Journal of Plant Physiology 215 (2017) 140–153

Contents lists available at ScienceDirect

Journal of Plant Physiology

journal homepage: www.elsevier.com/locate/jplph

Research Paper

Proteomic analysis of developing wheat grains infected by powdery mildew (*Blumeria graminis* f.sp. *tritici*)

Jie Li[a,1], Xi-wen Yang[a,1], Yong-chun Li[b], Ji-shan Niu[b], De-xian He[a,*]

[a] College of Agronomy, Henan Agricultural University/Collaborative Innovation Center of Henan Grain Crops/National Key Laboratory of Wheat and Maize Crop Science, Zhengzhou, China

[b] College of Agronomy, Henan Agricultural University/National Engineering Research Centre for Wheat, Zhengzhou, China

ARTICLE INFO

Keywords:
Grain development
Powdery mildew
Proteome
Wheat

ABSTRACT

Blumeria graminis f.sp. *tritici* (*Bgt*) infection greatly interferes with the normal source-sink relationships and always causes tremendous loss of yield and quality in wheat. To better understand the impact of this pathogen on grain development, proteome characterization during grain development in susceptible wheat cultivar Xinong 979 infected by powdery mildew was investigated by 2-DE and tandem MALDI-TOF/TOF-MS. Identification of 111 differentially expressed protein spots representing 85 unique proteins and six expression patterns showed a chronological description of wheat grain formation. Comparative proteome profiles indicated that 43 protein spots displayed significant abundance change, which is mainly involved in stress/defense responses, primary metabolism, and storage protein. The down-regulation of defense response-related proteins including alpha-purothionin, lactoylglutathione lyase, and alpha-amylase inhibitor CM16 in infected grains compared to control during seed filling might be related to the susceptibility of wheat to *Bgt*, while the enhanced expression of beta-amylase and glyceraldehyde-3-phosphate dehydrogenase and the down-regulation of ADP glucose pyrophosphorylase in infected grains probably resulted in the negative effects on yield formation. Our data reveal the complex grain metabolism mechanisms and defense responses during compatible interactions of wheat and *Bgt*, and provide valuable information for further understanding of the underlying molecular processes which can possibly yield novel strategies for breeding resistant cultivars and protection strategies in the field.

1. Introduction

Wheat is one of the most widely grown crops in the world and is a major food source for humans, livestock, and industrial raw materials. Starch and protein are two main storage compounds in wheat grain and they determine the main traits for grain yield and flour quality. The developmental process of wheat grains includes three distinct phases: cell differentiation, grain filling, and desiccation/maturation (Wan et al., 2007). The first stage determines the number of endosperm cells per grain, while the second stage accumulates the storage products in endosperm and thereby determines the latent grain weight and quality (Nadaud et al., 2010).

Grain is a major sink during crop reproductive stages, its growth and development depend on the delivery of photosynthetic fixed carbon. Sucrose is the main translocation form of photosynthate. The quantity of sucrose for export from source leaves largely depends on photosynthetic activity and the transient storage content in vacuole. Factors that affect source supply (e.g., low photosynthetic rate results in less sucrose) and sink demand (e.g., the development of pathogens on plant organs leads to competition between sinks) could be detrimental to yield formation. Wheat leaf diseases (e.g., powdery mildew and rust) interfere with the normal source-sink relationships of the plant and cause a great yield loss (Green et al., 2014). Powdery mildew of wheat is caused by *Blumeria graminis* f. sp. *tritici* (*Bgt*). The mycelium of the pathogen grows over the surface of leaves and shoots, penetrates the epidermal cells forming haustoria that divert nutrients away from the host plant and uses them for their growth. In colonized source organs (e.g., leaves), nutrient demand of the pathogen acts as an additional major sink competing with host sinks, thereby modifying the transport of photoassimilate (Biemelt and Sonnewald, 2006). Competitiveness

Abbreviations: 1-Cys Prx, 1-Cys peroxiredoxin; 2-DE, two-dimensional electrophoresis; AAT, aspartate aminotransferase; AlaAT, alanine aminotransferase; *Bgt*, *Blumeria graminis* f.sp. *tritici*; DI, disease indices; DPA, days post-anthesis; MALDI-TOF/TOF-MS, matrix-assisted laser desorption/ionization time-of-flight tandem mass spectrometry; TCTP, translationally controlled tumor protein

* Corresponding author at: College of Agronomy, Henan Agricultural University, Zhengzhou, Henan, 450002, China.
E-mail address: hedexian@126.com (D.-x. He).

[1] Contributed equally to this study and share first authorship.

http://dx.doi.org/10.1016/j.jplph.2017.06.003
Received 9 February 2017; Received in revised form 15 May 2017; Accepted 9 June 2017
Available online 10 June 2017
0176-1617/

between plant and fungal sinks has been examined in many studies. Sutton et al. (2007) found that sugars and amino acids taken up by leaf tissues were transferred to the fungal mycelium. Glucose in particular was taken up into infected regions with higher rates than into uninfected tissues. Enhanced glucose uptake was also observed in *Arabidopsis thaliana* source leaves with powdery mildew infection (Fotopoulus et al., 2003). Bancal et al. (2012) found that rust fungal sink had a competitive priority for photoassimilates over grain filling. Pathogen-induced alterations in source-sink relationships usually lead to a reduced photosynthesis (Lemoine et al., 2013). Comparison the metabolism consequence of susceptible and resistant barley leaves with powdery mildew infection indicated that hexose content was much higher in susceptible leaves than in resistant leaves and greater accumulation of hexose was accompanied by a greater extent of down-regulated expression of photosynthetic-related genes (Swarbrick et al., 2006). Because proteins are directly linked to cellular functions, proteomics combines with bioinformatics can reveal complex biological processes and metabolic pathways involved in the interaction mechanisms of plant and pathogen. For example, proteomic studies of conidia from the barley pathogen *Blumeria graminis* f.sp. *hordei* showed that quite a lot of proteins were involved in carbohydrate, lipid, or protein metabolism, implying that dormant conidia are prepared for the catabolism of storage compounds (Noir et al., 2009; Bindschedler et al., 2009). Several proteomic studies on wheat leaves with *Bgt* infection showed that extensive proteins related to carbon metabolism, defense responses, and photosynthesis were affected (Fu et al., 2016; Wang et al., 2012; Li et al., 2017). Moreover, a susceptible wheat line was found to have higher energy perturbation than a resistant near-isogenic line (Wang et al., 2012).

Wheat powdery mildew is a major leaf disease. Previous researches have provided abundant biological information about the impacts of powdery mildew on source organs. The pathogen does not directly infect grains, although some phenotypic results of grain responding to powdery mildew stress have been documented (Cao et al., 2014; Gao et al., 2014), the responses are poorly understood at the molecular level. The metabolism of sink tissues (grains) is different from that of source tissues (leaves), which possess various mechanisms for stress adaptation and the accumulation of reserves, e.g. starch and protein. As an advanced technology, proteomics could improve our understanding on developing grain responding to powdery mildew stress. Many reported proteomic studies on the interaction of grain development and pathogen infection have been focused on panicle diseases such as Fusarium head blight (Trümper et al., 2016; Yang et al., 2010), but proteomic study about the effects of leaf diseases including powdery mildew on grain development was reported only by Gao et al. (2014) which investigated two grain development stages (10 and 25 days post anthesis). This literature does not provide overall understanding of the molecular mechanism underlying developing grain response to powdery mildew infection. In the present study, we performed proteome analysis of grain development during powdery mildew infection by a comparative proteomic approach. Our results reveal the diverse metabolism changes and defense responses and provide new insights into the interaction of grain development and powdery mildew.

2. Materials and methods

2.1. Experimental design

The field trial was carried out at the Science Education Experimental Park of Henan Agricultural University (longitude 113.6° E; latitude 34.9° N) during the 2014–2015 cropping seasons under natural soil conditions. The test grain of Xinong 979, a highly productive and early maturing wheat cultivar, is susceptible to the predominant Chinese isolate E20 of *Bgt*, and was provided by Northwest Agriculture and Forestry University.

The plants were grown in six plots, each with an area of 3 × 4 m (Supplemental Fig. S1). Three plots were uninoculated plots (control) and three plots were artificially inoculated with *Bgt* race E20 every two days for a total of nine inoculations since 15 March. The methods of inoculation according to the description of Gao et al. (2014) with some modifications. In one method, pots with infected seedlings were placed in the expected inoculated plots (5 pots/plot) to initiate disease development. In the other method, numerous infected wheat leaves were scattered in the experimental area.

2.2. Assessment of the severity of powdery mildew in the field

The severity of the disease was assessed every five days post anthesis for a total of five times. Fifty plants were chosen by a five point sampling method from each treatment plot at each time as proposed by Gao et al. (2014). A modified 0–9 scale method was adopted for the assessment, in which scale 0 was defined as immune, scale 1–2 as highly resistant, scale 3–4 as moderately resistant, scale 5–6 as moderately susceptible, and scale 7–9 as highly susceptible (Li et al., 2011). The disease index (DI) can be expressed as follows:

$$DI = \frac{0 \times n0 + 1 \times n1 + \cdots + 9 \times n9}{9 \times (n0 + n1 + \cdots + n9)} \times 100$$

where n0, n1,…, n9 represent the number of wheat leaves with severity as 0, 1,…9, respectively.

2.3. Sample preparation and protein extraction

For the analysis, representative wheat ears that blossomed on the same day were marked. Grain samples of control and infected wheat with the same DI were collected from each plot and at each development stage based on thermal times according to the cumulative average daily temperatures, shown in Table 1. Collected grains were stored at −80 °C prior to analysis.

For 2-DE, proteins were extracted from grain samples with three biological replicates according to the method of Zhang et al. (2015). Extracted protein samples were suspended in a lysis buffer containing 2 M thiourea, 7 M urea, 4% 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate (CHAPS), and 20 mM DTT at room temperature for 2 h. Concentrations of total protein were determined using the Bradford assay (Bio-Rad) with the bovine serum albumin as the standard (Li et al., 2013).

2.4. 2-DE and images analysis

Each protein sample (800 μg) was loaded onto a ReadyStrip™ IPG Strip (pH 4–7, 24 cm, linear gradient, Bio-Rad, USA) and was passively rehydrated using a PROTEAN® IEF Cell (Bio-Rad Laboratories, Inc.). Then, isoelectric focusing and SDS-PAGE were conducted as described by Li et al. (2017). 2-DE experiments were performed with three biological replicates. Protein gels were strained in Coomassie brilliant blue G-250 solution, scanned at 300 dpi with a UMAX Power Look 2 100XL scanner (Maximum Tech, Taiwan, China). Quantitative intensity analysis of protein spots in gels was performed by the PDQuest software (version 8.0.1, Bio-Rad Laboratories, Inc.). First, protein spots on the gels were detected and quantified, and subtracted the background. The

Table 1
Details of grain samples harvested at post-anthesis period according to thermal time corresponding to cumulative average daily temperatures.

DPA	Date	°Cd
10	2014.04.22–05.02	238 °C
15	2014.05.02–05.07	342 °C
20	2014.05.07–05.12	457 °C
25	2014.05.12–05.17	589 °C
30	2014.05.17–05.22	741 °C