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Molecular diversity of 'flavescence dorée' phytoplasma strains in Slovenia

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Abstract

The 'flavescence dorée' (FD) phytoplasma is associated with the most devastating grapevine yellows disease 'flavescence dorée'. It belongs to the ribosomal group16SrV in which a high 16SrDNA identity among subgroup members exists. To investigate the genetic diversity of this phytoplasma in Slovenia a large analysis of strains in known and possible hosts and vectors was performed. The genotyping has revealed the occurrence of FD strains FD1, FD2 and FD3. Although isolates of FD2 and FD3 were detected in grapevines, those of FD2 prevailed. On the other hand, symptomatic as well as asymptomatic clematis (*Clematis vitalba*) plants were exclusively infected with FD3. Strains undistinguishable from FD1, FD2, FD3 and alder yellows were also detected in *Alnus glutinosa* and *A. incana*. In some alder samples mixed infections with different strain combinations were also demonstrated. FD2 strain was shown in the leafhopper vector of the disease *Scaphoideus titanus*. The FD1 strain was detected in the mosaic leafhopper *Orientus ishidae*. In addition, various specimens of this leafhopper were positive in PCR with a high presence of mixed infections.

Key words: 'Flavescence dorée', genetic clusters, *Vitis vinifera*, *Clematis vitalba*, *Alnus glutinosa*, *Alnus incana, Scaphoideus titanus, Orientus ishidae*, *Oncopis alni*.

Introduction

Europe is the world's major producer and exporter of grapevine (*Vitis vinifera*) cuttings and wine. This important economic segment is currently facing an epidemic threat associated with the 'flavescence dorée' disease, which has been known in France since the middle of the 1950s (Boudon-Padieu, 2002). The disease spread to other European countries and was detected for the first time in Slovenia in 2005. Since then the number of *foci* of the disease in Slovenia has been growing rapidly.

The agent associated with 'flavescence dorée' is FD phytoplasma, which is transmitted among grapevines by insects. The only known natural vector of FD is the leafhopper *Scaphoideus titanus*. However, some other leafhoppers e.g. *Orientus ishidae* (Mehle *et al.*, 2010) and *Dictyopara europea* (Filippin *et al.*, 2009) have been shown to harbour FD phytoplasmas. The latter was also shown to transmit FD from clematis (*Clematis vitalba*) to grapevine. The alder yellows phytoplasma (AldY), which is genetically related to FD, has been detected in more than 85% of alder trees in SW France (Malembic-Maher *et al.,* 2009). These findings support the hypothesis that these plants might be a natural source of FD. FD belongs to the ribosomal group 16SrV with high 16SrDNA identity among the subgroups (Lee *et al*., 2004). The phylogenetic analysis has shown the existence of three genetic clusters FD1, FD2 and FD3 (Arnaud *et al*., 2007, Filippin *et al.*, 2009). AldY strains do not form a homogenous phylogenetic group but are distributed in every cluster, except FD3 (Malembic-Maher *et al*., 2009). In order to study the prevalence and distribution of FD strains in Slovenia a molecular comparison of FD strains involved in former outbreaks in Slovenian vineyards, and strains from clematis and alder plants as well as from *Scaphoideus titanus*, *Orientus ischidae*, and *Oncopis alni* was carried out.

Materials and methods

Under the official survey of the Phytosanitary Administration of the Republic of Slovenia, 1,679 symptomatic grapevine (*V. vinifera*) samples were collected from 2002 to 2010. They were sampled in the vineyards from the three Slovenian winegrowing regions - SW Primorska, SE Posavje and NE Podravje. In addition, clematis, *Alnus glutinosa* and *A. incana*, as well as insect specimens of *S. titanus*, *O. ishidae* and *O. alni* were also collected. All samples were analyzed for the presence of FD with a real-time PCR procedure (Hren *et al.*, 2007). Further molecular characterization was than performed on the positive samples by PCR with FD9R1/FD9F1 primers followed by nested PCR with FD9F3b/ FD9R2 primers and RFLP. The purified nested PCR products were cloned into pGEM-T vector and sequenced.

Results

From tested grapevine samples, 123 were proved to be FD positive and 86 of them were further analyzed. Amplicon digestion with the restriction enzymes *Hpa*II, *Taq*I or *Alu*I revealed that the genetic cluster FD2 represented the 66.3% of all analyzed grapevine samples and that FD3 occurred in the 31.4% of the samples. We did not detect strains in the FD1 cluster in any grapevine sample. However, 13 dubious samples are still under investigation. From 69 tested clematis samples, 49 were FD positive and all of them had the FD3 profile. The incidence of FD infection in alder trees was very high and mixed infections were frequent. In alders all three genetic clusters were detected, as well as the AldY one (table 1).

The RFLP pattern of sample from *S. titanus* was identical to those of the reference FD92 from the genetic cluster FD2. Mixed infection in various samples from

Table 1. Distribution of FD clusters in plants and insects in Slovenia. Frequency of occurrence (%) in brackets; OWG, out of wine growing region.

	FD1 (%)	FD2 (FD-D) (%)	FD3 (FD-C) (%)	AldY (%)	Winegrowing region
Vitis vinifera	0	57 (66.3)	16 (31.4)	0	SW, SE, NE
Clematis vitalba	0	0	49 (100)	0	SW, SE, NE, OWG
Scaphoideus titanus	0	1 (100)	0	0	SE
Alnus glutinosa and *A. incana*	3 (16.7)	7 (38.9)	7 (38.9)	1 (5.6)	OWG
Orientus ishidae	3 (25.0)	6 (50.0)	3 (25.0)	0	OWG
Oncopis alni	1 (14.2)	0	3 (42.9)	3 (42.9)	OWG

O. ishidae and *O. alni* were common. However, the sequence analysis from the former demonstrates the presence of FD1, FD2 and FD3. In the latter the FD2 cluster has not been confirmed, but AldY was present (table 1).

Discussion

The molecular analysis of FD strains in Slovenia showed a high diversity of genetic clusters and their distribution among host plants, and established, as well as putative, insect vectors. As in France (Salar *et al*., 2009) the genetic cluster FD2 was the prevailing one in grapevine and was not related to any specific wine-growing region. The strains belonging to FD3 were detected in about one-third of the grapevine samples and were exclusively present in FD infected clematis plants. Clematis samples were collected in the vicinity of the FD infected vineyards, but also in those where FD had been never confirmed or even outside the winegrowing regions. Some of the infected clematis plants showed the symptoms, but the frequency of totally asymptomatic but infected plants was also very high. The collective evidence of FD3 distribution among clematis plants in Italy, Croatia and Macedonia (Filippin *et al*., 2009) as well as in Slovenia suggests that this plant species might constitute a wild reservoir of FD3 strains. High incidence of FD strains in alder trees in an agreement with the situation in France (Malembic-Maher *et al*., 2009) was detected. Alders hosted phytoplasmas from clusters FD1, FD2 and FD3 and AldY. Phytoplasmas from the same clusters with the exception of FD2 were detected in *O. alni*, the insect which has been confirmed as vector of Palatinate grapevine yellows (Maixner *et al*., 2000). FD was only detected in one out of 57 tested samples of *Scaphoideus titanus*. However, the strain belonged to the FD2 cluster. On the other hand, in the polyphagus leafhopper *O. ishidae*, the presence of both FD1 and FD2 was confirmed (Mehle *et al*., 2010). To shed light on the role of *O. ishidae* in the possible transmission of FD from reservoir host plants, or from grapevine to grapevine research is in progress.

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