Plant Physiology and Biochemistry 70 (2013) 311–317

Contents lists available at SciVerse ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Short communication

Gene expression and biochemical changes of carbohydrate metabolism in *in vitro* micro-propagated apple plantlets infected by '*Candidatus* Phytoplasma mali'

Filomena Giorno [a,*], Gea Guerriero [a,1], Matteo Biagetti [a], Anna Maria Ciccotti [b], Sanja Baric [a]

[a] Laimburg Research Centre for Agriculture and Forestry, Laimburg 6 – Pfatten (Vadena), 39040 Auer (Ora), BZ, Italy
[b] IASMA Research and Innovation Centre, Fondazione Edmund Mach, Via E. Mach 1, I-38010 San Michele all'Adige, TN, Italy

ARTICLE INFO

Article history:
Received 5 May 2013
Accepted 29 May 2013
Available online 13 June 2013

Keywords:
Malus x domestica
Apple proliferation
Gene expression
Carbohydrate metabolism
Stress response

ABSTRACT

'*Candidatus* Phytoplasma mali' (*Ca.* P. mali) is the disease agent causing apple proliferation (AP), which has detrimental effects on production in many apple growing areas of Central and Southern Europe. The present study investigated transcriptional and biochemical changes related to the sugar metabolism as well as expression of pathogenesis-related (PR) protein genes in *in vitro* micro-propagated AP-infected and healthy control plantlets with the aim of shedding light on host plant response to '*Ca.* P. mali' infection. Expression changes between infected and control plantlets were detected by quantitative real-time PCR analysis. The most significant transcriptional changes were observed for genes coding for pathogenesis-related proteins and for heat shock protein 70, as well as for some genes related to the sugar metabolism, such as a sorbitol transporter (SOT5), hexokinase, sucrose-phosphate synthase or granule bound starch synthase. Furthermore, biochemical analyses revealed that infected plantlets were characterized by a significant accumulation of starch and sucrose, while hexoses, such as glucose and fructose, and sorbitol were present at lower concentrations. In summary, the present analysis provides an overview of a gene set that is involved in response to phytoplasma infection and, therefore, it may help for a better understanding of the molecular mechanisms involved in phytoplasma–host plant interaction in apple.

1. Introduction

Phytoplasmas are plant pathogenic bacteria of the class Mollicutes, characterized by obligate parasitism, reduced genomes, limited number of metabolic pathways and the absence of a cell wall [1]. Due to the impossibility to grow phytoplasmas in axenic cultures, the molecular mechanisms of their pathogenicity and symptoms triggered in host plants are still poorly understood [1]. In plants, phytoplasmas are restricted to the nutrient-rich phloem tissue and transmitted from plant to plant by sap-sucking insect vectors or through grafts [1]. Most typical symptoms of phytoplasma-induced disease are small and/or bronze reddish leaves, enlarged stipules, leaf-rosette formations, virescent flowers with abnormal number of petals, and decreased fruit quality [1]. At the tissue level, host plants show anatomical aberrations such as callose accumulation on the plates of sieve tubes and in some cases phloem tissue proliferation and necrosis [2]. Reduced stomatal conductance has also been reported together with the inhibition of photosystem II (PS II) activity [3,4].

Molecular approaches mainly based on transcriptome analyses have shown that gene expression changes are associated with a wide array of symptoms observed in the diseased tissues [5–9]. Considerable and tissue specific modulations were found for genes related to the cell wall metabolism, which are most likely correlated to the anatomical aberrations found on the plates of sieve tubes in infected plants [2,6,10]. Jagoueix-Eveillard et al. [5] reported a down-regulation of a putative sterol-C-methyltransferase in periwinkle after infection with *stolbur* phytoplasma and associated this gene repression with leaf yellowing and stunting. Expression changes in genes related to developmental processes were also found in phytoplasma-infected plants and, correspondingly, abnormalities in flower organs were shown [1]. An overall decrease in

Abbreviations: APP, apple proliferation phytoplasma; PS II, photosystem II; PR, pathogenesis related; Hsp70, heat shock protein 70; qRT-PCR, quantitative reverse transcriptase real-time PCR.

* Corresponding author. Present address: Department of Molecular Plant Physiology, Radboud University Nijmegen, Heyendaalseweg 135, 6525 AJ Nijmegen, The Netherlands.
E-mail addresses: mgiorno@science.ru.nl, filomena.giorno@gmail.com (F. Giorno), guerrier@lippmann.lu (G. Guerriero), matteobiagetti@hotmail.com (M. Biagetti), annamaria.ciccotti@alice.it (A.M. Ciccotti), Sanja.Baric@provinz.bz.it (S. Baric).
[1] Present address: Centre de Recherche Public, Gabriel Lippmann 41, Rue du Brill L-4422 Belvaux, Luxembourg.

0981-9428/$ – see front matter © 2013 Elsevier Masson SAS. All rights reserved.
http://dx.doi.org/10.1016/j.plaphy.2013.05.040

transcript abundance of genes coding for proteins involved in the photosynthesis machinery was described in several investigations, suggesting a possible link to interferences with PS II activity [3,4,8]. This inhibition may also have an impact on the carbohydrate metabolism, particularly on the accumulation of soluble carbohydrates and starch, as observed in source leaves of plants infected by phytoplasmas [11–14]. Albertazzi et al. [6] and Hren et al. [8] have recently found that specific genes such as vacuolar invertase and sucrose synthase were up-regulated in phytoplasma-infected grapevine leaves, which may be linked to the negative interferences with sucrose metabolism [11,12]. Beside the transcriptional changes strictly related to the primary and secondary metabolism, other major alterations were found in the host tissues and included the activation of defence genes such as the *heat shock protein 70* (*Hsp70*) and genes coding for pathogenesis-related (PR) proteins, which are downstream components of systemic acquired resistance in plants [6,8].

Thus, the main objective of the present study was to investigate transcriptional changes occurring in apple tissue after phytoplasma infection, and to ascertain the hypothesis that biochemical changes related to carbohydrate metabolism may be associated to transcriptional modulations of specific gene classes in the diseased tissue. To this end, the expression profiles were monitored in *in vitro* micro-propagated apple plantlets infected with '*Candidatus* Phytoplasma mali' ('*Ca.* P. mali'), the disease agent of apple proliferation (AP) that can have serious impact on the yield of many apple cultivars [15,16]. Unravelling the transcriptional changes associated with carbohydrate metabolism will undoubtedly be an important step towards a better understanding of the molecular mechanisms involved in phytoplasma–host plant interaction and, in turn, may help to develop future strategies for the control of disease progression in apple.

2. Results

2.1. Analyses of gene expression changes of PR and Hsp70 genes

It was shown in previous studies that genes encoding for PR proteins and for Hsp70 were differentially expressed in response to biotic stresses [6,8,9]. Therefore, members of the *PR* family and the *Hsp70* gene were identified in the apple genome and used as marker genes. *PR-1a* gene was slightly down-regulated in infected plantlets with respect to the healthy controls, while no transcriptional changes were observed for the *TLP* gene encoding a thaumatin-like protein (Fig. 1). On the other hand, increased messenger RNA levels of *PR-6*, *PR-8*, *MALD1* and *Hsp70* genes in infected plants compared to healthy samples were found (Fig. 1).

2.2. Transcriptional changes in genes involved in carbohydrate metabolism and transport

Transcriptional changes of genes coding for sugar transporters and enzymes involved in carbohydrate metabolism were assessed by qRT-PCR. Increased messenger RNA levels in APP-infected plantlets compared to controls were observed for *sucrose-phosphate synthase 1* (*SPS1*), *hexokinase 2* (*HXK2*), *granule bound starch synthase Ia precursor* (*GBSSIa*) and *sorbitol transporter 5* (*SOT5*)

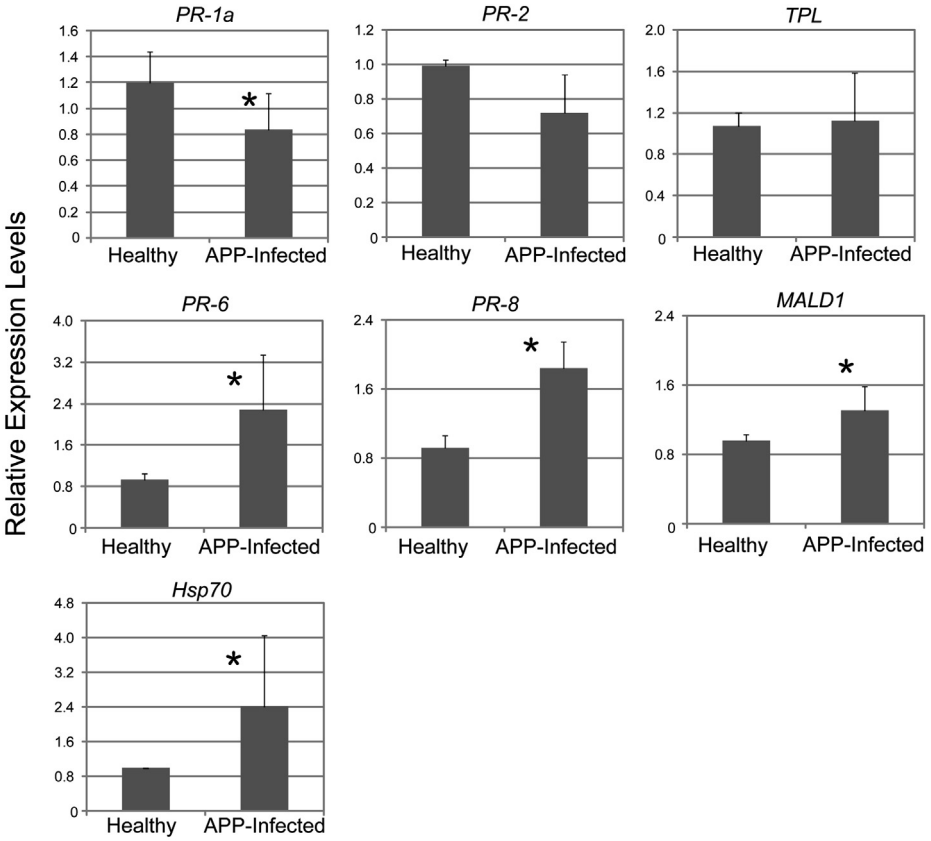

Fig. 1. Expression analyses of *PR* and *Hsp70* genes *in vitro* micro-propagated apple plantlets. Transcript levels of *PR* and *Hsp70* genes were analyzed by qRT-PCR in control (Healthy) and infected (APP-Infected) apple plantlets. The qRT-PCR analysis results were normalized using *EF1 alpha*, *Tip-41* and *IMPA9* as housekeeping genes. Each bar represents the average relative expression levels of three biological replicates. Statistical differences were determined using Student's *t*-test (*, $P < 0.05$).