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Poster abstract

NMR metabolomics to explore compositional changes in grape berry under heat stress

Marion Cariou¹, Catherine Deborde^{1,2*}, Valentina Parisi^{2,3}, Annick Moing^{1,2}, Daniel Jacob^{1,2}, David Lecourieux⁴, Fatma Lecourieux⁴, Pierre Pétriac^{1,2}

¹ Univ. Bordeaux, INRAE UMR1332 BFP, F-33140 Villenave d'Ornon, France

² Bordeaux Metabolome, MetaboHUB, PHENOME-EMPHASIS, F-33140 Villenave d'Ornon, France

³ Department of Pharmacy, Univ. Salerno, Via Giovanni Paolo II, 132, 84084 Fisciano SA, Italy

⁴ Univ. Bordeaux, INRAE, Bordeaux Sciences Agro, UMR1287 EGFV, ISVV, F-33140 Villenave d'Ornon, France

Climate change is increasing the frequency of heat waves in the vineyard that alter the metabolism and final composition of grape berries. Here, the metabolic response of short-term stress to high temperature was thus studied using fruiting cuttings of two *Vitis vinifera* L. varieties, Merlot and Cabernet Sauvignon, grown in a greenhouse. It is noteworthy that Merlot is known to be more sensitive to heat stress than Cabernet Sauvignon in the vineyard. The stress was localised in the cluster level, applied at the late veraison stage for 8 hours, and compared to a control condition. Subsequently, berries were harvested at 1, 2, 4 and 8 hours after the start of the experiment, and their skin and pulp profiled using ¹H-NMR metabolomics of semi-polar extracts.

1D ¹H-NMR spectra of pH-adjusted extracts were acquired with water presaturation at 500 MHz. The raw ¹H-NMR spectra were processed for ppm calibration, global and local baseline correction and local alignment using NMRProcFlow (v1.4.14) online tool (<https://nmrprocflow.org>). Each spectrum was divided into buckets, i.e. spectral variables, using Extraction of Relevant Variables for Analysis module (ERVA, resolution factor 0.0005, SNR >3) and normalised to total spectrum intensity after removing the pre-saturated residual water, methanol and solvent-impurities regions using NMRProcFlow to create the dataset matrix. After filtering with a SNR >10, this resulted in datasets of 276 variables quantified for the pulp, and 400 variables for the skin.

Twenty-five major metabolites were annotated on the 1D spectra, based on 1D and 2D NMR experiments of pulp and skin extracts and comparison with an in-house spectral database, including four soluble sugars and polyol, six organic acids, thirteen amino acids and two phenolics. Two-way ANOVA and ANOVA-PCA of the ¹H-NMR fingerprints showed that the effect of variety was more important than the effect of heat stress and that one variety, Cabernet Sauvignon, was more responsive to elevated temperature in terms of number of variables impacted. The annotated metabolite most affected by stress in Cabernet Sauvignon was *trans*-caftaric acid, decreased in the pulp, and arginine, decreased in the skin.

Overall, our preliminary results highlight spectral variables that could be used as biomarkers of the heat stress susceptibility and could further contribute to the identification of pathways affected by heat stress.

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