Bulletin of Insectology ***64*** *(Supplement): S211-S212, 2011*
ISSN 1721-8861

Phloem-specific protein expression patterns in apple and grapevine during phytoplasma infection and recovery

Rita MUSETTI, Federica DE MARCO, Khaled FARHAN, Rachele POLIZZOTTO, Simonetta SANTI, Paolo ERMACORA, Ruggero OSLER
Dipartimento di Scienze Agrarie e Ambientali, Università di Udine, via delle Scienze, 208, I-33100 Udine, Italy

Abstract

Recovery - complete remission of disease symptoms - has been reported in plants affected by phytoplasmas. The physiological basis for this phenomenon is not yet understood, but it seems associated to ultrastructural and biochemical modification of the phloem, the tissue where phytoplasmas live and spread. In this work we compared asymptomatic, phytoplasma-infected and recovered apple and grapevine leaf tissues by means of ultrastructural and gene-expression analyses, focusing on a possible role of specific phloem proteins in the plant defense-related processes. Preliminary results indicate that different occlusion mechanisms could interact in the phloem during phytoplasma symptomatic status and/or recovery.

Key words: apple, grapevine, phloem, phytoplasma, recovery.

Introduction

Recovery in grapevine and apple can occur also in single plants showing severe symptoms since several years, manifesting through a complete remission of the symptoms, associated to the disappearance of the phytoplasmas from the crown (Carraro *et al.*, 2004). The physiological basis for this phenomenon is not yet understood; it has been observed that in apple, apricot and in grapevine, recovery from phytoplasma-associated diseases was accompanied by an overproduction of hydrogen peroxide localised in the phloem tissues (Musetti *et al*., 2004, 2005, 2007). Musetti *et al*., (2010) reported an abnormal callose and phloem protein 2 (PP2) accumulation in the phloem of recovered apple plants associated to the up-regulation of two callose synthase- and three PP2-coding genes, supporting the hypothesis that recovered apple plants were able to develop resistance mechanisms depending on Ca^{2+} signal activities. Plugging mechanism in the phloem is carried out also by the activity of other specialized calcium-powered proteins, called Sieve Element Occlusion (SEO) proteins. Genes encoding SEO proteins are widespread among dicotyledonous plants (Rüping *et al*., 2010). Callose deposition and protein plugging can operate in parallel: while phloem specific proteins act quickly, callose acts slowly. Specific aim of this work was to compare leaf tissues in asymptomatic (H), phytoplasma-infected (symptomatic, D) and recovered (R) apple and grapevine plants by means of ultrastructural and gene-expression analyses, focusing on a possible role of specific phloem proteins (callose synthases, PP2 and SEO) in the infection establishment and/or in the recovery from phytoplasma-associated diseases.

Materials and methods

Grapevine (*Vitis vinifera* L. cv. Chardonnay) and apple leaf samples (*Malus* x domestica cv. Florina) were collected from H, D and R plants in late summer, when typical symptoms of the apple proliferation and the 'bois noir' infection appear in apple and grapevine, respectively. Plants were grown in experimental fields located in Friuli Venezia Giulia (North-East Italy). Total RNA was extracted from frozen leaves enriched in midribs using RNeasy Plant Mini Kit (Qiagen) with minor modifications. Total RNA was quantified spectrophotometrically (Nanodrop, Thermoscientific), treated with Turbo DNase (Ambion) and reverse transcribed using SuperScript® III Platinum® Two-Step qRT-PCR Kit (Invitrogen). Quantitative Real Time PCR analysis were performed on a DNA Engine OPTICON 2 instrument (Biorad) with 5 PRIME Master Mix (including SYBR Green). Genes coding grapevine callose synthases (*Vv*CaSy) were identified *in silico* from grapevine genome browser, while sequences of genes coding for SEO proteins (*Vv*SEO, *Md*SEO) were obtained from Rüping *et al*., (2010). Primers were designed using Primer3 software. Ultrastructural observations were carried out on small portions of leaf midribs processed as described in Musetti *et al*. (2010), using a Philips CM 10 transmission electron microscopy (TEM), operating at 80 kV.

Results

Seven sequences from *Vv*CaSy and two sequences from genes codifying for SEO proteins (*Vv*SEO and *Md*SEO) were identified and considered suitable for gene expression analyses. With regards to the expression of the genes encoding VvCaSy, four genes among seven showed, in this preliminary effort, an expression level one order of magnitude lower than others and thus not further characterized (data not shown). Among the more expressed genes, *CaSy0* appeared to be significantly induced in D and R plants, compared to healthy ones, while *CaSy1* and *CaSy6* were not influenced by the sanitary status of the plant. Anyway, this last gene showed a slight up-regulation in R plants (figure 1).

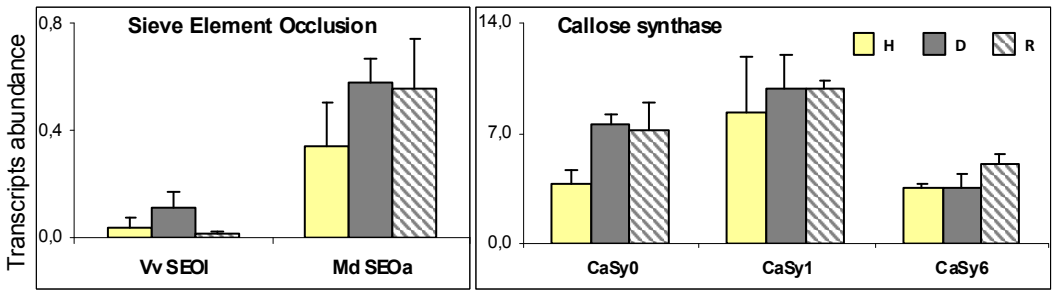

Figure 1. Relative expression levels of two genes coding for SEO protein (VvSEOl, grapevine and MdSEOa, apple) and three genes coding for grapevine CaSy. Mean expression values from three individuals for each plant group (H, D, R) plus standard errors are shown. Expression levels of the genes of interest are normalised on ubiquitin (UBQ expression level = 100) for grapevine and TEF1-α for apple.

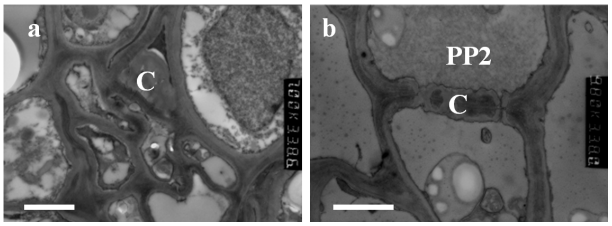

Figure 2. Micrographs of grapevine leaf tissue. (a) In the phloem of 'bois noir'-infected leaves, callose occlusion (C) is very evident. (b) In recovered plants, PP2 plugs are accumulated in the sieve tube lumen.

TEM observations revealed callose deposition mainly in sieve plates of D grapevines, while in R ones, PP2, forming big plugs, appeared to be a common ultrastructural trait fFigure 2). As regards SEO protein expression pattern, *Vv*SEOl and *Md*SEOa appeared influenced by the sanitary status of the plants, tending to have a higher transcription level in D samples (figure 1).

Discussion

Phytoplasmas colonize mostly the sieve tubes of the phloem, systemically in the plant. Upon phloem injury, sieve elements are occluded by combined callose-collar formation around sieve pores and protein plugging to prevent leakage of nutrients and pathogen invasion and spread. Identifying cellular modifications in host plants and changes of plant gene expression induced by phytoplasmas is critical for understanding how these pathogens cause diseases and consequently how plants react to their challenge, leading to recovery. In this work we performed preliminary analyses about modifications of phloem-specific protein expression patterns comparing H, D and R grapevine and apple plants. In both plant/phytoplasma associations, different occlusion mechanisms could take place in the phloem during phytoplasma symptomatic status and/or recovery. As already demonstrated in apple (Musetti *et al*., 2010), different isoforms of callose synthases are triggered in grapevine, during phytoplasma infection (CaSy0) or when recovery occurs (CaSy6). On the other hand, up-regulation of genes coding for SEO proteins in D plants point to their potential function in the rapid phloem occlusion mechanisms during phytoplasma spread in the host tissue.

Acknowledgements

This study was funded by Fondazione CARIPLO (Italy), Project AGER-Agroalimentare e Ricerca.

References

CARRARO L., ERMACORA P., LOI N., OSLER R., 2004.- The recovery phenomenon in apple proliferation-infected apple trees.- *Journal of Plant Pathology*, 86(2): 141-146.

MUSETTI R., SANITÀ DI TOPPI L., ERMACORA P., FAVALI M. A., 2004.- Recovery in apple trees infected with the apple proliferation phytoplasma: An ultrastructural and biochemical study.- *Phytopathology*, 94: 203-208.

MUSETTI R., SANITÀ DI TOPPI L., MARTINI M., FERRINI F., LOSCHI A., FAVALI M. A., OSLER R., 2005.- Hydrogen peroxide localisation and antioxidant status in the recovery of apricot plants from European stone fruit yellows.- *European Journal of Plant Pathology*, 112: 53-61.

MUSETTI R., MARABOTTINI R., BADIANI M., MARTINI M., SANITÀ DI TOPPI L., BORSELLI S., BORGO M., OSLER R., 2007- On the role of H_2O_2 in the recovery of grapevine (*Vitis vinifera* cv. Prosecco) from 'flavescence dorée' disease.- *Functional Plant Biology*, 34: 750-758.

MUSETTI R., PAOLACCI A., CIAFFI M., TANZARELLA O. A., POLIZZOTTO R., TUBARO F., MIZZAU M., ERMACORA P., BADIANI M., OSLER R., 2010.- Phloem cytochemical modification and gene expression following the recovery of apple plants from apple proliferation disease.- *Phytopathology*, 100: 390-399.

RÜPING B., ERNST A. M, JEKAT S. B., NORDZIEKE S., REINEKE A. R., MÜLLER B., BORNBERG-BAUER E., PRÜFER D., NOLL G. A., 2010.- Molecular and phylogenetic characterization of the sieve element occlusion gene family in *Fabaceae* and non-*Fabaceae* plants.- *BMC Plant Biology*, 10: 219.

Corresponding author: Rita MUSETTI (e-mail: Rita.Musetti@uniud.it), Dipartimento di Scienze Agrarie e Ambientali Università di Udine, via delle Scienze, 208, Udine, Italy.