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TaULP5 contributes to the compatible interaction of adult plant resistance wheat seedlings-stripe rust pathogen

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ABSTRACT

Adult plant resistance indicates that plant is susceptible to pathogen at seedling stage, but resistant at adult stage. Understanding the mechanism of the interactions between APR wheat plants and *Puccinia striiformis* f. sp. *tritici* (*Pst*) is important for the creation of strategies to improve cultivar disease resistance. In this study, a full-length cDNA was isolated from APR wheat cultivar Xingzi 9104 (XZ), and was designated as ubiquitin-like protein 5 (*TaULP5*). TaULP5 was likely to be located in the cytoplasm, with a percentage of 75.9% *Arabidopsis* protoplasts number. The expression of *TaULP5* was largely induced in the compatible interaction of wheat seedlings to *Pst*, while no obvious change was found in the incompatible interaction of wheat adult plants to *Pst*. Moreover, when *TaULP5* was knocked down, the wheat resistance at seedling stage to *Pst* was improved. In addition, knockdown of *TaULP5* increased the expression levels of some biotic stress-related genes, such as PR1 and PR2. It is the first time to confirm that ubiquitin-like protein could contribute to the compatible interaction of XZ to *Pst*, and the results will lay a foundation for understanding the mechanisms of different interactions between APR wheat plants and *Pst* at post-translational level.

1. Introduction

Wheat stripe rust, caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*), is one of the most destructive wheat diseases in the world. Breeding and rational utilization of disease-resistant varieties is the safest, the most effective, the most economical and the most environmentally sound method for controlling wheat stripe rust [5,8]. At present, wheat resistance to *Pst* can be broadly categorized into all-stage resistance (seedling resistance) and adult plant resistance (APR). Compared with seedling resistance, APR is much more stable and durable. APR indicates that plant is susceptible to pathogen at seedling stage, but resistant at adult stage. Understanding the mechanisms of different interactions between APR wheat and *Pst* is important for the strategies creation of improving cultivar disease resistance.

Post-translational modification of proteins through the ubiquitin-proteasome system (UPS) serves a critical regulatory role in most cellular processes [7,9,20,22]. Ubiquitins, as a group of highly conserved eukaryotic polypeptides 76 amino acids in length, play roles by attaching to lysine residues (or sometimes to other amino acids) of their target proteins via their C-terminal glycines [12,15]. Ubiquitin-like proteins (UBLs) are another important component of the UPS. And there is a resemblance between UBLs and ubiquitins in sequence [16]. All UBLs share a similar three-dimensional structure [25]. UBLs are ligated to their target proteins or other molecules by distinct but evolutionarily related enzyme cascades [18]. Host UBL pathways have been confirmed to play important roles in plant-pathogen interactions. For example, the ubiquitin-like protein ISG15 was found to be involved in the host antiviral immune defense [24,31].

Ubiquitin-like protein 5 (ULP5) is a recently identified component of the UPS. UBL5 was first identified in 2001 as an 8.5-kD protein product of a gene isolated from human adult iris cDNAs

Abbreviations: dpvi, days post virus inoculation; EF, elongation factor 1 alpha-subunit gene; EST, expressed sequence tag; hpi, hours post-inoculation; ORFs, Open reading frames; PcG, Polycomb group; PDS, phytoene desaturase gene; *Pst*, *Puccinia striiformis* f. sp. *tritici*; RT-PCR, Reverse-transcription polymerase chain reaction; UBLs, Ubiquitin-like proteins; UBP, ubiquitin-specific protease gene; ULP5, Ubiquitin-like protein 5; UPS, ubiquitin-proteasome system; VIGS, virus-induced gene silencing; XZ, Xingzi9104.

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[13]. Unlike other ubiquitins, ULP5 contains an ubiquitin super-fold with an electrostatic surface [23]. Meanwhile, the C-terminal peptide sequence of ULP5 is not a double glycine but a pair of tyrosines, followed with variable amino acid residues [29]. According to previous report, ULP5 may affect rat energy metabolism [2]. In addition, the ULP5 yeast ortholog ubiquitin-1 (Hub1) had been reported in *Saccharomyces cerevisiae*, and it was found to play a role in mRNA and pre-mRNA splicing [29]. However, the studies about the function of plant ULP5 were relatively backward. Little is known regarding the biological functions of plant ULP5, although many ULP5 genes have been predicted based on the ESTs and genomic information (NCBI) of some plants. Thus, whether ULP5 can participate in plant disease resistance, especially the interactions of APR plant and pathogen?

In this study, *TaULP5* was firstly isolated from APR cultivar Xingzi 9104 (XZ), and the gene function in the compatible interaction between XZ and *Pst* was explored. The results will lay a foundation for understanding the mechanisms of different interactions between APR wheat plants and *Pst* at post-translational level.

2. Materials and methods

2.1. Plant materials and treatments

XZ processes APR to *Pst* pathotype CYR32, that means it is susceptible to CYR32 at the seedling stage but resistant at the adult stage [19]. XZ and CYR32 used in this study were obtained from the Institute of Plant Pathology, Northwest A&F University, Yangling, Shaanxi, China. Wheat cultivation and *Pst* inoculation were performed as described in Ref. [10]. Leaves were collected at 0, 24, 48 and 120 h post-inoculation (hpi). After collection, all samples were immediately frozen in liquid nitrogen and stored at −80 °C.

2.2. RNA extraction and cDNA synthesis

Extraction of total RNA was performed with TRIzol reagent (Invitrogen, Carlsbad, CA) following the manufacturer's instructions. DNase I was used to remove the genomic DNA from total RNA. The total RNA was quantitated using a NanoDrop™ 1000 spectrophotometer (Thermo Fisher Scientific, USA). Three μg of total RNA was used to synthesize the first-strand cDNA using the RT-PCR system (Promega, Madison, WI, USA) with the Oligo (dT) 18 primer according to the manufacturer's instructions.

2.3. Gene isolation and sequence characterization

The sequence fragment identified from the transcriptome libraries of XZ challenged with *Pst* was used as a query probe to screen publically available wheat expressed sequence tag (EST) databases for the full length of target gene. Homologous wheat EST sequences were retrieved and assembled. Open reading frames (ORFs) in the assembled sequences were predicted by NCBI's ORF FINDER. Reverse-transcription polymerase chain reaction (RT-PCR) was used to amplify the full-length cDNA with specific primers (Table A.1). The PCR products were cloned into the pMD18-T Simple vector (TaKaRa Biotechnology) and sequenced with an ABI PRISM 3130xl Genetic Analyzer (Applied Biosystems, USA). The DNAMAN (version 5.2.2) program was used for phylogenetic comparison between the deduced protein sequences and the corresponding characterized proteins from other plant species.

2.4. Subcellular localization of TaULP5

The *TaULP5* ORF was PCR-amplified without stop codons using specific primers with *Hin*dIII and *Bam*HI restriction sites. The sequence fragment was then sub-cloned into PTF486, which contains the eGFP open reading frame. The recombinant vector PTF486-*TaULP5* was end-sequenced with an ABI PRISM 3130XL Genetic Analyzer (Applied Biosystems, USA). The primers used for vector construction were listed in Table A.1. Well-expanded leaves from 4 week-old *Arabidopsis thaliana* were used for protoplast preparation. Mesophyll protoplasts and reagents were prepared according to [30]. The PTF486-*TaULP5*-eGFP fusion construct was transformed into *Arabidopsis thaliana* mesophyll protoplasts using the method described by Ref. [30]. Protoplasts were incubated with plasmids at 25 °C in the dark for 16 h with gentle swirling at 40 rpm. eGFP fluorescence images of transformed protoplasts were obtained using confocal microscopy with a Nikon PCM2000 (Bio-Rad) laser-scanning confocal imaging system. The experiment was repeated every other day using the same batch of *Arabidopsis* plants for three times totally.

2.5. Quantitative RT-PCR (qRT-PCR)

The expression profiles of *TaULP5* and PR protein genes were determined using quantitative real-time PCR analyses with specific primers (Table A.1). These primers were first used to amplify the fragment by regular PCR. The cloned fragment was then sequenced to confirm primer specificity. The wheat translation elongation factor 1 alpha-subunit (EF) gene (GenBank Accession No. M90077) and cytoplasmatic ribosomal protein S13 gene (GenBank Accession No. AY736126) were selected as the internal reference genes for qRT-PCR analyses. Three independent biological replicates were used for each time point, as well as a non-template control, and the results were analyzed using the comparative threshold ($2^{-\Delta\Delta CT}$) method [21].

2.6. Functional analysis of TaULP5 in response to Pst infection

For virus-induced gene silencing (VIGS) vector construction, a 223-bp fragment was amplified using specific primers with *Pac*I and *Not*I restriction sites. The *TaPDS* sequence fragment in BSMV-*TaPDS* was replaced by the amplicon to generate the BSMV-*TaULP5* vector. The recombinant vectors were end-sequenced with an ABI PRISM 3130XL Genetic Analyzer (Applied Biosystems, USA). The primers used for vector construction were listed in Table A.1. The detailed VIGS procedures were performed as described by Ref. [27] with a minor revision. In vitro transcription products of the three BMSV genome sequences (α, β, γ) were diluted 30 times, and 0.5 μL of each was mixed in a 1:1:1 ratio. Next, 9 μL of FES buffer was added to the mixture. And then the mixtures were directly applied to the second leaves by rub inoculation with a gloved finger. BSMV-*TaPDS* was used to silence the wheat phytoene desaturase gene (PDS) as a positive control and FES buffer was used as a negative control. The wheat seedlings inoculated with BSMV were incubated in a growth chamber at 25 ± 2 °C. Nine days post inoculation (dpi), the base of the third leaves and the top of the fourth leaves were infected with CYR32. After *Pst* inoculation, the wheat seedlings were incubated in a growth chamber at 16 ± 2 °C. The *Pst*-inoculated leaves were then sampled at 0, 24, 48, 120 hpi for silencing efficiency analysis and histological observation. *Pst* infection types were examined at 15 dpi. Two independent biological replicates were performed for each treatment.

2.7. Histological observation

The samples were stained as previously described [28], and the stained leaf segments were observed to analyze the infection site, necrotic area, and hyphal length using an Olympus BX-51 microscope (Olympus Corp., Tokyo). No more than 5 different infection sites were examined on each randomly selected leaf segment, and a