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Research article

# Exogenous application of pectin-derived oligosaccharides to grape berries modifies anthocyanin accumulation, composition and gene expression

Daniel Villegas [a, *], Michael Handford [b], José Antonio Alcalde [a], Alonso Perez-Donoso [a]

[a] Pontificia Universidad Católica de Chile, Departamento de Fruticultura y Enología, Vicuña Mackenna 4860, PO Box 7820436, Santiago, Chile
[b] Universidad de Chile, Department of Biology, Faculty of Sciences, Universidad de Chile, Las Palmeras, 3425 Santiago, Chile

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ABSTRACT

Anthocyanins are secondary metabolites synthesized in grape berry skins via the phenylpropanoid pathway, with functions ranging from skin coloration to protection against pathogens or UV light. Accumulation of these compounds is highly variable depending on genetics, environmental factors and viticultural practices. Besides their biological functions, anthocyanins improve wine quality, as a high anthocyanin content in berries has a positive impact on the color, total phenolic concentration and, ultimately, the price of wine. The present work studies the effect of the pre-*veraison* application of pectin derived oligosaccharides (PDO) on the synthesis and accumulation of these compounds, and associates the changes observed with the expression of key genes in the phenylpropanoid pathways. To this end, pre-*veraison* Cabernet Sauvignon bunches were treated with PDO to subsequently determine total anthocyanin content, the anthocyanin profile (by HPLC-DAD) and gene expression (by qRT-PCR), using Ethrel and water treatments for comparison. The results show that PDO were as efficient as Ethrel in generating a significant rise in total anthocyanin content at 30 days after treatment (dat), compared with water treatments (1.32, 1.48 and 1.02 mg e.Mv-3G/g FW respectively) without any undesirable effect on berry size, soluble solids, tartaric acid concentration or pH. In addition, a significant alteration in the anthocyanin profile was observed. Specifically, a significant increase in the relative concentration of malvidin was observed for both PDO and Ethrel treatments, compared with water controls (52.8; 55.0 and 48.3%, respectively), with a significant rise in tri-hydroxylated forms and a fall in di-hydroxylated anthocyanins. The results of gene expression analyses suggest that the increment in total anthocyanin content is related to a short term increase in *phenylalanine ammonia-lyase* (*PAL*) expression, mediated by a decrease in *MYB4A* expression. A longer term increase in *UDP-glucose flavonoid 3-O-glucosyltransferase* (*UFGT*) expression, probably mediated by a rise in *MYBA1* was also observed. Regarding the anthocyanin profile, despite the increase observed in *MYB5A* expression in PDO and Ethrel treatments, no changes in *flavonoid 3′-hydroxylase* (*F-3′-H*); *flavonoid 3′5′-hydroxylase* (*F-3′5′-H*) or *O-methyltransferase* (*OMT*) could be related with the profile modifications described. Overall, this study highlights that application of PDO is a novel means of altering specific grape berry anthocyanins, and could be a means of positively influencing wine quality without the addition of agrochemicals.



## 1. Introduction

Anthocyanins are secondary metabolites synthesized in grape berry skins (and in some varieties in pulp or flesh) from *veraison* to maturity. Although their main function is berry skin coloration (red to blue color) (Boss and Davies, 2009; Kalt et al., 2003), these molecules are involved in a wide range of biological functions including antioxidant capacity, and protection against UV-light and pathogen attack (Takahama, 2004; Chalker-Scott, 1999). The

*Abbreviations:* PDO, pectin-derived oligosaccharides; TAC, total anthocyanin content; mg e.Mv-3G/g FW, milligrams of malvidin-3G equivalent per fresh weight; SS, soluble solids; TA, titratable acidity; dat, days after treatment; *PAL*, phenylalanine ammonia-lyase; *C4H*, cinnamate 4 hydroxylase; *4CL*, 4 coumarate:CoA ligase; *CHI*, chalcone synthase; *F3H*, flavanone 3 hydroxylase; *F3′H*, flavonoid 3′-hydroxylase; *F3′5′H*, flavonoid 3′5′-hydroxylase; *DFR*, dihydroflavonol 4-reductase; *LDOX*, leucoanthocyanidin dioxygenase; *UFGT*, UDP-glucose flavonoid 3-O-glucosyltransferase; *OMT*, O-methyltransferase; *MYB*, R2R3-MYB transcription factor.

* Corresponding author.
*E-mail addresses:* dlvilleg@uc.cl (D. Villegas), mhandfor@uchile.cl (M. Handford), jalcalde@uc.cl (J.A. Alcalde), agperez@uc.cl (A. Perez-Donoso).

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accumulation and proportion of these compounds depend on genetics (Dai et al., 2011; He et al., 2010; Rio-Segade et al., 2008), environmental factors and viticultural practices (Downey et al., 2006), with light and temperature the primary environmental factors capable of modifying the synthesis and accumulation of anthocyanins (Keller, 2010; Mori et al., 2005a,b; Roubelakis-Angelakis and Kliewer, 1986). These molecules, which are flavonoid compounds, are synthesized through the phenylpropanoid pathway (Fig. 1), which is widespread and extensively studied in different plant species. Consequently, several structural genes, encoding enzymes in the anthocyanin biosynthetic pathway, have been described (Borsani et al., 2010; Boss et al., 1996; Cultrone et al., 2010; Honda et al., 2002; Matus et al., 2008; Xie et al., 2011). Among these structural genes *PAL*, encoding phenylalanine ammonia-lyase (responsible for the first step in anthocyanin biosynthesis) and *UFGT*, encoding UDP glucose:flavonoid-3-O-glucosyltransferase (responsible for glycosylation of anthocyanidin) have been described as key genes (Fig. 1). The expression of these structural genes is in turn controlled by biosynthetic genes encoding R2R3MYB, basic helix-loop-helix (bHLH) and tryptophan-aspartic acid repeat (WDR) transcription factors (Hichri et al., 2010; Jeong et al., 2006). In grapes, several MYB-family proteins controlling various points of the phenylpropanoid pathway have been identified (Fig. 1). MYB4A transcription factor is described as an inhibitor of *C4H* and *4CL* expression (Colquhoun et al., 2011; Jin et al., 2000) and has also been suggested as an inhibitor of *PAL* expression (Cavallini et al., 2015), whereas MYBA1/2 transcription factors activate the expression of *UFGT* and *OMT* (a structural gene responsible for methylation downstream of anthocyanin glycosylation) (Bogs et al., 2007; Cutanda-Perez et al., 2009; Kobayashi et al., 2002; Walker et al., 1999). Another transcription factor, MYB5A trans-activates the expression of *F3′5′H* (responsible for deviation of the phenylpropanoid pathway to tri-hydroxylated anthocyanin forms) (Deluc et al., 2006, 2008).

Besides their importance in plant metabolism, anthocyanins have been extensively studied due to their beneficial effect on human health (De Pascual-Teresa and Sanchez-Ballesta, 2008; Jing et al., 2008; Maletić et al., 2009) as well of being an important determinant of quality in red wines (Fanzone et al., 2012; Mori et al., 2005b). In order to increase anthocyanin synthesis and accumulation, various viticultural strategies have been developed, such as defoliation or application of chemicals, which have not always led to the desired effect and/or are expensive and time consuming (Greer and La Borde, 2006; Smart and Robinson, 1991). An innovative practice has been the application of plant cell wall derivatives (more specifically, pectin-derived oligosaccharides; PDO) in order to increase anthocyanin content in grapes. In this way, Ochoa-Villareal et al. (2011) reported that application of PDO can promote color development in Flame Seedless table grapes through anthocyanin accumulation. However, the effect of PDO on the expression of multiple structural and regulatory genes of the phenylpropanoid pathway, and their influence on wine-making varieties, has not been evaluated to date. Therefore, we examined whether application of PDO promotes anthocyanin accumulation in Cabernet Sauvignon berries, determining changes in the profile of the different anthocyanins and associating the modifications observed (either in accumulation or in the anthocyanin profile) to changes in expression of structural and regulatory genes of the

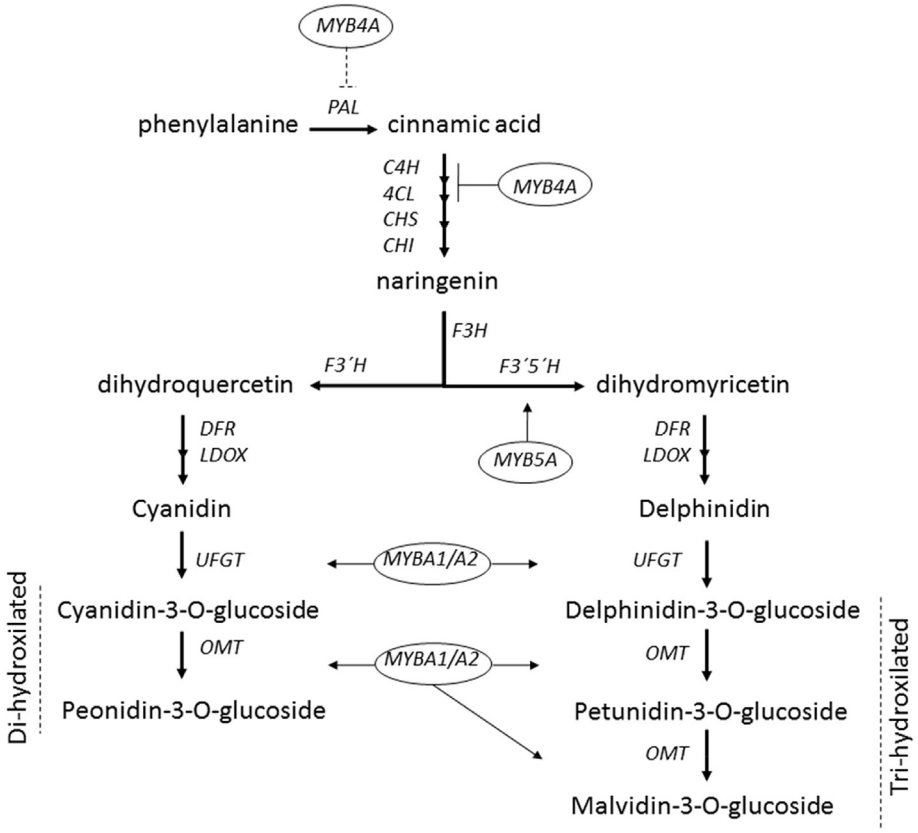


**Fig. 1.** Simplified overview of the phenylpropanoid pathway leading to the accumulation of di- and tri-hydroxylated anthocyanins in grape berries. Gene abbreviations: *PAL*, phenylalanine ammonia-lyase; *C4H*, cinnamate 4 hydroxylase; *4CL*, 4 coumarate:CoA ligase; *CHI*, chalcone synthase; *F3H*, flavanone 3 hydroxylase; *F3′H*, flavonoid 3'-hydroxylase; *F3′5′H*, flavonoid 3′5'-hydroxylase; *DFR*, dihydroflavonol 4-reductase; *LDOX*, leucoanthocyanidin dioxygenase; *UFGT*, UDP-glucose flavonoid 3-O-glucosyltransferase; *OMT*, O-methyltransferase; *MYB*, R2R3-MYB transcription factor. The dashed line over *PAL* indicates a possible negative regulation by *MYB4A*.