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RESEARCH ARTICLE

Comparative proteomics analysis of maize (*Zea mays*) leaves infected by small brown planthopper (*Laodelphax striatellus*)

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Abstract

Maize rough dwarf disease (MRDD) is a viral disease caused by brown planthopper infestation, and leads to great yield loss, especially in China. Comparative proteomics was performed using maize inbred line Zheng 58 and LN 287. MRDD pathogen was detected as rice black-streaked dwarf virus (RBSDV) by quantitative real time PCR (qRT-PCR) in Shandong Province, China. The modified trichloroacetic acid (TCA)/acetone method was used for soluble protein extraction from leaves. Two-dimensional electrophoresis (2-DE) analysis was performed on 24-cm long, pH 4–7 linear immobilized pH gradient (IPG) strips, and gels were stained with silver and coomassie brilliant blue. We identified 944 proteins expressed in RBSDV infected maize leaves by proteomics approaches. Among these, 44 protein spots that revealed a 1.5-fold difference in intensity were identified by mass spectrometry between mock-inoculated and RBSDV infected samples. Among these, 17 and 26 spots were up-regulated, and 27 and 18 spots were down-regulated in the virus infected samples of Zheng 58 and LN 287, respectively. Differential protein spots were analyzed by mass spectrometry identification, which could be divided into six categories. Furthermore, the expression of stress-related proteins was detected and confirmed by qRT-PCR. This study lays the foundation for further investigations, enabling the enhancement of MRDD resistance in maize.

Keywords: maize, MRDD, planthopper, 2-DE, quantitative real-time PCR

1. Introduction

Maize is an important feeding source and industrial raw material, which occupies an important position in the world economy. In recent years, maize rough dwarf disease (MRDD) has become a devastating disease, especially in China (Tao *et al*. 2013). This disease has caused severe yield losses, and there are currently no effective prevention and control strategies. MRDD was firstly reported in 1954 in China (Luan *et al*. 2012). An epidemic outbreak occurred in the 1990s, especially on the Yellow-Huai-Hai River Plain, which are summer maize-growing regions of China (Wang

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et al. 2006, Liu *et al*. 2014). Three pathogens, maize rough dwarf virus (MRDV), rice black-streaked dwarf virus (RBSDV) and Mal de Rio Cuarto virus (MRCV), could result in its phenomenon. The RBSDV, which belongs to the *Fijivirus* genus in the family of *Reoviridae*, was identified as the main virus of MRDD in China (Wang *et al*. 2006; Liu *et al*. 2014). The genome of RBSDV is a double-strand RNA that contains 10 linear genomic segments (S1–S10), according to polyacrylamide gel electrophoresis (PAGE) migration (Zhang *et al*. 2008). MRDD is transmitted in a persistent manner through the insect, brown planthopper (*Laodelphax striatellus*). To date, preventive measures such as chemicals or alterations on the sowing date, could not completely control MRDD. Furthermore, the progress of studies has been slow, which contributed to great natural variations in the amount of insects. Moreover, there is no ideal artificial inoculation method.

In order to investigate the invasion and defense mechanism of the *fijivirus*, it is important to understand its virus-host interaction. The proteomics technique has been confirmed to be a powerful tool for understanding the response of the host to pathogens in many species of plants (Slykhuis 1976; Brown *et al*. 2006; Afroz *et al*. 2011; Yang *et al*. 2011; Wu *et al*. 2013a). The identification of differentially expressed proteins during virus inoculation not only uncovers physiologically consistent protein patterns associated with the whole process, but also reveals the unexpected importance of some pathways. Especially for virus and other pathogens, proteomics has contributed to defining functional genes and proteins involved in plant-pathogen interactions. Wu *et al*. (2013a) obtained 24 new proteins with 2-dimensional electrophoresis (2-DE) analysis after sugarcane mosaic virus (SCMV) infection between resistant and susceptible maize ecotypes. After soybean mosaic virus infection, 16 proteins were potentially involved in protein degradation, defense signal transfer, reactive oxygen, and other metabolic pathways (Yang *et al*. 2011).

In the case of MRDD, Li *et al*. (2011) identified 91 different protein-related multiple metabolic/biochemical pathways between RBSDV infected and control plants using the proteomics method. Various resistance-related maize genes and cell wall- and development-related genes were found in RBSDV infected maize with oligomer-based microarrays (Jia *et al*. 2012). Comparative proteomics analysis of IR64 with near-isogenic rice mutants revealed 22 proteins that may be potentially associated with rice resistance to the brown planthopper (Sangha *et al*. 2013). The above research could provide an important clue for understanding the defense mechanism of insect-mediated diseases. Nevertheless, little is known on the molecular basis of the maize defense mechanism against this pathogen. The virus-host interaction of RBSDV infection remains unclear in maize.

The purpose of this study was to investigate the protein expression pattern of maize inbred lines, which had different resistances to RBSDV. Differentially expressed proteins were identified by 2-DE proteomics analysis. This enabled the investigators to explore the mechanism of disease resistance, and the interaction between the virus and plant. Furthermore, this would also enhance the understanding of the disease resistance mechanism, and provide a theoretical basis for maize resistant to MRDD breeding.

2. Materials and methods

2.1. Plant materials and inoculation with RBSDV

Two inbred maize lines (Zheng 58 and LN 287) were selected, because Zheng 58 was sensitive to RBSDV, which was the variant of Ye 478 (U8112×5003), and LN 287 was highly resistant to RBSDV，which was selected from US hybrid P78599. When the plants grew to the three-leaf stage, these plants were inoculated with small planthoppers captured from a winter wheat field where MRDD happened seriously. Each plant was inoculated by two planthoppers per seedling for 3 d, and transferred to the field in Jiaozhou Farm, Shandong Province, China. A total of 180 plants were planted for proteomics analysis, in which row spacing was 5 cm and line spacing was 60 cm. The control groups were shrouded with net sheds to prevent MRDD infection, while the treatment groups were exposed to its surroundings for a second inoculation, when planthoppers were prevalent at early June in the east of Shandong region. These field trials were repeated three times. After one month, leaves were sampled for real-time PCR to confirm the existence of RBSDV. In the field, the following phenotypes were observed in RBSDV sensitive plants: the height of the plant was significantly lower than the control; white waxy apophysis appeared behind the leaves; the color of the leaves was dark green. Leaves with obvious symptoms with Zheng 58 and leaves in the same position with LN 287 were sampled at 45, 60 and 75 d after sowing.

2.2. RNA extraction and quantitative real-time PCR (qRT-PCR)

In order to identify the inoculation degree of RBSDV in the molecular level, real-time PCR was performed. Total RNA was extracted from the leaves of inoculated and non-inoculated plants using TRIzol Reagent (TaKaRa, Japan), according to manufacturer's instructions. Then, total RNA was treated with RNase-free DNase I (TaKaRa) to remove the possible contamination of genomic DNA. RNA concentration was determined using Nanodrop 2000 (Thermo, USA). Approximately 2 μg of total RNA