JOURNAL OF PROTEOMICS 89 (2013) 124–140

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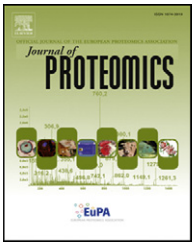

Comparative proteomic analysis of the plant–virus interaction in resistant and susceptible ecotypes of maize infected with *sugarcane mosaic virus*

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ARTICLE INFO

Article history:
Received 16 December 2012
Accepted 3 June 2013
Available online 14 June 2013

Keywords:
Compatible interaction
Incompatible interaction
Maize
Sugarcane mosaic virus
2D-DIGE

ABSTRACT

Sugarcane mosaic virus (SCMV) is an important viral pathogen and has caused serious losses in grain and forage yield. To identify candidate SCMV resistance proteins and to explore the molecular mechanisms involved in the plant–SCMV interaction, we conducted proteomic analyses of leaf samples from resistant and susceptible ecotypes of maize infected with SCMV. Proteins were analyzed by quantitative two-dimensional differential gel electrophoresis (2D-DIGE), and 93 protein spots showed statistically significant differences after virus inoculation. Functional categorization showed that SCMV-responsive proteins were mainly involved in energy and metabolism, stress and defense responses, photosynthesis, and carbon fixation. The majority of the identified proteins were located in chloroplast and cytoplasm based on bioinformatic analysis. Among these identified proteins, 17 have not been identified previously as virus-responsive proteins, and 7 were new and did not have assigned functions. Western blotting analyses confirmed the expression patterns of proteins of specific interest, and the genes encoding these proteins were further analyzed by real-time PCR. The results of this study showed overlapping and specific proteomic responses to SCMV infection between resistant and susceptible maize ecotypes. This study provides further insight into the molecular events during compatible and incompatible interactions between viruses and host plants.

Biological significance

Sugarcane mosaic virus (SCMV) is an important viral pathogen and has caused serious losses in grain and forage yield. However, little is known about host–SCMV interactions from the proteome perspective. This study analyzed proteomic changes in resistant and susceptible plants that are infected with SCMV using DIGE based proteomics. We identified 17 proteins that have not been identified previously as virus-responsive proteins, and 7 new proteins without assigned functions. These proteins are interesting candidates for future research, as they may be associated with new biological functions and play important roles in plant–virus interactions. Real-time RT-PCR analysis of genes encoding several proteins of

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http://dx.doi.org/10.1016/j.jprot.2013.06.005

interest provided indication on whether the changes in protein abundance were regulated at the mRNA level.

The results of this study showed overlapping and specific proteomic responses to SCMV infection between resistant and susceptible ecotypes. After inoculation, the proteins involved in energy and metabolism, stress and defense responses, photosynthesis and other four functional groups showed significant changes in both ecotypes, which suggested that SCMV infection influenced these physiological processes in both the resistant Siyi and the susceptible Mo17. However, the oxidative burst was more pronounced during incompatible plant–SCMV interactions, as compared to those defined as compatible. We also observed an increase of enzymes involved in glycolysis and gluconeogenesis pathways in the resistant maize ecotype Siyi, while decrease in the susceptible maize ecotype Mo17. In addition, there is a marked increase of guanine nucleotide-binding protein beta submit in the resistant Siyi, which suggests a possible involvement of G-protein associated pathways in the resistant responses of maize to SCMV. These observations may possibly reveal protein targets/markers that are useful in the design of future diagnosis or plant protection strategies and provide new insights into the molecular mechanism of plant–virus interactions.

1. Introduction

The increasing world population and unsustainable exploitation of natural resources have made food security a burning issue in the 21st century [1,2]. Although technological developments in agriculture have significantly increased crop yields, higher productivity is required to meet the growing demand for food now and in the future. Unfortunately, at present, there are multidimensional challenges for agriculture world-wide. Plant diseases caused by bacteria, fungi, viruses, and nematodes are some of the greatest constraints affecting agricultural productivity [3]. Recently, pathogenicity and defense-related genes and proteins expressed during phytopathogen infections have been identified using functional genomic strategies. However, there is still much to learn about the complexities of plant–pathogen interactions [4].

Sugarcane mosaic virus (SCMV) was first reported in Ohio, USA in 1963. It belongs to the sugarcane mosaic subgroup of the Potyviridae, together with maize dwarf mosaic virus (MDMV), Johnsongrass mosaic virus, Sorghum mosaic virus (SrMV), and Zea mosaic virus. SCMV infects maize, sorghum, sugarcane and other poaceous species throughout the world. For maize, the symptoms of SCMV infection occur first in the youngest leaves as an irregular, light- or dark-green mosaic or mottle that may develop into narrow, light-green or yellowish streaks along the veins. The mosaic or mottle often appears as dark green areas on a yellowish or chlorotic background. Early infection, before the fourth or fifth leaf stage, may cause moderate to severe stunting, a “bushiness” of the plant, and poor seed set, depending on the infected line. As infected maize grows and the temperature rises, the mosaic often fades and young leaves become more yellow [5]. Recently, a high incidence of SCMV and other plant virus co-infection was reported in China [6] and some other areas [7]. In addition, new strains or genome variations of SCMV continue to be reported in different countries [7–11]. Our laboratory has previously reported that while SCMV has been known for a long time, it remains a threat to the industry [12].

Genetic analysis on backcross five (BC5) progeny derived from the cross FAP1360A (resistant) × F7 (susceptible) confirmed that at least two dominant genes, *Scmv1* and *Scmv2*, were required for resistance to SCMV [13]. In order to further identify SCMV resistance candidate genes and obtain information about the molecular mechanisms involved in SCMV–plant interaction, expression profiling has been studied based on suppression subtractive hybridization and microarray technology [14,15]. A subset of genes differentially expressed in response to SCMV infection has been identified. The majority of these differentially expressed genes are related to cell rescue and defense, signal transduction, and transcription categories. Some of the genes identified are found to co-localize with SCMV resistance genes *Scmv1* and *Scmv2*. Meanwhile, little is known about host–SCMV interactions from the proteome perspective. Within the post-genomics fields of research, proteomics is very important as it focuses on proteins, which are the key functional units of the cell [16]. Recently, a number of plant proteomic studies have provided good overviews of the proteins present in a given tissue or organelle, or at a particular stage of development [17–19]. Differential gel electrophoresis (DIGE) is considered the first real quantitative proteomic differential display method, as there is less variation between gels and therefore, greater confidence in the results [20].

In the present study, we analyze the changes of protein profiles in a resistant and a susceptible maize ecotype during SCMV infection using a DIGE-based proteomic approach. Proteins that were highly and significantly changed are selected for identification by mass spectrometry combined with bioinformatics. These data provide a valuable resource for discovering novel proteins involved in the pathogen response. In the future, such proteins could be introduced into agronomically important species to create resistant crops. This study will contribute to a better understanding of the molecular basis of plant–virus interaction.

2. Materials and methods

2.1. Plant materials and virus infection

The maize (*Zea mays* L.) inbred line Siyi (resistant to SCMV) and Mo17 (susceptible to SCMV) were grown in a containment greenhouse under a constant temperature 24 °C and a