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Occurrence and diversity of phytoplasmas detected in clematis and their relationships with grapevine "flavescence dorée" phytoplasmas

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Abstract

A three-year survey conducted in Italy and in some of the Balkan countries to study grapevine yellows epidemiology showed that clematis plants were positive for phytoplasmas of the 16SrV ribosomal group that in grapevine is associated with "flavescence dorée" (FD), a quarantine disease. Molecular data from 16Sr RNA, *rpL22-S3* and *SecY* genes showed the presence of different FD phytoplasma isolates belonging to the 16SrV-C ribosomal subgroup. Infected clematis plants were found in areas where FD phytoplasma has never been detected in grapevine. Among all leafhoppers collected, only *Dictyophara europaea* were PCR-positive to the FD phytoplasma.

Key words: clematis, "flavescence dorée", grapevine.

Introduction

Clematis vitalba L. is a vine-like plant growing in fields, in underbrush and in woody areas of many countries worldwide. Recently, a "flavescence dorée" (FD) phytoplasma isolate was found in clematis plants in north-eastern Italy (FD-C type) (Angelini *et al,* 2004). FD-C is one of the phytoplasma types formerly identified in grapevine and associated with grapevine "flavescence dorée", which is a quarantine pest in Europe. Other FD phytoplasmas, all belonging to 16SrV-C and D subgroups, are present in grapevine in Europe, but so far none of them have ever been identified in other wild or cultivated plants except grapevine (Martini *et al*., 2002).

The aims of this study were: i) to verify the occurrence and geographic distribution of clematis plants positive for FD phytoplasmas in Italy and in some of the Balkan countries; ii) to molecularly characterize the isolates from clematis and compare them with grapevine phytoplasmas; and iii) to identify new potential insect vectors.

Materials and methods

A total of 349 clematis leaf samples were collected from 2004 to 2006 in northern and central Italy and in some Balkan countries (table 1). Leaves of symptomatic grapevines were collected from the same areas. Leafhoppers present in FD-infected clematis and grapevine plants were captured by sweep net, maintained in alcohol during the summer and subsequently classified.

DNA was extracted and amplified by nested PCR from plants and insects according to previously reported protocols (Angelini *et al,* 2001; Martini *et al,* 2002). PCR/RFLP assays were carried out using primers that amplify: i) the 16S-23S ribosomal rRNA genes; ii) the genes coding for ribosomal proteins L22 and S3; and iii) the FD9 region coding for SecY translocase protein. RFLP analyses were performed with *Taq*I, *Mse*I, *Alu*I, *Hpa*II and *Hpy*188I endonucleases. Nucleotide sequences in the *secY* gene were obtained for 11 clematis plants, 4 grapevines and 3 insects and compared with phytoplasma sequences from GenBank.

Results

Clematis plants positive for the FD phytoplasma were found in almost all the areas investigated (table 1). In total, 36% of the samples were positive using the PCR test and this included clematis plants in geographic regions where FD has never been reported, such as Macedonia, Croatia and some areas of central and north-eastern Italy.

Symptoms in phytoplasma positive clematis plants included a yellowing and reddening of leaves, but their association with phytoplasma presence was not always established (77%). A statistical analysis showed that the percentage of phytoplasma positive clematis plants was similar in vineyard areas and in areas far away from vineyards.

RFLP analyses used show that all clematis phytoplasmas belonged to the 16SrV-C subgroup. RFLP patterns identical to those of the FD-C isolate were present in almost all the positive plants. Some clematis samples showed peculiar RFLP patterns in the *secY* gene; nucleotide sequencing allowed to detect in these isolates nucleotide insertion and deletion.

Further sequence analyses revealed that clematis and grapevine plants from central Italy were positive for an isolate slightly different from FD-C. Isolates from clematis and grapevine in Serbia had a variation in the *secY* gene. A FD phytoplasma isolate identified in French

Table 1. Geographic origin and number of clematis samples analyzed, together with PCR results.

Country	Region	PCR results	
		N. of positive samples	N. of negative samples
Italy	Piedmont	9	3
Italy	Lombardy	3	8
Italy	Trentino Alto Adige	0	9
Italy	Veneto	52	92
Italy	Friuli Venezia Giulia	14	14
Italy	Liguria	0	1
Italy	Emilia Romagna	1	1
Italy	Umbria	2	0
Italy	Latium	0	1
Italy	Tuscany	4	5
Slovenia	Obalno-K.	1	0
Croatia	Istria	4	0
Croatia	Lika/Senj	0	1
Serbia	Nišavski	16	65
Serbia	Rasinski	8	6
Serbia	Beogradski	1	0
Serbia	Šumadijski	2	3
Serbia	Braničevski	0	4
Serbia	Borski	0	5
Serbia	Jablanički	3	2
Serbia	Pčinjski	0	1
Serbia	Sremski	0	1
Macedonia	Stip	3	0
Macedonia	Negotino	1	1
Macedonia	Kavadarci	2	0
TOTAL		126	223

grapevines (Arnaud *et al.*, 2007), but not yet detected in Italy, was present in five clematis plants collected in north-western Italy.

A total of 20 insect species were collected. Preliminary molecular analyses led to the identification of FD-C phytoplasma in *Dictyophara europaea* so additional specimens of the leafhopper were collected and tested. Almost 5% of the specimens contained the phytoplasma: 7 samples out of 133 collected in northern Italy (Veneto) and 7 out of 154 collected in Serbia. *D. europaea* specimens were always positive for an isolate undistinguishable at molecular level from those present in clematis and grapevine plants of the region where they were collected.

Discussion

FD phytoplasma isolates in the clematis plants analyzed in this work were not homogeneous at the molecular level, though all of them belonged to the 16SrV-C subgroup. The spatial distribution of FD phytoplasma isolates was mainly dependent on the environmental conditions.

A relationship between FD phytoplasmas present in clematis and in grapevine plants was found, which suggested a possible transmission of FD phytoplasmas from one species to the other. An exception was the FD phytoplasma isolate identified in clematis plants from Piedmont, which has never been identified in Italian grapevines so far and showed 100% nucleotide identity with a French isolate from Savoie (Arnaud *et al.*, 2007). Sequencing of a wider number of samples from all surveyed areas is needed to confirm the association between FD phytoplasma isolates in clematis and in grapevine plants.

The fact that in some regions the FD phytoplasma was present in clematis but it was not detected in grapevine, or only erratically, suggests that the original host of the FD-C isolate in Europe could be *C. vitalba*.

Because of the inability of *Scaphoideus titanus,* the known vector of FD, to transmit FD-C isolate from and to clematis (E. Boudon-Padieu, pers. comm.) and its short survival time on clematis (Toševski and Forte, unpublished data; E. Boudon-Padieu, pers. comm.), we looked for other potential vectors, more strictly associated with clematis. The percentage of infected *D. europaea* specimens in the field was notable and pointed out the possible role of this insect as vector, although no transmission trials have been carried out so far. In addition, this species was consistently captured on clematis plants and in vineyards, both in northern Italy and Serbia.

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corrigenda

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Page 327 in the 2nd column, paragraph 4 of results, lines 3-5 the sentence:

"Isolates from clematis and grapevine in Serbia had a variation in the *secY* gene."

has to be deleted.