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Proteomic analysis of differentially synthesized proteins in potato purple top phytoplasma-infected tomato plants

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Abstract

Potato purple top (PPT) is an emerging potato disease complex. The etiological agents of PPT are phloem-inhabiting, cell wall-less bacteria known as phytoplasmas. Tomato is an alternative host of these phytoplasmas. PPT phytoplasma-infected tomato plants exhibit symptoms including abnormal foliage development and formation of "big buds". In the present study, two-dimensional gel electrophoresis (2-DE) was performed to compare the proteomes of healthy and Columbia Basin PPT phytoplasma-infected 'Rutgers' tomato. Our results showed that the levels of at least 15 host proteins were significantly altered in response to PPT phytoplasma infection. The identities of these PPT-responsive proteins and their roles in phytoplasma pathogenesis are currently being examined.

Key words: phytoplasma, potato purple top, proteomics, two-dimensional gel electrophoresis.

Introduction

Potato purple top (PPT) is a potato disease complex associated with infection by diverse phytoplasmas belonging to at least five major 16Sr groups. The agent associate with Columbia Basin PPT disease is a phytoplasma affiliated with subgroup A (16SrVI-A) of the clover proliferation group (Lee and Bottner, 2004). Tomato is an alternative host of the Columbia Basin PPT phytoplasma. One of the most striking symptoms exhibited by PPT-infected tomato plants is the formation of "big bud", an aberrant floral structure with enlarged and fused sepals and absence of pistils, stamens, and petals (figure 1). Because phytoplasmas cannot be cultured *in vitro*, they remain one of the least characterized plant pathogens, and the underlying molecular mechanisms of their pathogenicity are poorly understood (Liefting *et al*., 2006). To learn how tomato plants respond to PPT phytoplasma infection and develop disease symptoms, we examined global gene expression profiles at the protein level using 2-dimentional gel electrophoresis. Our results revealed that the synthesized levels of at least 15 proteins were significantly altered in Columbia Basin PPT phytoplasma-infected Rutgers tomato plants. The identities of these differentially synthesized host proteins are currently being characterized, and the results are being correlated with our tomato microarray data. Findings from the current study will provide insights into phytoplasma-host interactions in tomato and should enhance our understanding of PPT phytoplasma pathogenesis.

Materials and methods

Tomato plants (*Lycopersicon esculentum* Mill. cv. "Rutgers") were grown in a greenhouse under 16 h light /8h dark photoperiod at 25°C and 70-80% humidity. Columbia PPT phytoplasma was graft-inoculated to healthy plants at the 8th true leaf stage. Leaf samples were collected, in triplicates, at three stages (0, 10, and 40 dpi). The leaf samples were frozen in liquid N_2 immediately and then stored at -80°C.

Protein extracts were prepared using a modified protocol of Damerval *et al*. (1986). Leaf samples were ground into powder in liquid N_2 then homogenized with 5 ml of ice-cold 10% TCA buffer. After centrifugation, the pellet was washed with 5 ml ice-cold acetone to remove the impurities (pigments and lipids) until the supernatant was colorless and the pellet turned white. The pellet was vacuum-dried and resuspended in a lysis buffer (7 M urea, 2 M thiourea, 2% CHAPS, 1% DTT and 1% pharmalyte). The resulting supernatant was used in subsequent experiments.

The first-dimensional separation was carried out with 17 cm IPG strips, using PROTEAN IEF Cell (Bio-Rad, CA). Equilibrated IPG strips were transferred onto 10% polyacrylamide gels and electrophoresis was performed on a Bio-Rad PROTEAN II xi Cell unit for the second-dimensional separation. The gels were stained using the Coomassie brilliant blue G250 and then viewed using Versa Doc imaging system Model 4000 (Bio-Rad). The 2d electrophoresis images and data were analyzed using the PDQuest software (Bio-Rad).

Results and discussion

IEF strips with a pH gradient of 4-7 were chosen for high-resolution first dimensional separation because, according to our pilot experiment, the pIs of most tomato leaf proteins were within this pH range. Protein profiles were reproducible among technical replicates of the same samples and among replicates from independent extractions. More than 300 protein spots were picked from each 2-DE gel by the PDQuest

software for comparison. As shown in figure 2, about 15 proteins were found to display differential accumulation patterns. Among them, seven proteins were up-regulated, and eight were down-regulated.

Figure 1. Symptoms of Columbia Basin PPT phytoplasma infection in tomato. (A): normal flowers on a healthy Rutgers tomato plant. (B): "big buds" on a phytoplasma-infected tomato plant. Due to early abortion of pistils, stamens, and petals, an abnormal floral structure, "big bud", is formed from enlarged and fused sepals. (In colour at www.bulletinofinsectology.org)

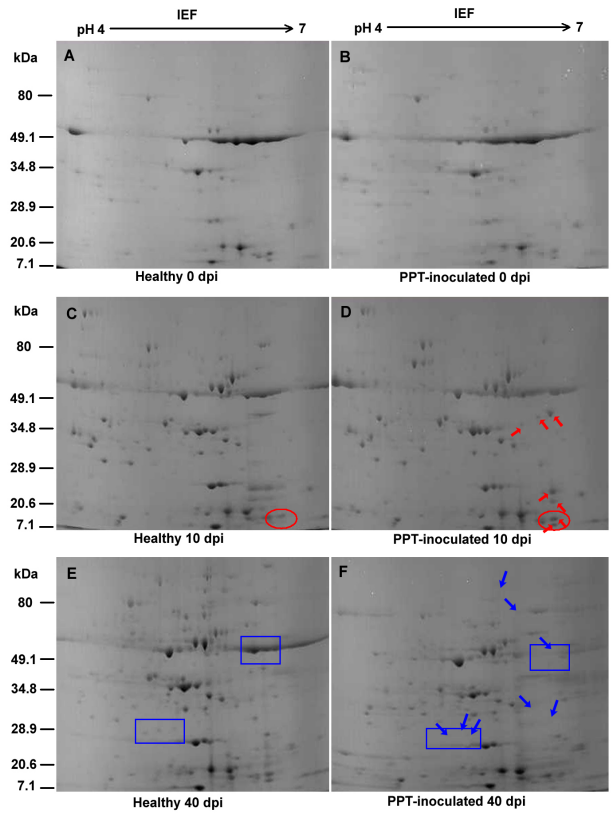

Figure 2. Representative 2-DE gels of Rutgers tomato leaf proteins. (A), (C) and (E): leaf proteins from healthy (mock-inoculated) plants. (B), (D) and (F): leaf proteins from Columbia Basin PPT phytoplasma-infected plants. Leaf samples were collected at 0, 10 and 40 dpi. Up- and down-regulated proteins are marked with red and blue arrows, respectively. Presumed PR proteins are marked by red circles. Presumed MADS-box family proteins are marked by blue rectangles. (In colour at www.bulletinofinsectology.org)

In this study, we examined host proteins that were differentially synthesized/accumulated in response to Columbia Basin PPT phytoplasma infection. A number of heavily stained protein spots with molecular weights (MW) around 14 kDa were present in samples from PPT-infected tomato plants while the corresponding spots were weakly stained in samples from mock-inoculated healthy plants at 10 dpi. Apparently, the levels of these 14 kDa proteins were much higher in infected plants than in healthy plants. Judging from their isoelectric point (pI) and MW values, these proteins could be members of a pathogenesis-related (PR) protein family. Such assumption was supported by results from our parallel tomato microarray experiments in which expressions of several PR protein genes were up-regulated in response to PPT phytoplasma infection, conceivably as part of plant basal defense against invading pathogens (van Loon and van Strien, 1999). At 40 dpi, the peak stage of flower development, a few proteins of approximately 46 kDa and 28 kDa were more heavily stained in healthy tomato plants than in PPT-infected plants. Two observations lead us suggest that down-regulation of these proteins might be responsible for flower deformation in PPT-infected plants: i) the MW values of these proteins coincide with those of MADS-box family proteins FA, DEF and TAG1, which are products of group B and group C floral homeotic genes (Theissen *et al*., 2000), and ii) our previous RT-qPCR experiments indicated that group B and group C floral homeotic genes were down-regulated in response to PPT phytoplasma infection in 'Rutgers' tomato. Findings and hypotheses reported in this communication are preliminary. The identities and roles of those differentially regulated, PPT phytoplasma infection/disease-related proteins are currently being characterized, with the goal of identifying host components that are involved in phytoplasma pathogenesis.

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