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Effect of pear decline phytoplasma on gene expression in *Catharanthus roseus*

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Abstract

Phytoplasmas are prokaryotes characterized by small genomes (530-1,350 kbp) and by a limited number of metabolic pathways. To investigate molecular mechanisms involved in pathogenesis, the differential display technique was applied to identify plant genes whose transcription was significantly modified in leaves of *Catharanthus roseus* infected by '*Candidatus* Phytoplasma pyri' phytoplasma. Out of the sixteen genes identified, eleven were up-regulated by phytoplasma presence, while five were down-regulated. Identified genes are mainly involved in plant defence/stress responses, signal transduction, protein metabolism and transport, transcriptional regulation, and plant cell wall structure.

Key words: differential display, host-pathogen interaction, stress response, plant pathogens.

Introduction

Phytoplasmas are small, pleomorphic prokaryotes of the class *Mollicutes* characterized by small genomes (530-1,350 kbp), with low G+C content, limited number of metabolic pathways, only one or two ribosomal RNA operons, a small number of tRNA, and the absence of a cell wall (Lee *et al*., 2000; Christensen *et al*., 2005).

They are an important group of plant pathogens that are responsible for hundreds of plant diseases world-wide. Phytoplasmas are localized in phloem sieve elements and transmitted from plant to plant by phloem sap-sucking insect vectors such as leafhoppers, planthoppers or psyllids (Weintraub and Beanland, 2006; Hanboonsong *et al*., 2002). Plants infected by phytoplasmas exhibits diverse and severe symptoms such as leaf yellowing, growth aberrations (proliferations, internode shortening, stunting), flower malformations (size reduction, virescence, phyllody) and generalized decline (Chang, 1998).

The pear decline (PD) phytoplasma recently classified as '*Candidatus* Phytoplasma pyri' (Seemüller and Schneider, 2004) causes an important disease in *Pyrus communis* fruiting cultivars. The molecular mechanisms involved in symptom development are largely unknown and, currently, the interactions between phytoplasmas and their host plant species are poorly understood.

The effects of PD phytoplasma on gene expression in order to elucidate the molecular mechanisms involved in host-pathogen interactions was studied. *Catharanthus roseus* G. Don., was chosen as a model of host plant. Gene expression of *C. roseus* plants infected with PD phytoplasma were studied using differential display technique (DDRT-PCR), which is a very suitable method to rapidly identify and isolate genes that are differentially expressed between two cellular populations, or within a single cell type under altered conditions (Carginale *et al*., 2004; Tessitori *et al*., 2007).

Materials and methods

Total RNA was isolated from 0.5 g frozen leaves of control and PD phytoplasma-infected samples of *C. roseus* using the RNeasy Plant kit (Qiagen). Differential Display was performed using RNA Image kit (GenHunter). DNA-free total RNA (0.5 µg) extracted from pooled leaves of control and PD-infected *C. roseus* was reverse-transcribed with MMLV-reverse transcriptase and a set of three anchored oligo(dT) primers. PCR amplification of cDNA fragments was performed using combinations of the anchored primers from the reverse-transcription step and 24 arbitrary upstream primers (AP), supplied by the manufacturer, giving a total of 72 possible combinations. PCR products obtained were separated by denaturing electrophoresis. After electrophoresis, gels were dried and then exposed to Fuji X-ray film for autoradiography. Differentially expressed cDNA bands were eluted and reamplified in a PCR reaction using the same anchoring and arbitrary primers used in the differential display reaction.

cDNA fragments were subcloned into the pCR4-TOPO vector. DNA inserts were sequenced and sequences obtained identified by on-line based FASTA program.

Reverse Northern dot-blot analysis was performed to confirm the differential expression of the identified cDNA bands. Cloned cDNAs to be analyzed were amplified by PCR and each sample was blotted in duplicate on nylon membranes Two single-stranded cDNA probes were prepared from 20 µg total RNA isolated from either control and PD-infected samples by reverse transcription. Equal amounts (5 X 106 cpm/ml) of each cDNA probe were heat-denatured and then hybridized separately to one of the two membranes. Dried membranes were exposed to phosphor screens (GE Healthcare) for 12 h and then scanned with a PhosphorImager apparatus (Storm Imaging System, GE Healthcare). ImageQuant software (GE Healthcare) was used for radioactive signal quantification.

Results

DDRT-PCR is a sensitive tool for identifying genes whose expression is altered under particular conditions. This technique has particular value when the species being studied lacks complete genomic sequence information. In this context, DDRT-PCR was used to perform a comparative gene analysis on *C. roseus* plants infected or not with '*Ca*. P. pyri'.

A total of thirty-two 5'-arbitrary primers were used, each of them together with one of the three 3'-anchored oligo(dT) primers (giving a total of ninety-six primer pair combinations), to amplify cDNAs obtained by reverse transcription of total RNA from control and PD-infected plants. Sixteen putative differentially expressed cDNA fragments were detected, eluted from the polyacrylamide gels, reamplified and cloned. Lengths of the cloned cDNA fragments ranged from 214 to 491 bp.

Because of the possibility of contamination of specific cDNA bands by heterogeneous sequences, five clones, obtained from each cDNA band on the gel, were examined. These colonies were randomly selected from each transformation, and the sequence of the cloned insert in each of the colonies was determined. The nucleotide sequences obtained were compared with sequences available in the EMBL Nucleotide Sequence Database using the Fastx3 search utility to recognize putative proteins that are encoded by these mRNAs.

Eleven of the sixteen genes identified appeared to be up-regulated following PD infection while the remaining five genes were down-regulated. The identified proteins encoded by the up-regulated genes were: cytochrome b, FIP37, WD-40 like, a subunit of a mitochondrial translocase, isopropylmalate synthase, LEA14, a protein containing a ZIM motif, ribosomal protein L27a, beta-glucosidase, a protein with unknown function, and Potyvirus VPg interacting protein.

E3 ubiquitin-protein ligase EDD1, putative copia-like polyprotein, Ras-related protein Rab11A, Ty3-gypsy retrotransposon, and a protein with hydrolase activity were the identified proteins encoded by the down-regulated genes.

Discussion

The identified genes are mostly related to plant defence/stress response, signal transduction, amino acid and protein metabolism, protein transport, cell wall structure, and other functions. Some of the identified genes (WD-40 like, ZIM protein, Potyvirus-interacting protein, EDD1) are transcription factors that coordinate downstream gene expression in signal transduction pathways.

Our results suggest that phytoplasmas cause disease symptoms by enhancing or repressing the expression of physiologically relevant host genes, and should serve as a basis for a more comprehensive analysis of gene expression patterns in phytoplasma-infected plants. The mechanisms by which phytoplasmas can alter host gene expression are not clear, but it appears that more than one mechanism could be responsible for gene activation/inhibition in host plants.

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