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TOWARDS A METABOLOMIC CHARACTERISATION OF THE GRAPEVINE RESPONSE TO FLAVESCENCE DORÉE INFECTION BY NMR AND LC-MS PROFILING.

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Flavescence dorée (FD) is a quarantine disease of grapevine, involving interactions between the plants, leafhopper vectors (phytophagous insect *Scaphoideus titanus* Ball, *Cicadellidae* family), and Flavescence dorée phytoplasma. This disease is a major threat to vineyard survival in different European grape-growing areas ^{1,2}.

Differences in susceptibility to FD between grapevine varieties exist, in terms of multiplication of the associated phytoplasma and its spread in the plant. Cabernet Sauvignon (CS) is highly susceptible to FD, whereas Merlot (M) is less susceptible ³. The objective of this project is to determine which primary and specialised metabolites are altered in grapevines following infection by FD phytoplasma under controlled conditions. The comparison between healthy and infected plants on the one hand, and highly susceptible and less susceptible varieties on the other hand, will make it possible to identify metabolites or pathways that are associated with improved resistance to FD.

Material & Methods

Plants of CS and M were infected with FD phytoplasma (FDp strain FD-PEY05) on a single leaf via the natural insect vector *Scaphoideus titanus* according to the protocol established in a high containment greenhouse ³. An equivalent number of plants were subjected to the healthy insects (Ms or CSs). Sampling took place 7-day (leaf in contact with insects Fi7dpi,) or 7-week post-inoculation (leaf in contact with insects Fi7wpi and distant leaf 5F7wpi). The leaves were harvested and immediately frozen in liquid nitrogen to quench metabolism. Phytoplasma titers were determined on 100 mg fresh powder of each leaf (adapted from ³). Metabolomic analyses were performed on a selection of the leaf samples.

Protocols for metabolomic analyses based on proton NMR (¹H-NMR), LC-LTQ/Orbitrap-HRMS, and LC-QqQ-MS for extraction, chemical analysis and processing, experimental data and metadata management were tested and adapted ^{4,5,6,7}. ¹H NMR spectra of pH-adjusted leaf hydromethanolic extracts were acquired ⁴ with water presaturation at 500 MHz and processed using the NMRProcFlow⁷ tool (<https://nmrprocflow.org/>). For both LC-MS, metabolites from methanolic extracts were separated by reverse-phase (C18) chromatography with a water/acetonitrile gradient. Untargeted LC-HRMS experiments were performed on a

UHPLC-ESI-LTQ/Orbitrap-MS system with an ESI probe operated in positive mode. Spectra were acquired at 30k resolving power in full scan and data dependent MS² with a 35% normalised collision energy. Raw data were processed using MS Dial v4.7⁸. Targeted LC-MS experiments were performed on an LC-QqQ-MS system with a multiple reaction monitoring (MRM) method in positive or negative mode depending on the metabolites. The specialised metabolites were quantified by using calibration curves built with standards⁶.

The phytoplasma titre data, metabolite quantification data or metabolomic signatures of leaf samples were managed with ODAM tool⁹.

Preliminary Results & Discussion

Visual observations of the NMR spectra revealed several tendencies: (i) 'insect bite' effects (Fi/5F comparison in M), (ii) 'grapevine variety' effects (CS/M comparison) in infected leaves (Fi7dpi) and (iii) 'infection' effects in M (healthy/infected comparison). These effects are reflected in a variation in the content of certain metabolites. Twenty-one primary metabolites including amino acids, sugars and organic acids were identified in leaf extracts spectra by 1D and 2D NMR and 15 specialised metabolites (flavonoids, stilbenes and cinnamic acids) by LC-MS. For the annotation of still unknown signals, further NMR and LC-MS acquisitions will be performed.

Multivariate and univariate statistical analyses were performed to mine the metabolomic profile data. Principal component analyses of ¹H-NMR data on primary metabolites, untargeted LC-HRMS and targeted LC-MS data both on specialized metabolites showed a discrimination of groups according to grapevine variety but also between the healthy and infected states. The spectral variables from untargeted analyses and specialized metabolites from targeted analysis differentiating the varieties or infection levels could be used as biomarkers of response to infection and should contribute to the identification of the pathways disrupted by infection.

Conclusion & Perspectives

These preliminary results are encouraging. The metabolome experiments and the univariate and multivariate statistical analyses of the metabolomic profiles will continue with the aim of highlighting metabolites and pathways that respond differentially between CS and M varieties in order to identify which ones are associated with better resistance to this disease. These data will allow a better understanding of the grapevine-FDp interactions and will be useful in the context of varietal selection.

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