MODELING FROM FUNCTIONING OF A GRAPE BERRY TO THE WHOLE PLANT

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Abstract:
Context and purpose of the study – Grape quality is a complex trait that mainly refers to berry chemical composition, including sugars, organic acids, phenolics, aroma and aroma precursor compounds. It is known that the composition and concentration of chemical compounds dynamically change along berry development and can be affected by genotypes (rootstock and scion), environment (light, temperature and water) and nutrient status (carbon and nitrogen). Moreover, the ongoing climate change is affecting the physiology of grapevine and ultimately wine quality and typicity. Therefore, a better understanding of the mechanisms controlling the accumulation of quality-related metabolites (both primary and secondary) in grape berry is essential to choose grapevine cultivars and viticultural practices best adapted to a given growth region. Process-based models can mechanistically integrate various processes involved in fruit growth and composition, and simulate the plant responses to weather and management practices, making them a promising tool to study the response of berry quality to those factors.

Material and methods – Three types of modeling approaches have been applied, including constraint-based flux balance analysis, process-based models, and 3D structure-functional models. These models were established, calibrated and validated based extensive experimental measurements in grapevines growing under contrast conditions, e.g. nitrogen limitation, modulation of leaf-to-fruit ratios, and light conditions. Fruit growth was measured in parallel with metabolite composition, enzyme activities, and whole plant growth processes, such as canopy photosynthesis, and transpiration. Moreover, *in silico* analysis was conducted to create virtual genotypes or to assess regulatory roles of model parameters.

Results– At cellular scale, we used constraint-based flux balance analysis model to investigate the flux modifications responsible for biosynthesis of anthocyanins in response to nitrogen limitation. At organ scale, we developed process-based models for sugar accumulation and anthocyanin composition in grape berries, which allowed us to determine the key processes responsible for these two important quality components. At the whole-plant scale, a 3D structure-functional model was developed to simulate water transport, leaf gas exchanges, carbon allocation, and berry growth in various genotype x environment scenarios. In the future, the interactions among the different scales of regulation will be further modelled to offer a model toolkit that allows more accurate predictions of grapevine growth and berry quality elaboration under changing environments and paving a way towards model-assisted breeding.

Keywords: Environmental adaptation, *Vitis vinifera*, berry quality, modeling.

1. Introduction

Grapevine is one of the most economically important fruit crops in the world, with its berries for various end-uses, i.e. fresh fruits, raisins, wine, etc. In France, viticulture accounts for 15% of the total value of agricultural production, with the vast majority (95%) of it devoted to wine production (36.7 million hL, source: FranceAgrimer, 2016). The growth of grapevine and elaboration of grape quality change dynamicly over berry development and are influenced by complex genotype x environment x management interactions (Coombe and McCarthy, 2000; Kuhn *et al.*, 2014; Poni *et al.*, 2018). Wine quality is tightly linked to berry composition at harvest, including sugar, organic acids, amino acids and a large array of secondary metabolites (polyphenols, aroma precursors, etc.) (Conde *et al.*, 2007). Organic acids (malic and tartaric), tannins, hydroxycinamates and the methoxypyrazine aromas are mainly accumulated during the first, vegetative growth phase of the berry development (Conde *et al.*, 2007; Coombe and McCarthy, 2000). In contrast, sugars (glucose and fructose, which determine wine alcohol content), anthocyanins and flavor compounds (thiols precursors, terpenes, etc…) are mainly accumulated during the second phase (ripening phase, the so-called véraison stage) (Conde *et al.*, 2007; Coombe and McCarthy, 2000). Both primary and secondary metabolism will be affected by climate change related factors (Lecourieux *et al.*, 2017; Martínez-Lüscher *et al.*, 2015; Rienth *et al.*, 2014; Rienth *et al.*, 2016; Sadras and Moran, 2012; Sadras and Petrie, 2011; Soar *et al.*, 2008; Sweetman *et al.*, 2014). Therefore, a better understanding of the mechanisms controlling the accumulation of quality-related metabolites (both primary and secondary) in grape berry is essential to choose grapevine cultivars and viticultural practices best adapted to a given growth region (Keller, 2010; van Leeuwen *et al.*, 2013).

Mathematical models can mechanically integrate various processes to reproduce the plant and fruit responses to climatic conditions and management practices, making them a promising tool to study the response of fruit quality to those factors (Beauvoit *et al.*, 2018; Génard *et al.*, 2010; Moriondo *et al.*, 2015). Many robust models have been developed to simulate biological processes and phenotypic responses of crops to environmental parameters, at various scales, from whole plant to metabolic pathways (Marshall-colon et al. 2017). At the plant or organ levels, models offer the possibility, by mechanically integrating various physiological processes (photosynthesis, respiration, carbon translocation and storage, growth, etc...), to describe how they causally interact and allow predicting how they respond to environmental factors. For example, models have been developed to simulate C3 and C4 photosynthetic processes (Wang *et al.*, 2017), transpiration and stomata conductance response (Buckley, 2017; Buckley and Mott, 2013; Deans *et al.*, 2017; Tardieu and Parent, 2017), phloem and xylem sap flows (Hall and Minchin, 2013; Hall *et al.*, 2017; Minchin and Lacointe, 2017), fruit growth (Fishman and Génard, 1998; Hall *et al.*, 2013; Liu *et al.*, 2007) and composition (Martre *et al.*, 2011). At the tissue, cellular and subcellular levels, metabolic models allow to compute and to predict metabolic fluxes in a given metabolic network, and are thus useful to investigate which steps or portions of a metabolic pathway is affected by an environmental factor For example, such models have been used to describe sucrose metabolism in sugarcane steams (Uys *et al.*, 2007) or in tomato (Beauvoit *et al.*, 2014; Colombié *et al.*, 2015).

Various mathematical models at different biological scales have been developed to model the interactions between rootstock and scion and to model the response of grape berry quality to environment (light, temperature and water) and trophic factors (carbon and nitrogen) (Fig. 1). Moreover, models are also used to dissect complex traits into simple biological relevant processes in order to assess genetic diversity and genetic determinisms for a better adaptation in the face of climate change. The objective of this work is to summarize different types of models that have been developed for grape at the laboratory of Ecophysilogy and Functional Genomics of Grapevine (EGFV) (Fig. 1) and beyond, in order to highlight their potential utilities for better understanding the control of berry growth and quality elaboration.

2. Modeling sugar accumulation in grape berry

Some attempts have been conducted to model sugar accumulation with a mathematical function, enabling the calculation of the onset, rate, and duration of sugar accumulation during the post-veraison stage. The model-assisted analysis found that the early maturity caused by climate change was mainly due to an early onset of ripening but without significant effect on ripening rate (Sadras *et al.*, 2008;

Sadras and Petrie, 2011). A process-based model has also been developed to dissect the relative contributions of carbon supply, sugar metabolism within the berry, and water dilution to sugar accumulation over berry development (Fig. 2) (Dai *et al.*, 2009). It well reproduced the effects of different leaf-to-fruit ratio on sugar concentration and showed that low leaf-to-fruit ratio not only affected carbon supply, but also modified the sugar metabolism and water dilution. This model had then been used to compare the regulation modes of sugar accumulation in three fleshy fruits with distinct sugar concentrations (Dai *et al.*, 2016). These fruits include grape, peach, and tomato, and different modes of regulation were identified, e.g. the higher soluble sugar concentration in grape than in tomato is a result of higher sugar importation, while the higher soluble sugar concentration in grape than in peach is due to a lower water dilution. These results provide novel insights into the drivers of differences in soluble sugar concentration among fleshy fruits.

3. Modeling anthocyanin metabolism and composition in grape berry

Anthocyanins determine the color of wine and are one of the important quality factors. The metabolism and composition of anthocyanins are influenced by various environmental factors and change dynamically over berry development (Poni *et al.*, 2018). It has been observed that low nitrogen can increase anthocyanin biosynthesis in grape berry (Hilbert *et al.*, 2003; Soubeyrand *et al.*, 2014). A constraint-based flux balance analysis model has been developed to investigate the flux distribution in grape cell in order to better understand the mechanisms underlying the nitrogen limitation induced anthocyanin biosynthesis (Fig. 3) (Soubeyrand *et al.*, 2018). To this end, the biomass composition of berry cells grown under different nitrogen levels were established with targeted metabolomics. The flux analysis showed that in low nitrogen-fed cells all the metabolic fluxes of central metabolism were decreased, whereas fluxes that consume energy and reducing power, were either, suggesting that anthocyanin biosynthesis acts as an energy escape valve in grape cells (Soubeyrand *et al.*, 2018).

Moreover, a process-based dynamic model was also developed to simulate the developmental anthocyanin composition in response to sugar supply and light conditions (Dai *et al.*, 2017). This model describes the flux partitioning by basic chemical reaction rules with total anthocyanin as input and reaction rate as parameters. It can successfully simulate the observed effects of sugar supply and light on anthocyanin composition. The model provides an alternative way of phenotyping by dissecting a complicated trait (anthocyanins) into developmentally stable traits (model parameters), and will be applied to cross progeny to identify potential genetic linkages with the model parameters.

4. Modeling vine functioning and berry growth

To better understand the mechanisms underlying fruit growth and its biochemical compositions, one needs to analyze the fruit and plant as an integrated system of interacting processes. Therefore it is necessary to integrate the fruit models (growth, sugar accumulation, anthocyanin composition etc) with a whole-plant model in order to capture the interactions between the fruit and parent plant as well as environmental factors. A 3D functional-structural grapevine model has been developed to couple the thermodynamics of water transport with leaf gas exchanges and integrate the biophysical berry growth model (Fig. 4). The newly developed whole-plant model can well reproduced the effects of canopy structure, radiation, temperature, CO_2, VPD, soil water content on leaf photosynthesis, transpiration, temperature and stomata conductance and leaf water potential (Zhu *et al.*, 2018a), as well as the berry growth and sugar concentration (Zhu *et al.*, 2018b). The robust performance of this model allows for modelling climate effects from individual plants to fields, and for modelling plants with complex, non-homogenous canopies. In addition, the model provides a basis for future modelling efforts aimed at describing the physiology and growth of individual organs in relation to water status. The water transport module of the 3D grapevine model has also been used to identify the effects of rootstocks on scion water use under water stress (Peccoux *et al.*, 2018). Model analysis revealed that the water-stress resistance rootstock differed mainly at root architecture and the biosynthesis of abscisic acid (Peccoux *et al.*, 2018).

5. Conclusion

Fruit growth and quality are a result of an integrative system that functions at different levels of the plant and combines metabolic networks and biophysical processes. The interest of developing integrative models is twofold: From the point of view of molecular biology, the existence of an integrated, multi-scale model could offer a useful framework to interpret omics data, in relation to environmental factors, developmental stages and agricultural practices; From an ecophysiological perspective, the integration of cellular and molecular levels can help refine plant models, shedding light onto the complex interplay between different spatial and temporal scales in the emerging system response (Beauvoit *et al.*, 2018). In the future, the interactions among the different scales of regulation will be further modelled to offer a model toolkit that allows more accurate predictions of grapevine growth and berry quality elaboration under changing environments and paving a way towards model-assisted breeding.

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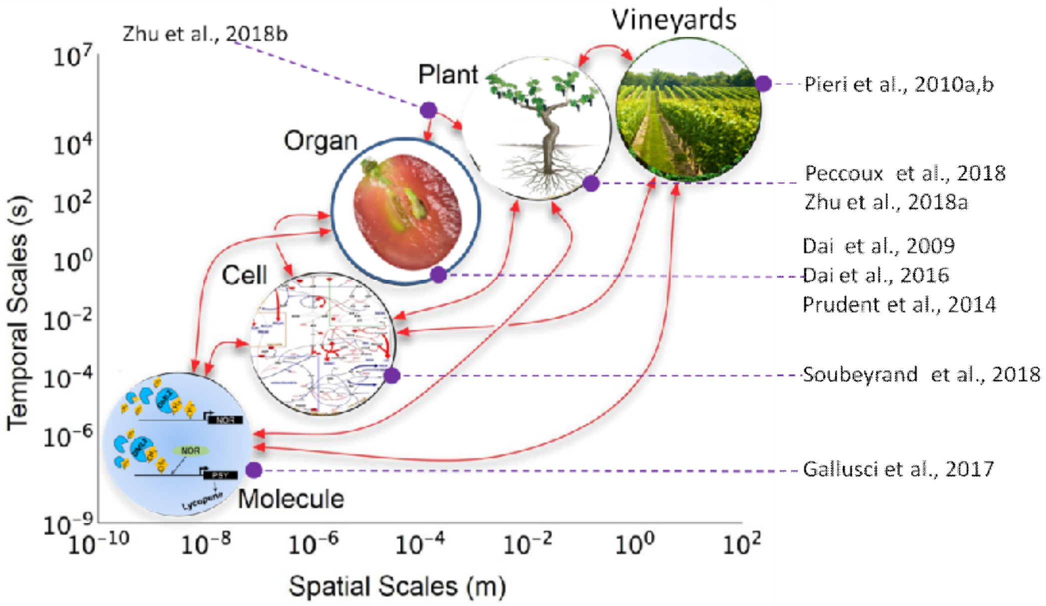

Fig. 1. Schematic representation of regulation of grapevine growth and the elaboration of grape berry quality at multi-scales. Main modeling results focused on each scale at laboratory EGFV are indicated. (The schema was modified based on Marshall-Colon et al., 2017. Front. Plant Sci 8:786)

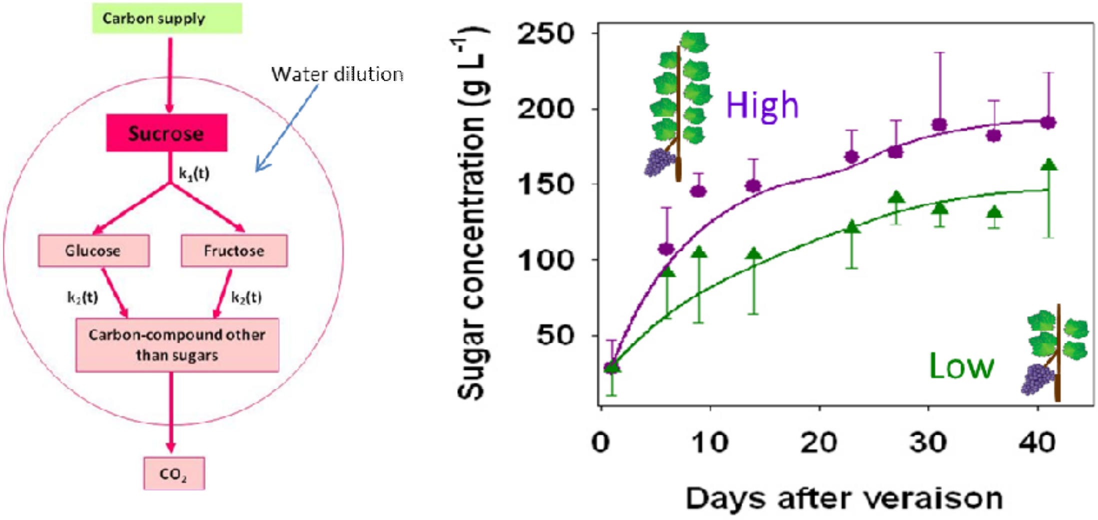

Fig. 2. Modeling sugar accumulation over berry development as a function of sugar importation, sugar metabolism, and water dilution. The model was able to well simulate the effects of different leaf-to-fruit ratio on sugar accumulation in grape berry (Dai *et al.*, 2009). Points are observed values and lines for model simulations.

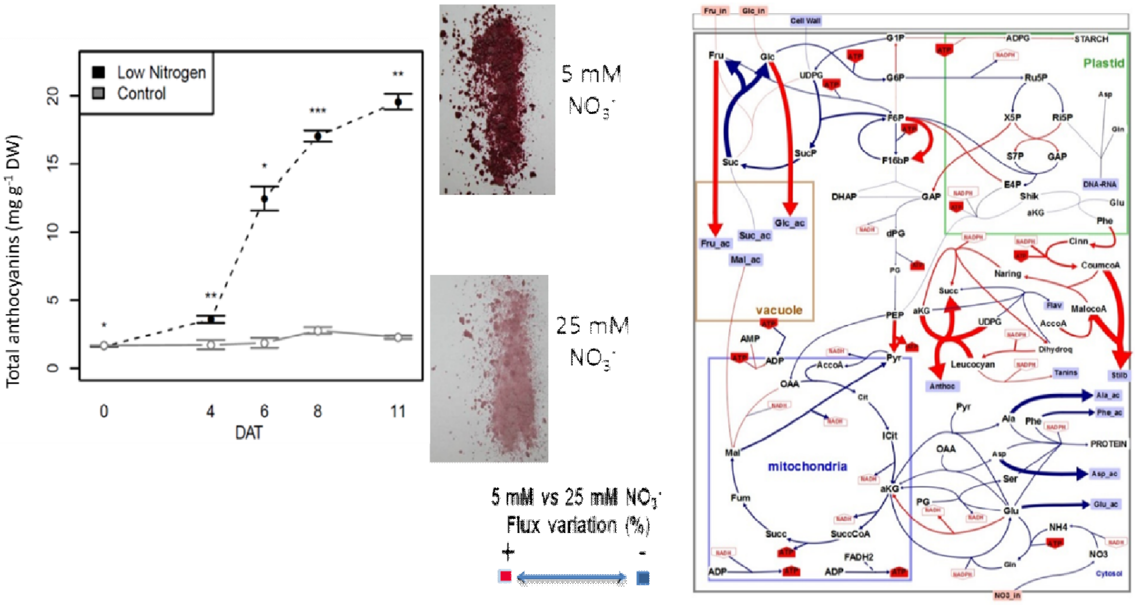

Fig. 3. Constraint-based flux balance analysis model for investigating the low nitrogen induced biosynthesis of anthocyanins (Soubeyrand *et al.*, 2018). The flux map indicated that anthocyanin biosynthesis acts as an energy escape valve in grape cells under low nitrogen conditions.

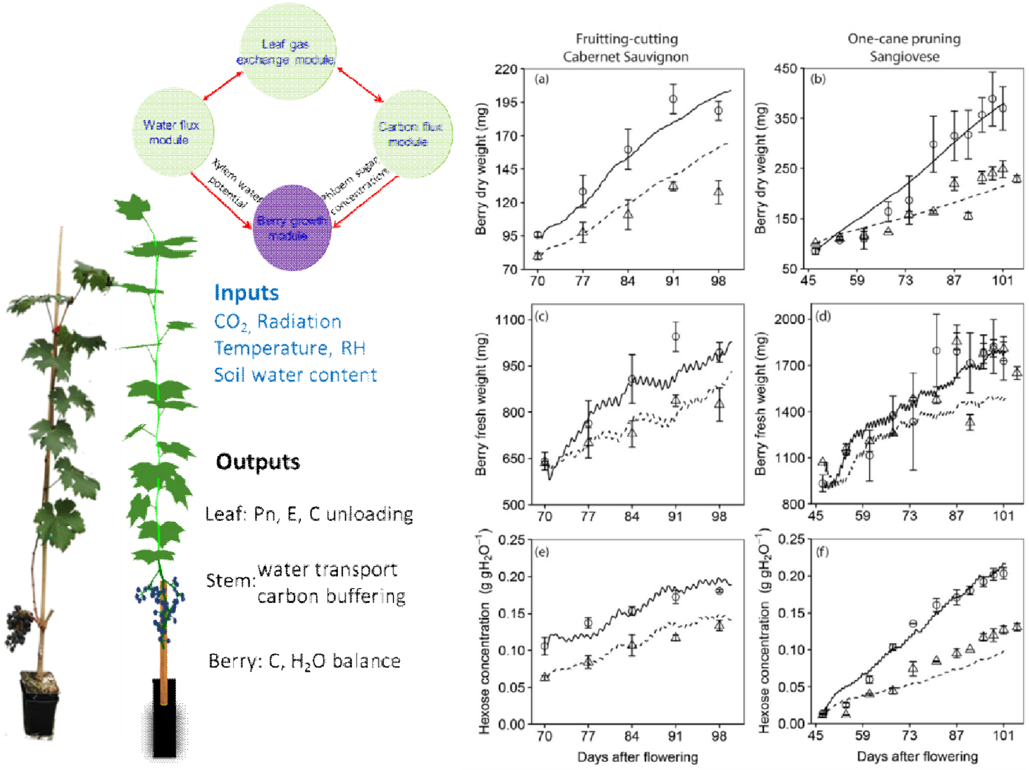

Fig. 4. A functional-structural plant model has been developed to simulate vine functioning and berry growth. The model includes 4 main modules including leaf gas exchange module, water flux module, carbon distribution module and berry growth module (Zhu *et al.*, 2018a; Zhu *et al.*, 2018b). The berry growth module was adapted from a biophysical fruit growth model describing water and carbon balances over fruit development (Fishman and Génard, 1998). The model can well simulate the effect of CO_2, light conditions and water stress on leaf transpiration and photosynthesis (Zhu *et al.*, 2018a), and the effect of leaf-to-fruit ratio on berry growth (Zhu *et al.*, 2018b). Points are observed values and lines for model simulations