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Phytoplasma induced changes in gene expression in poinsettia

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Abstract

Phytoplasma infection causes a desirable branching phenotype in the ornamental plant *Euphorbia pulcherrima* by releasing apical dominance. Four *E. pulcherrima* genes specifically regulated during phytoplasma infection were identified using differential display of cDNA-PCR products. According to BLAST searches the possible functions of the identified genes included a histidine-containing phosphotransmitter and a protein with similarity to an *Arabidopsis thaliana* protein with unknown function.

Key words: phytoplasma, plant hormone, poinsettia, gene expression.

Introduction

A branch-inducing factor in poinsettia (*Euphorbia pulcherrima*) has been known for several decades, but only in 1997 this graft transmissible factor was identified as a phytoplasma (Lee *et al*., 1997). While phytoplasmas are normally associated with yield and quality loss, infection in the ornamental *E. pulcherrima* produces a desirable, free-branching growth form. Other symptoms in *E. pulcherrima* include shorter internodes and less lobed leaves (Preil and Engelhardt, 1982). This phytoplasma also induces a free-branching phenotype in related species such as *E. milii* and *E. fulgens* (Nicolaisen, 2004).

The molecular mechanisms involved in the phytoplasma-plant interaction have only been the subject of a few studies: the host gene expression in *Catharanthus roseus* after challenge with phytoplasma was investigated by differential display RT-PCR (DD-RT-PCR) (Jagoueix-Eveillard *et al*., 2001) and twenty four genes were identified as deregulated. However, most of these did not show any significant homologies to known proteins in the GenBank. A subunit III of photosystem I and a ribulose 1,5-biphosphate carboxylase-oxygenase were identified together with proteins of unknown function. Carginale and co-workers (Carginale *et al*., 2004) investigated gene expression changes, also by DD-RT-PCR, and found only four genes that were differentially expressed in phytoplasma-infected *Prunus armeniaca* tissue (among those a heat shock protein and a metallothionein). Pacros *et al*. (2006) investigated gene expression levels of known floral development genes in a tomato-stolbur phytoplasma interaction which involves floral abnormalities and found several genes to be differentially regulated. The goal of this work was to identify *E. pulcherrima* genes deregulated during PoiBI infection. Gene expression in non-infected restricted-branching and phytoplasma-infected free-branching *E. pulcherrima* internodes was compared by DD-RT-PCR.

Materials and methods

Virusfree *E. pulcherrima* (Willd. ex. Klotzsch) cv. Lilo plants infected with poinsettia branching inducing (PoiBI) phytoplasmas or non-infected were grown in a greenhouse at 20/18 °C day/night and a day length of 15 h under insect-proof conditions. Plants were propagated vegetatively. Sampling was done shortly after the onset of lateral bud outgrowth. RNA was extracted as described (Salzman *et al*., 1999). Phytoplasma titers in individual plants were estimated using Q-PCR (Christensen *et al*., 2004).

DD-RT-PCR using 48 primer combinations was done using the Hieroglyph mRNA profile kit (Genomyx Corporation, Foster City, CA) according to the manufacturer's instructions. Putative differentially expressed genes from DD-RT-PCR were reamplified and cloned into pCR 2.1 TOPO (Invitrogen, CA, USA). The differential expression of identified genes from DD-RT-PCR or microarray experiments was confirmed by Q-RT-PCR using total RNA from internodes or leaves from 8 individual healthy plants and 8 infected plants using SYBR Green PCR Master Mix on an ABI Prism 7900 Sequence Detection System. An elongation factor 1α gene (acc. no. EF153173) Q-RT-PCR was performed alongside as internal standard. Relative transcript levels between healthy and infected tissue were calculated using the ΔΔCt method (Applied Biosystems).

Results and discussion

Initially, relative phytoplasma titers were estimated in individual plants using Q-PCR. The plant with highest level of infection had approximately 3 times higher phytoplasma titer than the least infected plant both in internodes and leaves (data not shown). Phytoplasmas were not detected in healthy plants.

RNA from infected vs. non-infected plants was subjected to DD-RT-PCR. From the DD-RT-PCR experiments 29 bands with differential intensity were excised and reamplified for confirmation of differential ex-

pression. Specific primers for these 29 genes were designed and Q-RT-PCR was performed on RNA from internodes and leaves from 8 individual healthy and 8 infected plants, this analysis was repeated once with another set of plants. The analysis confirmed that 2 genes, Ep11, and Ep60 showed differential expression in phytoplasma-infected internodes compared to non-infected tissue. Ep11 was upregulated approximately 2 fold whereas Ep60 was downregulated approximately 10 fold in infected internodes. The low number of truly deregulated genes may be caused by the fact that phytoplasma occurs in low titer in *E. pulcherrima*. As synchronized infection with phytoplasma is not possible, important phytoplasma-specific genes may not be regulated at the time of tissue sampling. Only a limited number of primer combinations were used in the DD-RT-PCR. Translation of Ep11 resulted in a hypothetical 150 aminoacid (aa) protein. Ep11 is highly similar to histidine-containing phosphotransmitters (HPt) from *A. thaliana* and other plants (75% aa identity and 88% similarity to a HPt-like protein acc. no. BAB01275). HPts are known to play a key role in the phosphorelay signal transduction pathway from the phytohormone cytokinin histidine kinase receptors to transcriptional response regulators in the nucleus (Sheen, 2002). Enhanced expression of cytokinin biosynthesis genes results in release of apical dominance (Medford *et al*., 1989), which is also observed in phytoplasma-infected *E. pulcherrima*, therefore, Ep11 may play a role in symptom expression in the free-branching phenotype of PoiBI infected *E. pulcherrima*. Studies of overexpression of HPt in arabidopsis young seedlings showed a phenotype of cytokinin hypersensitivity (Suzuki *et al*., 2002) whereas insertion mutants in multiple HPt genes in arabidopsis showed reduced sensitivity to cytokinin (Hutchinson *et al*., 2006). Translation of Ep60 resulted in a hypothetical 249 aa protein which was highly similar (56% identity and 75% similarity) to a putative arabidopsis gene (AT5G13140) with unknown function. Expression analysis of the AT5G13140, as displayed from the response viewer tool of Genevestigator (Zimmermann *et al*., 2004), suggests this gene is also down regulated by various treatments inhibiting growth including cold, salt and heat stress. The Ep60 homologue in arabidopsis is upregulated under high CO_2 conditions that would increase plant growth. The effect of phytoplasma on *E. pulcherrima* morphology (free-branching, shorter internodes and leaf shape) may originate from local concentration alterations in hormone levels or in changes in hormone perception. In particular, Ep11 may play a major role in the release of apical dominance as this gene is involved in cytokinin signal transduction and cytokinin is known to play a significant role in this process.

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