

Technical Note

## COMPARISON OF THE CHEMICAL DIVERSITY OF *VITIS ROTUNDIFOLIA* AND *VITIS VINIFERA* CV. ‘CABERNET SAUVIGNON’

### COMPARAÇÃO DA DIVERSIDADE QUÍMICA DE *VITIS ROTUNDIFOLIA* E *VITIS VINIFERA* CV. ‘CABERNET SAUVIGNON’

Marisa Maia<sup>1,2,\*</sup>, António E.N. Ferreira<sup>1</sup>, Jorge Cunha<sup>3</sup>, José Eiras-Dias<sup>3</sup>, Carlos Cordeiro<sup>1</sup>, Andreia Figueiredo<sup>2,\*</sup>, Marta Sousa Silva<sup>1,\*</sup>

<sup>1</sup>Laboratório de FTICR e Espectrometria de Massa Estrutural, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal.

<sup>2</sup>Grapevine Pathogen Systems Lab (GPS Lab), Biosystems and Integrative Sciences Institute (BioISI), Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal.

<sup>3</sup>Instituto Nacional de Investigação Agrária e Veterinária (INIAV), Quinta da Almoinha, 2565-191 Dois Portos, Portugal.

\* Corresponding author: Tel.: +351 217 500 075; e-mail: mfsilva@fc.ul.pt; aafigueiredo@fc.ul.pt; mrmaia@fc.ul.pt

(Received 18.11.2020. Accepted 21.12.2020)

#### SUMMARY

Grapevine is one of the most important fruit plants in the world, mainly due to its grapes and related products, with a highly economic and cultural importance. Every year, vineyards are affected by several pathogen outbreaks and the only way to control them is through preventive applications of agrochemicals every 12 to 15 days. This approach is not sustainable and not always effective. The *Vitis* genus comprise different species that exhibit varying levels of resistance to pathogens, thus the understanding of the innate resistance/susceptibility mechanisms of these different *Vitis* species is crucial to cope with these threats. In this work, an untargeted metabolomics approach was followed, using Fourier transform-ion cyclotron resonance mass spectrometry (FT-ICR-MS), to analyse the metabolic chemical diversity of two *Vitis* species: *Vitis rotundifolia* (resistant to pathogens) and *V. vinifera* cv. ‘Cabernet Sauvignon’ (susceptible to pathogens). Chemical formulas from both *Vitis* were used to build Van Krevelen diagrams and compositional space plots, which do not require full metabolite identification and provide an easy comparison method. Based only on these visualization tools, it was shown that the *V. rotundifolia* metabolome is more complex than the metabolome of *V. vinifera* cv. ‘Cabernet Sauvignon’. Moreover, the regions that present a higher density are associated to lipids, polyketides and carbohydrates. Also, *V. rotundifolia* metabolome presented a higher ratio O/C compounds.

#### RESUMO

A videira, devido às uvas e seus derivados, é uma das plantas de fruto mais importantes em todo o mundo, com elevada importância económica e cultural. Todos os anos, as vinhas são afetadas por diversos agentes patogénicos e a única forma de os controlar é a através do uso preventivo de fitoquímicos a cada 12-15 dias. Esta abordagem não é a mais sustentável e nem sempre é a mais eficaz. O género *Vitis* possui diferentes espécies que exibem diferentes níveis de resistência a patógenos, sendo por isso importante o estudo dos mecanismos de defesa de *Vitis* que são naturalmente resistentes/suscetíveis de forma a conseguir minimizar os impactos causados por estas ameaças. Neste trabalho, foi seguida uma abordagem de metabolómica não direcionada, usando espectrometria de massa de ressonância ciclotrónica de íão e transformada de Fourier (FT-IC-MS), para analisar a diversidade química de duas espécies de *Vitis*: *Vitis rotundifolia* (resistente a patógenos) e *V. vinifera* cv. ‘Cabernet Sauvignon’ (suscetível a patógenos). As fórmulas químicas obtidas de ambas as *Vitis* foram usadas para construir diagramas de van Krevelen e gráficos de composição elementar. Estas análises não requerem uma identificação absoluta do metabolito, permitindo uma rápida, fácil visualização e interpretação dos dados. A partir destas técnicas de visualização, percebeu-se que o metaboloma de *V. rotundifolia* é mais complexo que o metaboloma de *V. vinifera* cv. ‘Cabernet Sauvignon’. As regiões que apresentaram uma maior densidade de fórmulas químicas encontram-se associadas à classe dos lípidos, glúcidos e policetídeos. O metaboloma de *V. rotundifolia* apresentou compostos com rácio O/C mais elevado.

**Keywords:** *Vitis vinifera*, FT-ICR-MS, van Krevelen diagram, elemental composition.

**Palavras-chave:** *Vitis vinifera*, FT-ICR-MS, diagramas de van Krevelen, composição elementar.

## INTRODUCTION

The history of the grapevine is long and extremely complex with different theories and is present in the human culture since ancient times. Geographical and archaeological studies show that cultivation and domestication of grapevine appear to have occurred between the 7000 and the 4000 BC (García and Revilla, 2013; Fortes and Pais, 2016), with fermentation processes being developed since 6000 BC (Terral *et al.*, 2010). Despite its importance, only one grapevine species was domesticated, while the others remain practically wild.

The genus *Vitis* comprises two sub-genera: *Muscadinia* and *Euvitis* differing in morphological, anatomical and cytological characters. The *Muscadinia* sub-genera comprise three species, while the *Euvitis* includes *Vitis vinifera*, with the subspecies *sylvestris* (wild vines) and *vinifera* (or *sativa*), the domesticated one. A great majority of cultivars, now widely cultivated for fruit, juice and mainly for wine belong to *Vitis vinifera* subsp. *vinifera* (Sefc *et al.*, 2003; This *et al.*, 2006).

Due to its cultural and economic importance, *V. vinifera* is considered one of the most important fruit crops in the world, with a global market size of 31 billion euros (OIV, 2019). However, it is highly susceptible to different pathogens, such as *Plasmopara viticola* (Berk. & Curt.) Berl. & de Toni) Beri, et de Toni, *Erysiphe necator* (Schweinf.) Burrill and *Botrytis cinerea* Pers., the causal agents of downy, powdery mildew and gray mold, respectively, requiring preventive applications of chemical products for disease control. On the other hand, *Muscadinia* species exhibit varying levels of resistance to the pathogens. Understanding the innate molecular basis resistance/susceptibility mechanisms of these different *Vitis* species became crucial for the development of new *V. vinifera* varieties, more resistant to pathogens. Recently, our group showed that the metabolome of *V. vinifera* cultivars is different from other *Vitis* species (Maia *et al.*, 2020a) with different degrees of tolerance/susceptibility to fungal and oomycete related pathogens, highlighting the importance of chemical fingerprinting and its relevance in the identification of resistance/susceptibility-related biomarkers.

The ultra-high-resolution and ultra-high-mass accuracy Fourier Transform Ion Cyclotron Resonance mass spectrometry (FT-ICR-MS) is considered to be superior to any other analytical technique and is one of the best approaches to perform untargeted analysis of complex samples (Kuhnert *et al.*, 2020). Due to its characteristics, it allows the detection of a large number of analytes in a single experiment providing a chemical fingerprint of any given sample and a reliable information on the elemental composition of all analytes detected

(Wu *et al.*, 2004; Gougeon *et al.*, 2009; Kuhnert *et al.*, 2020). Such characteristics allow the characterization of different samples, eg: wine (Roullier-Gall *et al.*, 2017, 2018), black tea (Kuhnert *et al.*, 2010), coffee (Jaiswal *et al.*, 2012) and grapevine leaves (Becker *et al.*, 2013; Maia *et al.*, 2016, 2020a; Adrian *et al.*, 2017).

Untargeted analysis of complex samples generates very complex mass spectrum (Gutiérrez Sama *et al.*, 2018), making the analysis challenging due to the difficulty to easily represent and visualize the data. Hence the validation of graphical methods supporting the interpretation and comparison of FT-ICR-MS complex data is very important. Two types of graphical representation were developed, allowing a comprehensive interpretation of complex mass spectrometry data from untargeted metabolomics approaches, both based only on the identified chemical formulas: two-dimensional van Krevelen (VK) diagrams displaying H/C (hydrogen/carbon) versus O/C (oxygen/carbon) ratios (Van Krevelen, 1950) and compositional space plots that use double-bond equivalents (DBE) values (Wu *et al.*, 2004; Mann *et al.*, 2015; Kew *et al.*, 2017; Brockman *et al.*, 2018; Gutiérrez Sama *et al.*, 2018). Analyzing the elemental composition of the different samples allows the qualitative comparison between series of related samples in terms of their chemical complexity. Van Krevelen diagrams and compositional space plots convey simple, albeit qualitative information on the main molecular classes represented (Tziotis *et al.*, 2011; Roullier-Gall *et al.*, 2014, 2017; Adrian *et al.*, 2017; Gutiérrez Sama *et al.*, 2018). Moreover, in VK diagrams, the H/C ratio is related to the degree of separation, whereas the O/C ratio is related to oxidation (Wu *et al.*, 2004). In plants, the degree of oxidation of certain compounds and their saturation are extremely important as they can be associated to defense responses to biotic and abiotic stresses (Torres *et al.*, 2006). This tool has been mainly used in organic matter samples but due to its easy application to the analysis of complex samples, VK diagrams have recently been used to discriminate samples of biological origin. So far, these plots have been used to study the volatile profile of varietal olive oils from Alentejo region (Martins *et al.*, 2020), satreja essential oils (Maccelli *et al.*, 2020), bottle-aged Chardonnay wines (Roullier-Gall *et al.*, 2017) and for early detection of grapevine leaves' infection (Maia *et al.*, 2019).

The present work aimed to compare the chemical diversity of two *Vitis* species (*Vitis rotundifolia* and *V. vinifera* cv. 'Cabernet Sauvignon'), without any stress, with different resistance levels to pathogens, through an untargeted metabolomics approach. In this study, both van Krevelen (VK) diagrams and compositional space plots were applied to the comparison of *Vitis rotundifolia* and *V. vinifera* cv.

'Cabernet Sauvignon' metabolomes in order to identify visually differences between both metabolomes and associate their resistance/susceptibility to pathogens to these metabolic differences. Grapevine genotypes were selected according to their resistance/susceptibility towards pathogens and importance in the wine industry. *V. rotundifolia*, the best known *Muscadinia* species, originated in the south-eastern United States serves as a rootstock to cope with the high sensitivity of European grapevines to *Phylloxera* disease (Fortes and Pais, 2016). Also, *V. rotundifolia* is highly resistant to the different pathogens (<https://www.vivc.de/>). *Vitis vinifera* cv. 'Cabernet Sauvignon' is one of the most planted grapevine cultivar in the world, covering an area of 341000 ha, and one of the most widely distributed across the world, mainly grown in China, France, Chile, the United States, Australia, Spain, Argentina, Italy and South Africa (OIV, 2017). This cultivar, widely cultivated worldwide, is highly susceptible to different pathogens.

## MATERIALS AND METHODS

### Plant material

*Vitis vinifera* cv. 'Cabernet Sauvignon' and *Vitis rotundifolia* leaves were collected in the spring (May) from field grown plants belonging to the Portuguese Ampelographic Grapevine Collection (CAN, international code PRT051, established in 1988), at INIAV- Estação Vitivinícola Nacional (Dois Portos) (Veloso *et al.*, 2010). CAN occupy nearly 2 ha of area with homogeneous modern alluvial soils (lowlands) as well as well drained soil. For all accessions in the field, a unique cultivar rootstock was used - Selection Oppenheim 4 (SO4) and each accession come from one unique plant. The climate in this region is temperate with dry and mild summer. The degree of resistance of the genotypes was accessed through bibliographic searches following the classification of International Organisation of Vine and Wine (<https://www.oiv.int>) and *Vitis* International Variety Catalogue VIVC (<https://www.vivc.de/>). For plant material collection, the best possible health status was guaranteed. The third to fifth leaves, from the shoot to apex, were collected from seven fully developed plants and immediately frozen in liquid nitrogen. Leaves were stored at  $-80\text{ }^{\circ}\text{C}$  until analysis. Three biological replicates were considered for analysis. Plant material was ground in liquid nitrogen and used for metabolite extraction in the week after material collection.

### Metabolite extraction and FT-ICR-MS analysis

Metabolite extraction from *V. vinifera* cv. 'Cabernet Sauvignon' and *V. rotundifolia* leaves was performed following a previously developed protocol (Maia *et al.*, 2016), with minor

modifications (Maia *et al.*, 2020a). Briefly, after metabolite extraction with different solvents, the methanol fraction collected was diluted 1000-fold in methanol and analysed by direct infusion on an Apex Qe 7-Tesla Fourier Transform Ion Cyclotron Resonance Mass Spectrometer (FT-ICR-MS, Brüker Daltonics). Leucine enkephalin (YGGFL, Sigma Aldrich) was added to all replicates as internal standard ( $[\text{M}+\text{H}]^{+} = 556.276575\text{ Da}$  or  $[\text{M}-\text{H}]^{-} = 554.262022\text{ Da}$ ). For positive ion mode analysis (ESI<sup>+</sup>), formic acid (Sigma Aldrich, MS grade) was added to all replicates at a final concentration of 0.1% (v/v). Spectra were acquired at both positive (ESI<sup>+</sup>) and negative (ESI<sup>-</sup>) electrospray ionization modes and recorded between 100 and 1000 m/z, as previously described (Maia *et al.*, 2020a).

### Data processing and chemical formula analysis

For all mass spectra, single point calibration with leucine enkephalin was performed using Data Analysis 5.0 (Brüker Daltonics, Bremen, Germany). Peaks were considered at a minimum signal-to-noise ratio of 4. For each replicate of *V. vinifera* 'Cabernet Sauvignon' and *V. rotundifolia*, mass lists were extracted. The metabolomics data are available in figshare data repository (Maia *et al.*, 2020b). Putative assigned formulas were calculated using Data Analysis 5.0 smart formula tool following the upper formula ( $\text{C}_{78}\text{H}_{126}\text{O}_{27}\text{P}_9\text{S}_{14}\text{N}_{20}$ ) and lower formula ( $\text{C}_1\text{H}_1\text{O}_0\text{P}_0\text{S}_0\text{N}_0$ ). Formulas were exported to build van Krevelen (VK) diagrams, for compositional space analysis and determination of the elemental composition. The H/C ratio versus the O/C ratio for every compound in the sample were calculated and plotted, double bond equivalents (DBE) values were calculated (DBE =  $\text{C}-\text{H}/2+\text{N}/2+1$ ) based on the  $\text{C}_x\text{H}_y\text{O}_z\text{N}_n\text{S}_s$  molecular formula of each compound and plotted as a function of the number of carbon atoms. For elemental composition analysis, putative assigned formulas from each replicate were firstly combined and formulas presented only in one replicate were excluded. Only formulas presented in 2/3 replicates were considered for the analysis. Chemical formulas detected in each *Vitis* were divided in seven classes (CHO, CHOS, CHONS, CHOP, CHONP, CHONSP, OTHER) according to the chemical elements present: carbon, hydrogen, oxygen, nitrogen, sulphur, phosphorus and other.

## RESULTS AND DISCUSSION

There is an increasing demand for more sustainable agricultural practices. In fact, since 2009, guidelines from the European Union (Directive 2009/128/EC) demand a reduction and sustainable use of pesticides (Scoones, 2016). To cope with these demands, researchers have been trying to uncover the defense/resistance grapevine mechanisms through "omics" studies aiming to help producers and industries for a sustainable viticulture (Buonassisi *et*

al., 2017; Li and Yan, 2020). Hence, the comparison of different *Vitis* genotypes with different resistance/susceptibility levels towards pathogens may allow a better understanding of these mechanisms. Since the metabolome is the first to be affected by changing conditions and provides information of current state of the organism, the study of different grapevine genotypes metabolomes, without stress, may highlight their innate resistance/susceptibility capabilities (Maia et al., 2020a). Having this in mind, the metabolome of two *Vitis* species, with different resistance levels to various pathogens was analysed in order to compare their chemical diversity and possibly relate their resistance or susceptibility to pathogens.

An untargeted metabolomics analysis using FT-ICR-MS was performed and the number of peaks

detected in *V. vinifera* cv. ‘Cabernet Sauvignon’ in positive ionization mode was around 1600 peaks per replicate and in negative mode was around 750 (Table I). In *V. rotundifolia* around 1100 peaks were detected in ESI<sup>+</sup>-MS and around 600 peaks were detected in ESI-MS (Table I). Replicates results demonstrate a high analytical reproducibility of the data obtained, indicating that the analysis of the metabolome profile of each *Vitis* leaves appears to be sufficiently consistent to distinguish these two *Vitis* species with different resistance levels to various pathogens. To provide insight into metabolite diversity, chemical formulas were assigned to the detected masses of each *Vitis*. A total of 385 and 1227 different formulas were detected respectively in *V. vinifera* ‘Cabernet Sauvignon’ and *V. rotundifolia* (Table II).

**Table I**

Number of peaks detected with ESI<sup>+</sup>-MS and ESI-MS in both *Vitis* genotypes

<i>Vitis</i>	Replicates	Number of peaks detected	
		ESI <sup>+</sup> -MS	ESI-MS
<i>V. vinifera</i> ‘Cabernet Sauvignon’	CS 1	1642	768
	CS 2	1599	838
	CS 3	1631	710
<i>V. rotundifolia</i>	ROT 1	1162	649
	ROT 2	1091	558
	ROT 3	1092	607

**Table II**

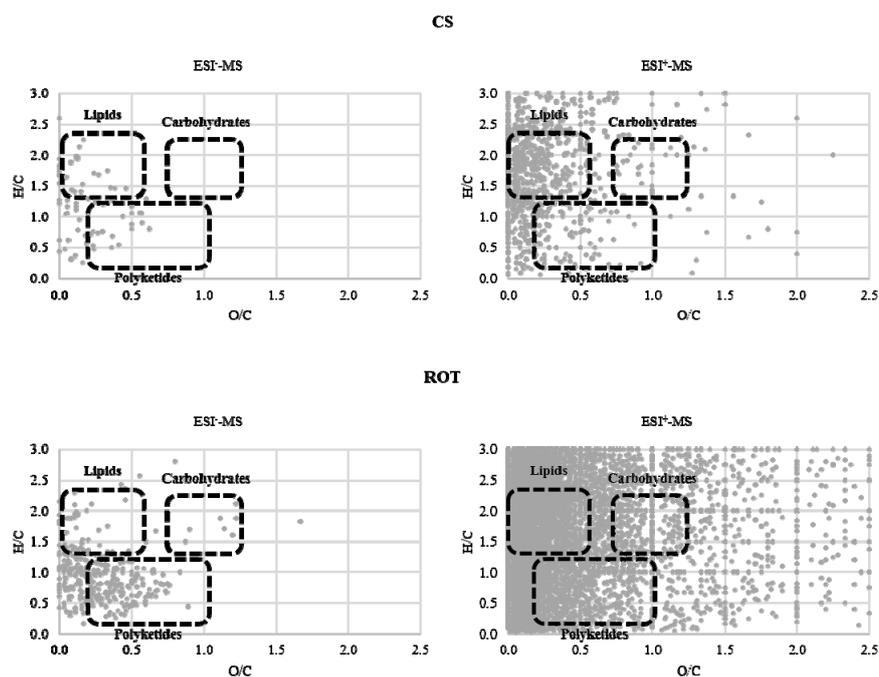
Number of elemental formulas detected in both *Vitis* according to their elemental composition: CHO, CHOS, CHON, CHONS, CHOP, CHONP, CHONSP and other

<i>Vitis</i>	Number of elemental formulas									Total
	CHO	CHOS	CHON	CHONS	CHOP	CHONP	CHONSP	Other	Total by Ionization mode	
<i>V. vinifera</i> ‘Cabernet Sauvignon’	8 <sup>♠</sup>	0 <sup>♠</sup>	9 <sup>♠</sup>	3 <sup>♠</sup>	3 <sup>♠</sup>	23 <sup>♠</sup>	1 <sup>♠</sup>	12 <sup>♠</sup>	59 <sup>♠</sup>	385
	6 <sup>♣</sup>	4 <sup>♣</sup>	38 <sup>♣</sup>	33 <sup>♣</sup>	14 <sup>♣</sup>	111 <sup>♣</sup>	45 <sup>♣</sup>	75 <sup>♣</sup>	326 <sup>♣</sup>	
<i>V. rotundifolia</i>	13 <sup>♠</sup>	0 <sup>♠</sup>	29 <sup>♠</sup>	2 <sup>♠</sup>	15 <sup>♠</sup>	88 <sup>♠</sup>	29 <sup>♠</sup>	19 <sup>♠</sup>	195 <sup>♠</sup>	1227
	20 <sup>♣</sup>	39 <sup>♣</sup>	121 <sup>♣</sup>	140 <sup>♣</sup>	55 <sup>♣</sup>	232 <sup>♣</sup>	174 <sup>♣</sup>	251 <sup>♣</sup>	1032 <sup>♣</sup>	

<sup>♠</sup> ESI-MS; <sup>♣</sup> ESI<sup>+</sup>-MS.

Compositional space plots and VK diagrams were generated for both genotypes and ionization modes

(Figure 1) highlighting a wide chemical diversity between the two *Vitis* genotypes under analysis.

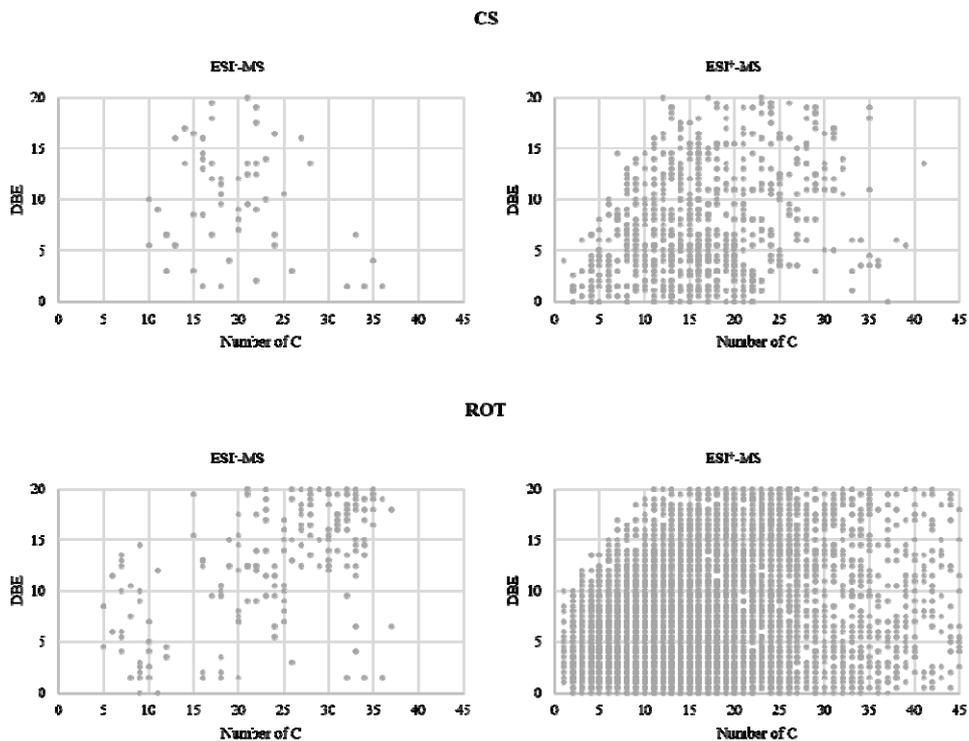


**Figure 1.** Van Krevelen diagrams of *V. vinifera* cv. ‘Cabernet Sauvignon’ (CS) and *V. rotundifolia* (ROT) in ESI-MS and ESI<sup>+</sup>-MS. Areas with the highest point density are highlighted for the three most important major classes of metabolites: lipids, polyketides, carbohydrates.

The positive ionization mode presented a higher number of peaks and consequently a higher number of chemical formulas (Table I and II) in both species analyzed. Empirical regions of metabolic classes of the chemical formulas detected were also created in each VK diagram. The regions that present a higher density are associated to lipids, polyketides and carbohydrates. The lipids class is one of the most represented in both *Vitis* genotypes analyzed. *V. rotundifolia* presented a higher number of compounds in the carbohydrate and polyketides region (Figure 1). In fact, polyketides are secondary metabolites involved in plant defense against pathogens. Within polyketides, flavonoids can be highlighted, considered to be one group of aromatic polyketides. In grapevine, flavonoids have already been identified as been associated to grapevine defenses against downy mildew (Buonassisi *et al.*, 2017; Chitarrini *et al.*, 2017; Nascimento *et al.*, 2019). Recently, our group also associated these compounds to resistant/tolerant and susceptible cultivars discrimination in no stress conditions (Maia *et al.*, 2020a). Concerning carbohydrates, these metabolites are important signaling molecules involved in biotic and abiotic stresses (Trouvelot *et al.*, 2014). Moreover, by comparing both samples, it is clear that *V. rotundifolia* presents a higher number of compounds with a higher O/C value, suggesting the presence of a higher number of oxidized compounds. Since plants do not possess mobile

defense cells, their innate immunity depends on an effective signal transduction between cells to activate defense responses. One of these signals is the production of reactive oxygen species (ROS) (Torres *et al.*, 2006; Frederickson Matika and Loake, 2014; González-Bosch, 2018). The majority of the studies regarding oxygenated species in plants are performed upon plant challenge with a pathogen and not at a constitutive level (Doke *et al.*, 1996; Figueiredo *et al.*, 2017; Nascimento *et al.*, 2019). Hence, the significance of this accumulation of oxidized compounds observed in *V. rotundifolia* must not be discarded and should be investigated in future experiments.

The DBE vs. the number of carbons were plotted for FT-ICR-MS data (Figure 2). *Vitis rotundifolia*, in both ionization modes, presented a larger number of compounds with a DBE values and with more carbon atoms in their structure. A recent study with *Vitis vinifera* cv. ‘Regent’ (a tolerant cultivar to pathogens) and *Vitis vinifera* cv. ‘Trincadeira’ (a susceptible cultivar to pathogens) infected with *Plasmopara viticola*, showed that, after infection the tolerant cultivar presented a higher content of unsaturated fatty acids which leads to a more fluid and permeable membrane and as a consequence to a better defense response to the pathogen (Laureano *et al.*, 2018). The results obtained, although without pathogen challenge, are consistent with that study.

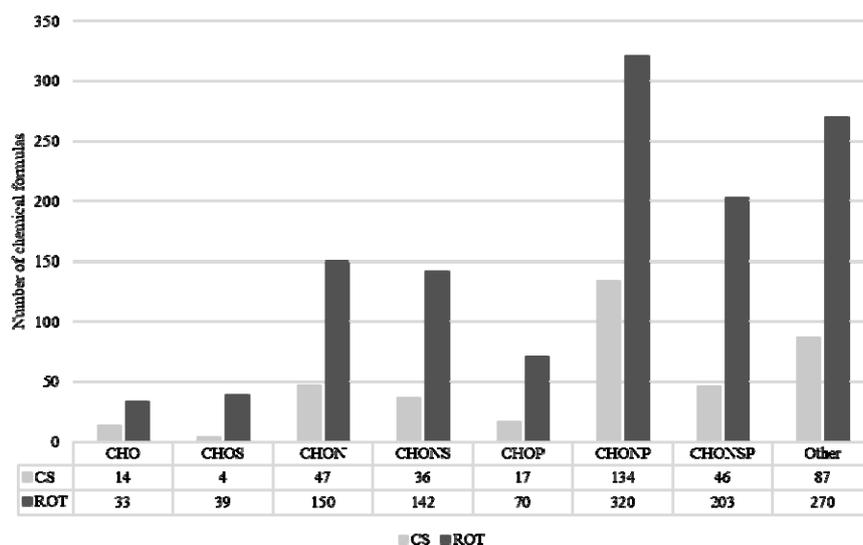


**Figure 2.** Compositional space plot of *V. vinifera* cv. 'Cabernet Sauvignon' (CS) and *V. rotundifolia* (ROT) in ESI-MS and ESI<sup>+</sup>-MS.

To better understand and investigate this phenomenon, future studies should be performed.

Elemental formulas of each compound detected were also investigated for both genotypes (Figure 3).

Significant differences between the two *Vitis* genotypes were observed, being CHON, CHONS, CHOP, CHONP and CHONSP the elemental formulas with the highest differences and more present in *Vitis rotundifolia* (Figure 3).



**Figure 3.** Chemical histogram of *V. vinifera* cv. 'Cabernet Sauvignon' (CS) and *V. rotundifolia* (ROT) according to the elemental formulas detected: CHO, CHOS, CHON, CHONS, CHOP, CHONP, CHONSP and other.

## CONCLUSIONS

In this work, two different *Vitis* genotypes that present different resistance levels towards pathogens were compared at the constitutive metabolic level through different visualization approaches. The results showed that both compositional space plots and VK diagrams allowed a fast comparison of chemical diversity between both metabolomes. Through these visualization techniques, it was shown that *V. rotundifolia* metabolome presented higher complexity than the metabolome from *V. vinifera* 'Cabernet Sauvignon'. Also, higher representation of compounds from the polyketides and carbohydrates groups was found in *V. rotundifolia*, which is in accordance with other studies. The presence of more oxidized compounds in the resistant *Vitis* at the constitutive level was also observed, and further studies should be conducted to better understand if their presence is associated to a higher capability to react upon pathogen challenge. These results open new insights into the study of constitutive compounds in grapevine through the analysis of complex untargeted data through visual tools.

## ACKNOWLEDGEMENTS

The authors acknowledge the support from Fundação para a Ciência e a Tecnologia (Portugal) through the projects PEst-OE/BIA/UI4046/2014, UID/Multi/04046/2019, UIDB/04046/2020, UIDP/04046/2020, PTDC/BAA-MOL/28675/2017, Investigator FCT programs IF 00819/2015 to Andreia Figueiredo and CEECIND/02246/2017 to Marta Sousa Silva, and the PhD grant SFRH/BD/116900/2016 to Marisa Maia. The authors also acknowledge the support from the Portuguese Mass Spectrometry Network (LISBOA-01-0145-FEDER-022125) and the Project EU\_FT-ICR\_MS, funded by the Europe and Union's Horizon 2020 research and innovation programme under grant agreement nr. 731077.

## REFERENCES

Adrian M., Lucio M., Roullier-Gall C., Héloir M.-C., Trouvelot S., Daire X., Kanawati B., Lemaître-Guillier C., Poinssot B., Gougeon R., Schmitt-Kopplin P., 2017. Metabolic fingerprint of PS3-induced resistance of grapevine leaves against *Plasmopara viticola* revealed differences in elicitor-triggered defenses. *Front Plant Sci.*, **8**, 101.

Becker L., Poutaraud A., Hamm G., Muller J.-F., Merdinoglu D., Carré V., Chaimbault P., 2013. Metabolic study of grapevine leaves infected by downy mildew using negative ion electrospray – Fourier transform ion cyclotron resonance mass spectrometry. *Anal. Chim. Acta*, **795**, 44–51.

Brockman S.A., Roden E.V., Hegeman A.D., 2018. Van Krevelen diagram visualization of high resolution-mass spectrometry metabolomics data with OpenVanKrevelen. *Metabolomics*, **14**.

Buonassisi D., Colombo M., Migliaro D., Dolzani C., Peressotti E., Mizzotti C., Velasco R., Masiero S., Perazzolli M., Vezzulli S., 2017. Breeding for grapevine downy mildew resistance: a review of “omics” approaches. *Euphytica*, **213**.

Chitarrini G., Soini E., Riccadonna S., Franceschi P., Zulini L., Masuero D., Vecchione A., Stefanini M., Di Gasparo G., Mattivi F., Vrhovsek U., 2017. Identification of biomarkers for defense response to *Plasmopara viticola* in a resistant grape variety. *Front. Plant Sci.*, **8**.

Doke N., Miura Y., Sanchez L.M., Park H.J., Noritake T., Yoshioka H., Kawakita K., 1996. The oxidative burst protects plants against pathogen attack: Mechanism and role as an emergency signal for plant bio-defence — a review. *Gene*, **179**, 45–51.

Figueiredo A., Martins J., Sebastiana M., Guerreiro A., Silva A., Matos A.R., Monteiro F., Pais M.S., Roepstorff P., Coelho A.V., 2017. Specific adjustments in grapevine leaf proteome discriminating resistant and susceptible grapevine genotypes to *Plasmopara viticola*. *J. Proteomics*, **152**, 48–57.

Fortes A.M., Pais M.S., 2016. Grape (*Vitis* species). In: *Nutritional composition of fruit cultivars*. 257–286. Simmonds M.S.J., Preedy V.R. (eds.), Academic Press.

Frederickson Matika D.E., Loake G.J., 2014. Redox regulation in plant immune function. *Antioxid. Redox Signal.*, **21**, 1373–1388.

García R.A.A., Revilla E., 2013. The Current status of wild grapevine populations (*Vitis vinifera* ssp *sylvestris*) in the Mediterranean Basin. The Mediterranean genetic code - grapevine and olive.

González-Bosch C., 2018. Priming plant resistance by activation of redox-sensitive genes. *Free Radical Bio. Med.*, **122**, 171–180.

Gougeon R.D., Lucio M., De Boel A., Frommberger M., Hertkorn N., Peyron D., Chassagne D., Feuillat F., Cayot P., Voilley A., Gebefügi I., Schmitt-Kopplin P., 2009. Expressing forest origins in the chemical composition of cooerage oak woods and corresponding wines by using FTICR-MS. *Chem. Eur. J.*, **15**, 600–611.

Gutiérrez Sama S., Farenc M., Barrère-Mangote C., Lobinski R., Afonso C., Bouyssière B., Giusti P., 2018. Molecular fingerprints and speciation of crude oils and heavy fractions revealed by molecular and elemental mass spectrometry: Keystone between petroleomics, metallopetroleomics, and petrointeractomics. *Energy & Fuels*, **32**, 4593–4605.

Jaiswal R., Matei M.F., Golon A., Witt M., Kuhnert N., 2012. Understanding the fate of chlorogenic acids in coffee roasting using mass spectrometry based targeted and non-targeted analytical strategies. *Food Funct.*, **3**, 976–984.

Kew W., Blackburn J.W.T., Clarke D.J., Uhrin D., 2017. Interactive van Krevelen diagrams - Advanced visualisation of mass spectrometry data of complex mixtures: Interactive van Krevelen Diagrams. *Rapid Commun. Mass Spectrom.*, **31**, 658–662.

Kuhnert N., Drynan J.W., Obuchowicz J., Clifford M.N., Witt M., 2010. Mass spectrometric characterization of black tea thearubigins leading to an oxidative cascade hypothesis for thearubigin formation. *Rapid Commun. Mass Spectrom.*, **24**, 3387–3404.

Kuhnert N., D'souza R.N., Behrends B., Ullrich M.S., Witt M., 2020. Investigating time dependent cocoa bean fermentation by ESI-FT-ICR mass spectrometry. *Food Res. Int.*, **133**, 109209.

Laureano G., Figueiredo J., Cavaco A.R., Duarte B., Caçador I., Malhó R., Sousa Silva M., Matos A.R., Figueiredo A., 2018. The interplay between membrane lipids and

- phospholipase A family members in grapevine resistance against *Plasmopara viticola*. *Sci Rep.*, **8**, 14538.
- Li Q., Yan J., 2020. Sustainable agriculture in the era of omics: knowledge-driven crop breeding. *Genome Bio.*, **21**, 154.
- Maccelli A., Cesa S., Cairone F., Secci D., Menghini L., Chiavarino B., Fornarini S., Crestoni M.E., Locatelli M., 2020. Metabolic profiling of different wild and cultivated *Allium* species based on high-resolution mass spectrometry, high-performance liquid chromatography-photodiode array detector, and color analysis. *J. Mass Spectrom.*, **55**, e4525.
- Maia M., Ferreira A.E.N., Nascimento R., Monteiro F., Traquete F., Marques A.P., Cunha J., Eiras-Dias J.E., Cordeiro C., Figueiredo A., Sousa Silva M., 2020a. Integrating metabolomics and targeted gene expression to uncover potential biomarkers of fungal/oomycetes-associated disease susceptibility in grapevine. *Sci. Rep.*, **10**, 15688.
- Maia M., Figueiredo A., Sousa Silva M., Ferreira A., 2020b. Grapevine untargeted metabolomics to uncover potential biomarkers of fungal/oomycetes-associated diseases. figshare. Dataset 12357314.v2. Available at: <https://doi.org/10.6084/m9.figshare.12357314.v2> (accessed on 15.06.2020).
- Maia M., Maccelli A., Nascimento R., Ferreira A.E.N., Crestoni M.E., Cordeiro C., Figueiredo A., Sousa Silva M., 2019. Early detection of *Plasmopara viticola*-infected leaves through FT-ICR-MS metabolic profiling. *Acta Hort.*, **1248**, 575–580.
- Maia M., Monteiro F., Sebastiana M., Marques A.P., Ferreira A.E.N., Freire A.P., Cordeiro C., Figueiredo A., Sousa Silva M., 2016. Metabolite extraction for high-throughput FTICR-MS-based metabolomics of grapevine leaves. *EuPA Open Proteom.*, **12**, 4–9.
- Mann B.F., Chen H., Herndon E.M., Chu R.K., Tolic N., Portier E.F., Chowdhury T.R., Robinson E.W., Callister S.J., Wullschlegel S.D., Graham D.E., Liang L., Gu B., 2015. Indexing permafrost soil organic matter degradation using high-resolution mass spectrometry. *PLoS One*, **10**.
- Martins N., Jiménez-Morillo N.T., Freitas F., Garcia R., Gomes da Silva M., Cabrita M.J., 2020. Revisiting 3D van Krevelen diagrams as a tool for the visualization of volatile profile of varietal olive oils from Alentejo region, Portugal. *Talanta*, **207**, 120276.
- Nascimento R., Maia M., Ferreira A.E.N., Silva A.B., Freire A.P., Cordeiro C., Silva M.S., Figueiredo A., 2019. Early stage metabolic events associated with the establishment of *Vitis vinifera* – *Plasmopara viticola* compatible interaction. *Plant Physiol. Biochem.*, **137**, 1–13.
- OIV, 2017. Focus OIV 2017: Distribution of the world's grapevine varieties. Available at: <http://www.oiv.int/public/medias/5888/en-distribution-of-the-worlds-grapevine-varieties.pdf> (accessed on 01.09.2020).
- OIV, 2019. World vitiviniculture situation: OIV statistical report on world vitiviniculture. Available at: <http://oiv.int/public/medias/6782/oiv-2019-statistical-report-on-world-vitiviniculture.pdf> (accessed on 01.09.2020).
- Roullier-Gall C., Hemmler D., Gonsior M., Li Y., Nikolantonaki M., Aron A., Coelho C., Gougeon R.D., Schmitt-Kopplin P., 2017. Sulfites and the wine metabolome. *Food Chem.*, **237**, 106–113.
- Roullier-Gall C., Signoret J., Hemmler D., Witting M.A., Kanawati B., Schäfer B., Gougeon R.D., Schmitt-Kopplin P., 2018. Usage of FT-ICR-MS metabolomics for characterizing the chemical signatures of barrel-aged whisky. *Front. Chem.*, **6**.
- Roullier-Gall C., Witting M., Gougeon R.D., Schmitt-Kopplin P., 2014. High precision mass measurements for wine metabolomics. *Front. Chem.*, **2**.
- Scoones I., 2016. The Politics of sustainability and development. *Annu. Rev. Env. Resour.*, **41**, 293–319.
- Sefc K.M., Steinkellner H., Lefort F., Botta R., Machado A.C., Borrego J., Maletić E., Glössl J., 2003. Evaluation of the genetic contribution of local wild vines to European grapevine cultivars. *Am. J. Enol. Vitic.*, **54**, 15–21.
- Terral J.-F., Tabard E., Bouby L., Ivorra S., Pastor T., Figueiral I., Picq S., Chevance J.-B., Jung C., Fabre L., Tardy C., Compan M., Bacilieri R., Lacombe T., This P., 2010. Evolution and history of grapevine (*Vitis vinifera*) under domestication: new morphometric perspectives to understand seed domestication syndrome and reveal origins of ancient European cultivars. *Ann. Bot.*, **105**, 443–455.
- This P., Lacombe T., Thomas M., 2006. Historical origins and genetic diversity of wine grapes. *Trends Genet.*, **22**, 511–519.
- Torres M.A., Jones J.D.G., Dangl J.L., 2006. Reactive oxygen species signaling in response to pathogens. *Plant Physiol.*, **141**, 373–378.
- Trouvelot S., Héloir M.-C., Poinssot B., Gauthier A., Paris F., Guillier C., Combiér M., Trdá L., Daire X., Adrian M., 2014. Carbohydrates in plant immunity and plant protection: roles and potential application as foliar sprays. *Front. Plant Sci.*, **5**.
- Tziotis D., Hertkorn N., Schmitt-Kopplin P., 2011. Kendrick-analogous network visualisation of ion cyclotron resonance Fourier transform mass spectra: improved options for the assignment of elemental compositions and the classification of organic molecular complexity. *Eur. J. Mass Spectrom.*, **17**, 415–421.
- Van Krevelen D.W., 1950. Graphical-statistical method for the study of structure and reaction processes of coal. *Fuel*, **29**, 269–228.
- Veloso M.M., Almandanim M.C., Baleiras-Couto M., Pereira H.S., Carneiro L.C., Feveiro P., Eiras-Dias J., 2010. Microsatellite database of grapevine (*Vitis vinifera* L.) cultivars used for wine production in Portugal. *Ciência Téc. Vitiv.*, **25**, 53–61.
- Wu Z., Rodgers R.P., Marshall A.G., 2004. Two- and three-dimensional van Krevelen diagrams: A graphical analysis complementary to the Kendrick mass plot for sorting elemental compositions of complex organic mixtures based on ultrahigh-resolution broadband Fourier Transform Ion Cyclotron Resonance Mass measurements. *Anal Chem.*, **76**, 2511–2511.