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Metabolic profiling of virus-infected transgenic wheat with resistance to *wheat yellow mosaic virus*

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ABSTRACT

Wheat (*Triticum aestivum* L.) is an important crop, and *wheat yellow mosaic virus* (WYMV) can cause a severe loss in wheat yield. A genetically modified (GM) wheat carrying a WYMV 72kD coding gene (Wheat 72kD) with resistance to WYMV has been constructed in a previous study. However, neither the influence of genetic modification on wild-type wheat (WT) metabolism nor the effect of WYMV-infection on the metabolic profiling in 72kD is clear. Gas chromatography-mass spectrometry (GC-MS) was used to detect the metabolic profiling in GM, WT, GM with WYMV-inoculation (GMV), WT with WYMV-inoculation (WTV) wheat, respectively. As a result, GM and WTV samples were close to each other on the principal component analysis (PCA) plot, indicating genetic modification and WYMV-infection might cause similar changes in wheat metabolism. Only 54 metabolites were annotated, and 16, 12, 17, and 14 metabolites were significantly different between GMV and GM, GMV and WTV, GM and WT, as well as between WTV and WT, respectively. Furthermore, overlapped metabolites were identified, including 3-chloro-4-hydroxybenzoic acid (CHBA), 3-sulfocatechol, S-mercaptocysteine, 1-chloro-2-nitrobenzene (2-chloronitrobenzene) and melarsoprol. In conclusion, genetic modification or/and WYMV-infection significantly affected the metabolism in wheat. This is the first report investigating the effects of WYMV-infection on metabolic profiling in GM wheat.

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1. Introduction

Wheat (*Triticum aestivum* L.) is one of the most important commercial crops and a major source of food worldwide. It is closely related to economic development, food supply, and human nutrition health. To improve the nutrition quality, the capability of drought resistance, insect resistance, disease resistance or anti-virus in plant, genetic modification (GM) has been widely utilized in agriculture [1,2]. Following the first transgenic wheat gained in 1992, nearly 200 cases of GM wheat have been reported [3–7].

W*heat yellow mosaic virus* (WYMV), which parasitizes in *Polymyxa graminis* and spreads depending on the spores of *P. graminis*, is a main cause of wheat yellow mosaic disease, and it can cause severe yield losses in wheat [8]. In the past years, GM wheat varieties with strong resistance to WYMV have been developed [9,10], such as GM wheat carrying a WYMV 72kD protein coding gene, which exhibits a strong resistance against WYMV [10]. Analyses have demonstrated that this GM wheat can steadily express WYMV 72kD gene in its offspring, and field trial indicated that they had enhanced resistance to WYMV [10]. However, there is still concern that genetic modification may introduce unintended and unforeseen effects into wheat, which may affect wheat metabolism and increase undesirable metabolites [11]. As wheat is an important crop, the biosafety of GM wheat should be evaluated strictly before large-scale application [12].

New methodologies have been developed to identify alterations in transgenic crops at different biological levels, including transcriptome, proteome, and metabolome. However, previous studies mainly focus on the comparative biosafety assessment of GM crops

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when compared with non-GM crops [13–17]. Little attention has been paid to the metabolic changes in virus-resistant GM wheat after virus infection, which happens frequently in practical. In this study, gas chromatography-mass spectrometry (GC-MS)-based metabolomics strategy was used to detect the metabolic profiling in GM wheat (72kD) and the corresponding wild-type (WT; Yang11) with or without WYMV-inoculation, respectively. The comparison between metabolic profilings of transgenic wheat, non-transgenic wheat, and wheat inoculated with WYMV may contribute to reveal the influences of genetic modification or/and virus infection on wheat metabolism.

2. Materials and methods

2.1. Plant materials

Seeds of the GM wheat (WYMV 72kD) and its WT control (Yang11) were provided by the State Key Laboratory for Agro-Biotechnology, National Center for Plant Gene Research, College of Agriculture and Biotechnology, China Agricultural University (Beijing, China). Virus source plants and wheat plants with and without WYMV-infection were obtained from Tianjin Entry-Exit Inspection and Quarantine Bureau (Tianjin, China).

2.2. Virus infection

Leaves from the virus source plants were grinded with 10 mL 0.2 mol/L phosphate buffer solution (PBS, pH 7.0) in a mortar, and the mixture was filtered through double-layered gauze. Thus, virus solution was generated, which was further diluted with 0.2 mol/L PBS (pH 7.0, dilution rate: 1:10). Seeds of 72kD and Yang11 were sowed in sterilized sand and cultured in an artificial incubator (11–13 °C; 16 h light/8 h dark cycle). Afterwards, wheat seedlings with 1–2 flatted true leaves were pulled out from sterilized sand and washed with double-distilled water. Seedlings were classified into 4 groups, namely, 72kD (GM group), 72kD with WYMV-inoculation (GMV group), Yang11 (WT group), and Yang11 with WYMV-inoculation (WTV group). After sprayed with carborundum, roots of wheat seedlings classified into the GMV and WTV groups were spread with WYMV solution, whereas roots of wheat seedlings in GM and WT groups were spread with PBS. Thereafter, roots were washed with double-distilled water, and seedlings were re-planted into the sterilized sand. After 20 h incubation in dark, seedlings were grown in a common condition (16 h light/8 h dark cycle; 12 °C for day and 11 °C for night). When the third leaf appeared at the three-leaf stage, the middle part of the second leaf of each seedling was utilized to prepare the sample for metabolic profiling.

2.3. Sample preparation

Leaf samples were obtained from wheat in GM (n = 8), GMV (n = 8), WT (n = 4), and WTV (n = 5) groups, and samples for metabolome profiling were prepared according to the methods described by Wu et al. [16] with a slight modification. Briefly, 18 ± 0.2 mg leaf sample was grinded with liquid nitrogen, and 1 mL isopropanol/acetonitrile/water (3:3:2, v:v:v) solution pre-cooled at −20 °C was added. The mixture was then shaken at 4 °C for 15 min. After centrifugation at 4 °C, 150 μL supernatant was generated and then desiccated using a SpeedVac concentrator (SPD111 V-230; Thermo Electron Corporation; MA, USA) at room temperature. Furthermore, derivatization was conducted using 2 μL C8-C40 n-alkanes mixture, 10 μL 40 mg/mL methoxyamine hydrochloride (Sigma-Aldrich, Deisenhofen, Germany) used as the methoxyamination reagent (30 °C, 90 min), 90 mL *N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide (MSTFA, Macherey & Nagel, Düren, Germany) plus 1% trimethylchlorosilane (37 °C, 30 min).

2.4. GC-MS analysis

GC-MS analysis was performed within 24 h after the sample preparation [16,18]. Briefly, 1 μL sample was injected in splitless injection mode into Aglient-Technologies 7683B series auto-sampler coupled to a 5975C Agilent mass-selective detector (Aglient-Technologies, CA, USA). The corresponding gas chromatograph was 7890A (Agilent) equipped with capillary column Rtx-5Sil MS (30 m, 0.25 mm id, 0.25 μm film thickness; Restek GmbH, Bad Homburg, Germany). The carrier gas was helium (1.0 mL/min), and the temperatures for injection, ion source, and transfer line were 230 °C, 200 °C, and 250 °C, respectively. The temperature program was: 70 °C for 5 min, 5 °C/min ramp to 350 °C, 330 °C for 5 min, fast cooling to 70 °C, followed by equilibration at 70 °C for 1 min. Mass spectra were recorded (50–400 m/z, two scans per second).

2.5. Data processing

2.5.1. Data pre-processing and metabolite identification

Background of all mass spectral data was firstly subtracted, and the resulting files were submitted to the Mass Profiler Professional (MPP) software (version 2.0, Agilent). The retention time drifts were corrected using the correlation optimized warping (COW) algorithm [19]. To resolve the problem of co-eluting peaks, deconvolution was performed using the Automated Mass Spectrometry Deconvolution and Identification System (AMDIS) software (version 2.66, National Institute of Standards and Technology (NIST); MD, USA), and intensity values of selected ions were utilized to quantify the co-eluting metabolites. Before statistical analysis, peak area of each metabolite was normalized, generating peak area percentage. To identify metabolites, mass spectra and retention index were aligned against the libraries NIST 2008 and Wiley 7 (Wiley, New York, USA) using the NIST Mass Spectral Search software (version 2.0, National Institute of Standards and Technology, Gaithersburg, MD, USA).

2.5.2. Principal component analysis (PCA)

PCA is an unsupervised multivariate method to visualize the dataset and display the similarity and difference. The dimensionality of complex data is reduced to what are called Principal Components (PC) that retain the maximal amount of variation within a sample [20]. Here, PCA was conducted using the R software (2011; R Development Core Team) to investigate the differences in metabolites among the samples based on the metabolite data.

2.5.3. Annotation of metabolites

Based on the public metabolite databases including Kyoto Encyclopedia of Genes and Genomes (KEGG; http://www.kegg.jp/), Human Metabolome Database (HMDB; http://www.hmdb.ca/), and PubChem Compound (http://www.ncbi.nlm.nih.gov/pccompound), all of the detected metabolites were annotated using MetaboSearch software, which is a tool for mass-based metabolite identification using multiple databases (http://omics.georgetown.edu/metabosearch.html) [21], and their related information were obtained.

2.5.4. Hierarchical clustering of the annotated metabolites

Based on the concentration of the annotated metabolites in each sample, hierarchical clustering analysis was performed using pheatmap package in R [22], and metabolites with similar

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