

O27. Flavescence dorée phytoplasma in northern Swiss vineyards is anthropogenic

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INTRODUCTION

Flavescence dorée (FD) is a quarantine disease associated with Flavescence dorée phytoplasma (FDp), which is mainly transmitted by the leafhopper *Scaphoideus titanus* Ball (Schvester et al., 1961). In 2015, the first two outbreaks of the disease were detected in canton of Vaud (Switzerland). Since then, seven additional foci have developed within a 30 km radius, leading to the uprooting of 1,6 ha of vineyard. In order to better understand the introduction and dissemination of the phytoplasma in the region, more than 660 positive samples from all outbreaks were genetically characterized using both known and newly identified variable *loci* allowing for a better study of FD epidemiology.

MATERIALS AND METHODS

Plant material (leaves) was collected from 2015 to 2022 during annual phytosanitary inspections. Total nucleic acids (TNA) from twelve plants representative of the different outbreaks were extracted using CTAB protocol (Anses) and sent to Macrogen for Illumina sequencing. Reads were assembled to FDp reference genome (Debonneville et al., 2022) with Bowtie2. SNPs were detected using Geneious 2023.0.4 and confirmed by Sanger sequencing.

TNA from symptomatic samples were extracted with the same CTAB protocol and presence of FDp was tested by quantitative PCR (qPCR) as previously described (Pelletier et al., 2009). Genetic characterization was done by multilocus sequence typing. *Loci* were amplified by PCR, products were sequenced forward and reverse using Sanger technology, sequences were *de novo* assembled and consensus were aligned using MUSCLE algorithm.

RESULTS AND DISCUSSION

From 2015 to 2022, samples of 2924 grapevines showing symptoms of yellowing were analyzed by qPCR. 28,8 % were found positive to FDp and 46,8 % to Bois noir phytoplasma.

A selection of representative samples from different outbreaks and years was used for multilocus genotyping. PCR and Sanger sequencing were done on 135 *map* samples, 77 *dnaK*, 648 *malG* and 43 *vmpA*. These *loci* were chosen for their proven variability of 3 to 12 SNPs per region as reported in literature (Arnaud et al., 2007; Rossi et al., 2019).

Nevertheless, the only gene showing variability in our samples was *malG*, with four SNPs. Indeed, all samples were infected by M54, *dnaK1* and cluster II *vmpA* genotypes. Two different *malG* genotypes were found and they were spatially correlated, *i.e.* the two *malG* genotypes were geographically gathered (four outbreaks of *malG* G3/G3 and five of *malG* G1/G3). However, these markers were not considered to be sufficient to trace FDp epidemic as they could not differentiate strains from the different outbreaks within the same *malG* genotype. Therefore, we used Illumina sequencing of samples from different *foci* with the same *malG* genotype to search for hypervariable genomic regions that are likely to discriminate among genetically similar strains. Two *loci* with one SNP each were detected in genes *ftsH5* and *ftsH17* in the Illumina datasets corresponding to *malG* G1/G3. The combination of these two SNP rolled out three different genotypes inside the *malG* G1/G3 group, leading to the discrimination of samples 100 m apart within the same outbreak. Nonetheless, no SNP was detected among Illumina datasets with *malG* G3/G3 genotypes. As a result, four different genotypes of FDp were found (Table 1), all of them in at least two non-contiguous plots.

Table 1 Different genotypes of FDp found in canton of Vaud.

Genotype	Gene					
	<i>map</i>	<i>dnaK</i>	<i>VmpA</i>	<i>malG</i>	<i>ftsH5</i>	<i>ftsH17</i>
A	M54	dnaK1	vmpAII	G1/G3	C	C
B	M54	dnaK1	vmpAII	G1/G3	C	G
C	M54	dnaK1	vmpAII	G1/G3	A	G
D	M54	dnaK1	vmpAII	G3/G3	C	C

Data suggest that FDp was initially introduced with planting material and progressively spread at the vineyard level through *Scaphoideus titanus*, which is present in Vaud since at least 2006. Actually, M54 is known to infect only grapevines, *S. titanus* and *Orientalus ishidae* (Malembic-Mahler et al., 2020; Casati et al. 2017). Indeed, the facts that M54 has never been found in other plant hosts and that *O. ishidae* (secondary vector) is extremely rare in the region (C. Linder personal communication) led us to exclude the landscape as a source of FDp in Vaud. Therefore, FDp outbreaks in Swiss vineyards in the north of the Alps are anthropogenic. This contrasts with the situation in the south of the Alps (Canton of Ticino), where landscape act as a reservoir of the disease (Oggier et al., 2023).

In conclusion, this work identified two additional hypervariable regions allowing to discriminate within G1/G3 haplotype in Vaud. It also suggests that in northern Swiss vineyards FDp was introduced *via* planting material without any influence from the environment. Thus, data highlight the importance of planting healthy vegetal material and therefore emphasize the need to use hot water treatment to provide phytoplasma-free grapevines.

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