



INTRAVARIETAL DIVERSITY: AN OPPORTUNITY FOR CLIMATE CHANGE ADAPTATION

Authors: Victoria SICHEL¹, Gautier SARAH¹, Loic LE CUNFF^{2,3}, Patrice THIS^{1,3}, Thierry LACOMBE^{1,3}, Charles ROMIEU^{1*}

¹UMR AGAP Institut, Univ Montpellier, CIRAD, INRAE, Institut Agro, F-34398 Montpellier, France.

²Institut Français de la Vigne et du Vin, F-34398 Montpellier, France.

³UMT Geno-Vigne®, IFV-INRAE-Institut Agro, F-34398 Montpellier, France.

*Corresponding author: charles.romieu@inrae.fr

Abstract:

Context and purpose of the study

Merlot grapevine is the second wine cultivar most planted in the world and especially in the Bordeaux wine region. This cultivar has many advantages in producing high quality wine; however, in the last decade, climate change has increased the sugar concentration in berries at harvest and shortened the maturation cycle. If this has been up to now a great opportunity to improve wine quality profile, we are touching the tipping point. High sugar concentration at harvest induces high alcohol content in wine which can negatively impact wine quality. There are many viticultural and oenological practices possible to limit this effect. In this study we focus on plant material through intra-varietal diversity of Merlot cultivar.

Material and methods

This study is based on three years of phenotyping a private collection of Merlot including 55 accessions and 3 ENTAV-INRA® registered clones. Each genotype is present in four blocks of 6 vines in the experimental plot. Among this collection, two extreme genotypes for sugar concentration, selected on 3 years of phenotyping, were phenotyped again on the fourth year at a larger scale. Separated individual berries were collected at 5 stages from green hard berries to mature berries. Sugar and organic acids were measured on each berry to compare both maturation cycles and to select triplicates of synchronized individual berries before transcriptomic analysis. Paired RNAseq reads were then aligned on high quality Merlot assembly and the counting table of gene expression was produced.

Results

Extreme genotypes have an average of 17g.L⁻¹ difference in sugar concentration at harvest which approximately represents 1%vol of alcohol in wine after fermentation. Whilst as expected thousands of genes were differentially expressed according to the advancement of ripening, statistical analysis only revealed 28 differentially expressed genes between the two genotypes throughout all developmental stages and gives an insight on genes potentially involved in the control of sugar concentration. Selection among intra-varietal diversity therefore appears as a tool to immediate adaptation but also offers possibilities in gene detection of very specific agronomical traits.

Keywords: Clones, Merlot, Sugar, Alcohol content, RNAseq



1. Introduction

Merlot comes from the Bordeaux wine region and is the second most planted red wine cultivar in the world [1]. It is particularly appreciated for its black fruit flavored wines with delicate and soft tanins along with its relatively early ripening date compared to other Bordeaux varieties [2], its high sugar concentration and its good maturity level. because of its precocity, Merlot is highly affected by climate change. Indeed, global warming and increased CO₂ levels in the atmosphere are affecting grapevine phenology and berry composition. In the past decades, potential alcohol has increased and total acidity decreased [3]. This has improved wine quality but could become negative in the near future because excessive alcohol content leads to less fruity wines and enhances bitterness mouth-feel [4-6].

There are many technical, oenological and viticultural practices available to reduce alcohol content in wine. This study focuses on the plant material, and more specifically on the genetic diversity available at clonal (i.e. intravarietal) level. Indeed, Merlot cultivar is a cross between Magdeleine Noire des Charentes (mother) and Cabernet franc (father)[7], whose unknown date of appearance seems more recent than traditional cultivars as Pinot noir. Since its creation Merlot has been vegetatively propagated by cuttings and grafted on american vines to resist phylloxera. In the 70's the French organism ENTAV (now IFV) and its partners selected twelve clones of Merlot which are marketed worldwide under ENTAV-INRA® trademark [8]. Apart from these widely propagated clones, Merlot genetic resource also exists in private conservation plots. Having not being subjected to a clonal selection process and certification framework, they can't be commercialized and are preferably called "accession" rather than "clones".

This experiment evaluates the phenotypic diversity available in a private collection of 55 Merlot accessions, identifies accessions that are more adapted to climate change and contributes to understand the physiological mechanisms underlying this adaptation.

2. Material and methods

Three years of phenotyping were performed on a private collection of Merlot containing 55 accessions and 3 clones in Bordeaux, France. Many agronomical traits were observed : phenology, vigor, tolerance to water stress, fertility, berry weight, yield, sugar content and acidity. This data was used to rank extreme accessions according to sugar concentration. Two groups of 6 accessions were selected, respectively exhibiting the lowest and the highest sugar concentration at harvest. These extremes were observed during a fourth year of phenotyping but this time including several preexisting generations of cuttings in order to insure the phenotypic stability. Ultimately this lead to identifying the two accessions that had the lowest and the highest sugar concentration level.

During this last year of phenotyping, batches of 10 single berries were sampled at 4 dates from green stage to harvest, each individual fruit being immediately enveloped in an aluminium foil and frozen in liquid nitrogen. A fragment of each berry was analyzed using HPLC to measure sugar and acid concentration. Results obtained allowed to select four successive biological triplicates of three well synchronized individual fruits on each genotype. From a physiological point of view, this sampling method is more reliable than mixing hundreds of unsynchronized berries in unknown variable

proportions [9, 10]. Gene expression levels were obtained through bioinformatics analysis before identifying which transcripts were statistically differentially expressed between both accessions.

3. Results and discussion

3.1. Phenotyping results

The statistical analysis demonstrated that the "accession" has a significant impact on sugar concentration at harvest (pvalue <0.05). The average difference between the two extremes is 17 g.L⁻¹ of sugar, i.e. the equivalent of 1%vol in finished wine. In addition, these results show significant differences in other characteristics related to the overall physiological balance of the plant. Correlations could be observed between sugar concentration and other agronomic measurements such as shoot weight, bunch volume, and deltaC13 (not shown here).

3.2. Berry kinetic comparison

Due to the asynchrony of berries, sugars and malic acid evolution during grape ripening were reconstructed (Figure 1) to adjust the phenological stages of each individual berry. Both accessions are initially close and the sugar accumulation phase does not seem to be discriminating. However, the switched off expression of genes affecting specific sugar transporters and aquaporins [10] in line with the increased concentration of tartaric acid indicates that the two accessions move apart during the wilting phase consequently to the physiological stop of phloem unloading in berries. C057 seems to lose more water and concentrates more rapidly than C008 (Figure 1).

3.3. Differentially expressed genes

All berries in the same cluster are asynchronous and have their own ripening rhythm [11]. The selection of individual berries allows to compare the two accessions at equivalent physiological stages and avoids the phenological bias triggering the expression of thousands of developmentally regulated genes [10]. Transcriptomic analyses led to the detection of 28 differentially expressed genes (DEGs) between the two extremes. The functions of these DEGs identified in this experiment are not yet fully elucidated and seem to be related to different aspects of the plant's developmental biology. Nevertheless, these genes directly or indirectly impact grape berry wilting and thus sugar concentration at harvest time. Most of these genes show differential expression between extreme genotypes throughout the ripening period, from the end of the herbaceous plateau to the terminal wilting phase (Figure 2).

Finally, the principal component analysis explained 84% of the variations in gene expression between the samples along two main axes, the first (68% of the variance explained) corresponding to the concentration of sugars, and the second (16% of the variance) to the expansion or wilting of the berries (Figure 3). The two accessions differ the most during this last phase of development. The gene expression thus confirms the previous phenotypic interpretation.

4. Conclusion

The accession significantly impacts sugar concentration at harvest and leads to a difference of approximately 1% vol of alcohol in wines. The wilting phase that follows sugar accumulation has a determining impact on the final sugar concentration at harvest and there are significant differences in behaviour at clonal level at this stage. In the future, it would be interesting to better understand the



functions of the differentially expressed genes identified in this study and use DNA sequencing data to identify causal mutations responsible for these transcriptomic and phenotypic differences.

5. Acknowledgement

This study is a result of a collaboration program between Petrus, INRAE, Institut Agro Montpellier and IFV. HPLC was performed in the biochemical phenotyping lab of AGAP institute at CIRAD in Montpellier, RNAseq was done by MGX Montpellier Genomix and bioinformatics analysis on MUSE MESO@LR cluster.

6. Literature cited

1. OIV: **Press Release**, <http://www.oiv.int/public/medias/5681/en-communiqu-depresse-octobre-2017.pdf> , **(Accessed 19 September 2018)**. 2017.
2. Parker A, de Cortázar-Atauri IG, Chuine I, Barbeau G, Bois B, Boursiquot J-M, Cahurel J-Y, Claverie M, Dufourcq T, Génry L *et al*: **Classification of varieties for their timing of flowering and veraison using a modelling approach: A case study for the grapevine species *Vitis vinifera* L.** *Agricultural and Forest Meteorology* 2013, **180**:249-264.
3. Van Leeuwen C, Darriet P: **The Impact of Climate Change on Viticulture and Wine Quality.** *Journal of Wine Economics* 2016, **11**(1):150-167.
4. Goldner MC, Zamora MC, Di Leo Lira P, Gianninoto H, Bandoni A: **EFFECT OF ETHANOL LEVEL IN THE PERCEPTION OF AROMA ATTRIBUTES AND THE DETECTION OF VOLATILE COMPOUNDS IN RED WINE.** *Journal of Sensory Studies* 2009, **24**(2):243-257.
5. Fontoin H, Saucier C, Teissedre PL, Glories Y: **Effect of pH, ethanol and acidity on astringency and bitterness of grape seed tannin oligomers in model wine solution.** *Food quality and preference* 2008, **2008 v.19 no.3**(no. 3):pp. 286-291.
6. Jordão A, Vilela A, Cosme F: **From Sugar of Grape to Alcohol of Wine: Sensorial Impact of Alcohol in Wine.** *Beverages* 2015, **1**(4):292-310.
7. Boursiquot J-M, Lacombe t, Laucou v, Julliard s, Perrin f-x, Lanier n, Legrand d, Meredith c, This p: **Parentage of Merlot and related winegrape cultivars of southwestern France: discovery of the missing link.** *Australian Journal of Grape and Wine Research* 2009, **15**(2):144-155.
8. PlantGrape: **Catalogue of Vines Cultivated in France, IFV – INRAE – l’Institut Agro | Montpellier SupAgro, 2009-2020.** 2020.
9. Shahood R, Torregrosa L, Savoi S, Romieu C: **First quantitative assessment of growth, sugar accumulation and malate breakdown in a single ripening berry.** *OENO One* 2020, **54**(4):1077-1092.
10. Savoi S, Torregrosa L, Romieu C: **Transcripts switched off at the stop of phloem unloading highlight the energy efficiency of sugar import in the ripening *V. vinifera* fruit.** *Horticulture Research* 2021, **8**(1).
11. May P: **From bud to berry, with special reference to inflorescence and bunch morphology in *Vitis vinifera* L.** *Australian Journal of Grape and Wine Research* 2000, **6**(2):82-98.

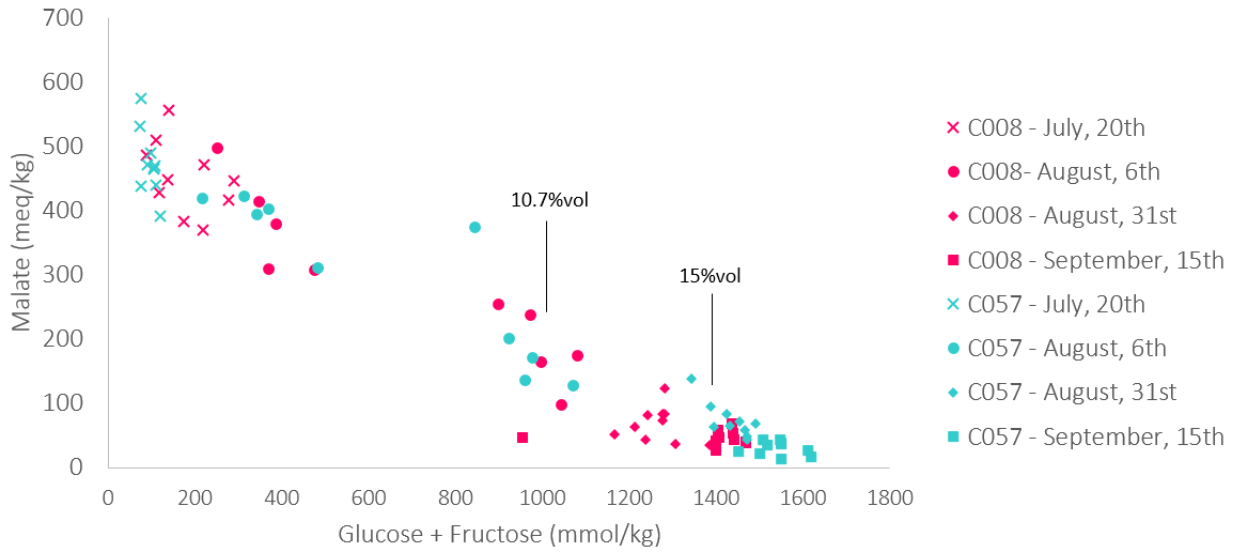


Figure 1 : Malate degradation in relation to sugar accumulation during ripening of individual berries on two Merlot accessions C008 and C057. The potential alcohol content (10.7% and 15%vol) has been added to the x axis according to the concentration of glucose and fructose.

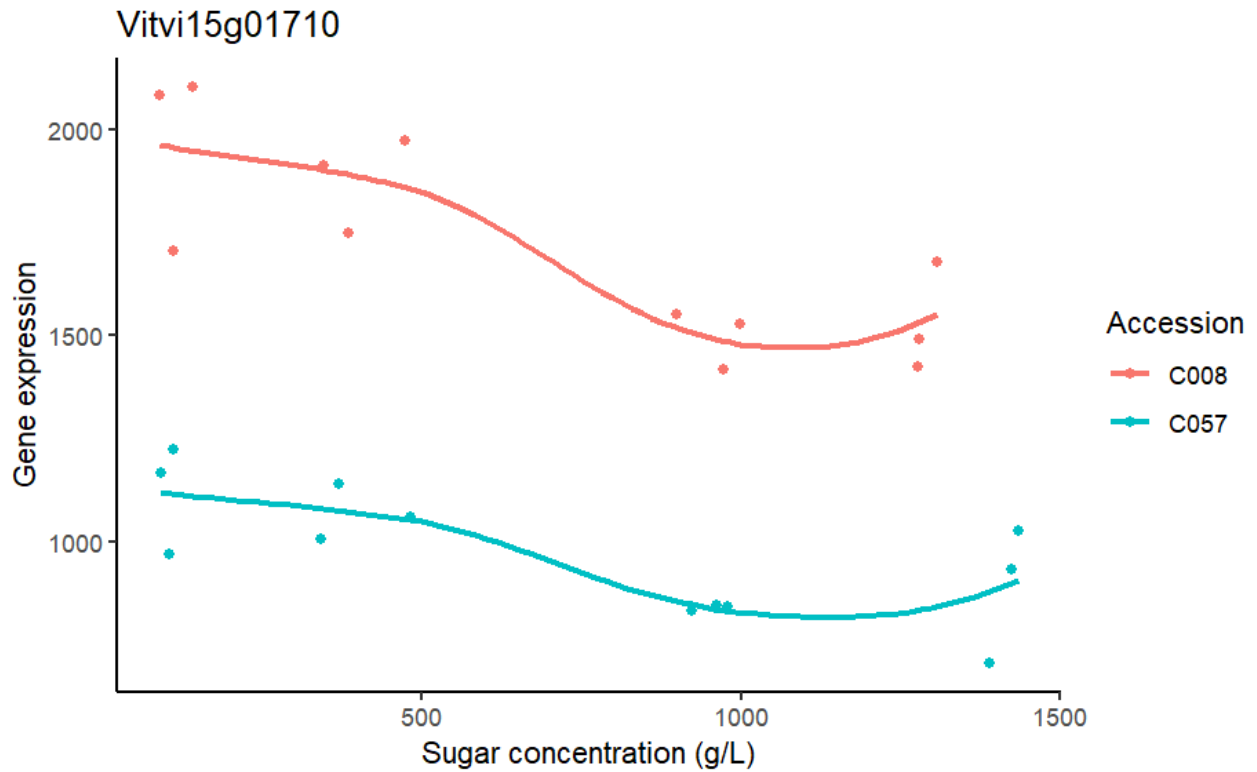


Figure 2: *Vitvi15g01710 - LOC100258557- anaphase-promoting complex subunit 11* expression according to sugar concentration in both extreme Merlot accessions.

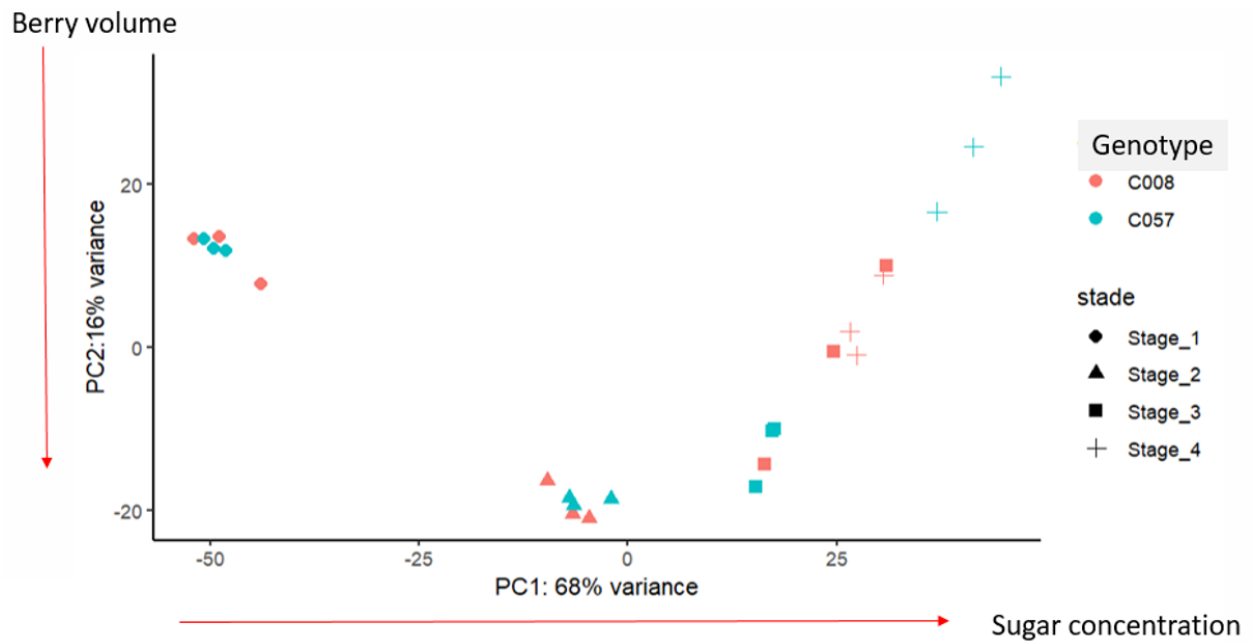


Figure 3: Principal component analysis of gene expressions on two extreme accessions of Merlot cultivar, each sample is a unique berry.