Bulletin of Insectology ***64*** *(Supplement): S49-S50, 2011*
ISSN 1721-8861

Nucleotide sequencing of *imp* gene in phytoplasmas associated to 'flavescence dorée' from *Ailanthus altissima*

Luisa FILIPPIN[1], Valentina DE PRA[1,2], Michela ZOTTINI[2], Michele BORGO[1], Elisa ANGELINI[1]
[1]*CRA-VIT Centro di Ricerca per la Viticoltura, Conegliano (TV), Italy*
[2]*Department of Biology, University of Padova, Padova, Italy*

Abstract

The molecular characterization of *imp* phytoplasma gene from 15 *Ailanthus altissima* plants infected by 'flavescence dorée' type 16SrV-C phytoplasmas revealed a high polymorphism of the pathogen in this tree in Northern Italy. Eleven different 'flavescence dorée' phytoplasma strains were identified in *A. altissima*, two of them having *imp* nucleotide sequence 100% identical to 'flavescence dorée' phytoplasmas found in plants of *Clematis vitalba* collected closely. This finding suggests a phytoplasma exchange between the two plant species.

Key words: clematis, epidemiology, grapevine.

Introduction

Ailanthus altissima (Mill.) Swingle (tree of heaven) is an invasive tree species, originally introduced from China, which arrived in Italy two centuries ago (Celesti-Grapow *et al*., 2009). Nowadays it is found ubiquitously in urban and rural areas not only in Italy, but also in the entire world, due to its efficient spreading.

Previous research works showed that in Italy it can be infected by phytoplasmas related to 'flavescence dorée' (FD) (Filippin *et al.*, 2008; 2010). FD is an epidemic disease of grapevine, mainly spread in Southern Europe, where it causes serious damages in vineyards and the quantitative and qualitative decrease of grape and wine production. FD and other related phytoplasmas have been identified also in alder (*Alnus glutinosa*) and in clematis (*Clematis vitalba*) (Angelini *et al.*, 2001; 2004; Filippin *et al.*, 2009).

The aim of this work was to understand the possible role of the tree of heaven in FD epidemics in Italy by means of molecular characterization of the FD strains in the *imp* gene, which is very polymorphic in the 16SrV subgroup phytoplasmas.

Materials and methods

One hundred and three leaf samples from *A. altissima* trees were collected in 2007-2010, mainly in Northern Italy (table 1). Each sample was made by a pool of leaves collected from different plants in the same restricted area. *C. vitalba* and *Vitis vinifera* plants growing close to the trees and showing phytoplasmas symptoms were sampled too.

DNA was extracted according to previously reported protocols (Angelini *et al.*, 2001). Real time and nested PCR/RFLP analyses on ribosomal genes were carried out in order to identify and characterize the phytoplasmas (Angelini *et al.*, 2001; 2007).

Phytoplasma *imp* gene from samples which tested positive was amplified by specific nested PCR (Da Rold *et al.*, 2010) and amplicons were double strand sequenced. Nucleotide sequences were compared with *imp* sequences from other FD phytoplasmas obtained in previous studies (Da Rold *et al*., 2010).

Table 1. *A. altissima* plants collected in the different geographical regions in Italy and number of samples found positive and negative for FD presence by molecular test.

Region	Province	PCR results	
		Positive samples	Negative samples
Friuli Venezia Giulia	Trieste	0	1
Friuli Venezia Giulia	Udine	3	9
Friuli Venezia Giulia	Pordenone	1	13
Venetia	Treviso	8	39
Venetia	Venice	0	4
Venetia	Vicenza	2	2
Venetia	Verona	0	1
Venetia	Rovigo	0	1
Lombardy	Milan	0	1
Lombardy	Brescia	0	1
Piedmont	Asti	0	1
Piedmont	Alessandria	1	0
Piedmont	Cuneo	0	1
Tuscany	Florence	0	1
Tuscany	Siena	0	2
Marche	Ancona	0	3
Latium	Rome	0	3
Latium	Frosinone	0	2
Apulia	Bari	0	3
Total		15	88

Results

Molecular analyses showed that 15 *A. altissima* samples out of 103 (about 15%) were infected with an FD-related phytoplasma (table 1). All infected trees were from Northern Italy, while the few plants collected in Central and Southern Italy were always FD-related phytoplasma-free. RFLP analyses on ribosomal genes showed that all detected phytoplasmas belonged to the ribosomal subgroup 16SrV-C, confirming previous preliminary data (Filippin *et al.*, 2010). No clear association between symptom and phytoplasma occurrence was observed.

The nucleotide sequencing of phytoplasma *imp* gene from *A. altissima* infected plants showed 11 different strains to be present, with a high degree of polymorphism among variants. The nucleotide identity among the most different strains was 69.4%, while the lowest aminoacid identity in the imp protein was 51.9%. None of the *imp* genetic variants found in *A. altissima* was 100% identical to other phytoplasma strains identified in alder and grapevine samples from the same geographic areas. In contrast, two FD-related phytoplasma strains from *A. altissima* collected in Friuli-Venezia-Giulia and Piedmont, respectively, showed the very same *imp* sequence found in FD-related phytoplasma infected clematis from the same areas.

Discussion

The finding of this study pointed out that phytoplasma exchange between clematis and the tree of heaven does exist. In contrast, the *imp* variants identified in *A. altissima* were similar but not identical to those found in grapevine. Further studies on a higher number of grapevine samples are needed to confirm these preliminary data.

The *imp* gene is demonstrated to be very useful as a molecular marker for epidemiology and traceability studies of FD-related phytoplasmas, due to its high polymorphism. Indeed, the sequencing of other less variable phytoplasma genes would have furnished less information useful for this study.

References

ANGELINI E., CLAIR D., BORGO M., BERTACCINI A., BOUDON-PADIEU E., 2001.- Flavescence dorée in France and Italy - Occurrence of closely related phytoplasma isolates and their near relationships to Palatinate grapevine yellows and an alder phytoplasma.- *Vitis,* 40: 79-86.

ANGELINI E., SQUIZZATO F., LUCCHETTA G., BORGO M., 2004.- Detection of a phytoplasma associated with grapevine 'Flavescence dorée' in *Clematis vitalba*.- *European Journal of Plant Pathology,* 110: 193-201

ANGELINI E., BIANCHI G. L., FILIPPIN L., MORASSUTTI C., BORGO M., 2007.- A new TaqMan method for the identification of phytoplasmas associated with grapevine yellows by real-time PCR assay.- *Journal of Microbiological Methods,* 68: 613-622.

CELESTI-GRAPOW L., PRETTO F., BRUNDU G., CARLI E., BLASI C., 2009.- *Plant invasion in Italy, an overview*, p. 22.- Palombi & Partner S.r.l., Roma, Italy.

DA ROLD G., FILIPPIN L., MALEMBIC-MAHER S., FORTE V., BORGO M., FOISSAC X., ANGELINI E., 2010.- The *imp* gene in 'flavescence dorée' and related phytoplasmas from grapevine, *Clematis* sp. and alder, p. 101. In: *18th International Congress of the IOM* (BROWN D. R., BERTACCINI A., Eds).- Chianciano Terme, Italy, July 11-16..

FILIPPIN L., BORGO M., LUCCHETTA G., MICHIELINI C., ANGELINI E., 2008.- Characterization of phytoplasmas in spontaneous plants in vineyard.- *Petria,* 18(2): 222-223.

FILIPPIN L., JOVIĆ J., CVRKOVIĆ T., FORTE V., CLAIR D., TOŠEVSKI I., BOUDON-PADIEU E., BORGO M., ANGELINI E., 2009.- Molecular characteristics of phytoplasmas associated with 'Flavescence dorée' in clematis and grapevine and preliminary results on the role of *Dictyophara europaea* as a vector.- *Plant Pathology*, 58: 826-837.

FILIPPIN L., BORGO M., ANGELINI E., 2010.- Identification of FD phytoplasma in plants of *Ailanthus altissima* in Italy, p. 14. In: *Current status and perspectives of phytoplasma disease research and management* (BERTACCINI A., LAVIÑA A., TORRES E., Eds).- Sitges, Spain, February 1-2.

Corresponding author: Elisa ANGELINI (e-mail: elisa.angelini@entecra.it), C.R.A. Centro di Ricerca per la Viticoltura, Viale XXVIII aprile 26, Conegliano (TV), Italy.