Rice is the leading food for more than three billion people worldwide. Ensuring rice quality is important to food security (Custodio et al. 2019; Li et al. 2019). Rice blast is one of the most serious diseases of cultivated rice, which was caused by Magnaporthe oryzae (Asibi et al. 2019; Jeon et al. 2020). Approximately 15% of rice yield is lost every year because of rice blasts in China (Shao 2018). Rice blast could occur at leaves, stems, nodes, and panicles throughout all stages of rice development (Shahriar et al. 2020). The leaf blast is initiated by the attachment of the spore of M. oryzae to the rice leaf surface, then the germ tube and appressorium emerge from the spore, eventually resulting in disease lesions (Wilson et al. 2009; Rijal and Devkota 2020).

For M. oryzae, many control methods, such as chemical control, breeding of disease-resistant varieties, elimination of overwintering pathogens, strengthening cultivation management, biological control, can be used (Chen et al. 2019; Shahriar et al. 2020). Among these, chemical control is the most effective technology for M. oryzae due to its highly effective sterilization ability. However, chemical control can cause severe environmental pollution (Zheng et al. 2020). In contrast, biological control is a relatively cost-effective and environment-protecting method, which has been successfully used to control M. oryzae (Kongcharoen et al. 2020; Xu et al. 2020).

Several studies have shown that the plant resistance toward M. oryzae can be increased by biotic substances by inducing defense responses (Rahman et al. 2014, 2015). Gómez-Vidal et al. (2009) found that the biocontrol agent fungi could modulate plant defense responses and energy metabolism in field date palms by promoting photosynthesis and energy metabolism. Signal transduction is associated with the resistance induced by biocontrol agents (Palmieri et al. 2012). Many antagonistic bacteria can biocontrol the rice blast (Tendulkar et al. 2007; Karthikeyan and Gnanamickam 2008). In particular, Streptomyces spp. is a well-known producer of bioactive compounds with antibacterial, antifungal, and antioxidant properties (Trejo-Estrada et al. 1998; Tendulkar et al. 2007). Streptomyces strains also can limit the development of various diseases by the induction of resistance genes expression or defense enzymes activities (Arseneault et al. 2014; Zhang et al. 2016; Xu et al. 2017). As per our previous studies, Streptomyces JD211 (JD211) have shown the antifungal activity to M. oryzae (Wang, Wei, Yang, Li, Tu, Ni, and Pan 2015b). And in the pot experiment, the disease index of M. oryzae was significantly decreased by powders of JD211 (Shao et al. 2017). After being treated with JD211, activation of defense responses in rice was found (Shao et al. 2017). Although JD211 appears to be a promising alternative strategy for controlling rice blast.
blast, the interaction among the JD211, rice, and pathogen is still unclear. And the key pathways or components involved in the JD211-induced defense mechanism need to be identified for a better understanding of how this method of the biocontrol function and how to enhance its efficacy.

The proteomic analysis is a powerful tool for investigating cellular changes in plants. Several proteins from rice are closely related to plant resistance using the proteomics approach (Kim et al. 2004; Chi et al. 2010; Wang et al. 2013). In the present study, we analyzed proteomic changes occurring in rice leaves in response to JD211 treatment and M. oryzae infection by the relative and absolute quantitation (iTRAQ) techniques, which aim to identify proteins pathways affected by the resistance activation. Our study identified a list of resistance-related proteins in rice leaves and revealed several important pathways associated with defense responses. These data provide important information for understanding the molecular mechanism involved in JD211-induced resistance and application clues for genetic breeding of crops with the improved M. oryzae resistance.

Materials and methods

Plant materials

All rice cultivars (ESR: Luliangyou 996) used in this study were grown in pots with soil. Before sowing, the powdery JD211 was mixed with soil at densities of 5 g/kg and incubated in soil for 7 days. Powders of JD211 with living spores were prepared, following the Wang et al. method (wang et al. 2015). Rice seedlings were grown indoors under natural daylight and the soil moist was kept, but there was no flowing water. For pathogen inoculation of rice leaves, spore suspension (10^5 spores/ml in 0.5% v/v Tween 20) was sprayed onto rice leaves at the seedling age of 20 days. After inoculation, all rice seedlings were incubated in the darkroom at 28°C for 2 days. There were four treatments: (1) Untreated control (the rice treated with sterile distilled water only). (2) JD211 treatment (the rice treated with JD211). (3) pathogen control (the rice treated with M. oryzae only). (4) JD211 + M, o treatment (the rice treated with JD211 and M. oryzae). For each treatment, there were five replicate plates.

Protein extraction and quantization

The protein extraction was done according to Isaacson et al. (2006) with some modifications. To extract proteins, the rice leaves were powdered with liquid nitrogen. Ground powders were homogenized in lysis buffer and centrifuged at 15,000 × g for 15 min. Then the supernatant was collected, and the protein concentrations were determined by BCA methods (Smith et al. 1985) and stored at −80°C for iTRAQ analysis.

Extracted proteins were separated by 12% SDS-PAGE to evaluate the protein quality. Three biological replicates were carried out for each sample.

Protein digestion and labeling

For each sample, 100 μg of proteins were reduced, alkylated, and trypsin-digested following Sacute et al. (Wisniewski et al. 2009). Samples were labeled with iTRAQ 8 plex Kit (AB SCIEX, USA) according to the manufacturer’s instructions. All labeled samples were mixed with an equal amount. The peptides from untreated control were labeled with 113 tags, the peptides from the JD211 treatment were labeled with 114 tags, the peptides from the pathogen control and the JD211 + M, o treatment were labeled with 115 and 116 Da tags, respectively. Then, the samples were dried in a vacuum freeze dryer for the iTRAQ analysis.

Fractionation

All iTRAQ-labeled samples were re-suspended with a 100 μL buffer A (2% acetonitrile) and pooled and purified using a strong cation exchange chromatography (SCX) column (Narrow-Bore 2.1 × 150 mm) from Agilent. Reversed-phase liquid chromatography (RPLC) was used on the Agilent 1200 HPLC System (Agilent). The parameter was Narrow-Bore 2.1 × 150 mm 5 μm with 215 and 280 nm UV detection. Separation was performed at 0.3 ml/min using a nonlinear binary gradient (Table 1), starting with buffer A and transitioning to buffer B (90% acetonitrile). Each segment was collected every 1 min for the 8–52 mins, mixed 4 or 5 tubes with 10 segments. Every segment was dried in a vacuum freeze dryer for the LC-MS/MS analysis.

LC-MS/MS analysis

The collected peptide fractions were resuspended in Nano-RPLC buffer A (0.1% formic acid, 2% acetonitrile). Then samples were separated by liquid chromatography (LC) using an Eksigent nanoLC-Ultra 2D system (AB SCIEX, USA). The samples were loaded on C18 nanoLC trap column (100 μm × 3 cm, C18, 3 μm, 150 Å) and washed by Nano-RPLC buffer A at 2 μL/min for 10 min. An elution gradient of 5–35% acetonitrile (0.1% formic acid) in 70 mins gradient was used on an analytical ChromXP C18 column (75 μm × 15 cm, C18, 3 μm 120 Å) with a spray tip. The LC fractions were analyzed using a Triple TOF 5600 mass spectrometer (AB SCIEX, USA). Mass spectrometer (MS) data acquisition was performed with a Triple TOF 5600 System (AB SCIEX, USA) fitted with a Nanospray III source (AB SCIEX, USA) and a pulsed quartz tip as the emitter (New Objectives, USA). Data were acquired using an ion spray voltage of 2.5 kV, curtain gas of 30 PSI, nebulizer gas of 5 PSI, and an interface heater temperature of 150°C. For information-dependent acquisition (IDA), survey scans were acquired in 250 ms, and 35 product ion scans were collected if they exceeded a threshold of 150 counts per second (counts/s) with a 2° to 5° charge-state. The total cycle time was fixed to 2.5 s. A rolling collision energy setting was applied to all precursor ions for collision-induced dissociation (CID). The dynamic exclusion was set for 1/2 of peak width (18 s), and the precursor was then refreshed off the exclusion list.
**iTRAQ protein identification and quantification**

The iTRAQ data were processed with Protein Pilot Software v. 5.0 (AB SCIEX, USA) against the Oryza sativa database using the Paragon algorithm (Shilov et al. 2007). The experimental data from tandem MS were used to match the theory data for protein identification. The database search parameters were the following: the instrument was Triple TOF 5600, iTRAQ quantification, cysteine modified with iodoacetamide, and biological modifications were selected as ID focus, trypsin digestion. For false discovery rate (FDR) calculation, an automatic decoy database search strategy estimated FDR using the PSPEP (Proteomics System Performance Evaluation Pipeline Software, integrated into the ProteinPilot Software). Only data with a false discovery rate (FDR) of less than 1% were used for subsequent data analysis. The peptides with a confidence of more than 95% were contained in iTRAQ labeling quantification, and proteins with at least one unique peptide were considered for further analysis. Finally, for differential expression analyses, fold change was calculated as the average ratio of 114/115 (A), 115/113 (C) and 116/113 (D), respectively. And proteins with a fold change larger than 1.2 or less than 0.83 and p-value less than 0.05 were considered significantly differentially expressed proteins (DEPs). P-values were calculated according to Student’s t-test.

**Bioinformatics analysis**

The Gene Ontology (GO) enrichment analysis of this experiment was based on the mainstream database David 6.7 (http://david.abcc.ncifcrf.gov/) and QuickGO (http://www.ebi.ac.uk/QuickGO) to describe GO classification annotation and enrichment analysis of the screening differential proteins. And proteins were classified based on molecular function, biological process, and cellular components. Genomes (KEGG) (http://www.kegg.jp/) annotation was also done for pathway analysis, and we assessed the interaction network for differentially accumulated proteins using sting software (http://string.embl.de/) database and Cytoscape (http://www.cytoscape.org/) software.

**Results**

To analyze proteomic changes associated with JD211-induced resistance in rice, when the seedling age was 23 days, leaf samples were collected from JD211-treated, pathogen-treated, untreated control, and JD211-treated plants before inoculation with M. oryzae. The rice proteome was analyzed using high-throughput 8-plex iTRAQ technique combined with a high-resolution LC-MS/MS SCIEX mass spectrometer. Using the Oryza sativa. fasta database, this study identified and quantified 4487, 4166, and 4209 rice proteins in the three technological replicates. And among these proteins, 3062 proteins were identified and quantified reliably. Most of them were located in chloroplast, plastid, thylakoid, chloroplast stroma, and plastid stroma (Figure 1).

**Identification of DEPs in rice responses to M. oryzae**

After the inoculation of M. oryzae, there were 278 proteins (164 up-regulated proteins, 114 down-regulated proteins) with significant changes in abundance in the pathogen control group compared to the untreated control group and we performed the pathway analysis on these DEPs using KEGG databases in rice plants. Our results demonstrated that 13 significant pathways associated with the up-accumulated proteins were enriched at the 5% significant level. Among these significant pathways, photosynthesis, photosynthesis-antenna proteins, metabolic pathways, glyoxylate and dicarboxylate metabolism, carbon metabolism, carbon fixation in photosynthetic organisms, ascorbate and aldarate metabolism, and glutathione metabolism were highly significant (p < 0.01) in the rice infected with M. oryzae (Figure 2a). There were 24 DEPs associated with photosynthesis, 20 proteins were up-regulated, such as ferredoxin-1, plastocyanin, cytochrome b6, chlorophyll a/b-binding protein, photosystem I iron-sulfur center and three photosystem I reaction centers subunit—most of them associated with electron transport. There also four proteins (photosystem II protein D1, photosystem II D2 protein, photosystem II CP47 reaction center protein, and photosystem II CP43 reaction center protein) down-regulated involved with the photosynthesis (Table 2), and photosystem II CP47 and CP43 reaction center protein were associated with light absorption.

Among the down-regulated proteins, for KEGG pathway enrichment analysis, 11 pathways were significantly enriched (p < 0.05) in the rice infected with M. oryzae. Porphyrin and chlorophyll metabolism, biosynthesis of amino acids, biosynthesis of secondary metabolites, and metabolic pathways were highly significant (p < 0.01). Porphyrin and chlorophyll metabolism pathways were the most highly significant, and the porphyrin and chlorophyll metabolism were the most significant ones (Figure 2b).

**Identification of DEPs in rice responses to JD211**

Compared with the untreated control group, 213 DEPs (115 up-regulated proteins; 98 down-regulated proteins) were identified in the JD211-treated group. There were 14 significant pathways after KEGG pathway enrichment analysis in up-regulated proteins (Figure 3a). Up-regulated proteins are mostly associated with the glyoxylate and dicarboxylate metabolism, photosynthesis, carbon metabolism, glutathione metabolism, ascorbate and aldarate metabolism, nitrogen metabolism, photosynthesis-antenna proteins, pyruvate metabolism, and metabolic pathways. Thirteen proteins involved in photosynthesis (Table 2), seven proteins (Chloroplast cytochrome f, Ferredoxin-1, Plastocyanin, Cytochrome b6-f complex iron-sulfur subunit, Chloroplast photosystem I reaction center subunit II-like protein, and Photosystem I reaction center subunit N) were increased, while six proteins were decreased, such as putative ATP synthase gamma chain, putative photosystem II 10 K protein ATP synthase epsilon chain, and ATP synthase B chain. Interestingly, the repression of four proteins that form photosystem II complex protein was also observed, but the abundance of these proteins in JD211 + M. o was higher than the pathogen control. Malate synthetase and isocitrate lyase are two key enzymes in the glyoxylate cycle (Table 3). Both of them were up-regulated by JD211 or M. oryzae, but the promotion of JD211 was higher than M. oryzae induced.

There were 15 significant pathways, including porphyrin and chlorophyll metabolism, carbon fixation in
photosynthetic organisms, metabolic pathways, carbon metabolism, biosynthesis of secondary metabolites, pentose phosphate pathway, and glyoxylate and dicarboxylate metabolism pathway in down-regulated proteins in the JD211 treated group compared with the untreated control group. Ten pathways were highly significant \( (p < 0.01) \) (Figure 3b). All proteins involved in porphyrin and chlorophyll metabolism were down-accumulated, but the repression of this process by \( M. \) oryzae was attenuated during JD211-induced resistance. Decreased abundance of proteins related to the pentose phosphate pathway (i.e. glyceraldehyde-3-phosphate dehydrogenase, and fructose bisphosphate aldolase) was also observed after the inoculation of \( M. \) oryzae.

**Identification of DEPs in rice responses to JD211 and \( M. \) oryzae**

A total of 158 DEPs (65 up-regulated proteins, 93 down-regulated proteins) were detected in the rice treated with JD211 and \( M. \) oryzae compared with the untreated control group. Proteins involved in glyoxylate and dicarboxylate metabolism, carbon metabolism, ascorbate and aldarate metabolism, metabolic pathways, fatty acid degradation, and biosynthesis of secondary metabolites had increased abundantly after being treated with JD211 and \( M. \) oryzae (Figure 4a). Proteins related to metabolic processes photosynthesis-antenna proteins, photosynthesis, metabolic pathways, and porphyrin and chlorophyll metabolism were also down-regulated in the rice treated with JD211 and \( M. \) oryzae. The proteins involved in the glyoxylate cycle (i.e. Malate synthetase and Isocitratelyase) were also up-regulated in JD211 + M.o treatment compared to the untreated control (Figure 4b).
they could cause oxidative damage to proteins, DNA, and lipids of the host. Several enzymes maintained the equilib-rium between production and scavenging of ROS. In our study, 27 ROS-related proteins had been identified, and most of them were up-accumulated (Table 4). Superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione peroxidase (GPX), and catalase (CAT) cooperated in removing ROS. Five APX, two SOD, one GPX, and one CAT were significantly up-regulated by JD211 or *M. oryzae*. Thioredoxin was known as a regulator to control the redox status of target proteins (Vieira Dos Santos and Rey 2006). Four thioredoxins were up-regulated in the pathogen control. But beta-1,3-glucanase-stress-inducible protein 1 was significantly down-regulated in the pathogen control. Three glutathione S-transferases were found in our data; thioredoxins were up-regulated in the pathogen control.

**Table 2. Differentially expressed proteins related to photosynthesis.**

| Accession number | Name                                                                 | Fold change | A - value | P - value | Fold change | A - value | P - value | Fold change | A - value | P - value |
|------------------|----------------------------------------------------------------------|-------------|-----------|-----------|-------------|-----------|-----------|-------------|-----------|-----------|
| Q7F471           | Photosystem II D2 p + 870:886 protein                                 | 0.968       | 0.318     | 0.629     | 0.000       | 0.735     | 0.002     |             |           |           |
| P0C364           | Photosystem II CP47 reaction center protein                          | 1.022       | 0.313     | 0.647     | 0.001       | 0.651     | 0.001     |             |           |           |
| P0C367           | Photosystem II CP43 reaction center protein                          | 1.087       | 0.039     | 0.687     | 0.000       | 0.855     | 0.000     |             |           |           |
| Q62BV1           | Putative photosystem II 10k protein                                  | 0.695       | 0.000     | 0.856     | 0.003       | 0.822     | 0.000     |             |           |           |
| P0C23            | ATP synthase epsilon chain                                           | 0.678       | 0.000     | 0.901     | 0.391       | 0.906     | 0.229     |             |           |           |
| Q7DN80           | ATP synthase subunit alpha                                           | 0.638       | 0.000     | 0.921     | 0.003       | 0.807     | 0.000     |             |           |           |
| Q62NK7           | Putative H(+)-transporting ATP synthase                              | 0.655       | 0.000     | 1.061     | 0.174       | 0.761     | 0.000     |             |           |           |
| Q48N1W           | Putative ATP synthase gamma chain 1, chloroplast (H(+)-transporting two-sector ATPase/F(1)-ATPase/ATPc1) | 0.768       | 0.000     | 1.073     | 0.014       | 0.861     | 0.003     |             |           |           |

**Proteomics analysis and identification of defense-related proteins**

Phenylalanine ammonia-lyase (PAL) is a key enzyme in the phenylpropanoid pathway (Vogt 2010). In our study, PAL was up-regulated by JD211 cooperated the inoculation of *M. oryzae*, but down-regulated after being infected with *M. oryzae*, suggesting that JD211 could increase the production of PAL. Chalcone-flavonane isomerase and two UDP-glycosyltransferases were associated with flavonoid biosynthesis. All of them were down-regulated after being treated with JD211 or pathogen. But the repression was lower in the rice-treated JD211 than the rice-infected pathogen. An isoflavone reductase, involved in the biosynthetic pathway of isoflavonoids phytoalexin (Cheng et al. 2015), was also repressed by the pathogen (Table 5).

Pathogenesis-related (PR) proteins had antimicrobial or insecticidal activity (Datta and Muthukrishnan 1999). Several PR proteins were induced after being treated with JD211. For example, thaumatin-like protein, which belongs to the PR 5 protein family (Kim et al. 2004), was activated by JD211, and the PR 5 was up-regulated by JD211 or pathogen. While beta-1,3-glucanase was activated by the pathogen or cooperated treated with JD211. Five heat shock proteins (HSP) were expressed at high levels in *M. oryzae*-infected leaves compared with the untreated control, indicating that *M. oryzae* activated the expression of the genes encoding HSP. Two inducible resistance proteins were also found. Dehydration-stress-inducible protein 1 was significantly up-regulated by JD211 and *M. oryzae*, while dehydration-stress-inducible protein 1 was down-regulated after pathogen infection. And a disease resistance response protein-like significant down-regulated in the pathogen control (Table 5).

**Proteomics analysis and identification of proteins in other pathways**

We also found other categories of proteins, including signal transduction and protein metabolism (Table 3), three
histones involved in protein modification were significantly changed in abundance, two of them were significantly up-regulated in JD211 + M. o treatment compared to the pathogen control, while repressed in the pathogen control compared to the untreated control. And three proteins (Elongation factor Tu, Elongation factor Ts, and Elongation factor 1-alpha) involved in protein biosynthesis were also up-regulated in JD211 + M. o treatment compared to the pathogen control. The proteins (Guanine nucleotide-binding protein subunit beta-like protein A and Small GTP-binding protein domain-containing protein) involved in signal transduction were also a significant increase in rice treated with JD211 and M. oryzae.

Discussion
As previously reported, JD211 had effects on the growth promotion of rice (Wang et al. 2015), and it also can
reduce the disease incidence of rice blasts (Shao et al. 2017). To better understand the cellular processes associated with JD211-induced resistance in rice seedlings, proteomic changes activated by JD211 were analyzed. These DEPs can be divided into three groups based on changes in the abundance of proteins. Group 1, proteins increased with JD211-induced resistance in rice seedlings, proteins related to defense response. Group 2, proteins increased by JD211 or cooperated inoculation of the pathogen; while proteins related to redox. Proteins increased by JD211 or cooperated inoculation mainly caused decreased abundance of proteins associated with the plant resistance (i.e. Phenylalanine ammonia-lyase, Putative UDP-glucosyltransferase, and Disease resistance response protein-like), indicating that induced resistance was associated with the proceeding in the chloroplast, such as light absorption.

**Rice proteins affected by M. oryzae**

Doke (1983) found the oxidative burst in potato tissues during the incompatible interaction induced by invading fungi or fungal wall components. Rapid production of ROS, also called oxidative burst, often happens to potential pathogen attack in plants. ROS could affect the cellular processes in plants infected with pathogens (Baker and Orlandi 1995) and have the ability to damage the host cell. Plants would produce the antioxidant enzymes to scavenge ROS after being infected by a pathogen (Das and Roychoudhury 2014); most redox-related proteins, belonging to group 2, were activated by M. oryzae. It is also seen in the cotton infected with necrotrophic pathogen *Rhizoctonia solani* (Zhang et al. 2016), and rice infected by Rice stripe virus (Wang et al. 2015).

* M. oryzae inoculation mainly caused decreased abundance of proteins associated with the plant resistance (i.e. Phenylalanine ammonia-lyase, Putative UDP-glucosyltransferase, and Disease resistance response protein-like), indicating that the rice disease resistance was repressed after the inoculation of the pathogen. While proteins’ response to abiotic stresses (five heat shock proteins) had increased.

### Table 4. Differentially expressed proteins related to defense response.

| Accession number | Name | Fold change | P-value | Fold change | P-value | Fold change | P-value |
|------------------|------|-------------|---------|-------------|---------|-------------|---------|
| Q0D2E3           | Phenylalanine ammonia-lyase | 0.944 | 0.343 | 0.681 | 0.000 | 1.229 | 0.002 |
| Q84T92           | Chalcone–flavone isomerase | 0.729 | 0.002 | 0.744 | 0.003 | 0.884 | 0.054 |
| Q6Y4Y1           | Putative UDP-glucosyltransferase | 0.793 | 0.003 | 0.876 | 0.002 | 0.847 | 0.031 |
| Q5VME5           | Putative UDP-glycosyltransferase (Os06g0287900 protein) | 0.675 | 0.007 | 0.598 | 0.001 | 0.834 | 0.016 |
| Q9FTN6           | Putative isoflavone reductase (Os01g0106300 protein) | 0.890 | 0.004 | 0.771 | 0.042 | 0.846 | 0.063 |
| Q7SGR1           | Putative pathogenesis-related thaumatin-like protein | 1.221 | 0.000 | 0.957 | 0.425 | 1.005 | 0.917 |
| Q2QND6           | Pathogenesis-related protein 5, putative, expressed (Os12g0569500 protein) | 1.314 | 0.009 | 1.252 | 0.000 | 1.060 | 0.000 |
| Q7F354           | Beta-1,3-glucanase | 1.300 | 0.094 | 1.912 | 0.028 | 1.685 | 0.006 |
| Q10NA9           | 70 kDa heat shock protein | 1.166 | 0.003 | 1.477 | 0.000 | 1.576 | 0.000 |
| Q2SN98          | 90 kDa heat shock protein | 1.343 | 0.000 | 1.460 | 0.000 | 1.336 | 0.001 |
| Q2QIV5          | 70 kDa heat shock protein | 0.996 | 0.000 | 1.323 | 0.014 | 1.194 | 0.023 |
| Q0DBJ9          | PREDICTED: stromal 70 kDa heat shock-related protein | 1.099 | 0.121 | 1.478 | 0.000 | 1.128 | 0.008 |
| Q6Z7V2          | 24.1 kDa heat shock protein, mitochondrial | 0.831 | 0.046 | 1.607 | 0.046 | 1.049 | 0.338 |
| Q6534           | Dehydration-stress inducible protein 1 | 1.162 | 0.059 | 1.715 | 0.053 | 3.870 | 0.014 |
| Q452S2          | Disease resistance response protein-like | 0.864 | 0.138 | 0.767 | 0.003 | 1.070 | 0.008 |
abundance in pathogen control, which was part of the plant’s basic resistance. PRs also induced after being infected with the pathogen.

Most of the carbon in a plant’s dry mass was fixed in photosynthesis, which is vital for the growth and survival of all plants. Proteins related to the electron transport (ferredoxin-1, Plastocyanin, and cytochrome b6) and carbon fixation (ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit) in the photosynthesis increased after being infected with the pathogen, but proteins involved in chlorophyll biosynthesis (Magnesium-chelatase subunit ChlI, Magnesium-chelatase subunit ChlD, and Uroporphyrinogen decarboxylase 1) and photosystem II had decreased abundance in the rice of the pathogen control treatment, a reduced abundance of proteins involved in the chlorophyll biosynthesis has been previously observed in the rice infected with rice stripe virus (Wang et al. 2015). During the plant infection, *M. oryzae* could produce the photosystem II inhibitor (Wilson et al. 2009; Chen et al. 2007). Bastiaans (1993) found that leaf blast in the rice could reduce the photosynthesis by repressing the assimilation rate at light saturation, and the chlorophyll plays a key role in light absorption, suggesting that *M. oryzae* inoculation may have a negative effect on chlorophyll biosynthesis and photosystem II complex protein biosynthesis. And the repression of chlorophyll biosynthesis may associate with disease lesions in rice leaves. Ballare (Ballaré 2014) suggested that the light could enhance the plant resistance by phytochrome B and presumably other photoreceptors. In other words, *M. oryzae* inoculation repressed the light absorption by down-regulated the proteins related to chlorophyll biosynthesis and light absorption; consequently, the rice resistance was decreased after infected *M. oryzae*. And the up-accumulated of proteins involved in the electron transport and carbon fixation was part of weak resistance in plants against the pathogen, and these proteins belong to group 3.

**Rice proteins affected by JD211 only or cooperated inoculation of *M. oryzae***

Similar to the pathogen control, proteins involved in electron transport and carbon fixation in photosynthesis were also increased in JD211 treatment, and proteins related to redox were activated by JD211 or cooperated inoculation of *M. oryzae*. Reduction in the abundance of proteins related to chlorophyll biosynthesis (Magnesium-chelatase subunit ChlI and Uroporphyrinogen decarboxylase 1) was attenuated by JD211, and the abundance of proteins in JD211 + *M. o* treatment was higher than JD211 treatment, indicating that JD211 enhance the disease resistance of rice by promoting the chlorophyll biosynthesis.

These ROS-related proteins also can be activated by JD211, but the changes in abundance of most ROS-related proteins were lower than pathogen-induced, suggesting that the response was just a primary resistance in rice against exotic bacteria rather than a disease resistance response. Seevers et al. (1971) suggested that ‘the association of peroxidase with resistance was a consequence of, not a determinant in, resistance.’ Whereas GDP-mannose 3,5-epimerase 1 and putative monodehydroascorbate reductase were significantly induced by JD211 after being inoculated with *M. oryzae*, both of them were involved in ascorbate biosynthesis (Doke 1983), indicating that ascorbate may play an important role in rice defense responses induced by JD211.

The phenylpropanoid pathway plays an important role in the plant defense, JD211 increased the abundance of proteins (Phenylalanine ammonia-lyase (PAL), Chalcone–flavanone isomerase, Putative UDP-glucosyltransferase, and Putative UDP-glycosyltransferase) involved in the phenylpropanoid pathway, then promoted the production of
phenylpropanoids to enhance the disease resistance of rice. The phenylpropanoid pathway provides many molecules that have important functions, such as coumarins, isoflavonoids, flavonoids, salicylic acid, and lignin (La Camera et al. 2004). Flavonoids play an important role in UV protection and photoprotection (Tretueter 2006), while salicylic acid is a signaling molecule in plant against the pathogen, and induces the defense PR gene production (Chen et al. 1995), which was consistent with PRs accumulation. Further analysis revealed that JD211 increased the disease resistance by promoting the production of phenylpropanoids.

The glyoxylate cycle could provide the carbon skeletons for carbohydrate synthesis in oilseeds (Eastmond and Graham 2001). And it also contributed to the defense reaction in soybean leaves (Cots et al. 2002). Our results show that malate synthase and isocitrate lyase were significantly up-regulated by JD211 only or cooperate inoculation of M. oryzae. JD211 could promote carbohydrate synthesis by the promotion of the glyoxylate cycle. JD211 treatment affected the proteins associated with signal transduction, indicating that the microbial recognition system was faster than that of the pathogen.

In conclusion, through an iTRAQ-based proteomic approach, we identified 213 DEPs in the JD211 treatment compared to the untreated control (A), 278 DEPs in the pathogen control compared to the untreated control (C), 158 DEPs in the JD211 + M, o treatment compared to the untreated control (D). Through a biological information function analysis of the DEPs, we found some pathways and molecular markers play an important role in JD211-induced resistance against M. oryzae. JD211 induced the production of phenylpropanoids and ascorbate and triggered a series of defense responses more quickly by promoting the signal transduction. Meanwhile, JD211 improved the energy synthesis for defense response. There also provided convincing evidence demonstrating the involvement of native immunity in response to M. oryzae in rice seedlings. Further work may major in proving the effectiveness of the specific protein in JD211-induced induced resistance.

Disclosure statement
No potential conflict of interest was reported by the author(s).

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