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Stolbur phytoplasma-infected tomato showed alteration of *SlDEF* methylation status and deregulation of methyltransferase genes expression

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Abstract

Phytoplasmas are phloem-restricted plant pathogenic bacteria belonging to the class *Mollicutes*. In stolbur phytoplasma-infected tomato, flower abnormalities have been associated with changes in the expression of floral development genes. In particular, *FALSIFLORA* (*FA*) is up-regulated whereas *SlDEFICIENS* (*SlDEF*) is down-regulated. To determine whether methylation is involved in down-regulation of *SlDEF*, the effect of phytoplasma infection on CpG methylation status of *SlDEF* was studied.

MSRE-PCR and bisulfite sequencing showed that *SlDEF* was hypermethylated in tomato infected with the stolbur strain PO. The chromomethyltransferase genes studied by RT-PCR were down-regulated. Moreover, PO-tomato treated with 5-Azacytidine showed flowers and fruits, suggesting that *SlDEF* was no more deregulated. These results suggest that the stolbur phytoplasma could be involved in the inhibition of the demethylation of *SlDEF*.

Key words: phytoplasma, floral development, methylation, tomato, plant response.

Introduction

Phytoplasmas are plant pathogenic wall-less bacteria, restricted to phloem sieve tubes, which induce plant disorders such as leaf yellowing, growth aberrations and/or flower malformations. Floral malformations in stolbur phytoplasma-infected tomato are reminiscent of those observed in *Arabidopsis* mutants affected in floral developmental genes. In infected tomato, *FA* (*LEAFY* ortholog) is up-regulated whereas *SlDEF* (*APETALA* 3) and *TAG* 1 (*AGAMOUS*) are down-regulated. It has been suggested that in *Arabidopsis*, *APETALA3* and *AGAMOUS* down-regulation may result from their hypermethylation (Finnegan *et al.,* 1996). Consequently in stolbur-infected tomato, the hypermethylation of the regulatory region of *SlDEF* may result in its down-regulation (figure 1). So the effect of stolbur phytoplasma infection on CpG methylation status of *SlDEF* was studied using two distinct isolates (C and PO) of stolbur phytoplasma, which induced different symptoms. In spite of similar multiplication rates in the plant, the two phytoplasma isolates (C and PO) induce distinct symptoms. Flowers from PO-infected plants (PO tomato) show virescence, phyllody and big bud symptoms, which are never observed in isolate C-infected tomato (C tomato) (Pracros *et al*., 2006).

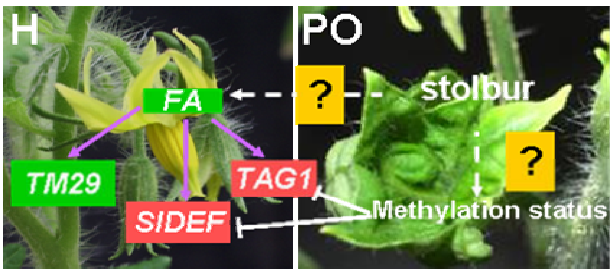

Figure 1. Possible involvment of methylation status of *SlDEF* in malformation of stolbur phytoplasma-infected tomato flowers.

Materials and methods

Plants

Tomato (*Solanum lycopersicum* cv Ailsa Craig). Plants were infected with the stolbur phytoplasma strains C and PO by grafting. In specific experiments, tomato seeds were treated with 10 or 50 µM of 5-Azacytidine prior infection.

RNA were extracted from tomato flower buds with Tri-reagent (SIGMA) and treated with DNase, following the supplier protocol.

Semi-quantitative RT-PCR were done with primers specific for each DNA methyltransferase genes. For each tube of RT, 1 µg of DNA, 0.55 µM of the 3' primer, 0.01 M DTT, 2.2 mM dNTP, 280 U of Rnase OUT were mixed in a final volume of 19 µl. After 5 min at 65 °C and 5 min at 45 °C, 1 µl (200 U) of Superscript II reverse transcriptase was added and incubated for 1 hour. Five min at 75 °C allowed the enzyme denaturation. PCR were done with 1 µl of cDNA, 0.35 mM $MgCl_2$, 0.9 mM dNTP, 2 µg/ml BSA, 4.4 µM each primer (3' and 5') and 2.2 U Taq DNA polymerase (Promega), in a final volume of 25 µl. Hybridization temperature depends on primers used.

Agarose gel analyses

RT-PCR products were eletrophoresed on a 1.5% agarose gel and stained with ethidium bromide. The intensity of the bands was measured with a FluorS and the associated software Quantity One (Biorad).

MSRE-PCR

DNA was purified from plants using the CTAB method (Murray and Thompson, 1980). Two µg of DNA were digested with *Mbo*I or *Sau*3AI restriction enzyme prior amplification with primers within the 5'end of *SlDEF*. Whereas *Mbo*I is insensitive to CpG methylation, *Sau*3AI does not cut at CGATC where the C is methylated, allowing the fragment to be amplified. PCR was carried out with 0.1 µg of DNA in a final volume of 25 µl.

Bisulfite sequencing

The EpiTect Bisulfite kit (Qiagen) was used following the supplier protocol. Bisulfite treatment converts C to T except when C is methylated.

Results

The PCR amplification after restriction of genomic DNA with *Mbo*I or *Sau*3AI, which are sensitive to whether the cytosine at the restriction site CGATC is methylated or not, shows that in the case of stolbur PO-infected tomato, there was a stronger amplification signal with *Sau3A1* digested DNA (as compared to *Mbo1*). This strongly suggests that CGATC sequences of *SlDEF* are hypermethylated in infected tomato.

In flower buds of stolbur phytoplasma-infected tomato, the bisulfite sequencing of the *SlDEF* promoter region, revealed a higher methylation status than in healthy ones.

The stolbur PO-infected tomato treated with 10 or 50 µM 5-azacytidine prior infection display early yellowing but nearly normal flowers.

Methylation is carried out by a wide variety of DNA methyltransferases (Dnmt) that are classified in three families: methylases (MET1), chromomethylases (CMT) and domain rearranged methyltransferases (DRM). Studying expression of seven distinct Dnmt of tomato by semi-quantitative RT-PCR, revealed that expression of class I and class III Dnmt was not affected by the stolbur phytoplasma infection. In contrast, the three Dnmt of the class II encoding the plant specific chromomethyltransferases (CMT) were dramatically down-regulated in PO-infected tomato and not in C-infected tomato.

Discussion

SlDEF gene is down-regulated in stolbur phytoplasma-infected tomato flower buds, in spite of the activation of the transcriptional factor FA. Because methylation is a common mecanism that repress gene expresion, it has been hypothesized that *SlDEF* repression was due to methylation, and further studied.

The results suggested that:

1) DNA from stolbur PO-infected tomato flower buds is selectively methylated at the regulatory region of *SlDEF*,
2) in 5-azacytidine treated plants, *SlDEF* is expressed even in the case of stolbur phytoplasma-infected plants,
3) in stolbur PO-infected tomato, unexpectedly, CMT genes are found to be down-regulated.

These data suggest that the stolbur phytoplasma could be involved in the inhibition of the demethylation of *SlDEF* during floral development.

Acknowledgements

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