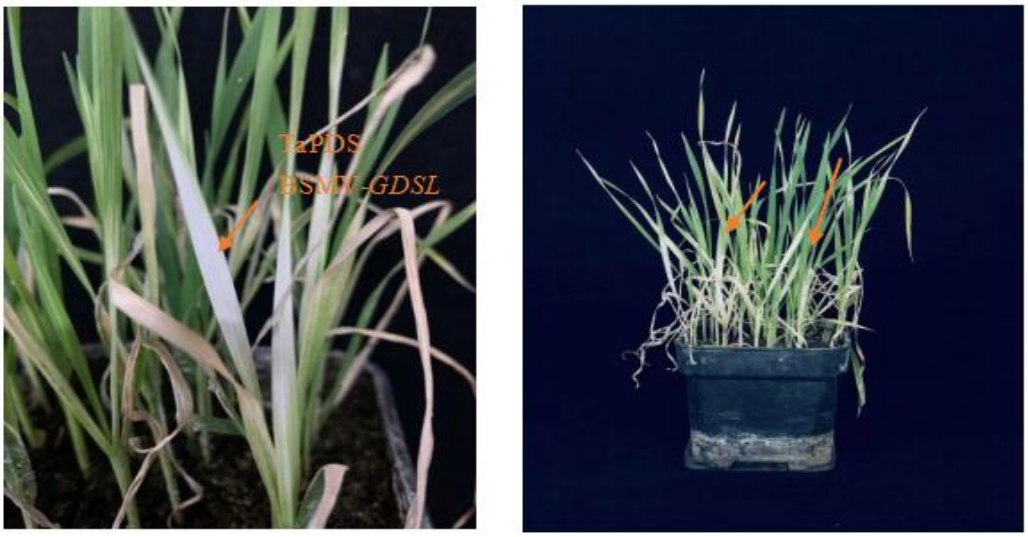

Figure 4.21. Phenotype of *TaPDS*.

(4) VIGS method was used to induce gene silencing on leaves for preliminary functional verification.

In order to verify the function of TaGDSL in wheat powdery mildew, the TaGDSL gene was silenced in common wheat Banong 207. When the fourth leaf of TaGDSL virus was fully expanded, the leaf was clipped and used to silence the plant for powdery mildew resistance identification. It was found that the lipid-related plastid protein TaGDSL gene was silenced in Banong 207 (BSMV: TaGDSL), the disease resistance of silenced plants (BSMV- γ) was increased compared with that of control plants (Fig. 4.22), indicating that TaGDSL gene may positively regulate resistance to wheat powdery mildew.

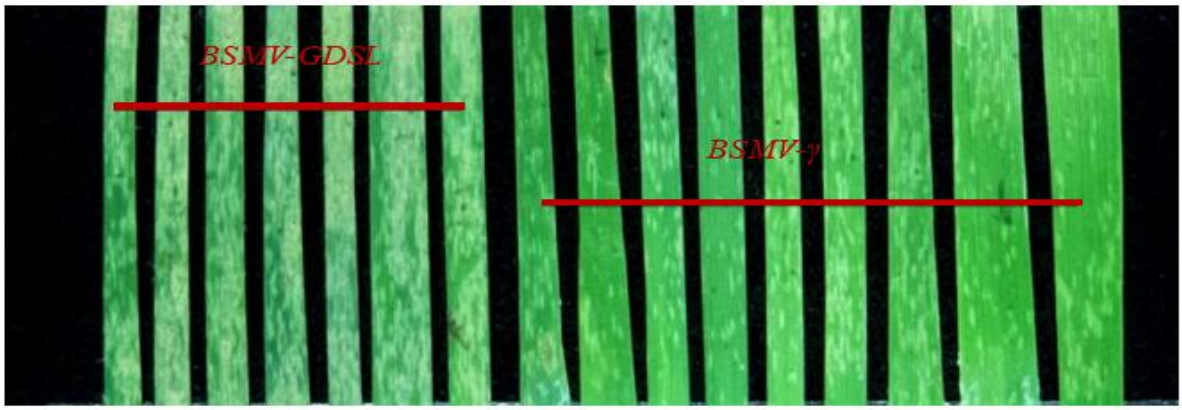

Figure 4.22. Functional analysis of *TaGDSL* by *BSMV-VIGS* in Bainong207.

The BSMV-TaGDSL refers to the occurrence of albino mildew inoculated 7 days after TaGDSL silenced in common wheat, and BSMV-γ refers to the occurrence of albino mildew inoculated in control inoculated with viral vector.

(5) Analysis of *TaGDSL* gene silencing efficiency.

About 7 days after inoculation, three silenced wheat and control wheat leaves were randomly selected for RNA extraction. After reverse transcription, quantitative PCR was performed to detect gene silencing efficiency. The specific primers and amplified sequences used for *TaGDSL* gene were conserved regions on A, B and D of wheat genome. Compared with the control group, the *TaGDSL* gene was effectively silenced, as shown in Figure 4.23. These results indicated that *TaGDSL* gene played a positive

regulatory role in wheat infected with powdery mildew spores and increased susceptibility when silenced.

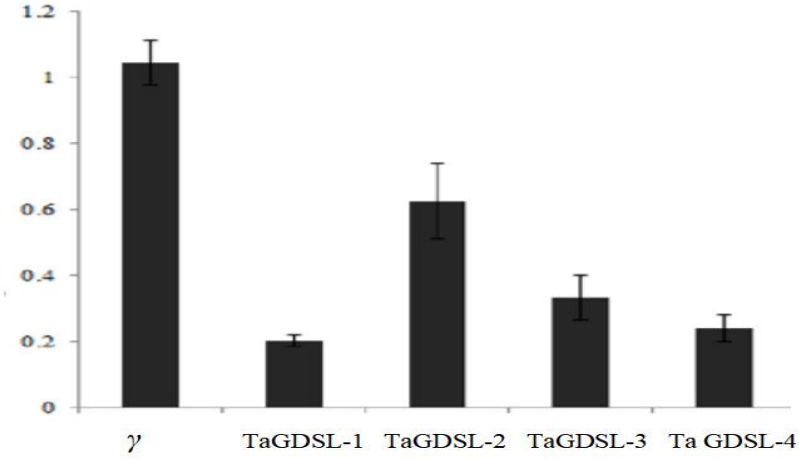

Figure 4.23. Analysis of relative expression of *TaGDSL* gene after genes silencing: γ = Control; *TaGDSL* gene silencing plants = TaGDSL-1, TaGDSL-2, TaGDSL-3, TaGDSL-4.

4.4.2. Construction of *TaGDSL* gene RNA interference (RNAi) vector and genetic transformation in wheat

(1) Construction of *TaGDSL* gene RNA interference (RNAi) vector.

① The common segments of *TaGDSL* gene in groups A, B and D were selected to design primers of RNAi fragment for interference to study gene function. Primers TaGDSL-RNAi1-PTCK303-F/ R and TaGDSL-RNAi2-PTCK303-F/ R were used to amplify the conserved region of *TaGDSL* gene as RNA interference sequence using wheat cDNA as template. Forward (RNAi1) and reverse (RNAi2) interference fragments of 285 bp *TaGDSL* gene fragment were obtained (Fig. 4.24a). The obtained bands were recovered, T-linked and sequenced, and the alignment analysis was consistent with the *TaGDSL* gene sequence obtained from NCBI, indicating that the sequence had been successfully cloned and named as TaGDSL-RNAi1 and TaGDSL-RNAi2 fragments, respectively.

② The above correctly sequenced TaGDSL-RNAi1 was connected to the linearized expression vector digested with *Bam*H 1 and *Kpn* 1 and transformed. The correct monoclonal bacterial solution was identified by colony identification and

sequencing analysis to extract the plasmid. The TaGDSL-RNAi-PTCK303 plasmid was digested with *Sac* 1 and *Spe* 1. The obtained TaGDSL-RNAi-PTCK303 linearized vector was ligated and transformed with TaGDSL-RNAi2, and the positive transmutation TaGDSL-RNAi-PTCK303 was identified as the recombinant RNA interference vector, which was further identified by *Sac* I and *Bam*H 1 double enzyme digestion. The expected gene fragment and intermediate vector length of 1269 bp could be cut out (Fig. 4.24b), indicating the successful construction of TaGDSL gene RNA interference (RNAi) vector.

③ The constructed and correctly identified TaGDSL-RNAi-PTCK303 plasmid and pTCK303 empty plasmid were transformed into GV3101, and the *TaGDSL* gene RNA interference fragment was amplified by PCR of *Agrobacterium tumfaciens* monoclonal colony transformed by TaGDSL-RNAi-PTCK303 plasmid to identify the transformation results. After electrophoresis, the fragment size was as expected (Fig.4.24c), indicating that the recombinant plasmid was successfully transformed into *Agrobacterium tumefaciens*. The Agrobacterium was successfully identified and transformed into wheat at embryonic stage of Bainong 207.

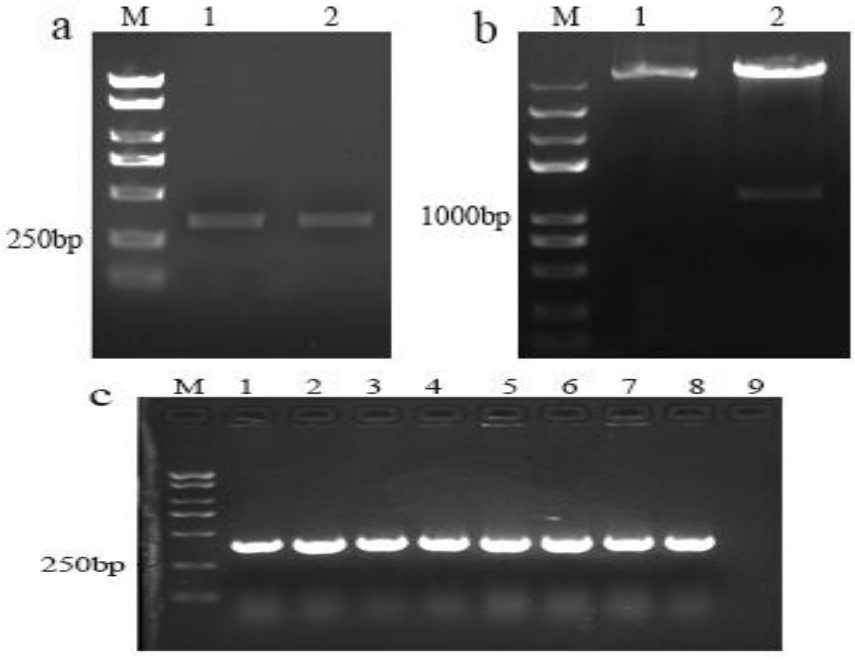

Figure 4.24. Construction of *TaGDSL*-RNAi-pTCK303 vector. (a = Products of wheat *TaGDSL* RNAi fragment amplified by PCR. b = Characterization of recombinant plasmid by restriction enzyme digestion. c = Positine dentification of *TaGDSL*-RNAi-pTCK303 plasmid by Agrobacterium monoclonal PCR verification. M - Standard 2000+ Marker, 1 - Amplified bands of *TaGDSL*-RNAi, 2 - ddH_2O).

(2) Genetic transformation of wheat.

In this study, the *TaGDSL* gene interference vector (TaGDSL-RNAi-PTCK303) was used to genetically transform wheat Penong 207 by *Agrobacterium tumefaciens*. GUS staining reagent was prepared by GUS staining kit. When transgenic wheat grew to the two-leaf phase, leaf tips were cut and placed in PCR tubes, and 30 μL GUS staining was added to remove the bubbles so that the leaf tips were fully exposed to GUS staining. The leaves were placed in a constant temperature incubator at 37 ℃ for 72 h, and then the leaf tips were decolorized with 75% ethanol. When the green color was completely removed, we found that some wheat leaf tips turned blue (Fig. 4.25), indicating that the transgenic plants corresponding to this leaf tip were positive plants. Subsequently, we further extracted wheat leaf DNA from plants that turned blue by GUS staining, and amplified wheat DNA with primer GUS F/ R, as shown in Figure 4.26. The wheat DNA corresponding to transgenic plants that can amplify the target band is a positive plant. The establishment of the wheat genetic transformation system provides a strong guarantee for the analysis of wheat gene function and the study of regulation mechanism.

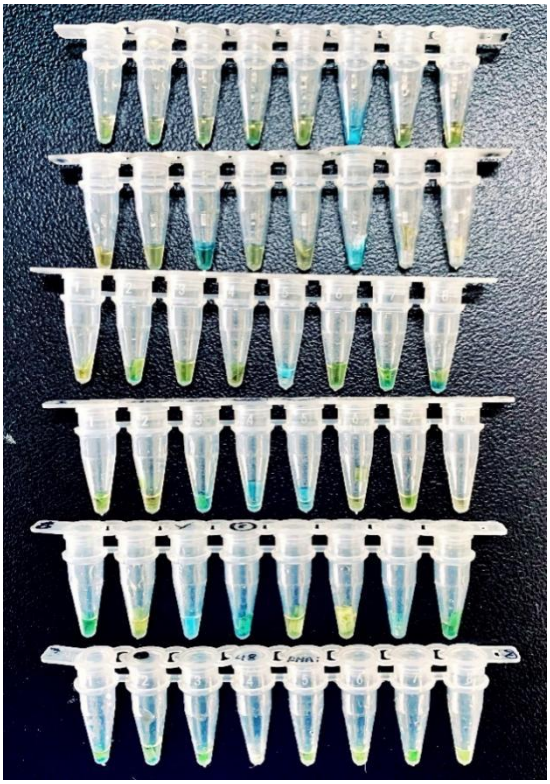

Figure 4.25. Gus staining identification of transgenic Wheat.

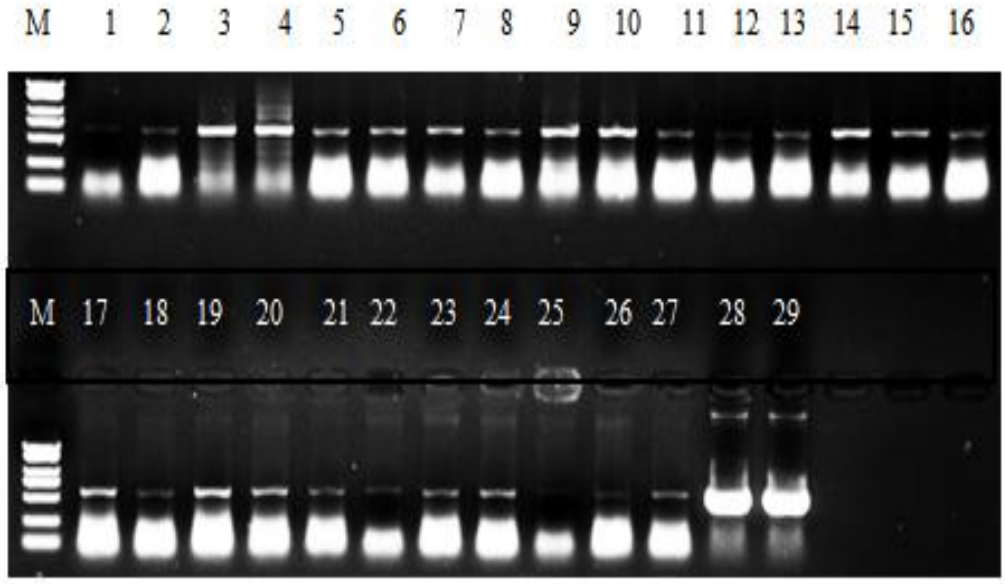

Figure 4.26. The PCR identification of Transgenic Wheat. 1-27: Identification of *TaGDSL* gene of wheat leaves by PCR; 28-29: Positive control.

(3) Identification of RNAi transgenic plants

The seeds of the identified T_0 generation wheat plants were harvested, and the harvested seeds were germed. After the T_1 generation seedlings grew out, RNA of the T_1 generation positive plants and normal plants were extracted respectively. After removing genomic DNA, the First-strand cDNA was synthesized by reverse transcription, and the relative expression of this gene in the positive plants and normal plants was detected. Real-time fluorescence quantitative (QPCR) results shows that the expression level of this gene in T_1 generation positive plants is reduced (Fig. 4.27). Among them, the expression of transgenic lines 1, 5 and 6 decreased significantly, so these three lines were propagated for further study.

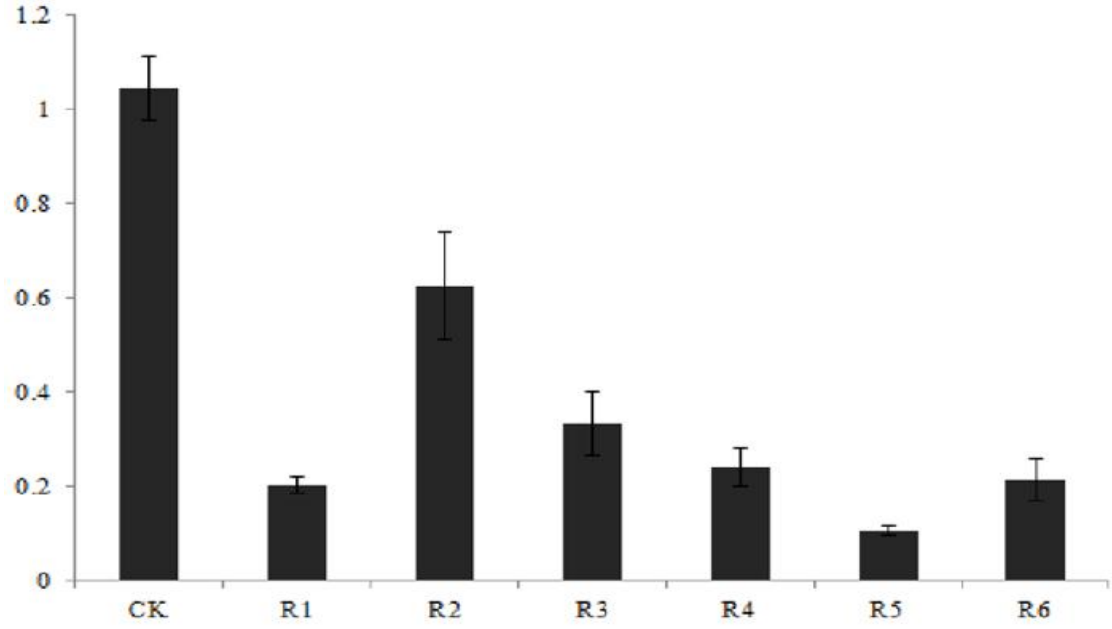

Figure 4.27. Quantitative analysis of *TaGDSL* in RNAi transgenic plants.

(4) Detection of disease resistance in T_1 generation of RNAi transgenic plants.

The identified T0 generation wheat plants were harvested and the harvested seeds were germinated. After the T1 generation seedlings were grown, further positive plants were identified and disease resistance was identified.

For disease resistance detection, in vitro leaf inoculation method was used. Leaves and normal leaves of T_1 generation plants with the same growth status were inoculated with mixed physiological races of Powdery fungi from Henan plot. The results were observed after the disease onset (Fig. 4.28). T_1 generation positive plants are more susceptible to powdery mildew than normal leaves. Repeated experiments showed consistent results, which further indicated that *TaGDSL* played a positive regulatory role in the regulation of powdery mildew fungi.

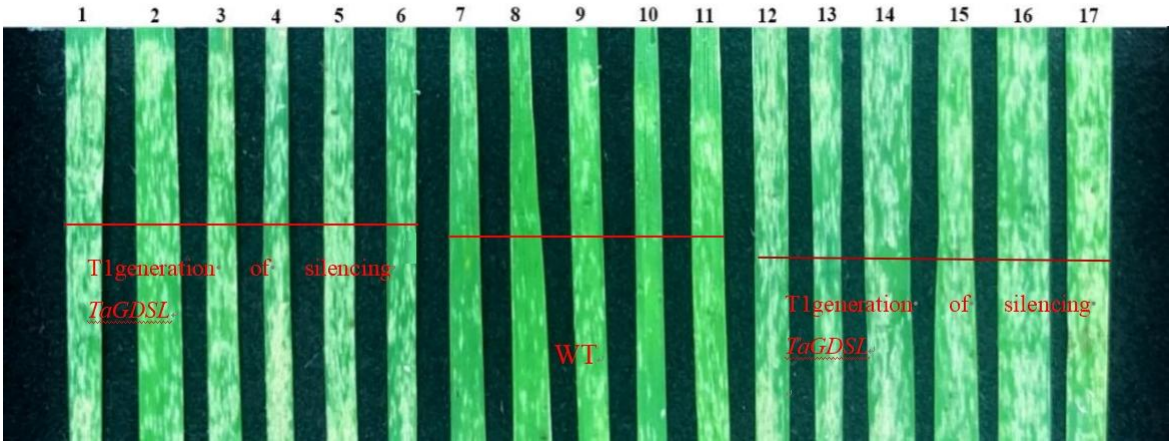

Figure 4.28. T_1 generation of *TaGDSL* silenced (RNAi) plants increased susceptibility to powdery mildew (1-6 and 12-17 = Leaves of RNAi-*TaGDSL* transgenic plants; 7-11 = WT - Wild type, Bainong207).

(5) Observation of spores on leaves of T_1 generation of *TaGDSL* silenced (RNAi) plants infected by powdery mildew.

The T_1 generation positive plants of Silencing (RNAi) *TaGDSL*, the wheat variety Bainong 207 without transgenic and the susceptible material Sumai 3 were planted. When the plants reached the 3-leaf stage, powdery mildew was inoculated. The leaves were taken at 24 h, 48 h and 72 h respectively. And then the mycelia growth and development were observed under a microscope after Coomassie brilliant blue staining and water decolorization. It was found that the growth rate of mycelia on the leaves of the RNAi positive plant was significantly higher than that of the WT (Wild type, non-transgenic plant), and the number of colonies was significantly increased (Fig. 4.29). Some of them could produce dense conidial chains, and the growth rate of mycelia on the leaves of the plants with interference expression was similar to that of Sumai 3 which was highly susceptible to powdery mildew. After *TaGDSL* silencing, plant susceptibility was enhanced, indicating that interference expression of *TaGDSL* could reduce wheat resistance to Powdery mildew.

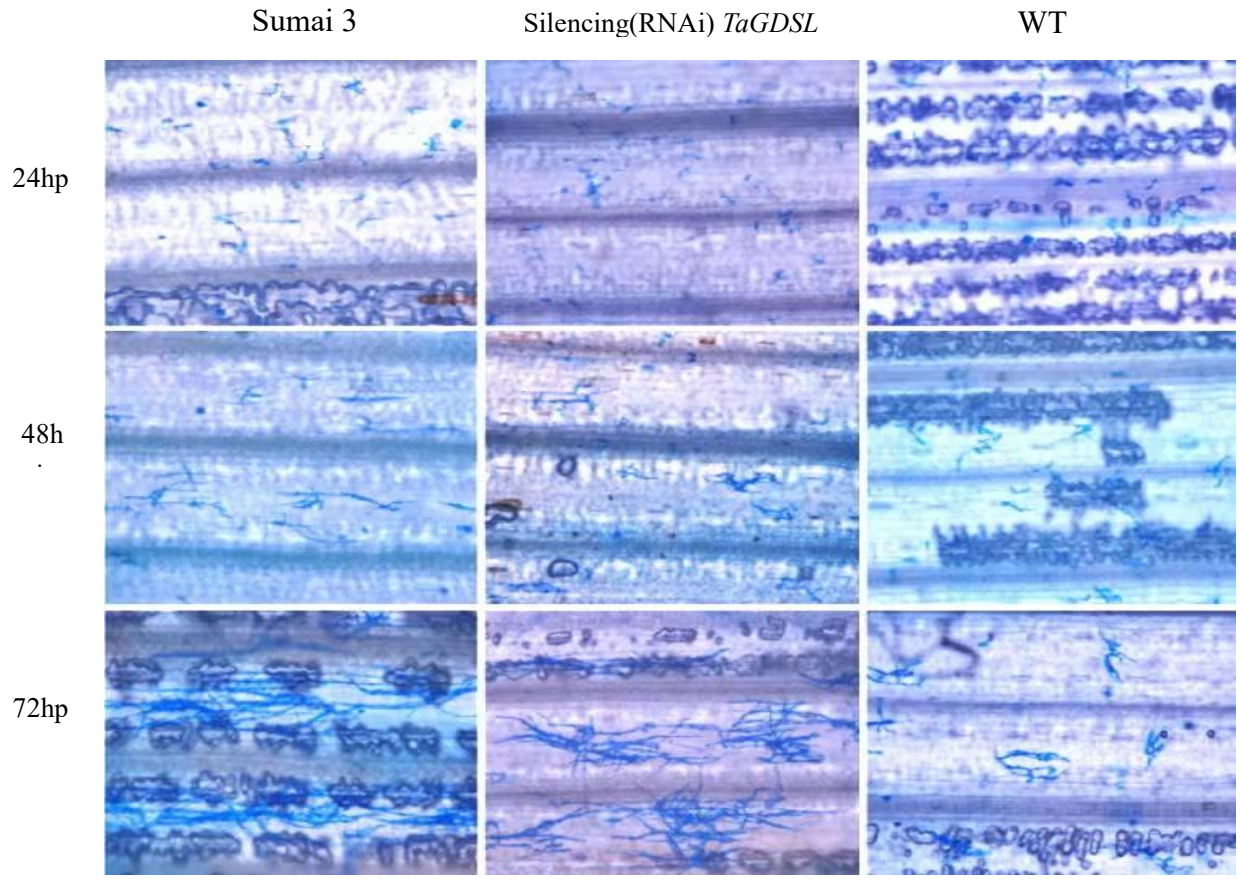

Figure 4.29. The development of powdery mildew in the transgenic plants and wild types (WT).

(6) The *TaGDSL* positively regulated physiological responses to powdery mildew. The silencing (RNAi) *TaGDSL* plants were grown in the seedling and maturity stage to test whether plants suppressed powdery mildew buildup in the wheat (Fig. 4.30). After inoculation with powdery mildew, at the maturation, straw biomass, grain, spikelet number, as well as 1000-grain weight of the RNAi lines were significantly more than WT lines (Table 4.1). In this study, it was found that transgenic T_1 positive plants that interfered with *TaGDSL* expression showed late ripening phenomenon before being infected with powdery mildew. The leaves of normal plants had all turned yellow and mature, while the leaves of transgenic positive plants were still green. These results suggest that *TaGDSL* also plays an important role in regulating plant growth and development and the transition from vegetative to reproductive growth. Overall, these findings indicate that silencing of *TaGDSL* could resist to powdery mildew in wheat plants.

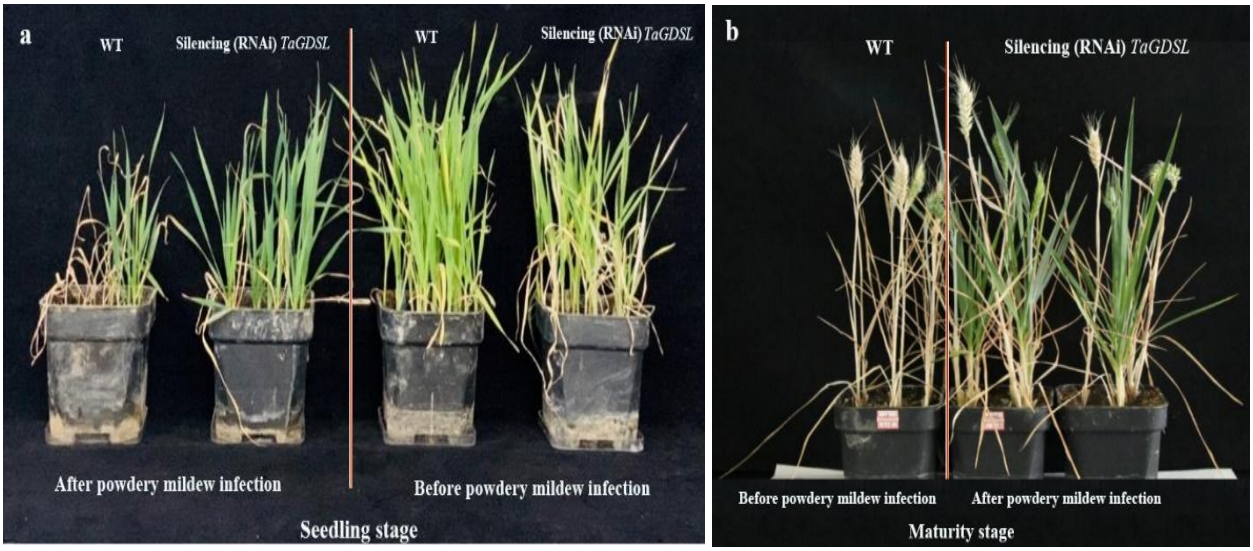

Figure 4.30. Phenotypic responses of silencing (RNAi) *TaGDSL* plants infected with Powdery mildew: a - seedling stage; b - maturity stage.

Table 4.1. The grain physical properties

Indicator	Wild type (WT) / per plant	RNA inferece (RNAi) / per plant	Over expression (OE) / per plant
Spikelet number (number)	8.7	17	6
Grains per Spikelet (number)	29.7	43.1	20.7
Grain length (mm)	5.38	7.43	4.75
Grain width (mm)	3.36	3.46	2.57
1000-grain weight (g)	38.63	54.26	27.62
Yield per plant (g)	13.47	25.73	5.32

Conclusions to chapter 4

The Lipid associated plastid protein (TaGDSL) obtained from yeast screen library. BSMV-VIGS technology and transgenic technology were used to verify that this gene was indeed associated with powdery mildew in wheat.

It can be predicted that *TaGDSL* protein is hydrophilic, and the dissolution of protein in aqueous solution is the result of the interaction between protein surface charge and protein.

Powdery mildew infection caused rapid upregulation expression of *TaGDSL*, the main site of powdery mildew in plants. This finding suggests a specific role of *TaGDSL* in powdery mildew tolerance.

BSMV: *TaGDSL*-inoculated plants exhibited strong resistance to powdery mildew, relative to wild type.

The silencing (RNAi) *TaGDSL* plants had enhanced powdery mildew resistance. Functional identification of the *TaGDSL* gene from wheat by the long -growth period assay proved that silencing of *TaGDSL* gene could resist to powdery mildew in wheat plants. After inoculation with powdery mildew, the straw biomass, grain, number of spikelets, and 1000-grain weight of the RNAi lines were significantly more than WT lines at the maturation.

These results support that *TaGDSL* is a negative regulator of powdery mildew resistance. our findings demonstrate that *TaGDSL* silencing is potentially useful since it can help generate genetically modified genotype materials with powdery mildew resistance in wheat.

CONCLUSION

The dissertation provides a theoretical generalization and a new solution to the scientific task of establishing the selection value of the source material of winter wheat, based on the trait of resistance to powdery mildew, determining the role of individual genes in the control of this feature.

1. Therefore, the authors identified the adult disease resistance of powdery mildew of 86 new wheat line.The experiment was conducted at the Wheat Test Base in Henan Province in 2020-2022.The test materials were from 45 relevant breeding units in China.There are 86 new wheat line.The test carried out powdery mildew resistance identification.And analyzing the source of disease resistance genes.

2. In the resistance test for powdery mildew in 2020, only 11.7% of the 86 new wheat line identified were performing well. There is no line of disease resistance in the adult stage that is immune or near immunized. There are 4 lines that are highly resistant to disease during adult stage. There are 2 lines with moderate resistance to disease resistance in adult stage. There are 4 lines that are moderately susceptible. They accounted for 4.7%, 2.3%, and 4.7% of the total identified materials, respectively, and the overall resistance was poor.

3. We use pedigree analysis and parental resistance to disease to derive disease resistance genes for varieties that are resistant to disease. It is speculated that the genetic sources of some powdery mildew resistant varieties may be Aikang58, Jimai 22, Yumai 34, Zhengmai 366, Liangxing 66, Zhoumai 16, Zhoumai 18, Yumai 47 and Xiaoyan 926A.

4. This study identifies the Lipid associated plastid gene *TaGDSL*. BSMV-VIGS technology and transgenic technology were used to verify that this gene was indeed associated with powdery mildew in wheat, and *TaGDSL* expression was down-regulated in susceptible varieties. Silencing of this gene increased wheat susceptibility, indicating that *TaGDSL* plays an important role in wheat defense against powdery mildew,

providing a reference for elucidating the mechanism of wheat resistance to powdery mildew.

5. We cloned and sequenced the *TaGDSL* gene in Bionong 64. The Lipid associated plastid protein (TaGDSL) was obtained, and the total length of *TaGDSL* cDNA was 1545 bp. Through the yeast two-hybrid experiment, we constructed the yeast expression vector with *TaGDSL* and *TaPm64* and co-transferred yeast. It was found that yeast could turn blue after culture on the four-deficient medium containing X-α-gal and ABA, which indicated that *TaGDSL* gene and *TaPm64* gene could interact in yeast. Then, we verified that *TaGDSL* and *TaPm64* genes could interact in tobacco leaves by bimolecular fluorescence complementation experiment.

6. *TaGDSL* gene exists in groups A, B and D on chromosome 6 of wheat, with high amino acid sequence similarity. Therefore, in the functional identification, the interference fragments were selected as the common fragments in the three genomes, so that the expression of the gene on the three genomes could be down-regulated.

7. Analysis of the tissue expression pattern of *TaGDSL* revealed that the gene was constitutively expressed.

8. Wheat leaves were treated with Powdery mildew fungus. qRT-RCR analysis showed that *TaGDSL* expression was induced by powdery mildew fungus, suggesting that *TaGDSL* gene was related to wheat resistance.

9. Since its discovery, VIGS technology has been widely used in the study of functional genes due to its short cycle, simple vector construction method and high silencing efficiency, and now it has become a rapid method to study gene function. In this study, A 250 bp fragment shared on groups A, B and D of *TaGDSL* was selected as the target fragment for silencing, and the BSMV-VIGS vector of this gene was constructed. After infecting wheat with wheat powdery mildew, it was found that compared with normal wheat, wheat plants with this gene silenced were significantly more susceptible to powdery mildew infection. It is concluded that *TaGDSL* gene is related to wheat powdery mildew resistance.

10. In order to further verify the function of this gene, RNAi vector was constructed and genetic transformation was carried out in wheat to achieve stable inheritance. The positive plants of T0 generation and T1 generation were identified by GUS staining and qRT-PCR, and the resistance of the positive plants of T1 generation was identified. The results showed that T1 plants were more susceptible to powdery mildew than normal plants. By qRT-PCR analysis of T1 generation positive plants, the expression of *TaGDSL* gene was silenced in different degrees in positive plants.

11. In order to further study the interaction between wheat and albino after *TaGDSL* silencing, the development of albino mycelia was observed, and samples were taken at different stages after inoculation. Through Coomassie brilliant blue staining, the growth status of mycelia in the leaves of T1 generation positive plants, sensitive cultivar Sumai 3 and AK 58 wheat was observed by microscope. The growth of spores on the leaves of T1 generation positive plants was significantly faster than that of wild type, even Sumai 3 with high susceptibility to powdery fungus. Therefore, it was determined that when the gene was silenced in wheat, the plant was more susceptible to powdery mildew. However, the specific pathway through which it affects the mycelium development of Powdery fungi still needs further study.

12. Previous studies have reported that the *GDSL* gene family has a variety of functions, and the main function is to participate in the regulation of plant growth and development. In this study, the protein expressed by *TaGDSL* was analyzed, and the protein expressed by *TaGDSL* was the same as the typical GDSL protein, with variable N-terminal sequence and highly conserved C-terminal sequence composition. It was speculated that *TaGDSL* affected the growth and development of wheat after powdery mildew infection. It was found that transgenic T1 positive plants that interfered with *TaGDSL* expression showed late ripening phenomenon before being infected with powdery mildew. The leaves of normal plants had all turned yellow and mature, while the leaves of transgenic positive plants were still green. Overall, these findings indicate that silencing of *TaGDSL* could resist to powdery mildew in wheat plants.

13. This study mainly verified the function of *TaGDSL* in wheat resistance to powdery mildew. After disrupting the expression of *TaGDSL*, it affected the resistance of wheat to powdery mildew by affecting the development of mycelia of powdery mildew.

PROPOSALS FOR BREEDING

1. In the programs for creating initial material of winter wheat with high resistance to powdery mildew use the following varieties: Baojingmai 166, Lingke 686, Xinong 837, Huimai858.

2. Some resistance genes against powdery mildew were suggested from Aikang 58, Jimai 22, Yumai 34, Zhengmai 366, Liangxing 66, Zhoumai 16, Zhoumai 18, Yumai 47 and Xiaoyan 926A.

3. The *TaGDSL* gene of wheat can be used to develop new resistant powdery mildew genotypes.

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APPENDIXES

Appendix Table 1. Wheat test lines and combination of Guanzhong irrigation district in Shaanxi province from 2020 to 2021

Number	Pedigree	Combination	Varieties origin
1	Shankenmai22	Xinong88/Xinong889	Crop Research Institute, Anhui Academy of Agricultural Sciences
2	Mengmai59	Zhoumai16/Aikang58	Henan Luohe Agricultural Science Institute
3	Guodao0366	Zhengmai366/Zhisheng5hao	Shaanxi Gaonong Seed Industry Co. LTD
4	Shandao198	Xinong979/Zhoumai16	Shaanxi Jufeng Seed Industry Co. LTD
5	Mengmai101	Zhoumai18/Mengxuan205-4	Henan Luohe Agricultural Science Institute
6	Huakenmai6hao	Ziyu111A-3/Xinong822	Shaanxi agricultural reclamation Dahua seed industry
7	Baojingmai166	Jimai22/4520	Henan Baojing Agricultural Technology Co. LTD
8	Baojingmai718	BJN171/BJM172	Henan Baojing Agricultural Technology Co. LTD
9	Kunmai8hao	Zhoumai22/Lianmai0358	Henan Kun Yu Seed Industry Co. LTD
10	Xinghuamai9hao	HD303/Hangxuan688	Xinxiang Agricultural Science Institute of Henan Province
11	Xinghuamai8hao	HD302/Hangxuan679	Xinxiang Agricultural Science Institute of Henan Province
12	Ronghua188	Xinong889/Xinong3517	Shaanxi Ronghua Agricultural Technology Co., LTD
13	Ronghua906	Xinong979/Xiaoyan22	Shaanxi Ronghua Agricultural Technology Co., LTD
14	Lingke686	Yumai34/Xinong686	Yang Ling Guorui Agricultural Technology Co., LTD
15	Xinong106	Zhoumai16/065	College of Agriculture, Northwest A&F University
16	Ronghua286	Xinong294/Xinmai26	Shaanxi Ronghua Agricultural Technology Co., LTD
17	Ronghua336	Luomai7239/Shanmai159	Shaanxi Ronghua Agricultural Technology Co., LTD
18	Ronghua116	Zhengmai366/Shanmai159	Shaanxi Ronghua Agricultural Technology Co., LTD
19	Lingmai1605	6103/Zhoumai18	Yang Ling Guorui Agricultural Technology Co., LTD
20	Xinong116	Xinong109/N1206	College of Agriculture, Northwest A&F University
21	Xinong837	Zhengmai366 (/ Xinong9814/D9401)	College of Agriculture, Northwest A&F University
22	Mingqi167	Xinong271/Zhoumai22	Yang Lingmingqi Wheat Crop Research Institute
23	Xianmai519	01-450/Zhoumai16	Xianyang Academy of Agricultural Sciences
24	Wunong144	Yu94-986/01333-1-5-6	College of Agriculture, Northwest A&F University
25	Wunong985	System selection	College of Agriculture, Northwest A&F University
26	Qiushi589	Wunong6/08Dasui	Weinan Qiushi Agricultural Technology Seed Co. LTD
27	Xinong161	Xinong979/Xinong3517	College of Agriculture, Northwest A&F University
28	Yongshun189	N0237-2-4-1-2/06804-2-3	College of Agriculture, Northwest A&F University
29	Xianmai569	Xinmai208 Variant strain	Xianyang Academy of Agricultural Sciences
30	Xinong168	Xinong4211/Zhoumai16	College of Agriculture, Northwest A&F University
31	Jvliang19	BainongAK58/22D	Shaanxi Province agriculture and animal husbandry improved seed farm
32	Xinong86	Xinong519/Shanmai159	College of Agriculture, Northwest A&F University
33	Xinong868	Zhengmai366/Zhoumai18	College of Agriculture, Northwest A&F University
34	Xinong619	Zhengmai366/Zhoumai18	College of Agriculture, Northwest A&F University
35	Weilong198	Xinong889//Youbang9987	Shaanxi Yangling Weilong Agricultural Technology Co. LTD
36	Enuo357	Nongda3471/Nuomai1	China Agricultural University
37	Xinong403	Shuangkang175/Shanmai99	College of Agriculture, Northwest A&F University
38	Xinong926	12L8012w/Xiaoyan22	College of Agriculture, Northwest A&F University
39	Xinong195	Aikang58/Zhengmai366	College of Agriculture, Northwest A&F University
40	Xinong627	Aikang58/Zhengmai366	College of Agriculture, Northwest A&F University

41	Xinong609	Aikang58/Xinong165	College of Agriculture, Northwest A&F University
42	Xinong105	Xinong501/Liangxing66	College of Agriculture, Northwest A&F University
43	Huimai858	Xinong979/02 (8) 8-3-7-0	Henan Huinong Seed Industry Co., LTD
44	Xinong911	Xinong389/Xinong9841	College of Agriculture, Northwest A&F University
45	Xinong537	BY9S30/Xinong979	College of Agriculture, Northwest A&F University
46	Xinong990	Shan481/Xiaoyan22	College of Agriculture, Northwest A&F University
47	Xinong33	Xiaoyan216/Wunong148	College of Agriculture, Northwest A&F University
48	Xinong158	2007140/Jiumai2	College of Agriculture, Northwest A&F University
49	Xinong936	Zhoumai26/Xinong1718	College of Agriculture, Northwest A&F University
50	Xinong333	Xiaoyan22D/Zhoumai16	College of Agriculture, Northwest A&F University
51	Xinong369	Xinong501/Xinong837	College of Agriculture, Northwest A&F University
52	Datang63	Zhongyu9037/H24-6	Shaanxi Datang Seed Industry Co., LTD
53	Qingyumai978	2006242-11/luo2706	Shaanxi Datang Seed Industry Co., LTD
54	Xian135	2004121-31/Xinong822	Shaanxi Datang Seed Industry Co., LTD
55	Shanyan582	Zhoumai16/Qingfeng197	Hanzhong Agricultural Science Research Institute
56	Shanyan816	Zhengmai9023/8219	Hanzhong Agricultural Science Research Institute
57	Xichun158	A8-18/01 (35) -1	College of Agriculture, Northwest A&F University
58	Xinong116	90 (107) /89 (330) /Aikang58	College of Agriculture, Northwest A&F University
59	Xinong837	3135E/Lankao906	College of Agriculture, Northwest A&F University
60	Mingqi167	Xinong383/W95-4	Yang Lingmingqi Wheat Crop Research Institute
61	Xichun519	Aikang58/Xumai1-97	College of Agriculture, Northwest A&F University
62	Dadi532	Taiyu28/Bainong64	Xi 'an Dadi Seedling Co. LTD
63	Datang69	Shannong981sp-10-12/Xinmai26	Shaanxi Datang Seed Industry Co., LTD
64	xiza 91	Fp98×Mp08	College of Agriculture, Northwest A&F University
65	Xinong943	Xiaoyan926A/Xiaoyan22	College of Agriculture, Northwest A&F University
66	Hangmai8	ZhongkehangtianH22/Xinong889	Shandong Jinan Yuhang Agricultural Development Co. LTD
67	Ximai303	Zhoumai16/Shannong757	Xianyang Academy of Agricultural Sciences
68	Xinong582	Xiaoyan22/Zhoumai22	College of Agriculture, Northwest A&F University
69	Xinong283	Xinong133/DH48	College of Agriculture, Northwest A&F University
70	Yanmai3568	Xiaoyan22/Yanmai9710	Xi 'an Yanliang District agricultural new variety experimental station breeding
71	Runmai28	Zhengmai9023/Yanzhan4110	Yicheng Runhe Crop Research Institute
72	Xichun375	08E47/Zhoumai22	College of Agriculture, Northwest A&F University
73	Xingmin213	0910/Hengguan35	Henan Fengwu Seed Industry Co. LTD
74	Xingmin213	(Xinong953/97-5)/SY01-1	Henan Fengwu Seed Industry Co. LTD
75	Mingyang670	34201/213 (6)	Xi 'an Mingyang Agricultural Technology Co. LTD
76	Qinxin358	PH85-4/918	Xi 'an Xinfeng Agricultural Technology Co. LTD
77	Shanza3hao	Zhoumai16/Xinong4211	College of Agriculture, Northwest A&F University
78	Xinong105	Xinong389/Xinong9841	College of Agriculture, Northwest A&F University
79	Xinong921	H18-5/Zhoumai26	College of Agriculture, Northwest A&F University
80	Weifeng163	DH-2/Zhoumai26	College of Agriculture, Northwest A&F University
81	Beilimai161	DH-2/Zhoumai26	College of Agriculture, Northwest A&F University
82	WeifengT03	Tianmai535Variant strain	College of Agriculture, Northwest A&F University
83	Xinong369	Zhoumai16/Lengmai9918	College of Agriculture, Northwest A&F University
84	Longmai838	Yumai47/Xiaoyan22	Jiangsu Tianlong Technology Co. LTD
85	Xinong369	Yumai49 Hybrid offspring	College of Agriculture, Northwest A&F University
86	Xinong369	34201/213 (6)	College of Agriculture, Northwest A&F University

Appendix Table 2 Primer setsused in this study

Name	Sequence (5'-3')*	Application
Pm46-F	TAGCTTGACCGGCCGGCTT	Cloning *Pm46*
Pm46-R	GGAGCAACACTTTTCAGACGGTG	
TaGDSL -F	GCCTGAACTAGCACGTGA	Cloning *TaGDSL*
TaGDSL -R	GACGGAAGCAGACACATAA	
pGFP-TaGDSL-F	CAGATCTGATGGGTTGTGAGAGGAAG	Cloning Subcellular localization construct pGFP-TaGDSL
pGFP-TaGDSL-R	GACTAGTCTCAGGCAGGCAGGCTAGG	
2YN-GDSL-PacI-F	CATTTACGAACGATAGTTAATTAAATGGGTTGTGAGAGGAAGCCG	LUC-TaGDSL
2YN-GDSL-SpeI-R	CACTGCCACCTCCTCCACTAGTGGCAGGCAGGCTAGGTGGA	
2YC-Pm46-PacI-F	CATTTACGAACGATAGTTAATTAAATGCCGGGCGGGGGGTTC	LUC-Pm46
2YC-Pm46-SpeI-R	CACTGCCACCTCCTCCACTAGTGACGGTGGCGTTCTTGCCGT	
qTaGDSL -F	CACCAACAAGCTCGCCCTCA	qPCR of *TaGDSL*
qTaGDSL -R	GGCCGGTCGATGATGTCCTG	
BMSV-TaGDSL-F	TAGCTGAGCGGCCGCCCCGGGTTCTCCCGCATGAAGGCCTGC	Cloning VIGS construct BSMV:

BMSV-TaGDSL-R	TAGCTGATTAATTAACCCGGGG CGCTGCCTTGGTACGGCAT	*TaGDSL*
RNAi- TaGDSL-F-1	GGGGTACCACTAGTGCGCTGC CTTGGTACGGCAT	Cloning silencing construct RNAi-*TaGDSL*
RNAi-TaGDSL -R-1	CGGGATCCGAGCTCTTCTCCCG CATGAAGGCCTGC	
OE- TaGDSL -F	GAGAACACGGGGGACTCTAGA ATGGGTTGTGAGAGGAAG	Cloning overexpression construct OE-TaGDS*L*
OE- TaGDSL -R	GAGCTCGGTACCCGGGGATCC TCAGGCAGGCAGGCTAGG	
TaAlpha-tubulin-F	ATCTCCAACTCCACCAGTGTCG	OE- TaGDSL
TaAlpha-tubulin-R	TCATCGCCCTCATCACCGTC	

Appendix Table 3. Results of Disease Resistance Identification of Wheat Test Lines from 2020 to 2021.

Line	Powdery mildew	
	Reaction	Type
CK1	9	ES
CK2	9	ES
1	9	ES
3	9	ES
9	9	ES
10	9	ES
11	9	ES
27	9	ES
28	9	ES
29	9	ES
30	9	ES
46	9	ES
49	9	ES
51	9	ES
52	9	ES
70	9	ES
72	9	ES
76	9	ES
34	9	ES
55	9	ES
60	9	ES
77	9	ES
79	9	ES
80	9	ES
81	9	ES
85	9	ES

Not: *ES: Easy susceptible*

Appendix Table 4. Results of Disease Resistance Identification of Wheat Test Lines from 2020 to 2021.

Line	Powdery mildew	
	Reaction	Type
CK1	9	ES
CK2	9	ES
4	3	MR
5	5	MS
8	3	MR
65	5	MS
61	5	MS
84	5	MS

Not: *ES: Easy susceptible*
MR: Medium resistance
MS: Medium susceptible

Patent of GDSL gene

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Certificate of Invention Patent

Title of Invention: TaGDSL, a wheat protein related to powdery mildew resistance, and its encoding gene and application
Inventor: Tao Ye, Wu LiuLiu, Li Chengwei, Hu Haiyan
Patent No.: ZL 2022 1 0579558.X
Patent application date: May 25, 2022
Patentee: Henan Institute of Science and Technology
Address: 453000 East Section of Hualan Avenue, Hongqi District, Xinxiang City, Henan Province
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Authorization proclamation No.: CN 114835789 A

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