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To cite this version:

Zhanwu Dai, Ghislaine Hilbert, Natalia Bobeica, Stefano Poni, Michel Génard, et al.. Mathematic model for simulating anthocyanin composition during grape ripening: another way of phenotyping. 10. International Symposium on Modelling in Fruit Research and Orchard Management, International Society for Horticultural Science (ISHS). INT., Jun 2015, Montpellier, France. 490 p., 10.17660/ActaHortic.2017.1160.54 . hal-02736834

HAL Id: hal-02736834

https://hal.inrae.fr/hal-02736834v1

Submitted on 2 Jun 2020

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Mathematic model for simulating anthocyanin composition during grape ripening: another way of phenotyping

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Abstract

Anthocyanins are responsible for grape color, an important quality factor for market acceptance. The relative proportion of cyanidin-based (cyanidin- and peonidin-derivatives) and delphinidin-based (delphinidin-, malvidin- and petunidin-derivatives) anthocyanins largely determines the color variation among red/purple/blue colored grape varieties. Anthocyanin biosynthesis is under complex regulation by nutrients, hormones, and environmental cues sensed by the berry. However, the physiological mechanisms underlying these regulations are poorly understood. A dynamic model was developed to simulate the developmental anthocyanin composition in two genotypes and different carbon and nitrogen conditions. This model describes the flux partitioning by basic chemical reaction rules with total anthocyanin as input and reaction rates as parameters. Data were gathered from two experiments, which studied the developmental changes in anthocyanin composition. The model was calibrated for two cultivars under a given growth condition and then validated by applying the model to other conditions for the same cultivar. The model can successfully simulate all the observed modifications in anthocyanin composition throughout berry ripening. This provides an alternative way of phenotyping by dissecting a complicated trait (anthocyanin content and composition) into developmentally stable traits (model parameters).

Keywords: climate change, color pigment, fruit model, fruit quality, *Vitis*

INTRODUCTION

Anthocyanins are responsible for grape color, an important quality factor for the resultant wine color and consequently wine typicity. A recent analysis in western Australian region clearly indicates that the anthocyanin content will be affected negatively in a projected warming climate condition, and more interestingly, different cultivars show genotype-specific magnitudes of response, with 'Shiraz' less sensitive than 'Cabernet Sauvignon' (Barnuud et al., 2013). This genotype specific response, opening up possibilities to select cultivars that can maintain wine typicity under climate change, may result from the differences of anthocyanin compositions in different genotypes. In fact, anthocyanins presented in red grapes are derived mainly from five anthocyanindins: cyanidin (Cy), delphinidin (Dp), peonidin (Pn), petunidin (Pt) and malvidin (Mv), with different patterns of hydroxylation, methylation, and acylation (Mazza and Francis, 1995). The relative composition of these anthocyanins has an important impact on the color hue and color stability of the resultant wines (Mazza and Francis, 1995) and, therefore, has been considered as fingerprint for cultivar identification and classification. The anthocyanin color changes progressively from red to blue with the increasing hydroxylation on the phenolic B ring (He et al., 2010).

Understanding the regulation of anthocyanin biosynthesis and especially the fine-tune of its composition is challenging. The quantity and composition of the anthocyanins in grape are strongly determined by genetic factors (Fournier-Level et al., 2009) and interact actively with various environmental conditions (Downey et al., 2006). Anthocyanins are synthesized

by the flavonoid pathway with coordinated regulation and most of the structural genes encoding enzymes in the pathway have been well identified in grapevine (Boss et al., 1996). However, the fine tune of anthocyanin composition by hydroxylation, methylation, and acylation is less studied. The present study aimed to develop a mathematic model to simulate the complex effects of genotype and environment interaction on the fine tune of anthocyanin composition in skin of grape during grape berry development. Ultimately, the newly developed anthocyanin model may serve as a phenotyping approach to estimate a set of parameter values for each individual in a progeny and couple with genetic analysis to gain a better understanding of the fine-tune and degradation of anthocyanins in grape berries.

MATERIALS AND METHODS

Model presentation

The schema of the model (inputs, variables, and parameters) was established based on the consensus of the anthocyanin biosynthesis pathway (Boss et al., 1996) and showed in Figure 1. Enzyme reactions were lumped to reduce the complexity while maintaining the key topology structure of the pathway. The transformation rate of an anthocyanin form to another was modeled as a quantity dependent reaction with a rate constant (r_i, i from 1 to 13). The variation in quantity of a given anthocyanin form at each time step is then described as the results of its biosynthesis, transformation to other forms, and degradation. For example, the variation in quantity of Pn-glc is described by an ordinary differential equation (ODE) as below:

$$\frac{dPn_glc}{dt} = r_1 Cy_glc - (r_9 + r_8 + kd)Pn_glc \tag{1}$$

where r_1, r_9, and r_8 are rate constants, kd is a degradation coefficient, Cy_glc and Pn_glc are quantity of the anthocyanins in berry. Fifteen ODEs are needed to describe all the 15 anthocyanin forms presented in Figure 1.

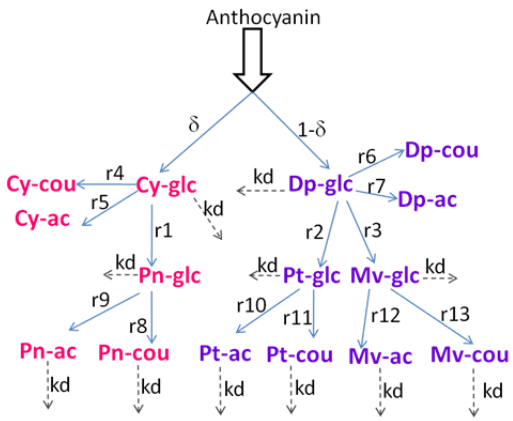

Figure 1. Simplified schema of anthocyanin biosynthesis used for the model. δ is an allocation coefficient between two branches of the metabolic pathway; r_i (i from 1 to 13) is the rate of transformation among metabolites; kd is degradation rate of anthocyanins. Abbreviations: Dp = delphinidin, Mv = malvidin, Pt = petunidin, Cy = cyanidine, Pn = peonidin, glc = glucoside, ac = acetate, cou = coumarate.

The influx of anthocyanin precursors ($\frac{dAP}{dt}$) into the anthocyanin biosynthesis pathway is needed as model input and is calculated based on the first order of derivation of

total anthocyanin content over berry development ($\frac{dTA_{obs}}{dt}$) and degradation as:

$$\frac{dAP}{dt} = \frac{dTA_{obs}}{dt} + kd\,TA \tag{2}$$

where TA_{obs} is total anthocyanins measured in the skin of a grape.

The developmental profile of each anthocyanin form is then calculated by integrate the ODEs and its concentration (mg/100gFW) is then calculated as:

$$[Anthocyanin_i] = \frac{100\,Anthcyanin_i}{FW_{skin}} \tag{3}$$

where FW_{skin} is the skin fresh weight (g) per berry.

Experimental data

Experimental data from two experiments, which studied the effects of carbon and nitrogen supply on anthocyanin composition during grape berry development, were used to calibrate and validate the model. Detailed experimental conditions were fully described in previous publications (Bobeica et al., 2015; Hilbert et al., 2003). Briefly, experiment 1 studied the response of anthocyanin composition in grape berries of 'Cabernet Sauvignon' to two leaf-to-fruit ratios, including 12 leaves per cluster or 3 leaves per cluster, which corresponded to carbon sufficient and limitation conditions, respectively. Berries were sampled nine times from veraison to maturity and separated into skin and pulp. The detailed anthocyanin composition in skin was analyzed with HPLC (Bobeica et al., 2015). Experiment 2 studied the effects of nitrogen supply on anthocyanin composition in grape berries of 'Merlot'. Three levels of nitrogen nutrient solutions (1.4, 3.6, and 7.2 mM nitrogen) were supplied from fruit set to leaf fall (Hilbert et al., 2003). Berries were sampled six times from veraison to maturity and anthocyanins measured as in experiment 1.

Model parameterization and evaluation

The model was implemented in R language, and all data analyses were done with R software (R Development Core Team, 2010). The ODEs of the model were numerically integrated by using the Euler method with a one-day time step. Model parameters were estimated for the 12 leave per cluster treatment of experiment 1 and the low nitrogen supply of experiment 2 through model calibration, by fitting the simulated outputs to the observations, using the nonlinear least squares method (nls function). The validation of the model was assessed with data from the remaining treatments. To assess the goodness-of-fit and the predictive quality of the model, two commonly used criteria, root mean squared error (RMSE) and relative root mean squared error (RRMSE), were adopted (Kobayashi and Salam, 2000).

RESULTS AND DISCUSSION

The observed anthocyanin fractions (Figures 2 and 3) did not cover all the possible anthocyanin forms presented in Figure 1. Therefore, the rate constants linked to the biosynthesis of the absent fractions were set to zero. The model correctly simulated the temporal profile of all the observed anthocyanin forms in 'Cabernet Sauvignon' under carbon sufficient condition with a RMSE of 13.7 mg/100 g FW and a RRMSE of 23.4% (Figures 2A and C). When the model was validated under carbon limitation condition by inputting the total anthocyanins of this condition and reaction constant estimated, RMSE and RRMSE were 2.9 mg 100 g^{-1} FW and 17.3%, respectively (Figures 2B and D). This suggests a good agreement between observed and predicted results. Moreover, the model well reproduced the effect of carbon limitation on the modification of anthocyanin composition: under

carbon sufficient condition, Mv-glc was much higher than Mv-ac and Pn-glc (Figure 2A); while under carbon limitation conditions, Mv-glc was similar to Mv-ac and Pn-glc was significantly reduced (Figure 2B).

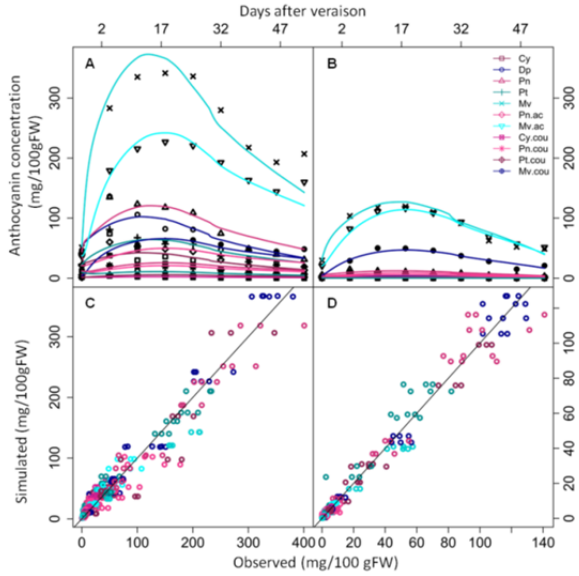

Figure 2. Comparison between observed (points) and simulated (lines) anthocyanin fractions during berry development under carbon sufficient (A, C) or limitation (B, D) conditions. The line 1:1 between observed and simulated results was added in C and D. Abbreviations of anthocyanin fractions are the same as in Figure 1.

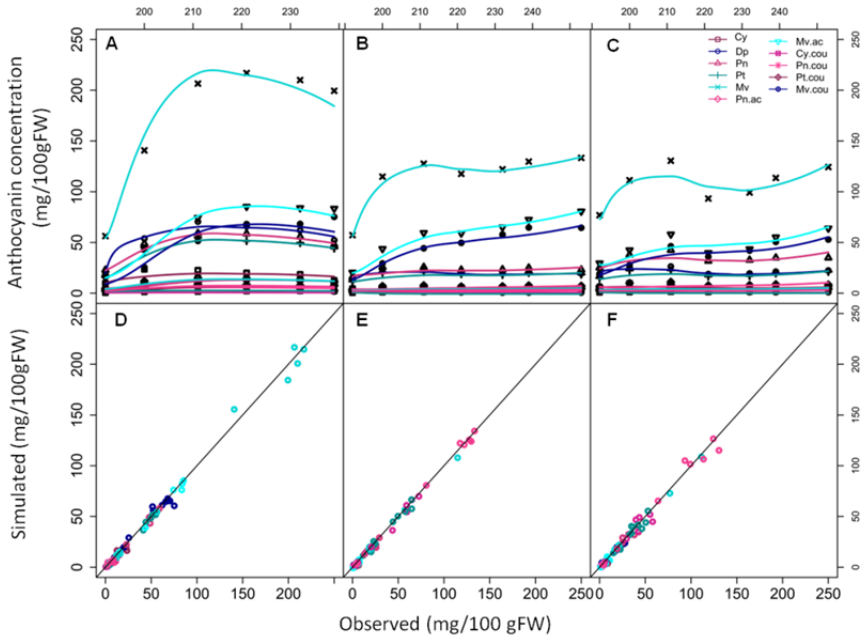

Figure 3. Comparison between observed (points) and simulated (lines) anthocyanin fractions during berry development under low (A, D), medium (B, E), or high (C, F) nitrogen supply conditions. The line 1:1 between observed and simulated results was added in D, E, and F. Abbreviations of anthocyanin fractions are the same as in Figure 1.

The model also correctly simulated the temporal profile of all the observed anthocyanin forms in 'Merlot' under low nitrogen supply condition with a RMSE of 3.9 mg 100 g^{-1} FW and a RRMSE of 11.6% (Figure 3A and D). Higher nitrogen supply reduced nitrogen concentration and increased the proportion of Pn-glc and Cy-glc (Figure 3B and C). The model was able to capture these modifications with RMSE of 2.3 mg 100 g^{-1} FW and RRMSE 11.1% for medium nitrogen supply (Figure 3B and E) and RMSE of 3.5 mg 100 g^{-1} FW and RRMSE of 17.1% for low nitrogen supply (Figures 3C and F).

Since it is known that different molecules of anthocyanins have different color hues and stabilities (He et al., 2010), it is important to study the alteration of anthocyanin composition in response to various growing conditions. In addition to carbon source limitation and nitrogen supply, light exclusion (Guan et al., 2014), water stress (Castellarin et al., 2007) and high temperature (Mori et al., 2007) are also known to affect not only the total anthocyanin content but also anthocyanin composition. Further efforts are warranted to test the validity of the model to simulate the effect of other environmental factors, such as light, temperature and water stress.

Mathematic models that represent biological processes with genetic parameters have been shown to be powerful in dissecting a complex trait into traits more physiologically relevant and stable over changing environments (Bertin et al., 2010). Coupling the model-dissected traits with QTL analysis has been successfully applied to a population of 20 introgression lines of tomato and dissected the trait sugar concentration into three relevant components, which strengthen the QTL analysis and provide novel insights into the regulation of tomato fruit sugar accumulation (Prudent et al., 2011). The present anthocyanin model is suitable to be applied as a phenotyping approach to estimate a set of parameter values for each individual in a progeny. Coupling the obtained parameter values as traits with QTL analysis will enable us to gain a better understanding of the fine tune and degradation of anthocyanins in grape berries.

ACKNOWLEDGEMENTS

This research was supported partly by a grant from the Environment and Agronomy department of the Institute National de la Recherche Agronomique (INRA). It also received funding from the European Community's Seventh Framework Program (FP7/2007-2013) under the grant agreement no. FP7-311775, Project INNOVINE.

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