Proteomic Analysis of UV-B-Induced Virulence-Mutant Strains of *Puccinia striiformis* f. sp. *tritici* Based on iTRAQ Technology

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The emergence of new physiological races of *Puccinia striiformis* f. sp. *tritici* (*Pst*) causing wheat stripe rust can lead to the loss of resistance of wheat cultivars to stripe rust, thus resulting in severe losses in wheat yield. In this study, after the germination of urediospores of three *Pst* strains including the original strain (CYR32, a dominant physiological race of *Pst* in China) and two virulence-mutant strains (CYR32-5 and CYR32-61) acquired from CYR32 via UV-B radiation, proteomic analysis based on isobaric tags for relative and absolute quantification (iTRAQ) technology was performed on the strains. A total of 2,271 proteins were identified, and 59, 74, and 64 differentially expressed proteins (DEPs) were acquired in CYR32-5 vs. CYR32, CYR32-61 vs. CYR32, and CYR32-61 vs. CYR32-5, respectively. The acquired DEPs were mainly involved in energy metabolism, carbon metabolism, and cellular substance synthesis. Furthermore, quantitative reverse transcription PCR assays were used to determine the relative expression of the 6, 7, and 1 DEPs of CYR32-5 vs. CYR32, CYR32-61 vs. CYR32, and CYR32-61 vs. CYR32-5, respectively, at the transcriptional level. The relative expression levels of one, five, and one gene, respectively, encoding the DEPs, were consistent with the corresponding protein abundance determined by iTRAQ technology. Compared with CYR32, the DEPs associated with energy metabolism, carbon metabolism, and cellular substance synthesis were up-regulated in the mutant strains. The results indicated that the virulence-mutant strains CYR32-5 and CYR32-61 had more tolerance to stress than the original strain CYR32. The results obtained in this study are of great significance for exploring the virulence variation mechanisms of *Pst*, monitoring the changes in *Pst* populations, breeding new disease-resistant wheat cultivars, and managing wheat stripe rust sustainably.

**Keywords:** *Puccinia striiformis* f. sp. *tritici*, virulence variation, proteomics, iTRAQ, UV-B radiation, wheat stripe rust

**INTRODUCTION**

Stripe rust (yellow rust), caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*), is a devastating wheat disease in wheat producing regions worldwide (Li and Zeng, 2002; Line, 2002; Chen, 2005; Wan et al., 2007; Chen et al., 2014; Wang et al., 2014). It can have a serious impact on wheat production, and can cause yield losses of 10–30% or even no yield once a disease outbreak or epidemic occurs.
Pst can mutate in many ways to produce new strains or physiological races (Ma et al., 1993; Li and Zeng, 2002; Jin et al., 2010; Hu et al., 2014; Kang et al., 2015). The emergence of new physiological races can lead to the loss of stripe rust resistance in wheat cultivars and cause periodic epidemics of the disease (Li and Zeng, 2002; Chen, 2007; Chen et al., 2014; Hu et al., 2014; Kang et al., 2015). Hence, exploring the mechanisms of virulence variation of Pst can provide an important basis for wheat breeding and control of stripe rust.

Ultraviolet-B (UV-B) radiation (280–320 nm) is a part of sunlight, and can affect living things on Earth’s surface. In most reported studies, UV-B radiation has been used as a stress condition, and many aspects of its effects on living organisms have been investigated, including physiological and biochemical effects (Fargues et al., 1996; Jansen et al., 1998; Fernandes et al., 2007; Wargent and Jordan, 2013; Braga et al., 2015), genetic effects (Kumar et al., 2004; Braga et al., 2015), and effects on proteins (Wu X. C. et al., 2011; Trentin et al., 2015; Gao et al., 2019). As the main propagules of wheat stripe rust, Pst urediospores, in high-altitude areas of northwestern China with dispersal with upper air flows, are affected by UV-B radiation, and virulence-mutant strains may be induced (Li and Zeng, 2002; Cheng et al., 2014; Hu et al., 2014; Kang et al., 2015).

Reported studies on the effects of UV-B radiation on Pst have focused on changes in the epidemiological components of Pst after UV-B radiation (Jing et al., 1993; Cheng et al., 2014), screening of virulence-mutant strains of Pst by UV-B radiation (Shang et al., 1994; Huang et al., 2005; Wang et al., 2009; Zhao et al., 2019), and random amplified polymorphic DNA (RAPD) analysis of the UV-B-induced virulence-mutant strains of Pst (Huang et al., 2005; Wang et al., 2009). An important study on Pst (Zheng et al., 2013) revealed the whole genome information of CYR32, a dominant physiological race of Pst in China, using high-throughput sequencing technology, which provided an important basis for the identification and functional verification of Pst proteins. More recently, a proteomics method based on isobaric tags for relative and absolute quantification (iTRAQ) technology, combining the isotope labeling method and tandem mass spectrometry, has been used to identify and quantify differentially expressed proteins (DEPs) between different proteomes, particularly in studies on plant-pathogen interactions (Fu et al., 2016; Xu et al., 2017; OuYang et al., 2018). Using this methodology, Zhao et al. (2016) investigated the difference between the proteomes of the urediospores of CYR32 before and after germination. The results showed that most of the DEPs were involved in biological processes such as carbon metabolism, energy metabolism, and transport. In our previous study (Zhao et al., 2018), the urediospores of three physiological races of Pst in China—CYR31, CYR32, and CYR33—were irradiated with a dose of UV-B radiation at which the relative lethal rate of the urediospores of each physiological race was 90%. Proteomic analysis of the irradiated urediospores, using methods based on iTRAQ technology, was then performed to explore the effects of UV-B radiation on Pst at the protein level. The results showed that most of the identified DEPs were mainly involved in energy metabolism, substance metabolism, and DNA biosynthesis. However, there are no reports on the differences in the level of protein expression between the original strains of Pst and the UV-B-induced virulence-mutant strains.

In our previous study (Zhao et al., 2019), two UV-B-induced virulence-mutant strains, CYR32-5 and CYR32-61, were screened from the UV-B-irradiated urediospores of CYR32 on the seedlings of the wheat cultivar Guinong 22. In this study, after germination of the urediospores of the original strain (CYR32) and the two UV-B-induced virulence-mutant strains (CYR32-5 and CYR32-61), proteins were extracted from the germinated urediospores and germ tubes, the DEPs among the proteomes of the three Pst strains were screened by using the proteomics method based on iTRAQ technology, and then the acquired DEPs were subjected to COG (Cluster of Orthologous Groups) annotations, KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway analyses, and quantitative reverse transcription PCR (qRT-PCR) assays. This study is of great significance for exploring mechanisms of Pst virulence variation, and it can provide a reference for wheat resistance breeding and control of wheat stripe rust.

MATERIALS AND METHODS

Materials

In the previous study (Zhao et al., 2019), the urediospores of the Pst physiological race CYR32 were irradiated with UV-B radiation under a radiation dose (the radiation intensity was 250 µW/cm², and the radiation time was 95 min) for which the relative lethal rate of urediospores was approximately 90% (i.e., the relative germination rate of urediospores was approximately 10%); then the irradiated urediospores were inoculated on the seedlings of Guinong 22 to screen virulence-mutant strains. Finally, two virulence-mutant strains named CYR32-5 and CYR32-61, with stable infection types on Guinong 22 throughout four successive generations, were obtained. In this study, the original strain of the Chinese physiological race CYR32 of Pst, and the two virulence-mutant strains (CYR32-5 and CYR32-61) were used. The wheat cultivar Mingxian 169, which is susceptible to all known Chinese physiological races of Pst, was used for Pst multiplication. Multiplication of the urediospores of each Pst strain was conducted in an artificial climate chamber (environmental parameters: light time, 12 h/d; light intensity, 10,000 lux; temperature, 11–13°C; and relative humidity, 60–70%) using the method described by Cheng et al. (2014).

Germination of Pst Urediospores

Fresh urediospores of each of the three strains CYR32, CYR32-5, and CYR32-61 were collected from the diseased leaves of Mingxian 169 seedlings. For each strain, a spore suspension with a concentration of 2.5 mg/mL was prepared with 0.2% Tween-80 solution. Then, 4 mL of the spore suspension was transferred into a Petri dish (16 cm in diameter) containing 1% water agar medium. The urediospores (10 mg) contained in the suspension were evenly scattered in the Petri dish. When the suspension was almost dry, the Petri dish was sealed with plastic wrap.
and incubated for 10 h at 9°C in a dark environment. After incubation, a small agar block was picked up and placed on a glass slide for microscopic observation. At least 300 urediospores were checked, and the germination rate of the urediospores was recorded. A urediospore with a germ tube longer than half of the diameter of the urediospore was regarded as germinated. If the germination rate of the urediospores was more than 90%, the urediospores and germ tubes on the surface of the water agar medium were gently collected with a cover glass. For each *Pst* strain, the urediospores and germ tubes collected from five Petri dishes (approximately 50 mg in total) were treated as a sample.

**Protein Extraction and iTRAQ Labeling**

In total, two samples of CYR32, three samples of CYR32-5, and three samples of CYR32-61 were acquired for protein extraction. Grinding tools were pre-cooled with liquid nitrogen, and then each of the prepared samples was pulverized with liquid nitrogen in a mortar and pestle. The pulverized sample was suspended in a trichloroacetic acid (TCA) and acetone solution (TCA:acetone = 1:10, w/v; pre-cooled at −20°C) and then precipitated for 2 h at −20°C. After centrifugation at 4°C under 20,000 × g for 30 min, the supernatant was discarded, and the precipitate was suspended in pre-cooled pure acetone, and then precipitated for 30 min at −20°C prior to centrifugation at 4°C under 20,000 × g for another 30 min. This process was repeated several times until the precipitate was substantially white. The precipitate was resuspended in a lysis buffer (8 M urea, 30 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 1 mM phenylmethylsulfonyl fluoride, 2 mM ethylenediaminetetraacetic acid, and 10 mM dithiothreitol) and sonicated for 5 min (pulse on 2 s, pulse off 3 s, power 180 W), followed by centrifugation at 20,000 × g for 30 min. The supernatant was collected and added to dithiothreitol to a final concentration of 10 mM. After incubation in a water bath at 37°C for 1 h, iodoacetamide was quickly added, to a final concentration of 55 mM. After incubation for 1 h in a dark environment, the supernatant solution was supplemented with a fourfold volume of pre-chilled acetonitrile, and then precipitated at −20°C for more than 3 h. After centrifugation at 4°C under 20,000 × g for 30 min, the precipitate was dissolved in 400 μL of digestion buffer to a final concentration of 50% triethylammonium bicarbonate (TEAB) and 0.1% sodium dodecyl sulfate (SDS). Subsequently, sonication was performed for 3 min with a pulse on time of 2 s and a pulse off time of 3 s at an ultrasonic power of 180 W, and centrifugation was performed for 30 min at 4°C and 20,000 × g. Finally, the supernatant was obtained, and the protein concentration was quantified using the Bradford assay (Supplementary Table 1).

From each sample solution, 100 μg of protein was transferred to a new clean centrifuge tube. The TEAB solution (0.1% SDS) was added to make the protein solution of each sample in the new tube up to the same volume. After adding 3.3 μL of trypsin (1 μg/μL) to the tube for protein digestion, the solution was incubated in a water bath at 37°C for 24 h. Subsequently, the tube was supplemented with 1 μL of trypsin (1 μg/μL), and the mixture in the tube was incubated in a water bath at 37°C for 12 h. After lyophilization of the mixture, 30 μL of TEAB (ddH2O:TEAB = 1:1, v/v) was added to the tube to dissolve the peptides. Using an iTRAQ® Reagent-8plex Multiplex Kit (Applied Biosystems, Foster City, CA, United States), peptides from the eight samples were labeled with the iTRAQ tags as follows: CYR32 (tags 113 and 114), CYR32-5 (tags 115, 116, and 117), and CYR32-61 (tags 118, 119, and 121), respectively (Supplementary Table 1).

The solution containing labeled peptides of each sample was diluted tenfold with buffer A (25% acetonitrile (ACN), 10 mM KH2PO4, pH 3.0), and the pH was adjusted to 3.0 with phosphoric acid. After centrifugation for 15 min at 15,000 × g, the labeled peptides in the supernatant were fractionated using a Phenomenex Luna SCX column (250 mm × 4.60 mm, 100 Å) with a high performance liquid chromatography (HPLC) system (Thermo Fisher Scientific, Waltham, MA, United States) at a flow rate of 1 mL/min. The system was equilibrated for 10–20 min with buffer A at a flow rate of 1 mL/min, prior to strong cation exchange fractionation. The HPLC gradient was as follows: 0–45 min, 100% buffer A; 45–46 min, 0–5% buffer B (25% ACN, 2 M KCl, 10 mM of KH2PO4, pH 3.0); 46–66 min, 5–30% buffer B; 66–71 min, 30–50% buffer B; 71–76 min, 50% buffer B; 76–81 min, 50–100% buffer B; 81–91 min, 100% buffer B. Absorbance was recorded at 214 nm. The eluted peptides were desalted with a Strata-X C18 column (Phenomenex, Torrance, CA, United States), and then dried by vacuum centrifugation at a low temperature.

**Nano LC-MS/MS Analysis**

The desalted, dried peptides were resuspended with solvent A (0.1% formic acid (FA) in H2O), transferred to an Acclaim Pepmap C18-reversed phase column (75 μm × 2 cm, 3 μm, 100 Å, Thermo Scientific), and then separated using a Dionex Ultimate 3000 Nano LC system with a C18 reversed phase column (75 μm × 10 cm, 5 μm, 300 Å, Agela Technologies) at a flow rate of 400 nL/min. The mobile phases were solvent A and solvent B (0.1% FA in ACN). The elution gradient was as follows: 0–10 min, 5% solvent B; 10–40 min, 5–30% solvent B; 40–45 min, 30–60% solvent B; 45–48 min, 60–80% solvent B; 48–55 min, 80% solvent B; 55–58 min, 80–5% solvent B; 58–65 min, 5% solvent B. Subsequently, 16 pre-isolated and purified components of peptides were detected using a Q-Exactive mass spectrometer (Thermo Fisher Scientific, Waltham, MA, United States) with set parameters as follows: polarity, positive ion mode; MS scan range, 350–2000 m/z; MS/MS scan resolution, 17,500; capillary temperature, 320°C; ion source voltage, 1,800 V; MS/MS acquisition modes, higher collision energy dissociation; normalized collision energy, 28.

**Proteomic Data Analysis**

The acquired raw mass spectrometry data were processed using the PD (Proteome Discoverer 1.3; Thermo Fisher Scientific, San Jose, CA, United States) software with the following parameters: mass range of parent ion, 350–6,000 Da; minimum number of peaks in MS/MS spectrum, 10; signal-to-noise ratio (S/N) threshold, 1.5. The spectra extracted using the PD software were searched against the Basidiomycota_UniProt database using Mascot 2.3.0 (Matrix Science, London, United Kingdom)
with the following identification parameters: fixed modification, carbamidomethyl (C); variable modification, oxidation (M), Gln→Pyro-Glu (N-term Q), iTRAQ 8 plex (K), iTRAQ 8 plex (Y), iTRAQ 8 plex (N-term); peptide tolerance, 15 ppm; MS/MS tolerance, 20 mmu; max missed cleavages, 1; enzyme, trypsin. Based on the Mascot search results and the extracted spectra, protein quantitative analysis was performed using the PD software with the following parameters: protein ratio type, median; minimum peptides, 1; normalization method, median; P-value, < 0.05; ratio, ≥ 1.2. In this study, proteins with at least two unique peptides, scores more than 70, P-values less than 0.05, and fold changes more than 1.2 or less than 0.83 were identified as DEPs between the different Ps strains. The fold changes of the up-regulated DEPs were more than 1.2, and those of the down-regulated DEPs were less than 0.83. For the convenience of expression, CYR32-5 vs. CYR32, CYR32-61 vs. CYR32, or CYR32-61 vs. CYR32-5 was used to represent the combination in which the proteome of the former was compared with that of the latter to screen DEPs.

Functional annotations of the proteins were carried out using the Blast2Go program1 (Conesa et al., 2005). The COG annotations of proteins were conducted using WebMGA2 (Wu S. T. et al., 2011). The annotations of the identified DEPs at the biological pathway level were performed using the KEGG database3. The KOBASE 2.0 software was used to annotate pathways by comparing similar protein sequences (Xie et al., 2011). Signal peptides were predicted and analyzed using the SignalP 5.0 program4 (Armenteros et al., 2019).

The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium5 via the iProX partner repository (Ma et al., 2019) with the dataset identifier PXD018136.

Relative Quantification of mRNA by qRT-PCR

Using the method described above, 20 mg of the germinated urediospores and germ tubes of each of the three strains CYR32, CYR32-5, and CYR32-61 were collected and treated as a sample. Total RNA from each sample was extracted using the RNeasy kit (Omega) according to the manufacturer's instructions, and then genomic DNA contaminants were removed by DNase I (RNase-free) treatment. The synthesis of the first-strand cDNA was performed by reverse transcription using the PrimeScript™ RT Master Mix Kit (TaKaRa, Dalian, China). Specific primers were designed for the selected DEPs according to their corresponding coding sequences using NCBI Primer-BLAST6 (Table 1). *Pst* β-tubulin gene TUBB (GenBank accession No. EG374306) was used as the reference gene, and the corresponding primer reported by Huang et al. (2012) (as shown in Table 1) was used in this study. SYBR Green real-time fluorescence quantitative PCR assays were performed using an Applied Biosystems ABI Model 7500 Real Time PCR system. The qRT-PCR reactions were carried out in a total 20 µL volume of reaction mixture containing 10 µL 2 × SYBR Premix DimerEraser, 0.6 µL forward primer (10 µM), 0.6 µL reverse primer (10 µM), 2.0 µL template DNA, 6.4 µL sterile purified water, and 0.4 µL 50 × ROX Reference Dye or Dye II. The amplification procedure was as follows: pre-denaturation at 95°C for 30 s, followed by 40 cycles of 95°C for 5 s, 55°C for 30 s, and 72°C for 30 s. The relative gene expression levels were calculated using the 2−ΔΔCT method described by Livak and Schmittgen (2001). The experiment was carried out with three biological replicates.

### Results

**Overview of the Proteomic Data of the Three Ps Strains**

A total of 337,731 spectra were acquired from the eight samples of the three Ps strains, and 52,290 spectra were matched by searching against the Basidiomycota_UniProt database using Mascot 2.3.0 (false discovery rate < 1%). In total, 8,882 peptides and 2,271 proteins were obtained (Supplementary Table 2).

**Acquired DEPs and COG Annotations**

The results of screening the DEPs in the urediospores and germ tubes after germination in three combinations, CYR32-5 vs. CYR32, CYR32-61 vs. CYR32, and CYR32-61 vs. CYR32-5, are shown in Figure 1. As shown in Figure 1, 59 DEPs were observed in the combination CYR32-5 vs. CYR32, of which 35 proteins were up-regulated and 24 proteins were down-regulated, and the DEPs E3JUB4 and Q9C1C1 (3.39%) were predicted to contain signal peptides. Seventy-four DEPs were observed in the combination CYR32-61 vs. CYR32, of which 43 proteins were up-regulated and 31 proteins were down-regulated, and the DEPs E3K3X8 (9.46%) were predicted to contain signal peptides. Sixty-four DEPs were observed in the combination CYR32-61 vs. CYR32-5, of which 40 were up-regulated and 24 were down-regulated, and the DEPs E3K302, E3KDD8, E3JSA3, and E3QJF7 (6.25%) were predicted to contain signal peptides. In total, 144 DEPs were observed in the three comparison combinations, of

| Accession | Forward primer (5′–3′) | Reverse primer (5′–3′) |
|-----------|------------------------|------------------------|
| TUBB      | ACAATGTCAACACGTGGCGGTTT | GTOGAAAGACATCGCTGCCCT |
| GI8253    | GCTTCTCTTCCGGCGGTGG | CATGTCACAGATGCGCCTGGT |
| F4S6X6    | TAGCAACGCGAGTACGACTAC | GTTCGTAGTTTCTACCTGGG |
| V2XNY1    | GAACATCGACGCTGATGACTAT | ATGACCCAGACTTATG |
| Q8C1C1    | AGATGATTGATGACGCGGTAAC | GAGAGGCCCTCACDGCCTTCA |
| F4S0Z3    | GCCATGACACACACAGACCAT | AACTTCTACCCAGACGGGT |
| F4R5N9    | GCTTCATGACGACGACGAGAAA | CTACGTCATACACCCAGGGT |
| E3L0W8    | GGATAGATGGGGGCGGTTGA | CTTCCGATCTAGGACCCCTT |
| D4QFJ2    | GGCATTTTCACAAACAGQGQT | ACTOCCACACTGGAGCATCT |
| J6EXB0    | CAGAACACGGGTGACATGCTG | CACCCCTCGTGCGCAGGTA |

1https://www.blast2go.com/
2http://weizhong-lab.ucsd.edu/metagenomic-analysis/server/cog/
3http://www.cbs.dtu.dk/services/SignalP/
4http://weizhong-lab.ucsd.edu/metagenomic-analysis/server/cog/
5http://protemecentral.proteomeexchange.org
6https://www.ncbi.nlm.nih.gov/tools/primer-blast/
which four were common in CYR32-5 vs. CYR32, CYR32-61 vs. CYR32, and CYR32-61 vs. CYR32-5. The DEPs that were predicted to contain signal peptides may be secretory proteins or membrane proteins.

All obtained DEPs were subjected to COG functional annotation, and then assigned to 25 categories that were represented by A–Z (Figure 2). Among all the DEPs, seven were annotated with multiple functions, and 22 were not annotated and had no annotation information. The four main functional categories were as follows: [J] translation, ribosomal structure and biogenesis (31, 21.68%), [O] posttranslational modification, protein turnover, chaperones (24, 16.78%), [C] energy production and conversion (17, 11.89%), and [U] intracellular trafficking, secretion, and vesicular transport (11, 7.69%). The detailed functional annotations of the up-regulated DEPs and the down-regulated DEPs are shown in Tables 2, 3, respectively. The acquired DEPs were classified into four major functional categories, including “information storage and processing,” “cellular processes and signaling,” “metabolism,” and “poorly characterized.” Of the up-regulated DEPs, 14, 43, 27, and 4 were involved in “information storage and processing,” “cellular processes and signaling,” “metabolism,” and “poorly characterized,” respectively, and 18 had no annotation information. Of the down-regulated DEPs, 23, 15, 18, and 5 were involved in “information storage and processing,” “cellular processes and signaling,” “metabolism,” and “poorly characterized,” respectively, and 7 had no annotation information.

**KEGG Pathway Enrichment Analysis of DEPs**

The biological pathways that the DEPs were involved in were investigated using the KEGG database, and 5, 7, and 7 significant enriched KEGG pathways (P < 0.05) were acquired for the DEPs of CYR32-5 vs. CYR32, CYR32-61 vs. CYR32, and CYR32-61 vs. CYR32-5, respectively (Figure 3 and Supplementary Table 3). The DEPs of CYR32-5 vs. CYR32 were involved in pathways including metabolic pathways, biosynthesis of secondary metabolites, carbon metabolism, ribosome, and pyruvate metabolism. The DEPs of CYR32-61 vs. CYR32 were mainly involved in the carbon metabolism pathway, calcium signaling pathway, and melanogenesis pathway. The DEPs of CYR32-61 vs. CYR32-5 were mainly involved in the ribosome pathway, melanogenesis pathway, and calcium signaling pathway.

**Transcriptional Analysis of DEPs**

To confirm the differences in protein abundance in each of the three combinations including CYR32-5 vs. CYR32, CYR32-61 vs. CYR32, and CYR32-61 vs. CYR32-5, after germination of the urediospores, qRT-PCR was used to investigate the relative expression levels of the genes encoding the DEPs identified by iTRAQ analysis. The relative expression levels of the genes encoding six proteins (G9B235, F4S8X5, V2XWY1, Q9C1C1, F4S0Z3, and F4RWN9) in CYR32-5 vs. CYR32, seven proteins (G9B235, F4S8X5, V2XWY1, Q9C1C1, E3L0W8, D4QFJ2, and J6EXB0) in CYR32-61 vs. CYR32, and one protein (F4S8X5) in CYR32-61 vs. CYR32-5 were determined, and the results are shown in Figure 4. In CYR32-5 vs. CYR32, the relative expression levels of the genes encoding Q9C1C1, G9B235, F4S8X5, F4RWN9, and F4S0Z3 were not consistent with the corresponding protein abundance based on iTRAQ data, but the relative expression of the gene encoding V2XWY1 was consistent with the corresponding protein abundance. In CYR32-61 vs. CYR32, the relative expression levels of the genes encoding Q9C1C1 and J6EXB0 were inconsistent with the corresponding protein levels, and the genes encoding G9B235, F4S8X5, V2XWY1, E3L0W8, and D4QFJ2 were consistent with the corresponding protein levels. In CYR32-61 vs. CYR32-5, the relative expression level of the gene encoding F4S8X5 at the transcriptional level was consistent with the corresponding protein abundance obtained via iTRAQ analysis.

**DISCUSSION**

In this study, using the proteomics method based on iTRAQ technology, a total of 144 DEPs were obtained from the germinated urediospores with germ tubes in the CYR32-5 vs. CYR32, CYR32-61 vs. CYR32, and CYR32-61 vs. CYR32-5 conditions. Nine DEPs, including E3JQF7, E3JSAS, E3JUB4, E3K302, E3K3X8, E3KDD8, E3KXP2, Q9C1C1, and R7SW21, were predicted to contain signal peptides. Peptidyl-prolyl cis-trans isomerase (PPI, E3JQF7) is a high-efficiency foldase. In biological cells, after ribosomal transcription, the peptide chain functions properly only through correct folding and positioning. PPI is associated with the correct folding of a protein (Lu et al., 2007). Heat shock protein 70 (R7SW21) can play an important role in the folding and stretching of newly synthesized polypeptides, can repair denatured proteins, and can prevent protein denaturation by up-regulating protein expression under
adverse conditions (Tsai and Gao, 2004). In the present study, both E3JQF7 and R7SW21 were up-regulated in CYR32-61 vs. CYR32, and E3JQF7 was up-regulated in CYR32-61 vs. CYR32-5, indicating that strain CYR32-61 may be more resistant to stress than strains CYR32 and CYR32-5.

The histone chaperone ASF1 (anti-silencing function 1) is a molecular chaperone of histone H3-H4. It has been reported that ASF1 plays important roles in the processes of DNA replication and repair in yeast (Le et al., 1997) and Arabidopsis (Zhu et al., 2011). In this study, ASF1 (E3L109) was up-regulated in CYR32-61 vs. CYR32-5, indicating that strain CYR32-61 may be more resistant to stress than strain CYR32-5.

G proteins are involved in various cellular activities, including regulation of intracellular Ca^{2+} concentration, protein synthesis, and the binding of ribosomes to the endoplasmic reticulum (Lagerstedt et al., 2005). The DEPs G9B235, H6QRQ7, and J9PGK7 acquired in this study were involved in G protein regulation. G9B235 was down-regulated in CYR32-5 vs. CYR32, J9PGK7 was up-regulated in CYR32-61 vs. CYR32-5, and H6QRQ7 and J9PGK7 were up-regulated, and G9B235 was down-regulated in CYR32-61 vs. CYR32. Furthermore, the relative expression level of G9B235 determined by qRT-PCR was consistent with the corresponding protein abundance obtained by iTRAQ technology in CYR32-61 vs. CYR32, but the former was inconsistent with the latter in CYR32-5 vs. CYR32. Therefore, the results indicated that there was little correlation between the expression levels at the transcriptional level and the protein level for some DEPs, which was consistent with a previous report by Zhao et al. (2016).

As an important component of the second messenger system, calmodulin plays a key role in the regulation of the calcium signal system, and is associated with physiological metabolism regulation, gene expression, and normal growth and development of cells (Chin and Means, 2000). In both CYR32-61 vs. CYR32 and CYR32-61 vs. CYR32-5, putative uncharacterized protein (E3L0W8) with the role of calcium ion binding and calmodulin (M5FTW6) were up-regulated, and the relative expression level of E3L0W8 determined by qRT-PCR was consistent with the protein level obtained by iTRAQ technology. Calmodulin (E3KLJ3) was up-regulated in CYR32-61 vs. CYR32-5.

In CYR32-5 vs. CYR32, three DEPs involved in energy production, including ubiquinol-cytochrome c reductase cytochrome c1 subunit (E3JWK6), ubiquinol-cytochrome c reductase iron-sulfur subunit (E3L519), and ATP synthase subunit beta (F4S0Z3), were up-regulated. E3JWK6 and E3L519 are components of complex III of the electron transport chain, and are associated with electron transport. F4S0Z3 is the β subunit of the F1 part of ATP synthase, and contains a site to catalyze ATP synthesis. In living organisms, the electron transport chain is a critical component of the electron transfer process, enabling the generation of ATP through substrate-level phosphorylation. Calmodulin (E3KLJ3) was up-regulated in CYR32-61 vs. CYR32-5. Therefore, the results indicated that there was little correlation between the expression levels at the transcriptional level and the protein level for some DEPs, which was consistent with a previous report by Zhao et al. (2016).

As an important component of the second messenger system, calmodulin plays a key role in the regulation of the calcium signal system, and is associated with physiological metabolism regulation, gene expression, and normal growth and development of cells (Chin and Means, 2000). In both CYR32-61 vs. CYR32 and CYR32-61 vs. CYR32-5, putative uncharacterized protein (E3L0W8) with the role of calcium ion binding and calmodulin (M5FTW6) were up-regulated, and the relative expression level of E3L0W8 determined by qRT-PCR was consistent with the protein level obtained by iTRAQ technology. Calmodulin (E3KLJ3) was up-regulated in CYR32-61 vs. CYR32-5.

In CYR32-5 vs. CYR32, three DEPs involved in energy production, including ubiquinol-cytochrome c reductase cytochrome c1 subunit (E3JWK6), ubiquinol-cytochrome c reductase iron-sulfur subunit (E3L519), and ATP synthase subunit beta (F4S0Z3), were up-regulated. E3JWK6 and E3L519 are components of complex III of the electron transport chain, and are associated with electron transport. F4S0Z3 is the β subunit of the F1 part of ATP synthase, and contains a site to catalyze ATP synthesis. In living organisms, the electron transport chain is a critical component of the electron transfer process, enabling the generation of ATP through substrate-level phosphorylation. Calmodulin (E3KLJ3) was up-regulated in CYR32-61 vs. CYR32-5. Therefore, the results indicated that there was little correlation between the expression levels at the transcriptional level and the protein level for some DEPs, which was consistent with a previous report by Zhao et al. (2016).

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### TABLE 2 | Functional annotations of the up-regulated differentially expressed proteins acquired in the combinations CYR32-5 vs. CYR32, CYR32-61 vs. CYR32, and CYR32-61 vs. CYR32-5.

| COG functional classification | Accession | Description | Treatment combination | Folder change |
|------------------------------|-----------|-------------|-----------------------|--------------|
| Information storage and processing (14) | | | | |
| [B] Chromatin structure and dynamics | E3L109 | Histone chaperone ASF | CYR32-61 vs. CYR32-5 | 1.31 |
| [J] Translation, ribosomal structure and biogenesis | E3K6N0 | 40S ribosomal protein S0 | CYR32-6 vs. CYR32 | 1.20 |
| | E3KQW3 | Eukaryotic translation initiation factor 3 subunit B | CYR32-5 vs. CYR32 | 1.22 |
| | E6RA36 | 60S acidic ribosomal protein, putative | CYR32-5 vs. CYR32 | 1.23 |
| | Q0Z8F6 | Elongation factor 1-alpha | CYR32-5 vs. CYR32 | 1.25 |
| | COL941 | Polyubiquitin-like protein | CYR32-61 vs. CYR32 | 1.27 |
| | E3KDZ2 | Ubiquitin-40S ribosomal protein S27a-2 | CYR32-61 vs. CYR32 | 1.28 |
| | IVR3 | Elongation factor 1-alpha | CYR32-61 vs. CYR32 | 1.32 |
| | E2D61 | Transcription elongation factor 1 alpha | CYR32-61 vs. CYR32-5 | 1.31 |
| | E3K5L7 | 40S ribosomal protein S19-A | CYR32-5 vs. CYR32 | 1.24 |
| | M7WRX9 | 60S ribosomal protein L3 | CYR32-5 vs. CYR32 | 1.21 |
| | P51997 | 60S ribosomal protein L25 | CYR32-5 vs. CYR32 | 1.37 |
| | E3JTG6 | Putative uncharacterized protein | CYR32-61 vs. CYR32 | 1.29 |
| | | | CYR32-61 vs. CYR32-5 | 1.21 |
| | [K] Transcription | | | |
| | E3L109 | Histone chaperone ASF1 | CYR32-61 vs. CYR32-5 | 1.31 |
| | Cellular processes and signaling (43) | | | |
| | [L] Intracellular trafficking, secretion, and vesicular transport | | | |
| | F4SA43 | Putative uncharacterized protein | CYR32-5 vs. CYR32 | 1.29 |
| | E3K017 | Putative uncharacterized protein | CYR32-61 vs. CYR32 | 1.27 |
| | F4R53 | Putative uncharacterized protein | CYR32-61 vs. CYR32 | 1.28 |
| | E3L3S6 | Putative uncharacterized protein | CYR32-61 vs. CYR32-5 | 1.22 |
| | L8P4K4 | Hsp70-like protein | CYR32-5 vs. CYR32 | 1.22 |
| | CYR32-61 vs. CYR32 | 1.21 |
| | F4RWN9 | Putative uncharacterized protein | CYR32-5 vs. CYR32 | 1.42 |
| | E2L9J3 | Uncharacterized protein | CYR32-61 vs. CYR32-5 | 1.21 |
| | CYR32-61 vs. CYR32 | 1.33 |
| | E3KQ6 | Putative uncharacterized protein | CYR32-61 vs. CYR32 | 1.22 |
| | E3KQ6 | Putative uncharacterized protein | CYR32-5 vs. CYR32 | 1.22 |
| | H6Q767 | Tubulin beta-1 chain, variant | CYR32-61 vs. CYR32 | 1.25 |
| | E3KQ3 | Actin-like protein 2/3 complex subunit 4 | CYR32-61 vs. CYR32-5 | 1.25 |
| | E3KNW6 | Profilin | CYR32-61 vs. CYR32-5 | 1.29 |
| | E3L6W8 | Putative uncharacterized protein | CYR32-61 vs. CYR32 | 1.33 |
| | J5QLA2 | Actin | CYR32-61 vs. CYR32 | 1.24 |
| | J9P8K7 | Beta-tubulin 1 | CYR32-61 vs. CYR32 | 1.28 |
| | CYR32-61 vs. CYR32 | 1.33 |
| | E3KQ26 | Putative uncharacterized protein | CYR32-61 vs. CYR32 | 1.37 |
| | [D] Cell cycle control, cell division, chromosome partitioning | | | |
| | E3KQ26 | Putative uncharacterized protein | CYR32-61 vs. CYR32 | 1.37 |
| | F4R5Y4 | Putative uncharacterized protein | CYR32-6 vs. CYR32 | 1.22 |
| | G7SB1 | Uncharacterized protein | CYR32-5 vs. CYR32 | 1.25 |
| | G7SB1 | Uncharacterized protein | CYR32-5 vs. CYR32 | 1.26 |
| | CYR32-61 vs. CYR32 | 1.25 |
| | [O] Posttranslational modification, protein turnover, chaperones | | | |
| | E3JZW9 | Putative uncharacterized protein | CYR32-61 vs. CYR32 | 1.41 |
| | COL941 | Polyubiquitin-like protein | CYR32-61 vs. CYR32 | 1.27 |
| | E3JZ49 | Putative uncharacterized protein | CYR32-61 vs. CYR32 | 1.23 |
| | E3JW9 | Putative uncharacterized protein | CYR32-61 vs. CYR32 | 1.30 |
| | F4R53 | Ubiquitin carboxy-terminal hydrolase | CYR32-61 vs. CYR32 | 1.22 |
| | G7EA3 | Uncharacterized protein | CYR32-61 vs. CYR32 | 1.21 |
| | I4YJ87 | Putative 26S protease regulatory subunit 6B | CYR32-61 vs. CYR32 | 1.20 |
| | R7SW21 | Heat shock protein 70 | CYR32-61 vs. CYR32 | 1.29 |

(Continued)
| COG functional classification | Accession | Description                                      | Treatment combination | Folder change |
|-------------------------------|-----------|--------------------------------------------------|-----------------------|---------------|
| Uncharacterized protein       | USH357    |                                                  | CYR32-61 vs. CYR32    | 1.27          |
| Putative uncharacterized protein | E3K302    |                                                  | CYR32-61 vs. CYR32-5  | 1.23          |
| E3 ubiquitin ligase complex SCF subunit sconC | E3L494    |                                                  | CYR32-61 vs. CYR32-5  | 1.21          |
| Hsp70-like protein            | L8WPK4    |                                                  | CYR32-5 vs. CYR32     | 1.22          |
| Uncharacterized protein       | Q9C1C1    | Cro r II                                         | CYR32-5 vs. CYR32     | 1.52          |
| Putative uncharacterized protein | E3KTD1    |                                                  | CYR32-61 vs. CYR32-5  | 1.60          |
| Putative uncharacterized protein | D6PWLO    |                                                  | CYR32-61 vs. CYR32    | 1.33          |
| Peptidyl-prolyl cis-trans isomerase | E3JJQF7   |                                                  | CYR32-61 vs. CYR32-5  | 1.21          |
| Peptidyl-prolyl cis-trans isomerase | E3KQ21    |                                                  | CYR32-61 vs. CYR32-5  | 1.79          |
| Putative uncharacterized protein | E3KFZ5    |                                                  | CYR32-5 vs. CYR32     | 1.40          |
| Calmodulin                    | E3KLJ3    |                                                  | CYR32-61 vs. CYR32-5  | 1.35          |
| Putative uncharacterized protein | F4RW3     |                                                  | CYR32-61 vs. CYR32-5  | 1.29          |
| Uncharacterized protein       | USH740    |                                                  | CYR32-5 vs. CYR32     | 1.38          |
| Calmodulin                    | M5FTW6    |                                                  | CYR32-61 vs. CYR32     | 1.21          |
| Putative uncharacterized protein | E3KFZ5    |                                                  | CYR32-5 vs. CYR32     | 1.42          |
| Putative uncharacterized protein | E3JWKL    | Ubiquinol-cytochrome c reductase cytochrome c1 subunit | CYR32-5 vs. CYR32    | 1.40          |
| Putative uncharacterized protein | E3K937    |                                                  | CYR32-5 vs. CYR32     | 1.23          |
| Ubiquinol-cytochrome c reductase iron-sulfur subunit | E3L519    |                                                  | CYR32-5 vs. CYR32     | 1.21          |
| ATP synthase subunit beta     | F4S823    |                                                  | CYR32-5 vs. CYR32     | 1.26          |
| Putative uncharacterized protein | F4S861    |                                                  | CYR32-5 vs. CYR32     | 1.26          |
| Putative uncharacterized protein | F4S8X5    |                                                  | CYR32-5 vs. CYR32     | 1.27          |
| Predicted protein             | B0CPP7    |                                                  | CYR32-5 vs. CYR32     | 1.21          |
| Pyruvate dehydrogenase e1 component alpha subunit | M5FPE0    |                                                  | CYR32-5 vs. CYR32     | 1.27          |
| Inorganic diphosphatase       | A8N2Q4    |                                                  | CYR32-61 vs. CYR32-5  | 1.22          |
| Putative uncharacterized protein | F4S8X5    |                                                  | CYR32-5 vs. CYR32     | 1.20          |
| Putative uncharacterized protein | E3K937    |                                                  | CYR32-61 vs. CYR32    | 1.27          |
| Putative uncharacterized protein | S7RH13    | Threonine synthase                               | CYR32-5 vs. CYR32     | 1.40          |
| Serine hydroxymethyltransferase | E6R529    |                                                  | CYR32-5 vs. CYR32     | 1.28          |
| Pyrroline-5-carboxylate reductase | E3JSA3    |                                                  | CYR32-61 vs. CYR32    | 1.42          |
| Aspartate aminotransferase    | E3KJR8    |                                                  | CYR32-61 vs. CYR32-5  | 1.53          |

(Continued)
transport chain and ATP synthesis are coupled together (Mitchell, 1961). A model for the coupling of electron transport and ATP synthesis in the combination CYR32-5 vs. CYR32 is shown in Figure 5. In this study, the acquired DEPs involved in the electron transport chain and in ATP synthesis were up-regulated, indicating that the processes of energy production during urediospore germination may be promoted in strain CYR32-5 compared to strain CYR32.

In the glycolysis pathway, glyceraldehyde-3-phosphate dehydrogenase can catalyze the formation of 1,3-diphosphoglycerate from 3-phosphoglycerate in the presence of NAD$^+$ and phosphoric acid; it is, hence, an important enzyme in glycolysis. In this study, D4QFJ2, annotated as glyceraldehyde-3-phosphate dehydrogenase, was up-regulated in CYR32-61 vs. CYR32 according to iTRAQ analysis, which was consistent with the relative expression level determined by qRT-PCR. The results indicated that, during urediospore germination, the glycolysis pathway (as shown in Figure 6) of virulence-mutant strain CYR32-61 may be promoted in comparison with that of strain CYR32. Glycolysis is the main process for the formation of ATP in carbohydrate metabolism. The results obtained in this study showed that the DEPs involved in the glycolysis pathway were up-regulated, further indicating that, during urediospore germination of the virulence-mutant strain CYR32-61 (as compared to the original strain CYR32), polysaccharide may be utilized to form ATP to promote the biological processes of urediospores. In addition, during glycolysis, the final product, pyruvate, can be irreversibly catalyzed by pyruvate dehydrogenase multi-enzyme complex (PDHc) to form acetyl-coenzyme A, which can be oxidatively decomposed in the tricarboxylic acid cycle. Pyruvate dehydrogenase E1
### TABLE 3 | Functional annotations of the down-regulated differentially expressed proteins acquired in the combinations CYR32-5 vs. CYR32, CYR32-61 vs. CYR32, and CYR32-61 vs. CYR32-5.

| COG functional classification                          | Accession | Description                              | Treatment combination | Folder change |
|--------------------------------------------------------|-----------|-------------------------------------------|-----------------------|---------------|
| Information storage and processing (23)                |           |                                           |                       |               |
| [B] Chromatin structure and dynamics                    |           |                                           |                       |               |
| E3JV96 Histone H2B                                      | CYR32-61 vs. CYR32 | 0.74                                    |
|                                                        | CYR32-61 vs. CYR32-5 | 0.76                                    |
| [J] Translation, ribosomal structure and biogenesis    |           |                                           |                       |               |
| E3KH0 40S ribosomal protein S22                        | CYR32-5 vs. CYR32 | 0.80                                    |
| F4RAC5 40S ribosomal protein S0                         | CYR32-5 vs. CYR32 | 0.83                                    |
| F4RB04 Putative uncharacterized protein                 | CYR32-5 vs. CYR32 | 0.82                                    |
| H6QUT2 Glutaminyl-tRNA synthetase                      | CYR32-5 vs. CYR32 | 0.82                                    |
| M5BM68 Elongation factor 1-alpha                       | CYR32-5 vs. CYR32 | 0.82                                    |
| B0D8C2 Predicted protein                               | CYR32-61 vs. CYR32 | 0.75                                    |
| E3KV5 Large subunit ribosomal protein L14e             | CYR32-61 vs. CYR32 | 0.79                                    |
| Q4PS60 Translation elongation factor 1-alpha           | CYR32-61 vs. CYR32 | 0.69                                    |
| Q9C1U7 Ribosomal protein L13A                          | CYR32-61 vs. CYR32 | 0.78                                    |
| E3JV99 Large subunit ribosomal protein L26e            | CYR32-61 vs. CYR32-5 | 0.81                                    |
| F4RJ44 Putative uncharacterized protein                | CYR32-61 vs. CYR32-5 | 0.82                                    |
| V5GFY5 60S ribosomal protein L10                        | CYR32-61 vs. CYR32-5 | 0.78                                    |
| D1MWJ6 Translation elongation factor 1-alpha           | CYR32-5 vs. CYR32 | 0.76                                    |
|                                                         | CYR32-61 vs. CYR32 | 0.74                                    |
| E3L6B3 Eukaryotic translation initiation factor 3 subunit A | CYR32-5 vs. CYR32 | 0.80                                    |
|                                                        | CYR32-61 vs. CYR32 | 0.79                                    |
| E3SKL7 40S ribosomal protein S19-A                     | CYR32-61 vs. CYR32-5 | 0.80                                    |
| M7WWM9 60S ribosomal protein L3                         | CYR32-61 vs. CYR32-5 | 0.77                                    |
| P61997 60S ribosomal protein L25                        | CYR32-61 vs. CYR32-5 | 0.57                                    |
| E3JQ18 60S ribosomal protein L36                        | CYR32-61 vs. CYR32 | 0.75                                    |
|                                                        | CYR32-61 vs. CYR32-5 | 0.80                                    |
| E3KT5 50S ribosomal protein L22                        | CYR32-61 vs. CYR32 | 0.78                                    |
|                                                        | CYR32-61 vs. CYR32-5 | 0.70                                    |
| F4S214 Putative uncharacterized protein                | CYR32-61 vs. CYR32 | 0.83                                    |
|                                                        | CYR32-61 vs. CYR32-5 | 0.77                                    |
| F4SA97 Putative uncharacterized protein                | CYR32-61 vs. CYR32 | 0.73                                    |
|                                                        | CYR32-61 vs. CYR32 | 0.75                                    |
| [K] Transcription                                      |           |                                           |                       |               |
| Cellular processes and signaling (15)                  |           |                                           |                       |               |
| [L] Intracellular trafficking, secretion, and vesicular transport |           |                                           |                       |               |
| E3KMW4 Putative uncharacterized protein                | CYR32-61 vs. CYR32 | 0.82                                    |
| F4S013 Clathrin heavy chain                            | CYR32-61 vs. CYR32 | 0.82                                    |
| F4RW09 Putative uncharacterized protein                | CYR32-61 vs. CYR32-5 | 0.79                                    |
| [Z] Cytoskeleton                                       |           |                                           |                       |               |
| K5WMR1 Uncharacterized protein                        | CYR32-61 vs. CYR32 | 0.78                                    |
| G9B335 Beta-tubulin                                   | CYR32-61 vs. CYR32 | 0.72                                    |
|                                                        | CYR32-61 vs. CYR32 | 0.78                                    |
| [D] Cell cycle control, cell division, chromosome partitioning |           |                                           |                       |               |
| E3JWZ6 Cyclin-dependent kinases regulatory subunit     | CYR32-61 vs. CYR32 | 0.83                                    |
|                                                        | CYR32-61 vs. CYR32 | 0.83                                    |
| [O] Posttranslational modification, protein turnover, chaperones |           |                                           |                       |               |
| F4ROCH7 T-complex protein 1 subunit alpha             | CYR32-61 vs. CYR32 | 0.83                                    |
|                                                        | CYR32-61 vs. CYR32 | 0.83                                    |
| F4S233 Putative uncharacterized protein                | CYR32-61 vs. CYR32-5 | 0.83                                    |
| V2XY1 Heat shock protein                              | CYR32-61 vs. CYR32 | 0.74                                    |
|                                                        | CYR32-61 vs. CYR32 | 0.68                                    |
| E3KTD1 Putative uncharacterized protein               | CYR32-61 vs. CYR32 | 0.77                                    |
| M7WQZ1 Heat shock 70kDa protein 1/8                    | CYR32-61 vs. CYR32 | 0.70                                    |
|                                                        | CYR32-61 vs. CYR32-5 | 0.72                                    |
| [T] Signal transduction mechanisms                     |           |                                           |                       |               |
| E3KCO7 Putative uncharacterized protein               | CYR32-61 vs. CYR32 | 0.75                                    |
| U5SH40 Uncharacterized protein                        | CYR32-61 vs. CYR32-5 | 0.78                                    |
|                                                        | CYR32-61 vs. CYR32 | 0.82                                    |
| [Y] Nuclear structure                                 |           |                                           |                       |               |
| E3KMW4 Putative uncharacterized protein               | CYR32-61 vs. CYR32-5 | 0.82                                    |
| F4RW09 Putative uncharacterized protein               | CYR32-61 vs. CYR32-5 | 0.79                                    |

(Continued)
| COG functional classification | Accession | Description | Treatment combination | Folder change |
|------------------------------|-----------|-------------|-----------------------|--------------|
| Metabolism (18)             |           |             |                       |              |
| [F] Nucleotide transport and metabolism | F4RN0 | Ribonucleoside-diphosphate reductase | CYR32-61 vs. CYR32 | 0.78 |
| [H] Coenzyme transport and metabolism | F4RPF7 | Putative uncharacterized protein | CYR32-5 vs. CYR32 | 0.71 |
| [I] Lipid transport and metabolism | E3K845 | Acyl-coenzyme A oxidase | CYR32-61 vs. CYR32 | 0.81 |
| | F4RPF1 | Putative uncharacterized protein | CYR32-61 vs. CYR32 | 0.78 |
| | E3KE31 | Putative uncharacterized protein | CYR32-61 vs. CYR32-5 | 0.80 |
| | E3JJU4 | Acyl-CoA dehydrogenase | CYR32-5 vs. CYR32 | 0.72 |
| | CYR32-61 vs. CYR32 | 0.82 |
| [C] Energy production and conversion | J6EXB0 | ADP, ATP carrier protein 2, (ADP/ATP translocase 2) | CYR32-61 vs. CYR32 | 0.81 |
| | E3KE31 | Putative uncharacterized protein | CYR32-61 vs. CYR32-5 | 0.80 |
| | E6RF63 | ADP, ATP carrier protein 2, mitochondrial (ADP/ATP translocase 2), putative | CYR32-61 vs. CYR32-5 | 0.79 |
| | B0CPP7 | Predicted protein | CYR32-61 vs. CYR32-5 | 0.66 |
| | M5FPE0 | Pyruvate dehydrogenase e1 component alpha subunit | CYR32-61 vs. CYR32-5 | 0.81 |
| | G7E585 | NAD-malate dehydrogenase | CYR32-5 vs. CYR32 | 0.58 |
| | CYR32-61 vs. CYR32 | 0.38 |
| | CYR32-61 vs. CYR32-5 | 0.75 |
| Poorly characterized (5)    |           |             |                       |              |
| [S] Function unknown         | E3KC3X8 | Putative uncharacterized protein | CYR32-61 vs. CYR32 | 0.76 |
| | F4RRL4 | Putative uncharacterized protein | CYR32-61 vs. CYR32-5 | 0.80 |
| | E3KV5U7 | Uncharacterized protein | CYR32-5 vs. CYR32 | 0.78 |
| [Q] Secondary metabolites biosynthesis, transport and catabolism | E3KWF2 | Putative uncharacterized protein | CYR32-5 vs. CYR32 | 0.71 |
| | Q4R0ZJ | D-arabinitol dehydrogenase 1 | CYR32-5 vs. CYR32 | 0.82 |
| | E3JV78 | Uncharacterized protein | CYR32-61 vs. CYR32-5 | 0.75 |
| | E3KN78 | Uncharacterized protein | CYR32-61 vs. CYR32-5 | 0.80 |
| | F4RMJ8 | Putative uncharacterized protein | CYR32-61 vs. CYR32-5 | 0.82 |
| | F4S9E0 | Putative uncharacterized protein | CYR32-61 vs. CYR32-5 | 0.83 |
| | G7E585 | Uncharacterized protein | CYR32-61 vs. CYR32-5 | 0.68 |
| | CYR32-61 vs. CYR32-5 | 0.75 |
| | CYR32-61 vs. CYR32 | 0.47 |
| No annotation (7)           |           |             |                       |              |
| | E3JUV8 | Putative uncharacterized protein | CYR32-61 vs. CYR32 | 0.83 |
| | E3KRI4 | Putative uncharacterized protein | CYR32-61 vs. CYR32-5 | 0.83 |
| | F4RYM8 | Putative uncharacterized protein | CYR32-61 vs. CYR32-5 | 0.81 |
| | A7KKN6 | Plasma membrane (H+)-ATPase | CYR32-61 vs. CYR32 | 0.81 |
| | E3LSL4 | Putative uncharacterized protein | CYR32-61 vs. CYR32 | 0.81 |
| | H6QS83 | Putative uncharacterized protein | CYR32-5 vs. CYR32 | 0.76 |
| | E3KSW4 | Glucose-repressible protein | CYR32-5 vs. CYR32 | 0.78 |

is a component of PDHc, and if its activity is inhibited, aerobic metabolism can be blocked, thus affecting the normal metabolism of living organisms (Danson et al., 1978). In CYR32-5 vs. CYR32, the expression of M5FPE0—an annotated as pyruvate dehydrogenase E1 component alpha subunit—was up-regulated, indicating that the formation of ATP during oxidative phosphorylation in the germinated urediospores and germ tubes may be promoted in strain CYR32-5 in comparison with strain CYR32.

Inorganic diphosphatase (A8N2Q4) mainly involved in DNA synthesis, can catalyze the conversion of one molecule of pyrophosphate into two molecules of phosphate, accompanied by the generation of high levels of energy (Kukko and Saarento, 1984). A8N2Q4 was up-regulated in CYR32-61 vs. CYR32 and
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**FIGURE 3 |** KEGG pathway enrichment of the differentially expressed proteins acquired in the combinations CYR32-5 vs. CYR32, CYR32-61 vs. CYR32, and CYR32-61 vs. CYR32-5 (P < 0.05).

**FIGURE 4 |** Comparison of encoding gene expression levels and protein levels of the selected enriched proteins identified by iTRAQ analysis in the combinations CYR32-5 vs. CYR32 (A), CYR32-61 vs. CYR32 (B), and CYR32-61 vs. CYR32-5 (C).
FIGURE 5 | Model for the coupling of electron transport and ATP synthesis. The differentially expressed proteins (marked in red) in the electron transport chain complex III, including ubiquinol-cytochrome c reductase cytochrome c1 subunit (E3JWK6) and ubiquinol-cytochrome c reductase iron-sulfur subunit (E3L519), were up-regulated in CYR32-5 vs. CYR32. The β subunit (F4S0Z3) of ATP synthase marked in red was up-regulated in CYR32-5 vs. CYR32.

FIGURE 6 | Glycolysis pathway. The glyceraldehyde-3-phosphate dehydrogenase marked in red was found to be up-regulated in CYR32-61 vs. CYR32.

It has been reported that UV-B radiation can induce virulence variation in *Pst* (Shang et al., 1994; Huang et al., 2005; Wang et al., 2009; Zhao et al., 2019). Similarly, in studies on two other important pathogens—*Puccinia triticina* (Neugebauer et al., 2018) and *P. graminis* f. sp. *tritici* (Zhang et al., 2017), causing wheat leaf rust and wheat stem rust, respectively, it has also been reported that mutation may be the main mechanism of virulence variation of the two pathogens, thus resulting in the loss of wheat resistance and severe losses of wheat yield. In this study, after the germination of urediospores, the expression levels of many proteins in UV-B-induced virulence-mutant strains, CYR32-5 and CYR32-61, were different from those in CYR32, the original strain. The changes in the related biological processes in CYR32-61 vs. CYR32-5 in this study. The results indicated that in comparison with strains CYR32 and CYR32-5, the energy metabolism pathway during urediospore germination may be promoted in strain CYR32-61, which may contribute to the progress in biological processes of urediospores.
which the DEPs are involved may result in changes in virulence phenotypes of \textit{Pst} and may be responsible for virulence variation. To further explore the virulence variation mechanisms of \textit{Pst}, it is of great importance to conduct studies by pathogen sequencing at the genome level and to investigate the metabolomics of \textit{Pst} after mutations.

**CONCLUSION**

In this study, using the proteomics method based on iTRAQ technology, a quantitative proteomic analysis of the germinated urediospores (with germ tubes) of the original strain, CYR32, and two UV-B-induced virulence-mutant strains, CYR32-5 and CYR32-61, was undertaken. A total of 2,271 proteins were identified in eight samples of the three \textit{Pst} strains, and 59, 74, and 64 DEPs were obtained in each of the three combinations, i.e., CYR32-5 vs. CYR32, CYR32-61 vs. CYR32, and CYR32-61 vs. CYR32-5, respectively. The identified DEPs were mainly involved in energy metabolism, carbon metabolism and cellular substance synthesis. The relative expression levels of the genes encoding some DEPs, quantified using qRT-PCR, were consistent with the corresponding protein abundance obtained via iTRAQ analysis. Compared with the original strain, CYR32, the DEPs involved in stress-related and energy metabolism were up-regulated in the virulence-mutant strains, indicating that the virulence-mutant strains, CYR32-5 and CYR32-61, were more tolerant to stress than the original strain. These results are of great significance for further studies on the mechanisms of \textit{Pst} virulence variation and for implementing control measures for wheat stripe rust.

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**DATA AVAILABILITY STATEMENT**

The datasets generated for this study can be found in the ProteomeXchange Consortium (http://proteomexchange.org) via the iProX partner repository with the dataset identifier PXD018136.

**AUTHOR CONTRIBUTIONS**

HW contributed conception of the study. HW and YaZ designed the experiments. YaZ, PC, and YuZ performed the experiments. YaZ and HW analyzed the data and wrote the draft of the manuscript. All authors contributed to manuscript revision, read and approved the final version of the manuscript.

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**SUPPLEMENTARY MATERIAL**

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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