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Gene expression and enzymatic activity of invertases and sucrose synthase in *Spiroplasma citri* or stolbur phytoplasma infected plants

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Abstract

Plant pathogenic *Mollicutes* induce multiple symptoms: yellowing, floral abnormalities or growth aberrations. *Mollicutes* are also responsible for impaired partitioning of photoassimilates in plants: accumulation of soluble sugar in source organs and depletion of sugar in sink organs. However, the molecular mechanisms of pathogenicity are poorly understood. For *Spiroplasma citri*, sugar metabolism play an important role in pathogenicity: a mutant unable to import fructose is non-pathogenic, whereas a mutant unable to import glucose is still pathogenic. These data suggest that key enzymes of sugar metabolism could be deregulated.

In plants, four main enzymes hydrolyse sucrose into glucose+fructose, and are susceptible to provide sugar to the mollicutes situated in the phloem sieve tubes: the vacuolar, cell-wall and neutral invertases, and the sucrose synthase. These enzymes are already known to be induced in response to biotic or abiotic stress. So, gene expression and enzymatic activity of these enzymes were studied in leaves of healthy or infected plants. Sugar concentration was also determined. Results showed that in contrast to gene expression, the enzymatic activities showed variation in the three pathosystems studied.

Key words: plant response, mollicute, sugar metabolism, invertase, sucrose synthase, enzymatic activity, RT-PCR.

Introduction

Plant pathogenic mollicutes, spiroplasmas and phytoplasmas, multiply in the phloem sieve tubes and are transmitted from plant to plant by phloem sap-feeding, leafhopper vectors. Previously, it has been shown that sugar metabolism play a key role in pathogenicity of *Spiroplasma citri*. Mutants unable to import fructose are virtually non-pathogenic. In contrast, those unable to use glucose induce severe symptoms, similarly to the wild-type strain, indicating that fructose and glucose are not equally involved (André *et al.*, 2004). *S. citri*-infected plants revealed an impaired partitioning of photoassimilates with an accumulation of soluble sugars, and in particular glucose, in source organs. Such an impaired carbohydrate partitioning has also been described in phytoplasma-infected plants, suggesting that key enzymes of sugar metabolism could be deregulated.

Four main enzymes are implicated in the sugar partition in plants: sucrose synthase, cell wall invertase, neutral invertase and vacuolar invertase. Those enzymes are known to be deregulated in plants in response to biotic or abiotic stress (Biemelt *et al.*, 2005). In *S. citri*-infected periwinkle, a model has been proposed in which increased vacuolar invertase activity would be responsible for glucose accumulation in source leaves.

Expression of invertase and sucrose synthase genes, was studied by semi-quantitative RT-PCR. Enzymatic activities of invertases, as well as sugar concentration in mature leaves, was determined for three pathosystems: stolbur phytoplasma-infected tomato, stolbur phytoplasma-infected periwinkle, and *S. citri*-infected periwinkle.

Materials and methods

Plants

Tomato, *Solanum lycopersicum* cv Ailsa Craig and periwinkle, *Catharanthus roseus*. Plants were infected with *Spiroplasma citri* GII3 (periwinkle) or with the stolbur phytoplasma (periwinkle and tomato), by grafting.

RNA were extracted from mature or young leaves with Tri-reagent (SIGMA) and treated with DNase, following the supplier protocol. Semi-quantitative RT-PCR were done with primers specific for each enzyme and each plant. For each tube of RT, 1 µl of RNA, 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, 200 U of Superscript II reverse transcriptase were 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 and 2.2 U Taq DNA polymerase (Promega), in a final volume of 25 µl. Hybridization temperature depends on the primer used. Each experiment was repeated at least three times.

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 software Quantity One (Biorad).

Enzymatic activity assays

The method derived from Pelleschi (1997), is based on the measurement of NADH absorption at 340 nm, knowing that the amount of NADH is proportional to the sucrose hydrolysed; 0.3 g of leaves were taken for each pathosystem.

Determination of sugar concentration: sucrose, glucose and fructose concentrations in plant leaves were determined for using the R-BIOPHARM kit (Saccharose/D-Glucose/D-Fructose).

Results

Primers design

Primers for tomato genes were designed from sequences available in the databanks. Sequence of invertases and sucrose synthase periwinkle genes were first amplified with degenerated primers. Then, specific primers for the four genes were designed from the sequences of the amplification products.

Gene expression

Semi-quantitative RT-PCR concerning the four genes were done for the three pathosystems both on young and mature leaves. In most case, no significant deregulation was observed. Cell-wall invertase was slightly over- expressed (<15%) in young leaves of periwinkle infected by *S. citri* or stolbur phytoplasma. In contrast, neutral invertase was equally expressed in all pathosystems.

Enzymatic activities

A higher activity was detected for (1) the vacuolar invertase in stolbur phytoplasma-infected tomato, (2) the cell-wall invertase in stolbur phytoplasma and *S. citri* infected mature leaves and (3) the neutral invertase in all pathosystems (table 1).

Sugar concentration

As expected from previous data, sucrose and glucose accumulate in mature leaves of infected periwinkles.

Discussion

In contrast to gene expression, the enzymatic activities showed variation in nearly every pathosystem studied.

The two host plants reacted slightly differently when infected by the same mollicute, whereas the same plant reacted similarly when infected by *S. citri* or stolbur phytoplasma. Young and mature leaves seemed to react nearly in the same way. Concerning the enzymatic activities, only the neutral invertase showed an increased activity in all three pathosystems. Our results contrast with those from tomato infected by *Pseudomonas syringae* where the cell-wall invertase gene is up-regulated, or from maïze where the vacuolar invertase activity is increased after a stress (Biemelt *et al*., 2005; Kim *et al*., 2000).

According to these data, the neutral invertase of tomato and periwinkle seems to respond to infection by the two mollicutes *S. citri* and stolbur phytoplasma (figure 1).

Sucrose hydrolysis by the neutral invertase would provide fructose in the cytoplasm. Diffusion to the sieve tube would make fructose availaible for *S. citri* growth. However, whereas *S. citri* use fructose preferentialy over glucose, it is unknown whether phytoplasmas use fructose or glucose. The neutral invertase would also provide glucose and/or fructose for phytoplasma growth.

Table 1. Enzymatic activities measured in the three pathosystems and compared to the healthy plants data. + up-regulation, - down-regulation, = no deregulation, yL young leaves, mL mature leaves.

	Tomato / Stolbur		Periwinkle / Stolbur		Periwinkle / *S. citri*	
	yL	mL	yL	mL	yL	mL
Vacuolar Invertase	+	+	ND	-	-	-
Cell Wall Invertase	-	+	+	+	-	+
Neutral Invertase	+	+	+	+	+	+

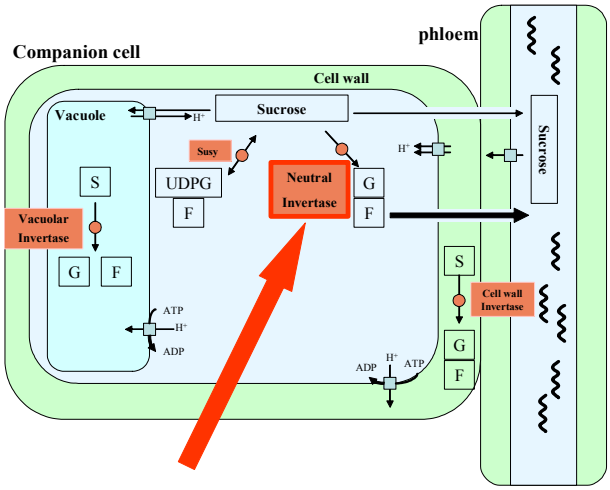

Figure 1. Putative role of neutral invertase in the plant response to mollicute infection. S: saccharose, G: glucose, F: fructose.

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