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Research article

Proteomic analysis of the compatible interaction of wheat and powdery mildew (*Blumeria graminis* f. sp. *tritici*)

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ABSTRACT

Proteome characteristics of wheat leaves with the powdery mildew pathogen *Blumeria graminis* f. sp. *tritici* (*Bgt*) infection were investigated by two-dimensional electrophoresis and tandem MALDI-TOF/TOF-MS. We identified 46 unique proteins which were differentially expressed at 24, 48, and 72 h post-inoculation. The functional classification of these proteins showed that most of them were involved in photosynthesis, carbohydrate and nitrogen metabolism, defense responses, and signal transduction. Upregulated proteins included primary metabolism pathways and defense responses, while proteins related to photosynthesis and signal transduction were mostly downregulated. As expected, more antioxidative proteins were activated at the later infection stage than the earlier stage, suggesting that the antioxidative system of host plays a role in maintaining the compatible interaction between wheat and powdery mildew. A high accumulation of 6-phosphogluconate dehydrogenase and isocitrate dehydrogenase in infected leaves indicated the regulation of the TCA cycle and pentose phosphate pathway in parallel to the activation of host defenses. The downregulation of MAPK5 could be facilitated for the compatible interaction of wheat plants and *Bgt*. qRT-PCR analysis supported the data of protein expression profiles. Our results reveal the relevance of primary plant metabolism and defense responses during compatible interaction, and provide new insights into the biology of susceptible wheat in response to *Bgt* infection.

1. Introduction

Powdery mildew of wheat is caused by *Blumeria graminis* f. sp. *tritici* (*Bgt*), which infects the aerial parts of wheat and seriously affects yield and quality (Cao et al., 2014). Average yield losses ranged from 10% to 15%, and in severe cases up to 30% (Bowen et al., 1991; Briceñofélix et al., 2008). The utilization of resistant varieties and fungicides is an effective method of controlling wheat powdery mildew. However, the appearance of new virulence genes within the pathogen population has led to the loss of host resistance and efficacy of many fungicides (Mcgrath, 2009; Oberhaensli et al., 2011). Plants possess innate immune systems to protect themselves from pathogens. The activation of plant defense responses by applying inducers may be an alternative strategy by which to combat pathogens (Tayeh et al., 2015). Therefore, the knowledge of susceptible plant defenses against phytopathogenic pathogens becomes important. The literature has indicated that the activation of plant defense responses involves a massive redistribution of energy, and efficient energy supply is a prerequisite for plant defense against pathogen infection (Rojas et al., 2014). The primary metabolism pathways of host play a critically important role in supporting cellular energy requirements for defense responses (Bolton, 2009). Energy-associated metabolism and defense responses show a coordinated stimulation of expression in response

Abbreviation: Bgt, *Blumeria graminis* f. sp. *tritici*; CBB, Coomassie Brilliant Blue; GS, glutamine synthetase; hpi, hours post infection; ICDH, Isocitrate dehydrogenase; MAPK5, mitogen-activated protein kinase 5; NDPK2, nucleoside diphosphate kinase 2; ROS, reactive oxygen species; RuBisCO, ribulose bisphosphate carboxylase/oxygenase; SAM, S-adenosyl methionine; SAMS, S-adenosylmethionine synthetase; 2-DE, two-dimensional electrophoresis.

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to biotic stresses (Less et al., 2011). Transcriptome analysis indicated that 3014 and 2800 genes displayed differential expression patterns in susceptible wheat cultivar Jingdong 8 and its near-isogenic resistant line (carrying a single Pm resistance gene *Pm30*), respectively, at 12 h post *Bgt* inoculation; these genes are mainly involved in metabolic processes and defense responses (Xin et al., 2012). Similar findings were seen in wheat line N9134 during stripe rust and powdery mildew challenge by large-scale transcriptome comparison (Zhang et al., 2014).

Although analysis of gene expression enhanced our understanding on the response of wheat to *Bgt*, many questions remain unclear because of the existence of the regulation of gene expression at transcriptional and translational levels (Quirino et al., 2010). The transcriptional changes can be poorly correlated with corresponding proteins. Because proteins are directly linked to cellular functions, technologies aimed at studying protein changes in plant tissues are an excellent complement. Proteomics combined with bioinformatics can explain many complex biological processes involved in cellular metabolism and defense mechanisms with pathogen invasion (Butt and Lo, 2007). Fu et al. (2016) analyzed the proteome change of leaves from *Bgt*-inoculated resistant wheat line N9134 and found differentially accumulated proteins mainly involved in stress response and primary metabolic pathways. The proteome analysis of resistant wheat line N0308 during *Bgt* infection showed that most of the upregulated proteins were also related to defense-related proteins and carbohydrate metabolic proteins (Mandal et al., 2014). Identification of proteins in susceptible and resistant near-isogenic wheat (lines JD8 and JD8-Pm30) leaves after *Bgt* inoculation revealed that fewer proteins were induced in JD8-Pm30 than JD8, and the *Pm30* gene might play a role in the decrease of protein number in incompatible interaction (Wang et al., 2012a).

To date, many investigations have focused on the interaction between resistant wheat and *Bgt*. There is a gap of knowledge on the responses of susceptible wheat to *Bgt* infection. We therefore carried out an investigation on the proteomic profiles of wheat leaves from the susceptible cultivar Xinong 979 by 2-DE and MALDI-TOF/TOF-MS at different time points following inoculation with *Bgt*. The results reveal the complex modulation of primary metabolism and defense responses, and provide an insight into the molecular mechanism of susceptible wheat plants in response to *Bgt*.

2. Materials and methods

2.1. Experimental materials, inoculation and pathogen infection

Wheat (*T. aestivum* L.) cultivar Xinong 979, provided by Northwest Agriculture and Forestry University, was used in the experiments. The susceptibility level of Xinong 979, a highly productive and early maturing wheat cultivar, to powdery mildew was estimated at a 6 on a scale ranging from 0 (immune) to 9 (highly susceptible) (Gao et al., 2014). The cultivar is susceptible to the predominant Chinese isolate E20 of *Bgt*.

For the experiments, Xinong 979 seeds were planted in 10 cm pots with a 16 h light/8 h dark photoperiod at 18 °C. The pots were covered with non-opaque and breathable hoods (hoods were made of mulch film with a wire skeleton) to prevent being contaminated by other fungi. Inoculation was performed according to the method described by Fu et al. (2016). One group of twelve-day-old wheat plantlets were sprayed with water and then artificially inoculated by dusting *Bgt* E20 conidia from sporulating seedlings. The other group of the same stages of seedlings served as the control and was mock-inoculated, i.e. only spraying of water on wheat leaves. Leaf samples of the two groups were respectively collected at 24, 48, and 72 hpi, and immediately frozen in liquid nitrogen, then stored at −80 °C for protein extraction. The experiments were repeated to obtain three biological replicates. Infected and uninfected leaf samples at three time points composed a biological replicate. Each sample of 0.5 g was excised from the middle part of the first leaf from 15 to 18 wheat plants.

For microscopy, inoculated leaf samples were collected with three biological replications at three time points (24, 48 and 72 hpi). These leaf tissues were cut into a length of 1 cm, and decolorized in ethanol/trichloromethane (3:1, v/v) with 0.15% (w/v) trichloroacetic acid (TCA) for 20 min at 70 °C. Then these leaf segments were stained using Coomassie Brilliant Blue (CBB) R-250 (0.6%) for 20 min and rinsed with distilled water, observed under a microscope (Model DM750M, Leica Microsystems, Wetzlar, Germany).

2.2. Protein extraction

Protein samples with three biological replicates were extracted by TCA precipitation with some modifications (Zhang et al., 2016). Leaf tissue (0.5 g) was ground in a prechilled mortar and pestle with liquid N_2, suspended in 10% w/v TCA in acetone with 0.07% v/v β-mercaptoethanol, and incubated at −20 °C for 2 h. After centrifugation for 30 min at 12,000 rpm at 4 °C, the pellets were washed with prechilled acetone containing 0.07% β-mercaptoethanol, incubated at −20 °C for 1 h, and then centrifuged at 4 °C. The pellets were washed three times in ice-cold acetone containing 0.07% β-mercaptoethanol, and centrifuged again at 4 °C. The pellets were vacuum-dried and solubilized in lysis buffer (2 M thiourea, 4% 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate (CHAPS), 8 M urea, and 20 mM DTT) at 25 °C for 2 h. In order to remove insoluble materials, the suspension was centrifuged at 14,000 rpm for 40 min at 25 °C. Concentrations of total protein were determined using a Bradford assay with bovine serum albumin as the standard.

2.3. Two-dimensional electrophoresis and image analysis

For two-dimensional electrophoresis (2-DE) separation of proteins samples, each protein sample (800 μg) was loaded onto a ReadyStrip™ IPG Strip with a length of 24 cm and a pH gradient of 4–7 (Bio-Rad Laboratories, Inc., Hercules, CA, USA) and rehydrated passively with 450 μL of protein solution containing 0.5% (v/v) immobilized pH gradient (IPG) buffer (pH 4–7) for 12–16 h at 20 °C. The first-dimension isoelectric focusing (IEF) was carried out using a PROTEAN® IEF Cell (Bio-Rad Laboratories, Inc.) at 20 °C with six steps: 250 V for 130 min, 250 V for 90 min, 500 V for 90 min, 1000 V for 2 h, 9000 V for 5 h, and 9000 V for 10 h with a total of 99 kVh and a constant 500 V for the last 12 h. Before the second dimension protein separation, each strip was incubated for 15 min in 10 ml "equilibration buffer I" consisting of 2% (w/v) SDS, 6 M urea, 0.375 M Tris-HCl (pH 8.8), 0.01% (w/v) bromophenol blue, 20% (v/v) glycerol, and 2% (w/v) DTT. Then, buffer I was washed off from the strips and the strips were place in "buffer II" for 15 min, which consisted of 2% (w/v) SDS, 6 M urea, 0.375 M Tris-HCl (pH 8.8), 0.01% (w/v) bromophenol blue, 20% (v/v) glycerol, and 2.5% (w/v) iodoacetamide.

Second dimension protein separation was performed by 12% vertical sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gels. These gels were fixed in 40% (v/v) ethanol and 10% (v/v) acetic acid for 2 h. The protein spots in gels were visualized by staining with a staining solution containing 20% (v/v) ethanol, 0.12% (v/v) CBB G-250, 10% (v/v) phosphoric acid, and 10% (w/v) ammonium sulfate.

Gel images were scanned at 300 dpi with a UMAX Power Look 2,