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CLONING AND BIOINFORMATICS ANALYSIS OF WHEAT POWDERY MILDEW RESISTANCE RELATED GENE TAGDSL

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Powdery mildew disease of wheat is caused by Blumeri agraminis F. sp. tritici. It is one of the primary fungal diseases of wheat. Therefore, it is of great significance to explore and utilize the broad spectrum anti-powdery mildew genes and study their resistance mechanism and molecular mechanism for effectively resisting the harm of powdery mildew. Material is wheat variety Bainong207 supplied by Henan Provincial Research Center of Food Crop Genome Editing Engineering Technology. PmD-19T vector, E. coli 5α, and Agrobacterium GV3101 strain were purchased from Takara Biological Company. PCR apparatus, electrophoresis apparatus, agarose gel electrophoresis imager, autoclaving cooker, water bath cooker, ultra-clean workbench, etc. RT-PCR cloned the entire length of the TaGDSL gene. Bioinformatics analysis of the sequence showed that the total length of ORF was 1269bp, encoding 423 amino acids, with a molecular weight of 38.99 kD and an isoelectric point of 8.19. In addition, the TAGDSL gene has a transmembrane domain, a signal peptide, and the protein is hydrophilic. GDSL lipase is involved in plant physiological metabolism and local and global immunity. It is of great significance for improving disease resistance and yield of wheat. Based on the previous research, this experiment cloned the full-length sequence of a wheat GDSL gene, which enriched the members of the plant GDSL lipase family. She provided a basis for the subsequent exploration of its function and mechanism of action in the resistance to powdery mildew by modern molecular biology methods and the study of its gene function. The TAGDSL lipase gene of wheat was closely related to the durum wheat gene and barley gene by phylogenetic tree analysis. At the same time use bioinformatics method to forecast the gene can be more intuitive and more comprehensive understanding of its structure and properties, for the subsequent use of modern molecular biology methods to explore its function and role in the resistance to powdery mildew mechanism to provide effective basis, and for creating varieties of wheat powdery mildew resistance gene source and theoretical basis.

Key words: *wheat, powdery mildew, TAGDSL gene, bioinformatics analysis, varieties of wheat powdery mildew resistance gene.*

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Introduction. Wheat (*Triticum aestivum* L.) is a worldwide food crop with strong adaptability and wide distribution. About 35 %–40 % of the world's population use wheat as the primary food, providing about 20 % protein and 21 % food calories for humans (Wang et al., 2017). As the world's largest country in wheat planting and output, China plays an important role in ensuring domestic food security and affects international food security (He et al., 2018). Wheat yield has been affected by diseases and insect pests, among which powdery mildew is one of the most critical diseases. It is distributed in the global scope, and the harm is greater. Wheat powdery mildew is made up of Gramineae brinell type wheat powdery mildew specialization *Blumeria graminis* F. sp. tritici *(Bgt)* is a specific fungus, cause of disease is one of the main fungus diseases of wheat (Johnson et al., 1979), in Asia, Africa, and Europe and the United States. have occurred worldwide diseases, especially in China, almost wheat-growing areas almost is powdery mildew, seriously affected the yield and quality of wheat in our country, since 1991, the year of wheat powdery mildew were to maintain the high incidence area. As of 2016, the area of wheat affected by powdery mildew disease has reached 7.4 million ha (Zou et al., 2017), which can yield 50 % in severe cases (Alam et al., 2013).

Powdery mildew fungus is a living parasitic fungus with the characteristics of a short life cycle, easy long-distance transmission of spores, and intense sexual recombination ability. Ascomidia and conidia of wheat powdery mildew

fungus are the leading carriers of disease transmission. In suitable temperature and humidity conditions, conidia on self-growing wheat seedlings completed over summer and infected early autumn wheat seedlings and caused disease. The specific process is as follows: Conidium by airflow to wheat leaf, about 30 minutes germination form Primary germ tube and Appressorial germ tube, 12 to 15 hours AGT elongation form into Apical appressorium to destroy the cell wall into skin cells, then form a finger in the host cell haustoria absorb nutrition to produce the secondary suction device and conidium chain, a new cycle of conidium infection (Edwards, 2002; Zhang et al., 2005). The powdery mildew fungus mainly infects the leaves of wheat. When the fungus is more serious, it can also infect other parts, such as the stalk, leaf sheath, and the ear of wheat. The photosynthetic capacity of wheat infected with powdery mildew decreased, while the ability of respiration and transpiration increased, thus reducing the accumulation of nutrients and increasing the consumption, resulting in significantly smaller wheat ears, reduced number of grains, smaller grains, and seriously reduced yield. After powdery mildew infection, Wheat plants are prone to lodging, with dry leaves and a fast death rate, which seriously affects wheat's average growth and development (Yu et al., 2013; Griffey et al., 1993). In the past 40 years, due to the improvement of wheat production conditions and the variation of virulence structure of the pathogen, wheat powdery mildew has rapidly spread from the local areas in the southwest and southeast coastal areas of China to almost all wheat areas in the country, causing considerable losses to China's grain production (Liu et al., 2016). Due to the demand and yield of wheat in China's crops at the forefront, to ensure its quality, spraying pesticide chemical reagent has become the primary prevention and control method because of some common diseases. Some prevention and control methods use different tillage methods, but whether spraying chemical reagents or changing tillage methods, their application will be limited by objective climate and environmental protection reasons (Qiu Lina, 2019; Shen et al., 2019). At present, the main ways to control powdery mildew are the use of fungicides and the cultivation of disease-resistant varieties, and the spraying of fungicides has caused pesticide residues and environmental pollution, so the cultivation of disease-resistant varieties of wheat has become the key to solve this problem. At present, both wild-type and artificially bred varieties resistant to wheat powdery mildew are scarce, so it is urgent to breed resistant varieties quickly and efficiently. With the rapid development of biotechnology, it is the best way to develop crop resistance genes from a micro perspective and promote the breeding of new varieties of crops, which is in line with the principle of green development. However, the variation of powdery mildew bacteria is relatively rapid, and it is easy to lose the single resistance of the variety (Zeng Fansong, 2017). Wheat breeding disease-resistant varieties are the prevention and control of wheat powdery mildew, guarantee safety in production is the most economical and effective method of mining using broad-spectrum powdery mildew resistance gene and study its disease-resistant mechanism, is the premise and guarantee for cultivating disease-resistant varieties (Kuraparthy et al., 2007), therefore resistance genes of digging, wheat resistance-related gene expression regulation, has important significance to the harm of effective resistance to powdery mildew.

Plant GDSL lipase is a large gene family that plays essential biological functions in plant growth, development, morphogenesis, lipid metabolism, and defense response (Kondou et al., 2008; Takahashi et al., 2010; Hao et al., 2014). The expression of the GDSL lipase gene can respond to both abiotic and biological stresses. Plant GDSL lipase gene expression can be induced by bacteria, salicylic acid, ethylene, jasmonic acid, and other hormones, as well as abiotic stress factors, suggesting that they may be involved in plant disease resistance and stress response (Oh et al., 2005; Kram et al., 2008; Lee et al., 2003). The performance of plant disease resistance results from the interaction between the disease resistance gene of the host plant and the pathogenic gene of the pathogen under the influence of specific environmental conditions (Shao et al., 2009). GDSL lipase can destroy the structural integrity of the actual fungus spore in the plant body through the transgenic node or direct connection of the signal and restrict its normal reproduction (Naranjo et al., 2009). GDSL lipase can induce plant disease resistance to fungi, and GLIP-1 mutant plants show stronger sensitivity to saprophytic fungal brassicas than wild-type plants (Zuo et al., 2005; Yuan et al., 2015). Wheat will encounter various biological and abiotic stresses during the growth process, such as wheat powdery mildew fungus, which will seriously harm the growth and development of wheat. When plants are under disease stress, a large number of reactive oxygen species (ROS) will be produced in cells (Lee et al., 2009), which will affect the average growth and development of plants. The antioxidant enzymes in cells can remove ROS and maintain the REDOX balance of cells (Tian et al., 1991; Wang et al., 2001).

The relationship between wheat GDSL lipase and powdery mildew is rarely reported. How this gene causes resistance to wheat powdery mildew is still unclear. Therefore, in this study, the gene was cloned, and its biological information was analyzed to find the disease-resistance mechanism. It was expected to lay a foundation for understanding the molecular mechanism of wheat powdery mildew and breeding disease-resistant varieties.

Materials and Methods. *Material.* Wheat variety: *Bainong207* supplied by Henan Provincial Research Center of Food Crop Genome Editing Engineering Technology. PmD-19T vector, *E. coli* 5α, and Agrobacterium GV3101 strain were purchased from Takara Biological Company. PCR apparatus, electrophoresis apparatus, agarose gel electrophoresis imager, autoclaving cooker, water bath cooker, ultra-clean workbench, etc.

RNA extraction method. RNA extraction process (refer to Takara RNA extraction kit).

Cloning and sequencing analysis of TAGDSL gene. The full-length sequence fragment of the *TaGDSL* gene was obtained at NCBI. Primer Premier 6 software was used for Primer design. The upstream Primer *TaGDSL*-F:

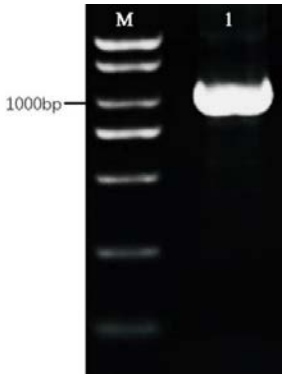
GCCTGAACTAGCACGTGA; The downstream primer *TaGDSL*-R: TTATGTGTCTGCTTCCGTC; The cDNA of *Bainong207* was used as the template for PCR amplification, and the gel was cut according to the DNA recovery kit and the PCR product was recovered. The pMD-19T clone vector was linked and transformed into *E. coli* 5 cells. The cells were coated and cultured overnight.

Bioinformatics analysis of TaGDSL gene. Sequences with high similarity were downloaded through BLASTP alignment on the NCBI website. A neighbor-joining phylogenetic tree was constructed by combining the downloaded sequences with Mega 7.0. The protein-coding region (CDS) was found according to the cloned *TAGDSL* gene sequence and translated into protein using BioXM2.7. Use online tools ProtParamExPASy site to analyze the protein's basic physical and chemical properties, including the theory of amino acid composition relative molecular weight, and pI value. They were using ProSWEETale tools to analyze protein hydrophobicity. Using TM pred tool to predict transmembrane region and across the membrane direction and signal peptide analysis.

Results. *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 Fig. 1. The results show that the extraction effect is good and the integrity is good. The OD260/280 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.

M = standard2000 + Marker; 1-2 = RNA samples
Fig. 1. RNA detection by electrophoresis

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 (Fig. 2). 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.



M = standard2000 + Marker; 1 = Amplified band
Fig. 2. *TaGDSL* gene PCR amplification

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. 3). A phylogenetic tree was constructed by using MEGA7.0 (Fig. 4). In the figure, wheat *TaGDSL* was most closely related to durum wheat *TDGDSL* and barley *HVGDSL*.

Ta = *Triticum aestivum*; TD = *Triticum durum*;
Hv = *Hordeum vulgare*; Zm = *Zea mays*
Fig. 3. Homologous analysis
of GDSL amino acid sequences

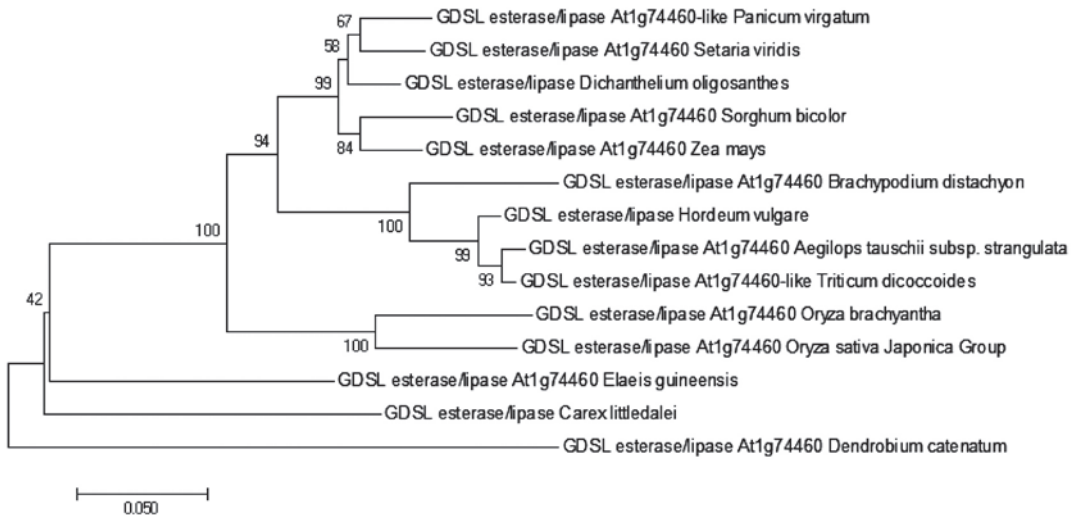


Fig. 4. Phylogenetic tree analysis of *GDSL* genes in other species

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 38.99kD, and the theoretical isoelectric point Pi was 8.19 (Fig. 5).

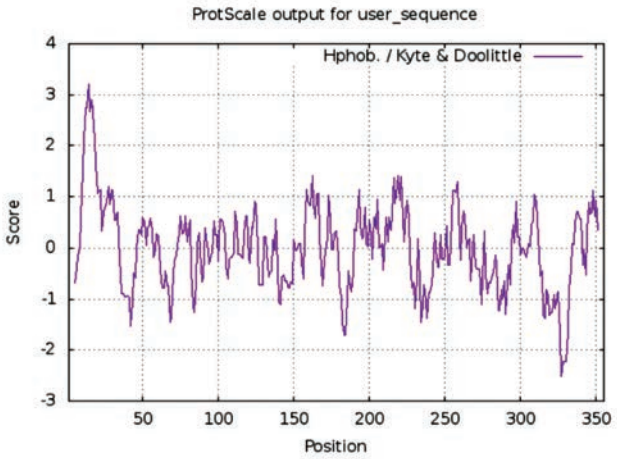


Fig. 5. Hydrophilic and hydrophobic analysis of *TAGDSL*

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. 6).

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. 5). The hydrophobic and hydrophilic regions coded by *TaGDSL* appear alternately and evenly distributed. Therefore, we predicted that the *TaGDSL* protein was hydrophilic.

Discussion. *GDSL* lipase is a multi-gene family that exists widely in the plant kingdom. It has hydrolase activity and can hydrolyze various lipids (Ling, 2007). In recent years, more than 1100 members of *GDSL* lipase have been found in

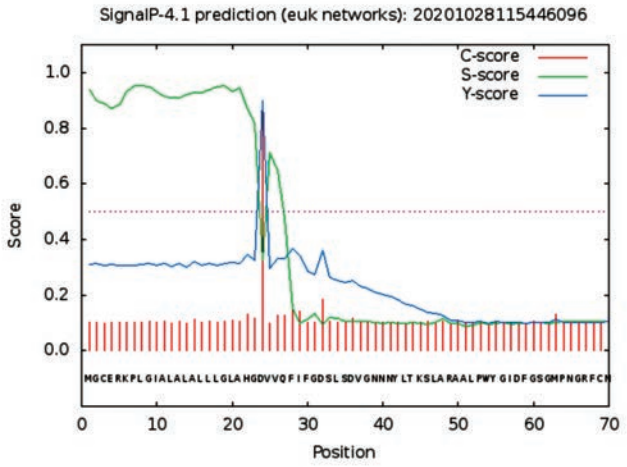


Fig. 6. Transmembrane region analysis of the *TAGDSL* gene

Arabidopsis thaliana, rice, maize, grape, and poplar, among which 108 family members have been found in Arabidopsis thaliana and 96 in grape (Beisson et al., 1997; LING H, 2008). *GDSL* lipase plays an essential role in plant responses to biotic and abiotic stress overruns (Huang et al.,2015; Su et al, 2007). *GDSL* lipase has broad-spectrum resistance to various pathogens (Hong et al., 2008). Plants can use multiple layers of the immune system to resist pathogen attacks (Han et al., 2006). In previous studies, the *GDSL* gene was related to wheat powdery mildew resistance, but the specific gene information was unknown.

In this study, the *GDSL* gene sequence of wheat was successfully cloned by RT-PCR (Feeney et al., 2005). Sequence analysis showed that the ORF length of the gene was 1269bp, encoding 423 aminoacids, the predicted molecular weight was 38.99 kD, and the theoretical isoelectric point PI was 8.19. As predicted (Pruitt et al., 2014; Morgulis et al., 2006). Isoelectric point can be used for the separation of amino acids. The *TAGDSL* gene has a transmembrane region (Fig, 7) and a signal peptide, which is predicted to be

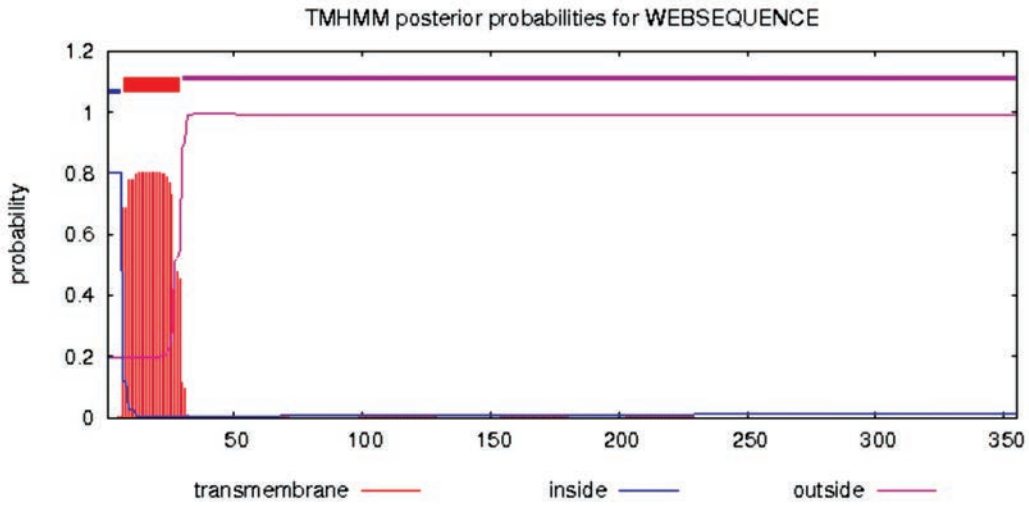


Fig. 7. Transmembrane domain prediction of TAGDSL gene

synthesized in the endoplasmic reticulum. In the *TAGDSL* protein-coding, hydrophobicity and hydrophilicity occur alternately. Hydrophilic amino acids are evenly distributed in the whole peptide chain, and hydrophobic amino acids are excessive. Therefore, we predict that the *TAGDSL* gene is hydrophilic and that the dissolution of the protein in an aqueous solution result from the interaction between the surface charge and ions of the protein in aqueous solution water molecules. Too high or too low ionic strength in solution will destroy the hydration layer on the protein surface and promote protein polymerization and precipitation.

To further study the evolutionary relationship of *GDSL* in different species, it was concluded that the wheat *TAGDSL* of wheat was closely related to the *TDGSL* of durum wheat and the *HVGDSL* of barley with the closest relationship. The results of this study provide data and a basis for further research on the biological functions of *TAGDSL*.

Conclusions. Wheat, as a major food crop, has become the main goal of breeding to improve its yield and stress resistance. GDSL lipase is involved in plant physiological metabolism and local and global immunity, which is of great significance for improving disease resistance and yield of wheat. Based on the previous research, this experiment cloned the full-length sequence of a wheat GDSL gene, which enriched the members of the plant GDSL lipase family, and provided a basis for the subsequent exploration of its function and mechanism of action in the resistance to powdery mildew by modern molecular biology methods and the study of its gene function. The bioinformatics analysis showed that wheat GDSL gene has a complex function, and further study is needed to fully clarify the function of this gene, which will provide the genetic source and theoretical basis for the creation of wheat powdery mildew resistant varieties.

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Клонування та біоінформаційний аналіз стійкості пшениці до борошнистої роси за допомогою TAGDSL гена

Збудником борошнистої роси пшениці є Blumeri agraminis F. sp. tritici. Це одна з основних грибкових хвороб пшениці. Тому дуже важливо виявити та використати проти борошнистої роси гени широкого спектру дії, а також вивчити механізм їх стійкості та молекулярний механізм для ефективної протидії шкідливості борошнистої роси. Матеріалом є сорт пшениці Bainong207, що поставляється Науково-дослідним центром з технології редагування геному культур у провінції Хенань. Вектор PmD-19T, E. coli 5α та штам Agrobacterium GV3101 були придбані у біологічної компанії Takara. Були задіяні ПЛР-апарат, апарат для електрофорезу, електрофорез з агарозним гелем, автоклав, плита на водяній бані, надчистий робочий стіл тощо. RT-ПЛР клонувала всю довжину гена TaGDSL. Біоінформаційний аналіз послідовності показав, що загальна довжина ORF становила 1269 п.о., яка кодує 423 амінокислоти, з молекулярною масою 38,99 кДт та ізоелектричною точкою 8,19. Крім того, ген TAGDSL має трансмембранний домен, сигнальний пептид, а також гідрофільний білок. Ліпаза GDSL бере участь у фізіологічному обміні рослин щодо локального та глобального імунітету. Це має велике значення для підвищення стійкості до хвороб та врожайності пшениці. На підставі попередніх досліджень цей експеримент клонував повнорозмірну послідовність гена GDSL пшениці, яка збагатила представників сімейства ліпаз GDSL рослин. Вона стала основою для подальшого дослідження її функції та механізму дії на стійкість до борошнистої роси за сучасними методами молекулярної біології та вивчення функції її генів. Ген ліпази TAGDSL пшениці був тісно пов'язаний з геномом твердої пшениці та геномом ячменю через філогенетичне дерево. У той же час використання методу біоінформатики для прогнозування гена може дати більш повне розуміння його структури та властивостей, при подальшому використанні сучасних методів молекулярної біології для вивчення його функції та ролі у механізмі стійкості до борошнистої роси задля забезпечення ефективної основи, а також для створення сортів з генами стійкості до борошнистої роси пшениці та теоретичної бази.

***Ключові слова:** пшениця, борошниста роса, ген TAGDSL, біоінформаційний аналіз, гени стійкості до борошнистої роси сортів пшениці.*