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Effects of exogenous indole-3-acetic acid on proteomic profiles of potato purple top phytoplasma-infected tomato plants

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Abstract

Phytoplasmas are cell wall-less bacteria that cause numerous diseases in diverse crops worldwide. Phytoplasma-infected plants often exhibit symptoms suggestive of hormone disorders. Indole-3-acetic acid (IAA), a naturally-occurring auxin, is involved in multiple essential plant growth and developmental processes. It has been shown that exogenous application of IAA can effectively remit symptoms caused by phytoplasma infections. The present study was designed to learn whether exogenously applied IAA would modify phytoplasma-induced changes in host gene expression profiles at the protein level and to understand the role of IAA in phytoplasma pathogenesis.

Key words: phytoplasma, tomato, IAA, proteomics.

Introduction

Phytoplasmas are a group of small, cell wall-less bacteria responsible for numerous serious and destructive diseases in agriculturally and environmentally important plant species (Lee *et al*., 2000). Plants infected by phytoplasmas exhibit an array of symptoms believed to result, at least in part, from hormonal imbalance. Indole-3-acetic acid (IAA) is the principal auxin that regulates growth and development of plants. Previous studies demonstrated that exogenous application of auxins on phytoplasma-infected periwinkle plants could induce symptom remission or even phytoplasma elimination (Ćurković-Perica, 2008), supporting the notion that plant hormones play a crucial role in phytoplasma pathogenesis. In the present study, we conducted a comparative proteomic analysis, using two-dimensional gel electrophoresis (2-DE), of healthy (mock-inoculated), PPT-infected, and IAA-treated/PPT-infected tomato plants. Our preliminary data revealed that, compared with healthy tomato plants, the 2-DE protein profile was altered in PPT phytoplasma-infected tomato plants. Exogenous application of IAA partially restored the normal profiles of certain PPT-responsive proteins. Findings from this study will aid us to understand the role of IAA in phytoplasma pathogenesis and the mechanism of exogenous IAA-induced phytoplasma disease symptom remission.

Materials and methods

Plant material and phytoplasma inoculum

Columbia Basin PPT phytoplasma (a member of subgroup 16SrVI-A) and its alternative host 'Rutgers' tomato were used as a pathogen-host pair. Symptomatic or healthy control shoots were grafted onto eight-week-old healthy plants in the greenhouse.

IAA application

200 ml of 0.114 mmol/L IAA solution (ethanol/water, 1/1000, v/v) was applied twice *via* root drenching. Exogenous IAA application was made at seven and nine days post-grafting inoculation. Water was withheld from the plants for two days before each application.

Protein extraction and 2-DE

Leaf samples from healthy, PPT-infected and IAA-treated/PPT-infected tomato plants were collected at 1-d and 14-d after the second IAA application. Proteins were extracted from leaves on the branch next to the graft union by a method modified from Damerval *et al.* (1986). Briefly, fresh leaf tissues (0.5 g) were triturated in a pre-cooled mortar with liquid nitrogen and homogenized with a TCA-acetone extraction buffer (10% w/v TCA in acetone containing 0.07% v/v β-mercaptoethanol). The homogenates were incubated overnight at -20°C. After centrifugation at 10,000 rpm for 1 h at 4°C in a microfuge, the pellets were washed with ice-cold acetone containing 0.07% v/v β-mercaptoethanol to remove pigments and lipids until the supernatant became colorless. Protein pellets were dried under vacuum before solubilization in 2 M thiourea, 8 M urea, and 2% CHAPS (w/v) by sonication. Insoluble tissue debris was removed by centrifugation at 13,200 rpm for 30 min. Concentrations of the protein extracts were measured using Bio-Rad Protein Assay Kit (Bio-Rad, CA).

For iso-electric focusing (IEF), 300 µg proteins were loaded. IPG strips (17 cm, pH 4-7, Bio-Rad) were rehydrated with 200 µL rehydration buffer (2 M thiourea, 8 M urea, 2% w/v CHAPS, 1% w/v DTT, 0.2% w/v Bio-Lyte 4-6/5-7, 0.001% w/v bromophenol blue). IEF was performed using Protean IEF Cell (Bio-Rad) at 20°C with 50 µA/strip by applying the following program: 50 V for 12 h, 250 V for 30 min, 1000 V for 1 h, 10,000 V for 5 h, and 10,000 V for 60 kV h. After IEF, the proteins in the strips were denatured with an equilibration buffer (1.5 M tris-HCl pH 8.8, 6 M urea, 20% glycerol,

2% sodium dodecyl sulfate [SDS], 2% DTT) for 15 min and then incubated with the same buffer containing 2.5% iodoacetamide instead of DTT for an additional 15 min. The strips were transferred onto 10% polyacrylamide gels, and SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed using a PROTEAN® II xi Cell (Bio-Rad) at 5 mA/gel for 45 min and 20 mA/gel until the bromophenol blue dye front reached the bottom of the gel. The gels were stained with Coomassie Brilliant Blue G-250 and scanned using a VersaDoc Imaging system (Bio-Rad). Stained gels were analyzed by PDQuest 2-D analysis software (Bio-Rad).

Results and discussion

Since phytoplasmas cannot be cultured in cell-free medium, these organisms remain one of the least characterized plant pathogens. The mechanisms of phytoplasma pathogenicity are particularly poorly understood. Proteomic technology provides a new approach to study interactions between phytoplasma and plant host, offering clues to a better understanding of phytoplasma pathogenesis (Ji *et al*., 2009).

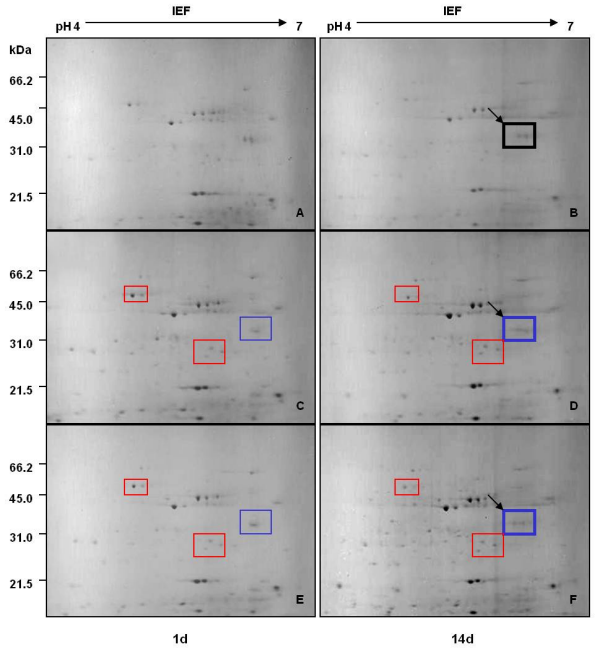

Figure 1. Representative proteomic profiles of tomato leaf tissues in response to PPT infection and IAA treatment. Proteins were extracted from leaves of healthy (A and B), PPT-infected (C and D), and IAA-treated/PPT-infected (E and F) plants. Examples of up- and down-regulated proteins are marked with red and blue rectangles, respectively. Proteins inside the heavy boxes are further discussed in figure 2.
(In colour at www.bulletinofinsectology.org)

To gain insights into the effects of exogenous IAA on proteomic profiles of PPT phytoplasma-infected tomato, 2-DE gels of leaf proteins from healthy, PPT-infected, and IAA-treated/PPT-infected tomato plants were compared (figure 1). Most of the tomato leaf proteins were concentrated in the range of pH 4-7 and molecular weight (MW) 21.5 - 66.2 kDa. With Coomassie Brilliant Blue stain, the total number of protein spots resolved was about 300 on each 2-D gel, suggesting that the proteomic approach has a great potential to unveil significant information regarding differentially synthesized proteins in PPT-infected *v.s.* IAA treated/PPT-infected plants. An analysis of the 2-D gels using the PDQuest software, with visual verification, revealed that levels of approximately 10% of the total leaf proteins were altered.

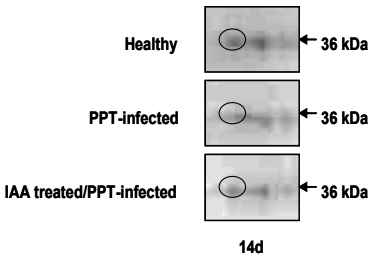

Figure 2. Close-up views of a 36 kDa protein exhibiting differential synthesis/accumulation patterns in PPT-infected *vs* IAA-treated/PPT-infected plants.

In IAA-treated/PPT-infected plants, the levels of certain PPT-responsive proteins partially restored to levels comparable to those of healthy controls. As shown in Figure 2, a protein of approximately 36 kDa was significantly down-regulated in PPT-infected plants; however, in IAA-treated/PPT-infected plants, the synthesis/accumulation level of the protein was similar to that of healthy plants. Currently, the identities and roles of these PPT- and IAA-responsive proteins are being characterized and correlated to the data from our parallel microarray and RT-qPCR experiments. Our preliminary results have indicated that these PPT-responsive proteins may be involved in hormone homeostasis and plant basal defense against pathogen infections.

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