Insights into physiological responses of different grapevine varieties to Flavescence Dorée infection: an integrated approach

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INTRODUCTION

Flavescence dorée (FD) is a severe and widespread quarantine disease that affects grapevines in various European viticultural regions (Boudon-Padieu *et al.*, 2002). It is caused by a phytoplasma (FDp), and grapevine varieties exhibit different levels of susceptibility to the disease. Cabernet Sauvignon (CS) is highly susceptible whilst Merlot (M) is less susceptible (Eveillard *et al.*, 2016). Previous studies have shown that phytoplasma infections lead to significant transcriptomic and metabolomic changes in grapevines (Bertazzon *et al.*, 2019; Dermastia, 2019; Margaria *et al.*, 2014; Prezelj *et al.*, 2016). The purpose of this study is to better decipher the differences of response to FDp infection of various grapevine cultivars, and identify biomarkers associated with reduced susceptibility. This was achieved under controlled conditions integrating transcriptomics, metabolic profiling and physiological studies.

MATERIALS AND METHODS

For all experiments, grapevine plants were inoculated with FDp strain FDPEY-05 (Papura *et al*., 2009) in a high-confinement greenhouse as described in Eveillard *et al.* (2016). FDp-infected or non-infected *Scaphoideus titanus* were placed for a one-week transmission on the fifth leaf from the apex. One week post-inoculation (wpi) and ten wpi, leaves were collected from each plant and immediately frozen in liquid nitrogen. Plant DNA extractions and phytoplasma absolute qPCR quantifications were performed as in Eveillard *et al*., 2016. For transcriptomic analysis, RNA extraction and reverse transcription were performed as in Dufour *et al.* (2016) followed by a high-throughput qPCR method (Fluidigm ©) with the pathways-targeted NeoViGen and Biostim chips (Bodin *et al*., 2020; Bodin *et al*., 2023; Dufour *et al*., 2016). For metabolic profiling, ethanolic extraction was performed on lyophilized samples. Soluble sugars, organic acids, free total amino acids, starch, proteins and cellular residues were quantified through enzymatic methods and via spectrophotometric/fluorescent assays. Physiological data, *i.e.* stomatal conductance and chlorophyll a fluorescence, have been acquired with the LI-COR 600 System in automatic mode. All statistical analyses were performed using R Studio (version 4.3.2.).

RESULTS AND DISCUSSION

No phytoplasma was detected in the leaves of the grapevines that were exposed to non-infected insects. Phytoplasma titers at 1 wpi were non statistically different among the grape varieties tested. However, titers were significantly higher in CS than in M at 10 wpi.

Transcriptomic analysis revealed specific gene expressions for each plant condition. Indeed, principal component analysis (PCA) separated infected and healthy plants on the first dimension and varieties on the second dimension. For example, infected-M showed overexpression of genes involved in jasmonic acid pathways, while healthy-M modulated the genes related to salicylic acid and ethylene pathways.

Results using the LI-COR 600 system also suggested different responses depending on the

susceptibility of grapevine varieties. Indeed, in M and CS, chlorophyll fluorescence tended to increase

in M but decreased in CS in infected leaves. The stomatal conductance significantly decreased in infected leaves at 10 wpi, as compared to leaves exposed to non-infected insects.
Finally, preliminary enzymatic assays indicate that, at 1 wpi, carbohydrate levels increased in infected CS as compared to healthy ones, while they decreased in infected M.
Altogether, these results are in line with studies showing significant transcriptomic differences between healthy and phytoplasma-infected plants, as well as between highly susceptible and poorly susceptible varieties (Bertazzon *et al.*, 2019; Margaria *et al.*, 2014). Additionally, the reduction in chlorophyll fluorescence in infected cultivars, directly linked to photosystem II activity, is in agreement with findings indicating a decrease in chlorophyll content (Teixeira *et al.*, 2020).

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