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Towards understanding the mechanisms of resistance to grapevine Flavescence dorée

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Abstract. Flavescence dorée (FD) is a very serious grapevine disease, classified as quarantine in Europe, where it appeared in the middle of the last century. It is associated with the presence of phytoplasmas, transmitted in the vineyard by a leafhopper of American origin, *Scaphoideus titanus*. FD causes severe wine production losses and often leads to plant death. There are currently no alternative solutions to insecticide treatments against the vector and uprooting of diseased vines. This paper summarizes the research conducted by CREA in the recent years to understand the grapevine mechanisms of susceptibility and resistance to FD and to identify innovative and more sustainable solutions to control FD. On the one hand, studies involving the observation of symptoms in the field, the presence, and the movements of the phytoplasma in different plant organs, and the comparison of transcriptomic and metabolic responses between highly susceptible and more resistant varieties revealed interesting mechanisms associated with these characteristics, and clearly different among varieties. In parallel, a vine population has been created by crossing susceptible and resistant varieties, which is currently being both genotyped and phenotyped in the vineyard, with the aim of identifying the genetic basis of the resistance. The results of these studies can be exploited for a more durable and sustainable vineyard management in the future.

1. Introduction

Flavescence dorée (FD) of grapevine is a strongly epidemic quarantine pest in the European Community, transmitted by the leafhopper *Scaphoideus titanus.* The disease is caused by specific phytoplasmas, classified in the phylogenetic group 16SrV [1]. They are phloem obligate parasites, very difficult to cultivate in controlled conditions.

FD leads to very serious damages to grape and wine industry, ranging from a lower yield of berries to plant death. The current effective strategies to control and limit FD epidemics are based on insecticide treatments against the vector and uprooting of the infected plants. However, in a view of a more sustainable agriculture, they should be associated with modern approaches, such as the elicitation of grapevine defences or / and the selection of resistant plant material.

Intraspecific differences in susceptibility to FD have already been observed both from field experience and in controlled conditions [2]. Indeed, some varieties, such as Chardonnay and Pint gris, show very serious damages when infected by FD, while most American rootstocks and very few European varieties, such as Tocai Friulano and Moscato bianco, possess resistance or tolerance to FD and/or to its vector.

In the present work we reported our studies of the last 5 years on this topic: 1) a comparative early transcriptomic profiling among Chardonnay and T. friulano in *in vitro* plantlets inoculated or not with FD [3]; 2) a field, metabolic and gene expression study on the defence strategies in T. friulano [4]; 3) the phenotyping of part of a F1 crossing population among Chardonnay and T. friulano [5]; 4) the creation of grapevine genetic linkage maps to find out quantitative trait *loci* (QTL) responsible for resistance and susceptibility to FD [6].

2. Comparative early transcriptomic profiling among Chardonnay and Tocai friulano revealed unique and very different plant responses induced by FD infection

In this work, RNA-Seq was used to compare early transcriptional changes occurring during the interaction between the FD phytoplasma, its vector *S. titanus* and the grapevine plants belonging to two different cultivars, one very susceptible to the disease (Chardonnay) and the other partially resistant (T. friulano).

The study was carried out with *in vitro* micro-propagated vine plants, where healthy or infective *S. titanus* individuals were caged for 3 days to inoculate the phytoplasma. The transcriptomic profiles were compared between the two experimental theses and the control plants without vectors after 3- and 6-days.

The comparison of the constitutive transcriptomic profiles highlighted that the two grapevine cultivars possess very different genetic background, and suggested the existence of passive defence strategies against the insect and/or the phytoplasma in T. friulano. Indeed, higher expression of many genes associated to known defence mechanisms, such as those encoding for putative microbe-associated molecular pattern (MAMP) receptors, PR (pathogen-related) -proteins, the metabolism of jasmonic acid (JA) and phenylpropanoids, was clearly evidenced in this variety at the basal constitutive status.

The attack of the infective vector prompted in T. friulano early and substantial transcriptomic changes, which lead to the rapid erection of active defence, above all the remodelling of the cell wall. On the opposite, in Chardonnay, the most susceptible variety, the response was delayed and mainly consisted in the induction of phytoalexin synthesis.

Finally, the healthy insect feeding activated in both cultivars a fast defence reaction mediated by the jasmonic acid and ethylene, that was surprisingly not detected in Chardonnay in presence of the phytoplasma-infected vector (Fig. 1). This suggests that the phytoplasma is able to decrease, silence and block the Chardonnay defence mechanisms.

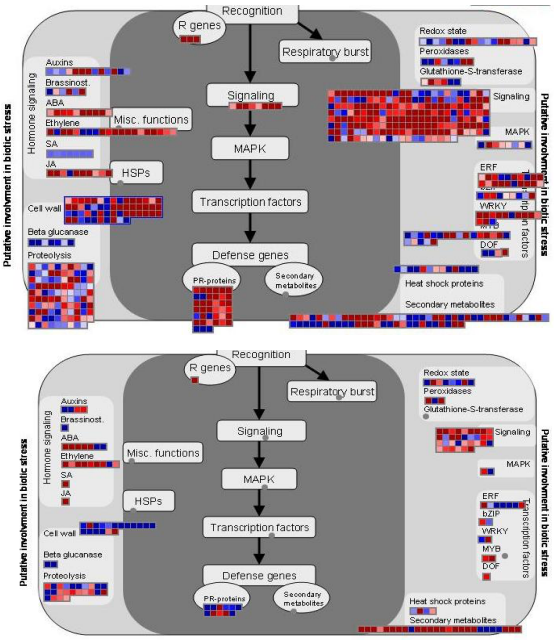

Figure 1. Transcriptomic response of Chardonnay to the feeding of the vector *Scaphoideus titanus* after 3 days, represented by MapMan. Above: feeding by healthy insects: many defence patterns are activated compared to the control. Below: feeding by the infective insects: most of the defence patterns activated above are now repressed. Figure from [3].

3. A successful defence strategy in T. friulano provides compartmentation of grapevine FD phytoplasma

In this work, the mechanisms occurring within T. friulano FD-infected plants were investigated in a 3-year field experiment, in order to identify the phytoplasma distribution in the plant and the metabolic pathways involved in the defence strategy to counteract the phytoplasma spread. Visual observation of symptoms, FD diagnosis in several parts of the plants, gene expression studies and metabolite analyses were carried out.

In T. friulano FD-infected plants the symptoms remained enclosed near the area where they appeared at the beginning of the season in the first year after the inoculation, while in a close Pinot gris vineyard they spread to all the canopy along the time.

PCR focused to detect the presence of FD phytoplasma in the wood showed that in T. friulano symptomatic plants the phytoplasma was totally absent in the secondary phloem of the trunk during all the year. Moreover, though it could be present in July in the 2-years old arms close to the symptomatic leaves, it disappeared in November. On the opposite, in Pinot gris, which is a very susceptible variety, the FD phytoplasma was identified in the trunk and in 2-years old arms, with a higher titre than in T. friulano (Figure 2).

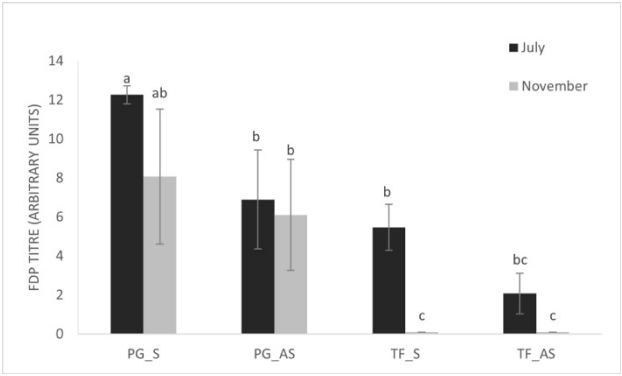

Figure 2. Titre of Flavescence dorée phytoplasma in symptomatic (S) and asymptomatic (AS) parts of the 2-years old arms in Tocai friulano (TF, more resistant cv) and Pinot gris (PG, more susceptible cv). Each value represents the mean ± SE of 5 to 10 biological replicates. Significant differences are indicated by different letters (Student Newman Keuls test, $P \leq 0,05$). Figure from [4].

Within T. friulano FD-infected plants, different modulation of defence genes and accumulation of defence metabolites were demonstrated in 1-year canes with or without FD phytoplasma presence and symptoms.

Indeed, symptomatic portions showed strong activation of salicylate-mediated responses, with higher expression levels of *PR1, PR2, PR5,* and *WRKY70* genes. Similarly, also the JA pathway was triggered, with *LOX, AOS, PR4,* and *PR6* genes more expressed than in asymptomatic canes. Moreover, a greater accumulation of trans-resveratrol was detected in symptomatic canes.

On the opposite, asymptomatic 1-year cane portions close to symptomatic ones showed activation of

jasmonate-mediated response and a higher content of ε-viniferin, the active form of resveratrol.

The polyphenol pathway showed that the genes coding for stilbene synthase 1 and 48 were more expressed in the 1-year canes, symptomatic or not, growing from the FD-infected 2-years old arms.

In conclusion, probably the activation of the jasmonate-mediated response and the increase of ε-viniferin in the asymptomatic 1-year cane portions close to symptomatic ones are the key mechanisms to contain and limit the spread of the pathogen and the disease within the T. friulano plants.

4. Phenotyping of a F1 crossing population among Chardonnay and T. friulano

In 2011 and 2017, Chardonnay was crossed with T. friulano producing an F1 offspring of over 600 individuals, which were grafted, multiplied, and finally planted in vineyard with 24 plants per individual, randomly distributed. The aim was to obtain a segregant population for studying the resistance and susceptibility traits and identifying the QTL (Quantitative Trait *Loci*) associated to these features. During 2019 and 2020 growing seasons, thousand *S. titanus* were used to infect the plants, and in the following 3 years phytosanitary surveys were carried out in the field, together with genotyping (see chapter 5).

In this work we report the visual observations of the symptoms, carried out in July and November, and the phytoplasma titre on a part of this population.

Seven F1 genotypes that displayed an intermediate level of disease severity between T. friulano and Chardonnay were selected for the measurement of the mean relative FD phytoplasma titre in the trunk of the infected plants using qPCR. The results showed that the severity of the infection was positively correlated with the titre of the bacterium (Figure 3).

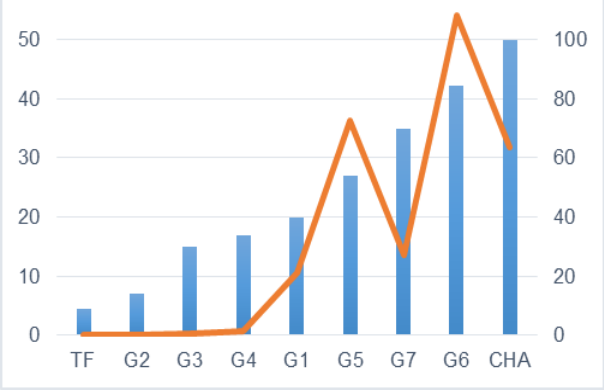

Figure 3. Severity of Flavescence dorée disease (bars, in percentage) and mean Flavescence dorée phytoplasma titre (line) evaluated on the trunk of seven F1 individuals (named G1 – G7) and the two parentals (TF, Tocai friulano and CHA, Chardonnay).

Indeed, as disease severity increased, a higher FD phytoplasma titre was detected, while F1 individuals that exhibited low disease severity hosted in general a lower titre of the pathogen, similarly to T. friulano.

In field in July the following feature were evaluated: plant vigour; symptom severity on canopy; rubberiness, presence of necrotic spots and absence of leaves on symptomatic canes; downward rolling and yellowing on symptomatic leaves. A robust negative correlation was observed between vigour and symptom severity in all the 3 years ($p<0.01$), while vigour was negatively and significatively correlated with downward rolling of the leaves and rubbery consistency only in some years.

In September the following features were evaluated: symptom severity on canopy; lignification; number of symptomatic, dried, and leafless canes; desiccation of cane apexes. A strong positive correlation was always observed between symptom severity and the number of symptomatic canes ($p<0.001$), which confirmed the validity of the observation. Interestingly, non-lignified canes were always negatively and significatively correlated with dried canes ($p<0.05$). Other correlations were significative only in one year: for example, in 2021 the percentage of non-lignified canes was positively corelated with symptom severity ($p<0.01$) and percentage of symptomatic canes ($p<0.05$).

In conclusion, useful phenotype traits associated to FD disease resistance and susceptibility were identified, and a high variability of these traits was observed in the F1 population, which is a promising basis for the QTL identification.

5. A grapevine genetic linkage map to find out quantitative trait *loci* (QTL) responsible for resistance and susceptibility to FD

The aim of this work was the genotyping of the F1 individuals of the same crossing population previously described (chapter 4), in order to identify genetic markers randomly distributed in the genome which are useful to create a linkage map.

At first a screening with SSR (Simple Sequence Repeats) was carried out using the DNA extracted from the F1 population samples to identify self-fertilized individuals, that were thus discarded. A total of 184 selected individuals and the two parents were then genotyped by GBS (Genotyping by Sequencing) [7] and data were subjected to bioinformatic analyses.

The GBS analysis produced 472 million reads, with an average read pair count per sample of 2.4 million, and 122,049 polymorphisms were found. Nineteen individuals were discarded due to their high proportion of missing data. SNPs (Single Nucleotide Polymorphisms) were filtered to obtain a high-quality dataset of polymorphic markers, and finally 8,556 SNPs and ten SSRs were retained and used for building the linkage map.

The *consensus* linkage map displayed 2,923 markers, 201 being co-localized. All markers were adequately grouped and ordered in the expected 19 linkage groups. The total length of the map was 1336.05 cM. SNP density among the 19 chromosomes was variable (Figure 4).

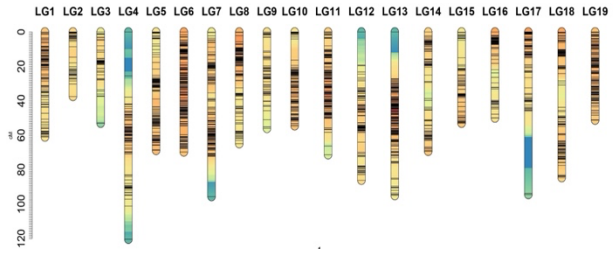

Figure 4. High-density *consensus* map of F1 population (*Vitis vinifera* 'Chardonnay' x 'Tocai friulano'). The X-axis represents the 19 grapevine linkage groups, while the Y-axis represents the genetic position of SNPs and SSR. Different colours correspond to SNP density.

Almost all chromosomes included a region with a higher presence of markers, while the largest lack of markers was in chromosome 2.

The total number of markers, the cumulative length of the map, and the mean interval between *loci* showed comparable results with the densest maps published so far, suggesting successful clustering and sorting of the high-quality SNP dataset. In future, the *consensus* map, together with the parental genetic maps of Chardonnay and T. friulano cultivars, obtained from the same GBS data, will be used for QTL identification.

6. Conclusions and perspectives

All the reported research activities have a common aim, *i.e.* to try to understand and unravel the metabolic, physiologic, and genetic mechanisms responsible for the susceptibility and resistance of grapevine varieties to FD disease.

The results obtained so far have shown that T. friulano, a partially resistant cultivar, possess specific features that enable the plant to successfully react to FD attacks, both by means of passive and active defence responses. Moreover, once the plant is infected, other peculiar reactions allow the compartmentation of the phytoplasma and, thus, avoid its spread all over the plant. On the opposite, the response defence of the very susceptible Chardonnay variety has been demonstrated to be manipulated by the phytoplasma itself, which provides signals that allow for repression of the JA/ET-mediated response reaction induced by the vector feeding.

Studying the segregant F1 population derived from the two varieties with opposite behaviour should allow us to uncover the QTLs associated to these interesting features. Genotyping and phenotyping of the F1 individuals are ongoing, and are foreseen to last other years, due to the pluriannual nature of grapevine and to the impossibility to cultivate and inoculate the pathogen in controlled conditions.

Though it could be a long way, the identification of the resistance genes and mechanisms and their use to obtain less susceptible grapevine plants could be one of the means to overcome the widespread and extensive damages caused by FD, especially nowadays that more sustainable strategies for grapegrowers and the environment are needed.

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