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Symptom expression and '*Candidatus* Phytoplasma prunorum' concentration in different *Prunus* species

Marta MARTINI, Paolo ERMACORA, Gabriele MAGRIS, Francesca FERRINI, Nazia LOI
Department of Agriculture and Environmental Sciences, University of Udine, Udine, Italy

Abstract

A SYBR® Green I real-time PCR assay has been used for specific detection and quantification of '*Candidatus* Phytoplasma prunorum' in different *Prunus* species such as *P. armeniaca*, *P. salicina*, *P. persica* and *P. tomentosa* grown in a screenhouse and infected by means of the vector. Infection level of '*Ca.* P. prunorum' in plant samples, expressed as '*Ca.* P. prunorum' GU per ng of plant DNA, was achieved by the standard curve quantification method choosing *rplV* (*rpl22*) gene as target for phytoplasma quantification and plant 18S rDNA to normalize the data. Visual inspections of the plants maintained in the screenhouse and qualitative molecular data demonstrated that the species *P. armeniaca* and *P. salicina* are the most susceptible and sensitive, that *P. persica* is less susceptible but quite sensitive and finally that *P. tomentosa* is less susceptible and quite tolerant. Quantitative molecular data for the first time demonstrated, for the first time, that '*Ca.* P. prunorum' titre increases during the vegetative season, and that symptom expression is correlated with its infection level.

Key words: ribosomal protein gene, SYBR® Green I real-time PCR, European stone fruit yellows, symptom expression.

Introduction

'*Candidatus* Phytoplasma prunorum' is associated with European stone fruit yellows, a quarantine phytoplasma disease present mainly in Europe and also in Turkey (Sertkaya *et al.*, 2005). Together with '*Ca.* P. mali' and '*Ca.* P. pyri', it belongs to a major ribosomal group, the apple proliferation (AP) phytoplasma group (16SrX) (Marcone *et al.*, 2010).

European stone fruit yellows (ESFY) is a destructive phytoplasma disease that has a wide range of host plants among cultivated and spontaneous stone fruits, which have large differences in terms of symptom expression and susceptibility (Marcone *et al.*, 2010). Detection of '*Ca.* P. prunorum' is carried out by molecular methods, and real-time PCR represents the most recent innovation for detection and quantification of phytoplasmas.

The present study is part of a wider project to improve knowledge about the dynamics of diffusion and colonization of ESFY on 13 different *Prunus* species grown in semi-controlled conditions (screenhouse) and infected by means of the vector. The species that resulted to be the most susceptible to ESFY in the experiment conditions were *P. salicina*, *P. armeniaca*, *P. persica* and *P. tomentosa*. The aims of the present work were to evaluate '*Ca.* P. prunorum' concentration in the four different *Prunus* sp. during the vegetative season and to verify a possible correlation between symptom expression and '*Ca.* P. prunorum' titre.

Materials and methods

Symptom expression had been monitored monthly during the vegetative season. In June 2009, for qualitative analyses, leaves were collected randomly from all plants of four species *Prunus salicina*, *P. armeniaca*, *P. persica* and *P. tomentosa* grown in a screenhouse. For quantitative analyses, plant material was collected in July and September from plants of the four species which gave positive results in the qualitative analysis. Total DNAs were extracted from phytoplasma infected plants using a CTAB extraction method modified (Martini *et al.*, 2009) from Doyle and Doyle (1990). The presence of '*Ca.* P. prunorum' in plants was assayed by qualitative '*Ca.* P. prunorum' specific real-time PCR assay (Martini *et al.*, 2007).

'*Ca.* P. prunorum' was quantified by SYBR® Green I real-time PCR as the number of '*Ca.* P. prunorum' genome units (GU)/ng of plant DNA according to Martini *et al.*, 2007. Ribosomal protein (rp) gene *rplV* (*rpl22*) and 18S rDNA were chosen respectively as targets for amplification of '*Ca.* P. prunorum' and plant DNA. A standard curve was established by 1:10 serial dilutions of a plasmid containing rp genes of LNp phytoplasma, starting from 1 ng/µl to 1 fg/µl in 20 ng/µl of total DNA from healthy periwinkle. To quantify plant DNA a standard curve was prepared with 1:10 serial dilutions of total DNA from a healthy plant of each of the four species starting at 50 ng/µl to 5 pg/µl. Statistical analyses were performed with the INSTAT GRAPHPAD software package using an ANOVA two-tailed.

Results

Results of symptom expression monitoring and qualitative analysis (June) are reported in table 1.

Table 1. Number of symptomatic and '*Ca.* P. prunorum' infected plants (*: mild symptoms).

Prunus sp.	Symptomatic plants	Phytoplasma positive plants
P. armeniaca	7/10	8/10
P. salicina	9/9	9/9
P. persica	2/9	3/9
P. tomentosa	3/10*	5/10

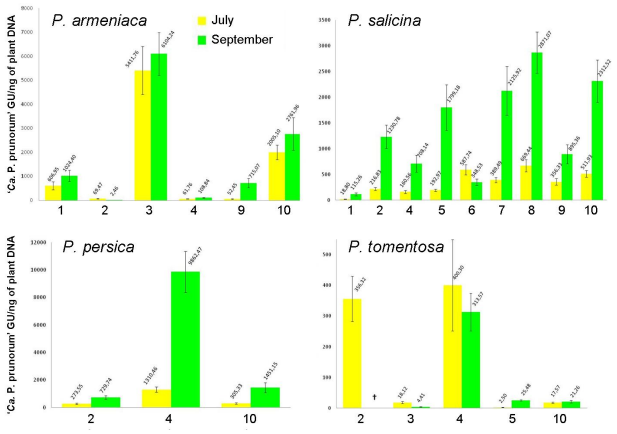

Figure 1. '*Ca.* P. prunorum' concentration (phytoplasma GU/ng of plant DNA) in each *Prunus* sp. plant in July and September.

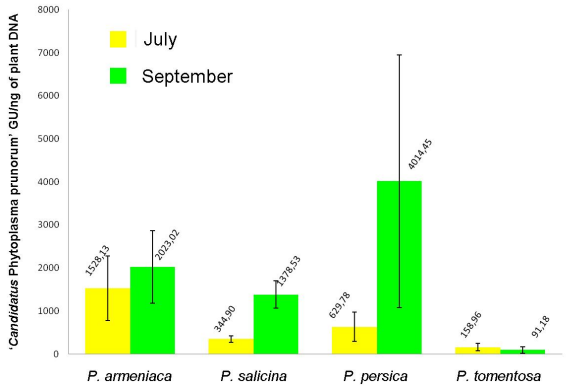

Figure 2. Comparison of the average of '*Ca.* P. prunorum' concentration (phytoplasma GU/ng of plant DNA) among the four *Prunus* sp. in July and September.

The four *Prunus* sp. showed the typical symptoms of the disease, even with slight differences in severity between species and between plants within the same species. Qualitative real-time PCR analysis demonstrated that the number of '*Ca.* P. prunorum' infected plants was higher than the number of symptomatic plants.

All data on '*Ca.* P. prunorum' infection level in July and September, obtained by quantitative real-time PCR analyses for each plant of the four *Prunus* species, are reported in figure 1. For the great majority of plants the phytoplasma concentration was higher in September than in July. Moreover, the phytoplasma concentration values in *P. salicina* appeared to be the most uniform.

Comparing the average of infection level of '*Ca.* P. prunorum' obtained from the four species (figure 2), the average differed significantly in September comparing to July respectively in *P. armeniaca* (1.3 times higher, P= 0.0337) and *P. salicina* (2.05 times higher, P=0.0064).

Discussion

In *P. armeniaca*, *P. salicina* and *P. persica*, but not in *P. tomentosa,* the infection level of '*Ca.* P. prunorum' increased during the vegetative season from July to September, when the plant metabolism is higher and the symptoms become more evident.

Regarding susceptibility, *P. armeniaca* and *P. salicina* appeared to be the most susceptible, whereas *P. persica* and *P. tomentosa* were the least. Concerning symptom expression the species *P. armeniaca*, *P. salicina* and *P. persica* demonstrated a higher sensitivity compared to *P. tomentosa*, which confirmed its tolerant behaviour. A higher *Prunus* sp. sensitivity correlated with a higher average of '*Ca.* P. prunorum' infection level; moreover plants of the four species, showing heavier symptoms in September correlated with the highest values of '*Ca.* P. prunorum' concentration in the same period. All these data demonstrated that, in our case, a correlation exists between symptom expression and phytoplasma concentration.

To our knowledge, it is the first time that quantitative molecular data have demonstrated how and how much the phytoplasma concentration changes during the vegetative season, and that symptom expression is correlated with phytoplasma infection level.

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Corresponding author: Marta MARTINI (e-mail: marta.martini@uniud.it), Department of Agriculture and Environmental Sciences, Via delle Scienze 208, 33100 Udine, Italy.