Metabolomic discrimination of grapevine water status for Chardonnay and Pinot noir

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Abstract

The vine water status has been widely explored, as it strongly affects grapevine physiology and grape chemical composition. Most of the studies have focused on its impact of either physiological traits, or berry composition in major metabolites, or traits involved in wine quality. In this study, grapevine water status has been assessed through carbon isotope discrimination, on juices from berries of *Vitis vinifera* cv. Pinot noir (PN) and Chardonnay (CH), during 3 vintages (2019, 2020 & 2021). A total of 220 samples were collected from 5 countries worldwide. Measured $\delta^{13}C$ (‰) varied from -28.73 to -22.6 for PN, and from -28.79 to -21.67 for CH. The same grape juices have been analysed by Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FT-ICR-MS) leading to the detection of up to 6276 elemental compositions. Multivariate statistical analyses revealed hundreds of elemental compositions significantly link to the observed gradients of water status. Abscisic acid (ABA) and various derivatives are examples of chemical markers, which are part of the complex fingerprints, regardless of the variety. Our results provide an unprecedented representation of the metabolites involved in the water status regulation at the grape level. It could contribute to a better knowledge of the grapevine mitigation strategy in a climate change context.

Introduction

Climate conditions have a major impact on grape characteristics and subsequently wine quality (Santos et al., 2020; van Leeuwen et al., 2004). Grapevine water status is known to affect grape quality during the fruit growing and ripening periods, and a moderate water deficit is often required for providing high quality wines. Such moderate water stress is usually associated with a reduction of the berry weight and of the titratable acidity, but it increases the total soluble solids (TSS), total anthocyanins and the concentration in polyphenols for red wines (Mirás-Avalos & Intrigliolo, 2017; Romero et al., 2010). However, above a certain threshold, these beneficial effects are no longer observed. This issue is becoming particularly important in a context of climate change for wine producing regions where irrigation is not allowed for mitigating the impact of important water deficits. Understanding the consequences of evolving climate conditions on grape characteristics and rationalizing the related compositional evolution is therefore required for the possible identification of new mitigation strategies.

Beyond the numerous targeted molecular and oenological analyses of grapes, recent years have seen the development of more integrated approaches designed for a better characterization of the complex factors affecting the grape berry characteristics (Deluc et al., 2009). Yet, a more comprehensive description at the metabolite level is still needed.

Metabolomics, considered as the quantitative analysis or description of all low molecular weight metabolites in specified cellular, tissue or biofluid compartments (Nicholson et al., 1999). It offers the possibility to comprehensively address the complex interplay between environmental factors, genetic factors and viticultural practices. These factors actually govern the instantaneous chemical composition of grape berries, and cannot

be simply reduced to the sum of individual chemical traits (Roullier-Gall et al., 2014). Besides, untargeted ultra-high-resolution metabolomics allow the identification of measured masses at the formula level, hence revealing a vast chemical diversity and allowing a better understanding of the biochemical mechanisms involved (Gougeon et al., 2009; Roullier-Gall et al., 2014; Schmitt-Kopplin et al., 2019)
Here, we have recorded ultra-high-resolution mass-spectra (FT-ICR-MS) and $\delta^{13}C$ (‰) measurements of grape juices collected at harvest over three successive vintages from five distinct regions world-wide, in order to draw correlations between water status and chemical compositions.

Materials and methods

During 3 vintages (2019, 2020,2021), grapes of Pinot noir and Chardonnay have been collected over 13 wine producing regions in France, Italy, Germany, Portugal and Argentina with contrasted climatic conditions. Each sampling consisted in 300 berries representative of a given plot, from which 2 pools of 100 berries were randomly picked. Each pool of 100 berries was then manually pressed, and the juice was kept frozen for subsequent analyses.
The water potential was evaluated by measuring $\delta^{13}C$ (‰) following the protocol described in Gaudillère et al. (2002). All samples have been measured in duplicate on a varia Micro Cube elemental analyser with a continuous flow mode associated with an IsoPrime (Elementar) isotope ratio mass spectrometer. USGS40 (IAEA, Vienna) was used as standard, and Vienna Pee Dee Belemnite (VPDB) international reference was used as reference.
Prior to metabolomics analysis, samples were centrifuged and filtered using 1ml SPE Bond ElutTM C18 cartridges (Agilent). Samples were diluted 1/20 in methanol, prior to analysis. Mass spectra were acquired using a 12T FT-ICR-MS, Bruker SolariX ultra high-resolution mass spectrometer (Bruker Daltonics, Bremen, Germany), in negative ionization mode, over a 92 - 1000 m/z mass range. Raw Spectra have been calibrated using Compass DataAnalysis 4.2 (Bruker Daltonics, Bremen, Germany) and peaks with a signal-to-noise ratio (S/N) of at least 3 have been considered. A matrix was then obtained by aligning all spectra within a range of 0.5 ppm and molecular formulae were assigned using an in-house developed software tool.
For both the Chardonnay and the Pinot noir matrices, masses detected in less than 5% of the spectra were removed. For each mass, zero values have been replaced with 2/3 of the minimum intensity for this mass. All statistics and graphics output have been done using the R statistical software version 4.2.0 (R Core Team, 2013). A PLS model has been built using the ‘ropls’ package (Thévenot et al., 2015), with the metabolomic dataset as predictor and the $\delta^{13}C$ values as response. Measured m/z have been annotated using the CHEBI database and annotated masses have been classified using ‘classyfireR’ (Djoumbou Feunang et al., 2016).

Results and discussion

Over the 3 vintages, 86 vineyards (49 Pinot noir & 37 Chardonnay) have been sampled from 13 viticultural regions representing a broad range of climatic conditions from high altitude and warm climate in Argentina (Mendoza) to colder climate (Burgundy, Alsace). For Chardonnay, 45 samples were analyzed for the 2019 vintage, 54 for 2020 and 13 in 2021. For Pinot noir, 76, 49 & 19 samples were analyzed for the 2019, 2020, 2021 vintages, respectively.
Measured $\delta^{13}C$ values ranged from -28.73 to -22.6 (‰) for Pinot noir, and from -28.79 to -21.67 (‰) for Chardonnay (Figure. 1) corresponding to nil to severe water deficit, following classes proposed by van Leeuwen et al. (2009)., Most of the samples show no water deficit, except for some regions in France 2019 and 2020, Rheinghau and Douro Valley. Among various factor, this can probably be due mostly to the impact of irrigation and irrigation methods (Zufferey et al., 2017), plant materials (Berdeja et al., 2014), soils (van Leeuwen et al., 2009) and training (Zufferey & Murisier, 2006),
In French wine producing regions, $\delta^{13}C$ values were the highest in 2020 with a maximum value of -21.66 ‰ for Chardonnay and -22.65 ‰ for Pinot noir and the lowest in 2021(CH_{max} = -26.25 ‰; PN_{max} = -26.17 ‰) compared to the 2019 vintage (CH_{max} = -23.82 ‰; PN_{max} = -23.82 ‰). Indeed, 2020 was warm in France, with early harvest date whereas 2021 was a rainy vintage. Altogether, and independently of the origin of the water status, the $\delta^{13}C$ values cover a wide range of conditions encounter by grapevine which can possibly lead to contrasted metabolomics compositions of the grape juice.
FT-ICR-MS analyses of grape juices led to the identification of 3296 m/z values for Chardonnay and 3744 for Pinot noir, to which unique elemental formulas could be assigned. 2901 m/z values are common to the two grape varieties. For both Chardonnay and Pinot noir, elemental formulas mostly corresponded to CHO compounds (1233 and 1410, respectively), then CHON (634 and 747, respectively), CHONS (536 and 609, respec-

tively) and CHOS (420 and 473, respectively). Such elemental formulas classification encompassed fatty acids, amino acids, sugars, phenolic compounds or organic acids and proved to be of interest for the understanding of the chemical diversity of grape juice and wine (Gougeon et al., 2009).
Partial Least Square regressions (PLS) performed on both datasets are presented in Figure 2. A clear separation of the $\delta^{13}C$ values according to metabolomics composition is highlighted for each grape variety. The model can explain 23.21% of the $\delta^{13}C$ variability for Chardonnay, with R^2Y of 0.981 and a Q^2Y of 0.842. For Pinot noir 23.03% of the variability is explained (R^2Y = 0.986 and Q^2Y = 0.842). When considering PLS models, R^2Y represents the amount of Y inertia explained by the model, the Q^2Y parameter is interpreted as the predictive performance of the model by cross-validation. Q^2Y and R^2Y near one denote the quality of the model and confirm the relevance of the two constructed models. Variable importance in projection (VIP) aims to estimate the importance of the m/z loadings in the projection. Correlation coefficients with the PLS first component have been computed and used to identify possible fingerprints and biomarkers. Elemental formulas with a "VIP" value above 1 and correlation coefficient with an absolute value above 0.6 have been selected.

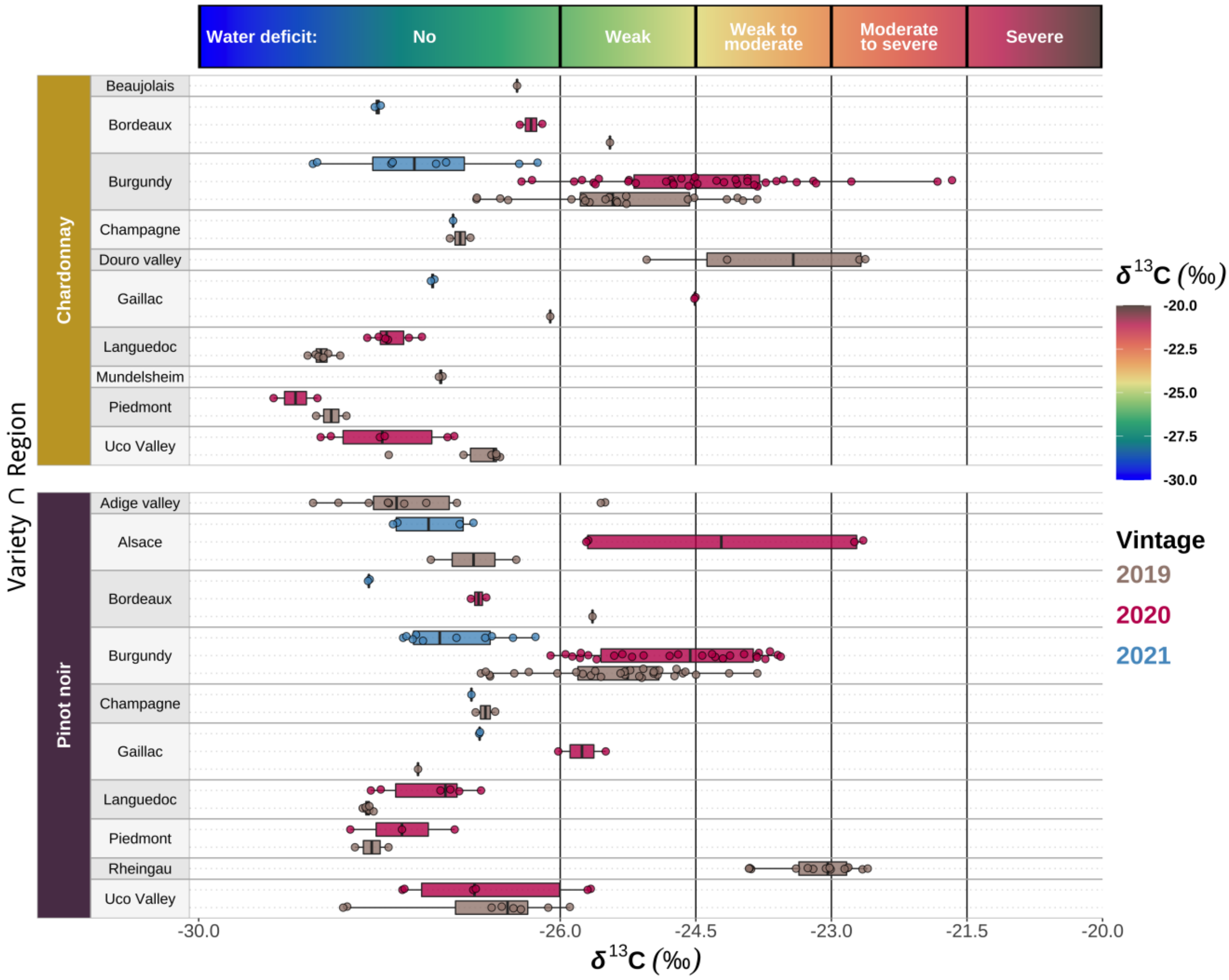

Figure 1. Boxplot of the $\delta^{13}C$ values for Chardonnay and Pinot noir grape juices from 13 wines producing regions and for 3 vintages**.**
Water deficit classes from $\delta^{13}C$ are taken from (Leeuwen et al., 2009).

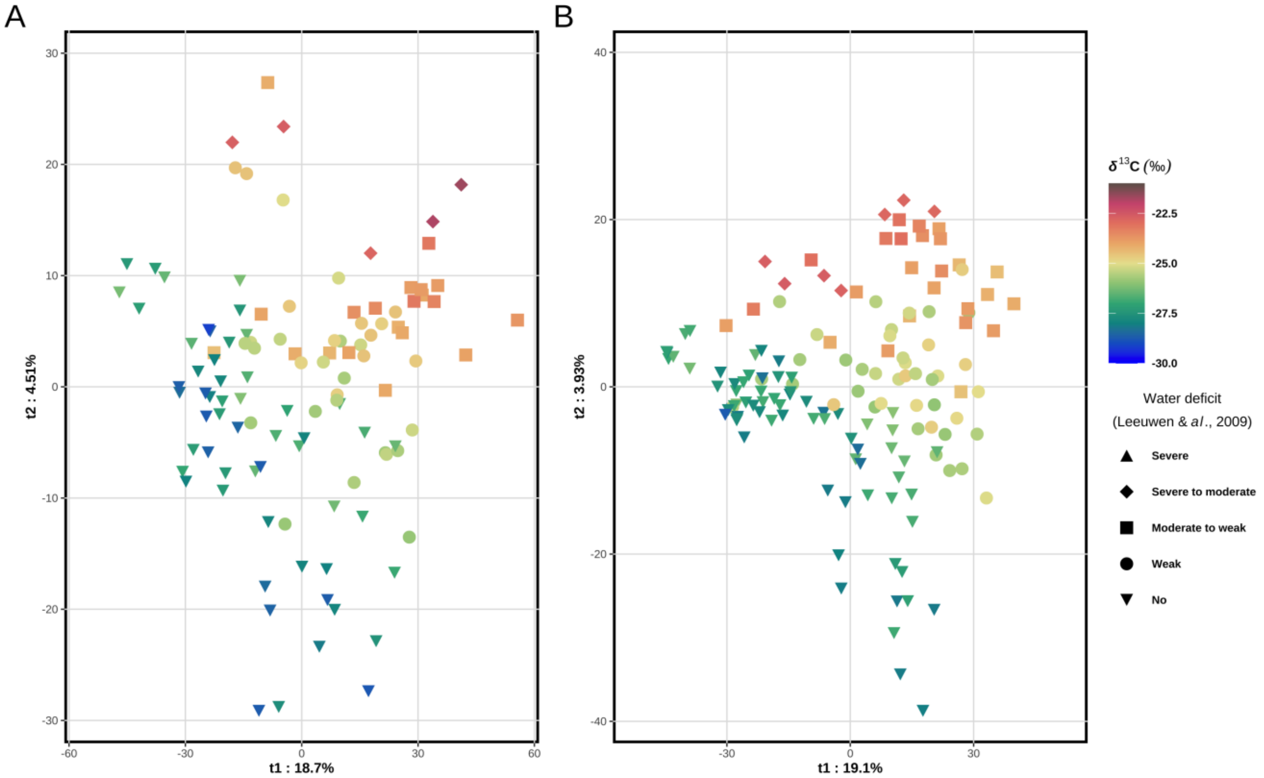

Figure 2. PLS models for the classification of (A) Chardonnay and (B) Pinot noir grape juice $\delta^{13}C$ values, according to metabolomics data.
The quality of the model has been evaluated using permutation (n=5000) of response variables ($\delta^{13}C$) and sevenfold cross-validation. (A) R2Y = 0.981, $p < 0.001$; Q2 = 0.842, $p < 0.001$. (B) R2Y = 0.986, $p < 0.001$; Q2 = 0.825, $p < 0.001$.

Table 1. Chemical class of the VIP>1 and with | ρ | >0.6

	CHO	CHON	CHONS	CHOS	Other	Total
Chardonnay	506	82	14	34	15	636
Pinot noir	531	71	32	47	25	706
Present in both	418	48	6	24	4	499

Multivariate statistical analysis has led to the identification of 636 discriminant elemental formulas for Chardonnay and 706 for Pinot noir. These elemental formulas were mostly found in the CHO chemical space (Table 1). Of these formulas, 269 and 279 masses could find a match in the CHEBI database (Hastings et al., 2016) for the Chardonnay and Pinot noir respectively. From these annotated masses, a classification of putative compounds has been performed with 'classyfireR'. Such tool emphasized 4 elemental formulas for both varieties linked to abscisic acid and derivatives, for both varieties. Putative assignments from the CHEBI database assigned these formulas to phaseic acid, abscisic acid, abscisic acid-glucopyranosyl ester and dihydrophaseic acid-glucoside. ABA and derivatives were positively correlated with the water deficit. The ABA pathway has been widely described for its key role in water stress regulation, as shown for instance by Deluc et al. (2009) for the Chardonnay grape variety. The 'classyfireR' classification also highlighted the presence of a high number of Glycosyl compounds, (41 for Pinot noir and 39 for Chardonnay), Sugar acids and derivatives (PN=19; CH=21), or Flavonoid O-glycosides (PN=7; CH=13). Glycosylated compounds have been described to play an important role in plants response to oxidative stress (Behr et al., 2020) and thus might be of interest (Laxa et al., 2019) to understand the underlying mechanism of the water status response in vine.

Conclusion

This study aimed to combine untargeted ultra-high-resolution mass spectrometry (FT-ICR-MS) and carbon isotopes discrimination ($\delta^{13}C$) on grape juices of two widely cultivated variety, i.e., *Vitis vinifera* cv. Pinot noir and Chardonnay. The analyzed samples represented a large variety of climatic conditions with an important gradient of $\delta^{13}C$ values. Hundreds of discriminant elemental formulas and key chemical families involved in the response to the water status, have been identified. ABA derivatives consistently appeared correlated to higher water stress. Our work has successfully linked untargeted metabolomics with water status measurement, showing the interest of this method for the comprehensive analysis of complex interactions beetween environmental conditions, vine and wine.

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