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Proteomic analysis of the impacts of powdery mildew on wheat grain

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ABSTRACT

Powdery mildew of wheat is one of the major foliar diseases, causing significant yield loss and flour quality change. In this study, grain protein and starch response to powdery mildew infection were investigated. Total protein, glutenin and gliadin exhibited a greater increase in grains from infected wheat, while the content of total starch and amylopectin was decreased. Comparative proteomic analysis demonstrated that the over-abundant protein synthesis-related proteins might facilitate the accumulation of storage proteins in grains from infected plants. The significant increase in triticin, serpin and HMW-GS in grains from infected wheat might relate to the superior gluten quality. In addition, overabundant carbohydrate metabolism-related proteins in grains from infected wheat were conducive to the depletion of starch, whereas the decreased abundance of ADP glucose pyrophosphorylase might be related to the deficiency of starch synthesis. These results provide a deeper understanding on the change of wheat quality under powdery mildew infection.

1. Introduction

Wheat is one of the "top three" cereal crops that provide a large proportion of nutrients for humans and livestock. The major components of mature wheat grain are starch, protein, and cell wall polysaccharides. Starch accounts for 70–80% of wheat grain dry weight, is a major determinant of wheat yield, which is comprised of amylose and amylopectin (Hurkman et al., 2003). The ratio of amylose to amylopectin greatly influences some starch physicochemical properties that are very important for the end-use of the product (Zeng, Morris, Batey, & Wrigley, 1997). Another important storage product of wheat grain is protein, accounts for 10–15%, whose composition is the key to flour quality. Of total wheat grain proteins, 80% are prolamins, including gliadins and glutenins; while the non-prolamin part, accounts for 15–20%, including albumins and globulins (Tasleemtahir, Nadaud, Chambon, & Branlard, 2014). Prolamins are the major storage proteins and determine the viscoelasticity of dough. In contrast to prolamins, non-prolamins contain essential amino acids that are very important for human health, such as lysine, aspartate, tryptophan and methionine. Grain quality is a complex trait with various determinants, including nutritional value, cooking quality, and physical appearance. These factors are generally associated with physicochemical properties of crop starch and protein.

Powdery mildew is a foliar disease that has a global distribution but in particular in regions with dry and cool climates, e.g. China, Europe, and the Southern region of South America. In recent decades, the disease has become more serious because of higher plant densities, nitrogen fertilizers, and irrigation (Singh et al., 2016). In China, wheat powdery mildew was first detected in 1927, has tended to be severe since the late 1970s, and has become an important disease by the 1980s (Liu, & Shao, 1994). Yield losses due to the disease infection were 10–15%, and in severe cases up to 30–40% (Wang, et al., 2012). In other areas of the world the disease also causes great commercial yield losses, such as 5–17% in North Carolina, 10–15% in Russia (Singh et al., 2016). The disease interferes with the normal source–sink relationships of plant, changes the translocation and distribution of photoassimilate, ultimately resulting in the loss of yield and changes in grain starch and protein compositions. It has been reported that compared with fungicide treated, test weight and grain protein content were significantly lower, while sedimentation value was higher in grains from infected plants (Samobor, Vukobratović, & Jošt, 2006); however, there is a reverse result indicated a positive correlation between grain protein content and the disease severity (Cao, Duan, Zhou, & Yong, 2012). These studies focused on phenotypic results of grain protein responding

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to powdery mildew stress, the understanding at molecular level is poor.

As an advanced technology, proteomics is a pivotal tool to unravel the change mechanism of grain quality under powdery mildew stress. Now, two-dimensional gel electrophoresis (2-DE) coupled with mass spectrometry (MS) proteomic approaches have been widely used in grain quality research in different plant species, including rice (Gayen, Paul, Sarkar, Datta, & Datta, 2016), coffee (Livramento et al., 2016), and wheat (García-Molina, et al., 2017). Further, a significant study on the proteomics of grain proteins response to environment stresses has been carried out, such as proteomic studies on wheat grains response to the increased air CO_2 concentration (Fernando et al., 2015). However, application of proteomics strategy in investigating the change mechanism of wheat grain quality under powdery mildew stress has not been reported yet. Therefore, in the present study, comparative proteomics was used to reveal the mechanism of wheat grain quality change at protein level. The objectives of the current study were to test the following hypotheses: (1) powdery mildew infection will modify mature wheat grain proteome and (2) changes in wheat grain proteome lead to the changes of the composition and content of grain starch and protein.

2. Materials and methods

2.1. Experimental design and the severity of powdery mildew

The experiment was conducted under field conditions in the Science Education Experimental Park of Henan Agricultural University (longitude 113.6°E; latitude 34.9°N) during the 2014/2015 seasons under natural soil conditions. A highly productive, strong gluten, widely planted in China, and powdery mildew susceptible wheat cultivar, Xinong 979, was employed in the test. Six plots were used for this experiment, each with an area of 3 m × 4 m. Three plots were uninoculated plots (control), and the rest of plots were artificially inoculated with powdery mildew. The methods of initiating powdery mildew occurrence based on the description of Li, Yang, Li, Niu, and He (2017).

Disease severity was assessed every 5 days post anthesis for a total of five times. Each time, fifty plants were selected using a five points sampling method from each infected plots. The disease assessment is based on a modified 0–9 scale method, in which 0 = no disease and 9 = severe infection (Sheng & Duan, 1991). The average disease index (DI) was calculated according to the following equation:

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

where n0, n1, …, n9 represents the number of the leaves with disease severity grade 0, 1, …, 9 respectively.

2.2. Sample preparation

During the flowering period, representative wheat ears that started to blossom at the same day were marked. These marked ears were sampled and shelled for grains at maturity stage. Grain samples of control and infected wheat that were used for the analysis of total starch and protein concentration and corresponding composition concentration. These samples were oven-dried at 80 °C to a constant weight, and then ground, filtered through an 80-mesh (180 μm) screen.

2.3. Starch composition assays

Amylose and amylopectin concentration were analyzed with a coupled spectrophotometer assay (Gao, Niu, Yang, He, & Wang, 2015). Briefly, 100 mg of milled grain sample was stirred with 0.5 M KOH for 15 min at 100 °C, and then diluted, adjusted pH to 3.5 with 0.1 M HCl. Next, I_2–KI reagent was added to the solution. After blending for 20 min, the mixture was monitored with a UV-6300 spectrophotometer at 430, 550, 620 and 730 nm. The absorption peak values of purified amylose (Sigma, USA) reacted with I_2-KI reagent were 620 and 430 nm, whereas those of amylopectin were 730 and 550 nm. The sum of amylose and amylopectin was total starch concentration. The ratio of amylose/amylopectin was calculated.

2.4. Protein composition analysis

Wheat grain protein composition was determined by successive solvent extraction as described by Zhou, Jiang, Dai, Jing, and Cao (2006). The Kjeldahl method was used to determine the total N concentration and the N concentration of all compositions (Gao, He, Niu, Wang, & Yang, 2014). Protein concentration was calculated by multiplying N concentration by 5.7.

2.5. Protein extraction

For 2-DE, protein samples with three biological replicates were prepared. Grain samples of 500 mg were ground to fine flour, and further ground for 30 min in extract buffer containing 100 mM Tris–HCl (pH 8.8), 1% sodium dodecyl sulfate (SDS), and 10 mM fresh dithiothreitol (DTT). Then centrifuged, the supernatants were added equal volume of phenol, shaken gently for 30 min, and centrifuged. The samples were collected from below the phenol phase. Protein solutions were precipitated with five-fold volumes of cold ammonium acetate/methanol for 2 h at −20 °C. Then centrifuged, the pellets were washed thrice in ice-cold acetone containing 5 mM DTT. After freeze-drying, the pellets were suspended in lysis buffer containing 2 M thiourea, 7 M urea, 4% 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate (CHAPS), and 20 mM DTT at the room temperature for 2 h. Removed the insoluble material by centrifuging. Concentration of total protein was measured by the Bradford assay (Bio-Rad) with bovine serum albumin as the standard (Li, et al., 2017).)

2.6. 2-DE and images analysis

800 μg of protein sample was loaded onto a ReadyStrip™ IPG Strip (pH 4–7, 24 cm, Bio-Rad, USA) and rehydrated passively for 12–18 h at 20 °C by a PROTEAN® IEF Cell (Bio-Rad Laboratories, Inc.). After isoelectric focusing (IEF), protein samples were separated on the second-dimension electrophoresis. All strips were loaded on 12% vertical homogeneous sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels. After electrophoresis, all gels were stained using Coomassie brilliant blue (CBB) G-250 solution, then destained in double-distilled water.

The gels were scanned at 300 dpi with a UMAX Power Look 2, 100XL scanner (Maximum Tech, Taiwan, China). Gel images were analyzed by the PDQuest software (version 8.0.1, Bio-Rad Laboratories, Inc.) to determine spots that were overabundant in infected or control samples. Spots on the gels were detected and quantified, and subtracted the background. Only spots that were present in three biological replications were analyzed. Protein spots expressed by ≥1.5-fold with a $p < 0.05$ in three biological replicates were considered as differentially expressed proteins.

2.7. Protein identification by mass spectrometry

Protein spots of varied intensities were manually excised from the 2-DE gel stained with Coomassie Brilliant Blue and transferred to 1.5 ml microcentrifuge tubes. The digested protein spots were destained for 20 min in 30 mM potassium ferricyanide/100 mM sodium thiosulfate (1:1 v/v) and washed with Milli-Q water until the gels were destained. The protein spots were incubated in 0.2 M NH_4HCO_3 for 20 min and then lyophilized. Each spot was digested overnight in 12.5 ng/μl trypsin in 25 mM NH_4HCO_3. Each peptide was extracted three times with 60% acetonitrile (ACN)/0.1% trifluoroacetic acid (TFA). The extracts were pooled and dried completely by a vacuum centrifuge.