AN ORTHOGONAL METABOLOMIC APPROACH ON THE VAUD SWISS VINEYARD TO ASSES THE "TERROIR EFFECT"

Lise Bréant[1], Guillaume Marti[1], Agnes Dienes-Nagy[2], Vivian Zufferey[2], Johannes Rösti[2], Fabrice Lorenzini[2], Katia Gindro[2], Olivier Viret[2] and Jean-Luc Wolfender[1].

1 School of pharmaceutical sciences, University of Geneva, University of Lausanne, 30 Quai Ernest-Ansermet, 1211 Geneva 4, Switzerland
2 Swiss Federal Research Station Agroscope Changins-Wädenswil, Route de Duillier, P.O. Box 1012, CH-1260 Nyon, Switzerland

1. Introduction

The so called "terroir effect" is one of the most important that influence the vine-fruit-wine continuum. This effect is however challenging to evaluate. In addition to climate, soil also marks a major impact to the "terroir effect" but little is known about its main contributing factors. A recent study conducted in the Vaud viticultural area has shown that vine nitrogen content appeared to be one of the most important parameter that influence this continuum [1]. The nitrogen content seems positively correlated with well appreciated organoleptic attributes.
In order to confirm this result a large scale metabolomic investigation of white and red wines around the Geneva lake has been undertaken. Three grape varieties, ten Vaud vineyards (Switzerland) and five vintages (2006-2010) have been investigated in this study (**Fig. 1**).

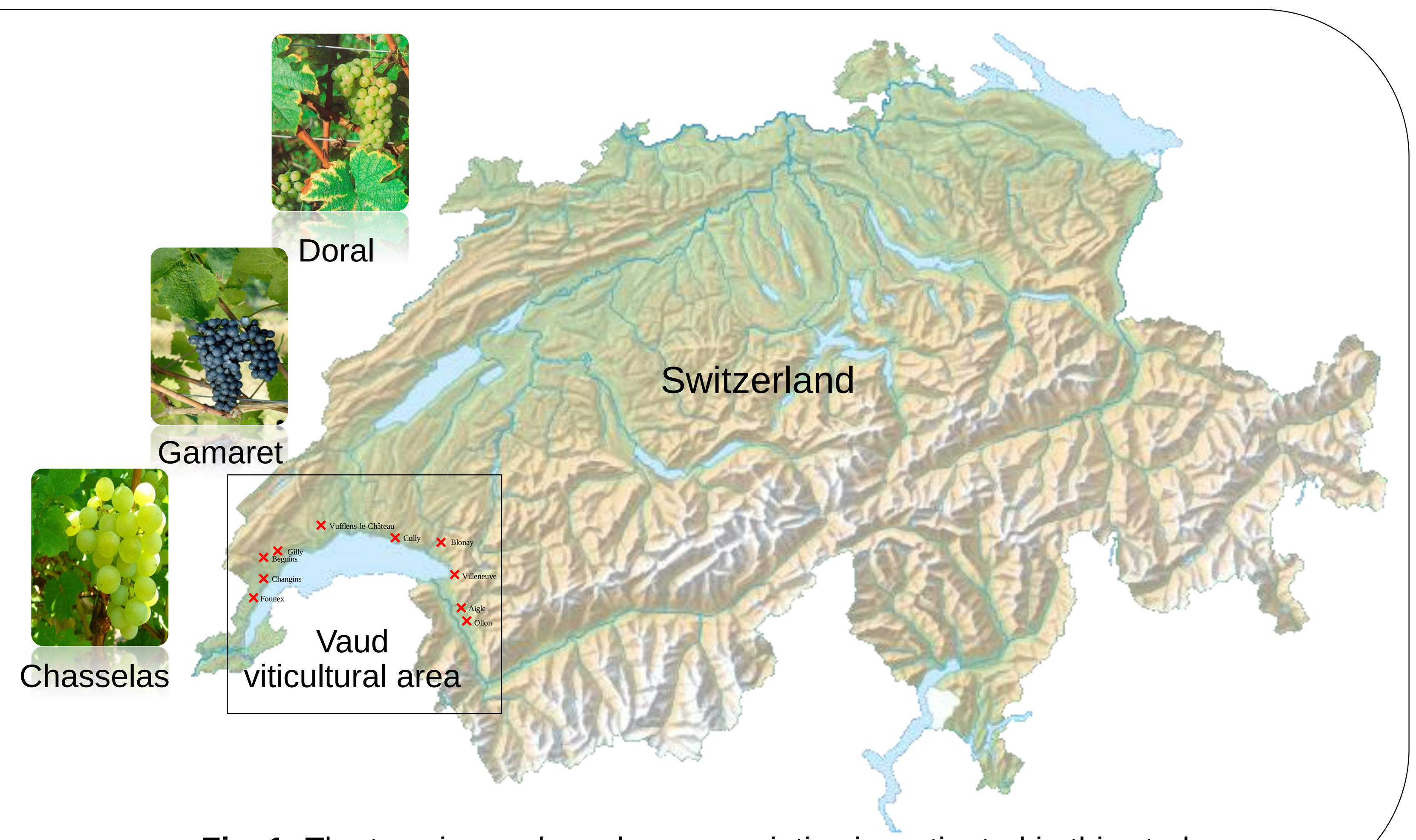


Fig. 1: The ten vineyards and grape varieties investigated in this study.

2. Nitrogen influences on vine-fruit-wine continuum

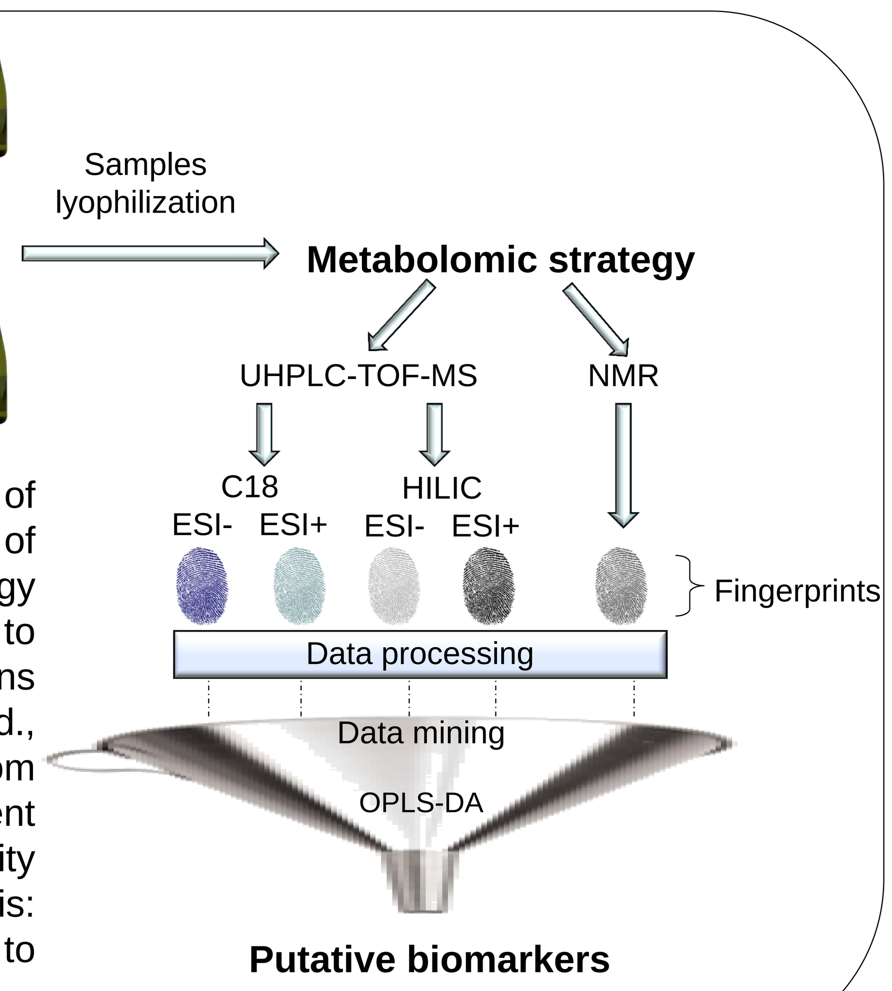
In order to understand this continuum a concentration of assimilable nitrogen has been supplied *in vivo* on vine leaves during the grape maturation.

A comprehensive MS based metabolomic strategy has been apply to highlight subtle but significant biochemical changes [2] related to nitrogen supply. MS fingerprints were recorded in both positive and negative UHPLC-TOF-MS with either hydrophilic interaction (HILIC) or reversed phase liquid chromatography.

Several putative biomarkers correlated to nitrogen supply could be highlighted by supervised data mining and identified by means of their accurate mass, and fragmentation pattern (**Fig. 2**).

Experimental protocol

Fig. 2: Experimental protocol consisting of pulverization (treated) or not (control) of nitrogen on the vines and metabolomic strategy apply to the lyophilized wine samples in order to identify putative biomarkers. Gradient conditions for C18 column (BEH acquity 50 × 1 mm i.d., 1.7 µm) analysis: Water/ACN + 0.1% FA from 98/2 to 2/98 in 5 min at 300 µl/min. Gradient conditions for HILIC column (BEH acquity amide 50 × 1 mm i.d., 1.7 µm) analysis: Water/ACN + NH4 acetate buffer from 5/95 to 50/50 in 5 min at 300 µl/min.



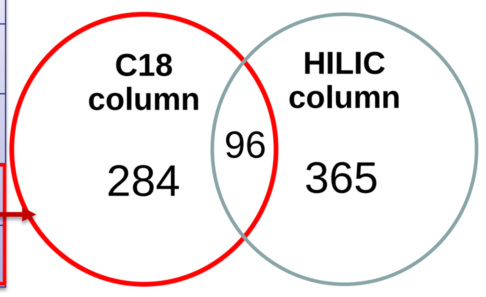
3. Fingerprints of lyophilized wines

Two different rapid profiling methods (fingerprinting) have been used to generate matrix for multivariate data analysis. 284 and 365 features (ESI+) were detected respectively in reverse phase (C18) and hydrophilic interaction (HILIC) UHPLC-TOF-MS (~ 5 min) (**Fig. 5 a and c**). Only 15 % of overlapping signals where detected in both analytical methods showing the complementarity of the approach (**Fig. 5 b**).

a)

	ESI(-)	ESI(+)
Threshold (counts)	250	1000
	Features detected	
C18 column	178	284
HILIC column	573	365

b)



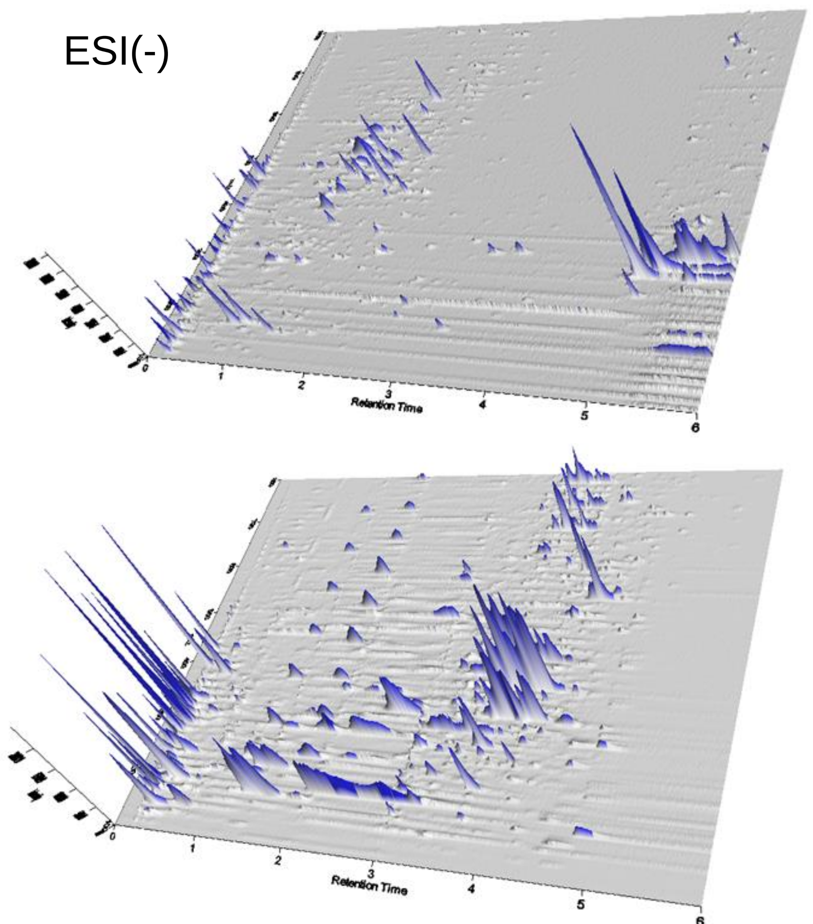
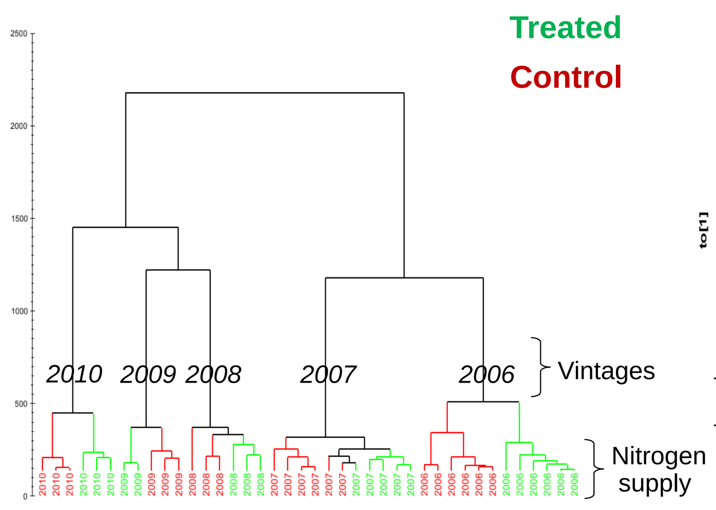


Fig. 4: a) Comparison of features obtained within C18 and HILIC column in positive and negative ESI modes; b) Venn diagram for positive ESI mode; c) UHPLC-TOF-MS chromatogram (BPI-3D ion map) of lyophilized white wine of Doral grape variety using a C_{18} (up) or HILIC (bottom) columns.

4. Chemometric analysis of the UHPLC-TOF-MS data

The hierarchical cluster analysis (HCA) of the LC-MS dataset for both column used displayed two levels of classification. The first one is linked to the vintage and the second one to the nitrogen treatment. An orthogonal partial least squares discriminant analysis (OPLS-DA) allowed the discrimination of wine samples according to the nitrogen supply with a significant level of confidence (**Fig. 6**). Biomarkers highlighted by the OPLS-DA are positively correlated with nitrogen supply (**Fig. 7**).



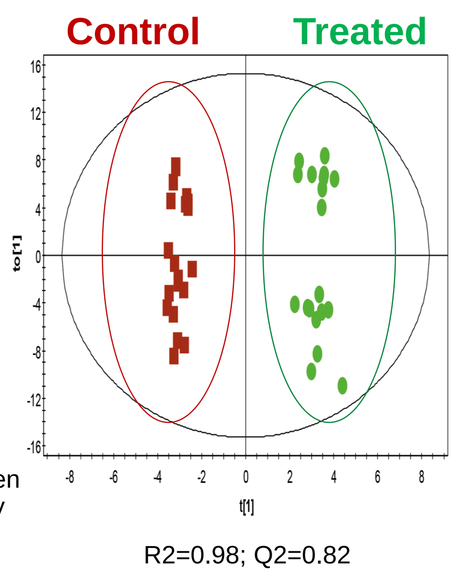


Fig. 6: HCA (left) and OPLS-DA (right) of the five vintages of white wine of Doral grape variety obtained with C18 column in ESI-.

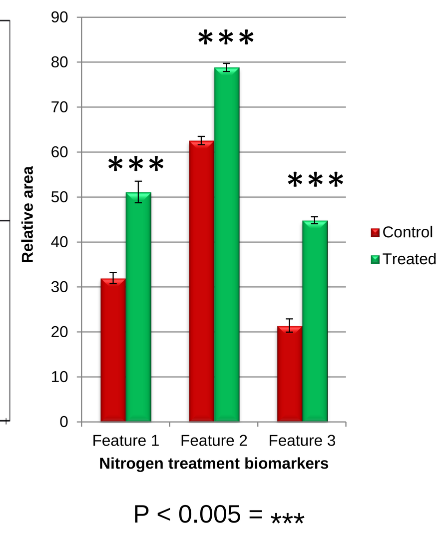


Fig. 7: Positively correlated biomarkers.

5. Conclusion

The metabolomic strategy devised provide detailed fingerprints with orthogonal and complementary detection methods. MVDA enabled a wine sample discrimination correlated to the nitrogen treatment. Different biomarkers were highlighted that were significantly up regulated upon nitrogen supply. The identification of these biomarkers is underway, their characterization and quantification might help to determine 'terroir' characteristics that favor well appreciated organoleptic quality. A large scale study with the same procedure will focus on Vaud vineyards which possess vine nitrogen content variations due to climatic conditions and pedologic attributes.

6. References

1. J-S. Reynard *et al. J. Int. Sci. Vigne Vin*. **2011**, *45*, 211-221.
2. G. Glauser, *et al. J. Chromatogr. A.* **2008**, 1180, 90-98.