Journal of Plant Physiology 219 (2017) 22–27

Contents lists available at ScienceDirect

Journal of Plant Physiology

journal homepage: www.elsevier.com/locate/jplph

Research Paper

# Vanillylacetone up-regulates anthocyanin accumulation and expression of anthocyanin biosynthetic genes by inducing endogenous abscisic acid in grapevine tissues

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ARTICLE INFO

*Keywords:*
Vanillylacetone
Abscisic acid
Anthocyanin
Maturation
Grapevine

ABSTRACT

We investigated the effect of vanillylacetone (VA) on anthocyanin accumulation with aim of improving grape berry coloration. Spraying *Vitis vinifera* cv. Muscat Bailey A berries with VA at veraison increased sugar/acid ratio, an indicator of maturation and total anthocyanin accumulation. To elucidate the molecular mechanism underlying the effect of VA on anthocyanin accumulation, *in vitro* VA treatment of a grapevine cell culture was carried out. Endogenous abscisic acid (ABA) content was higher in the VA-treated cell cultures than in control at 3 h after treatment. Consistent with this, the relative expression levels of anthocyanin-synthesis-related genes, including *DFR*, *LDOX*, *MybA1* and *UFGT*, in VA-treated cell cultures were much higher than those in control, and high total anthocyanin accumulation was noted in the VA-treated cell cultures as well. These results suggest that VA up-regulates the expression of genes leading to anthocyanin accumulation by inducing endogenous ABA. In addition, VA increased total anthocyanin content in a dose-dependent manner. Although VA treatment in combination with exogenous ABA did not exhibit any synergistic effect, treatment with VA alone showed an equivalent effect to that with exogenous ABA alone on total anthocyanin accumulation. These findings point to the possibility of using VA for improving grape berry coloration.

## 1. Introduction

Grape and its products, particularly wine, are important economically and the color of grape berries and wine is a crucial factor affecting product price. Grape berry coloration is influenced by such environmental factors as ambient temperature (Mori et al., 2007), sunlight (Chorti et al., 2010), and water (Castellarin et al., 2007), and adverse weather conditions result in poor berry coloration. To maintain or improve berry coloration, girdling (Koshita et al., 2011), leaf removal (Hunter et al., 1991; Matsuyama et al., 2014; Tardaguila et al., 2010), and abscisic acid (ABA) treatment (Katayama-Ikegami et al., 2016; Kitamura et al., 2007; Koyama et al., 2010; Mori et al., 2005; Peppi et al., 2008; Sandhu et al., 2011) have been examined.

Grape berry color is determined mainly by the amount of anthocyanins, which are classified as flavonoid molecules. The anthocyanins are synthesized *via* the phenylpropanoid biosynthesis pathways (Fig. 1). In the pathways, phenylalanine is converted to *p*-coumaroyl-CoA in phenylpropanoid biosynthesis pathway, and the *p*-coumaroyl-CoA is converted to colored anthocyanidin by leucoanthocyanidin dioxygenase (*LDOX*) in flavonoid biosynthesis pathway. Finally, the colored anthocyanidins are converted to anthocyanins, which are more stable water soluble plant pigments and show deeper color than anthocyanidin, by UDP glucose flavonoid 3-*O*-glucosyl transferase (*UFGT*) in anthocyanin biosynthesis pathway. Thus, *UFGT* is the key enzyme for the biosynthesizing anthocyanins (anthocyanidin-3-glucoside) from anthocyanidins by glycosylating anthocyanidins (Ford et al., 1998) and its expression is regulated by *MybA1* (Kobayashi et al., 2005). Therefore, the transcription levels of the genes encoding these enzymes determine grape berry color and red wine appearance. On the other hand, ABA triggers berry ripening (Coombe and Hale, 1973). ABA up-regulates the expression of anthocyanin-synthesis-related genes, such as *UFGT*, and increases anthocyanins in grape skin (Ban et al., 2003; Jeong et al., 2004).

Vanillylacetone (synonym, zingerone; IUPAC name, 4-(4-hydroxy-3-methoxyphenyl) butan-2-one), denoted by VA in this study, belongs to the phenolic alkanone group (Fig. 2). VA is a pungent component in ginger (*Zingiber officinale*) and is widely known as a nontoxic and

*Abbreviations:* VA, vanillylacetone; ABA, abscisic acid; MybA1, myeloblastosis transcription factor A1; UFGT, UDP, glucose flavonoid 3-*O*-glucosyl transferase; CHS, chalcone synthase; CHI, chalcone isomerase; F3′H, flavonoid 3′-hydroxylase; F3′5′H, flavonoid 3′,5′-hydroxylase; DFR, dihydroflavonol 4-reductase; LDOX, leucoanthocyanidin dioxygenase

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http://dx.doi.org/10.1016/j.jplph.2017.09.005
Received 15 February 2017; Received in revised form 12 September 2017; Accepted 16 September 2017
Available online 20 September 2017
0176-1617/ 

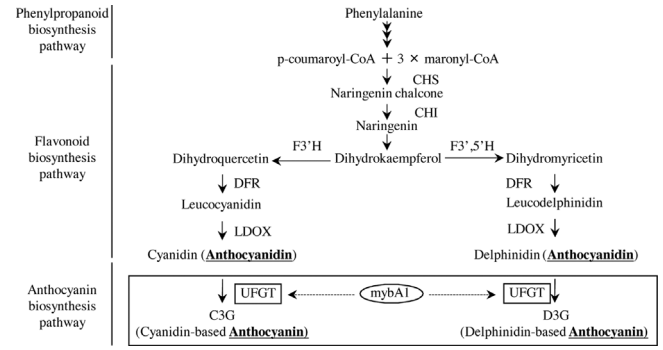


**Fig. 1.** Biosynthesis of anthocyanin in grape. Anthocyanins are synthesized from Anthocyanidins by UFGT. CHS, chalcone synthase; CHI, chalcone isomerase; F3′H, flavonoid 3′-hydroxylase; F3′5′H, flavonoid 3′,5′-hydroxylase; DFR, dihydroflavonol 4-reductase; LDOX, leucoanthocyanidin dioxygenase; MybA1, myeloblastosis transcription factor A1; UFGT, UDP glucose flavonoid 3-*O*-glucosyl transferase. C3G, cyanidin-3-glucoside; D3G, delphinidin-3-glucoside.

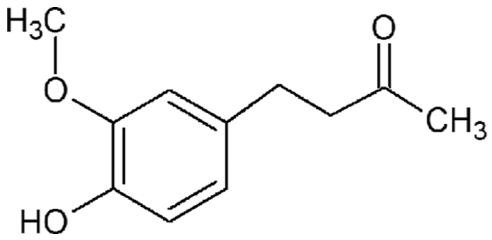


**Fig. 2.** Chemical structure of vanillylacetone.

inexpensive bioactive natural product that exerts various pharmacological activities in animals, including anti-inflammatory, antidiabetic, antilipolytic, antidiarrheal, and antispasmodic activities (Ahmad et al., 2015). In the course of our investigation of the effects of various bioactive natural products on anthocyanin synthesis, we found that VA strongly up-regulated *MybA1* and *UFGT* (Enoki, personal communication). However, there are few reports of the effect of VA on anthocyanin synthesis in plants. Therefore, we conducted a molecular evaluation of VA in grapevine to understand how VA affects anthocyanin synthesis.

The objective of this study was to assess the effect of VA on anthocyanin synthesis in grape berries and to elucidate the molecular mechanism underlying the effect of VA on anthocyanin synthesis using grape cell cultures. We found that VA up-regulates the expression of genes involved in total anthocyanin accumulation by inducing endogenous ABA.

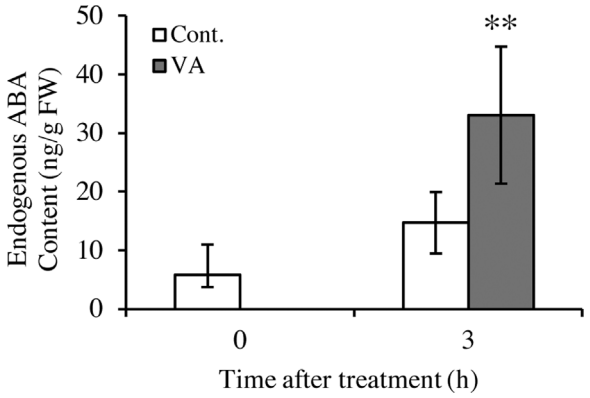


**Fig. 4.** Effects of vanillylacetone treatment of VR cell cultures on endogenous ABA content at 0 and 3 h 1 mM VA was applied. Error bars indicate mean ± S.E (n = 8). ** indicate significant difference compared with control treatment (without VA) for each time-point at $P < 0.01$ (*t*-test).

## 2. Materials and methods

### 2.1. Plant materials

*Vitis vinifera* cv. Muscat Bailey A grapevines growing in the experimental vineyard of The Institute of Enology and Viticulture, University of Yamanashi, Yamanashi, Japan were used.

The *V. vinifera* VR cell line (PRC00003), which has high anthocyanin-synthesizing ability, was provided by research institution of RIKEN Bio-Resource Center (RIKEN BRC), Experimental Plant Division, Tsukuba, Japan. VR cell cultures were maintained in semi-solid medium specified by RIKEN BRC. The medium was sterilized by autoclaving at 121 °C for 15 min (1.06 kg cm$^{-2}$) and gelified with 0.25% gellan gum. Only white VR cells not colored red were subcultured every two weeks in an incubator at 27 °C in the dark.

### 2.2. VA treatment of grape clusters at veraison

Grape clusters were sprayed with 1 mM VA (Tokyo Chemical Industry,

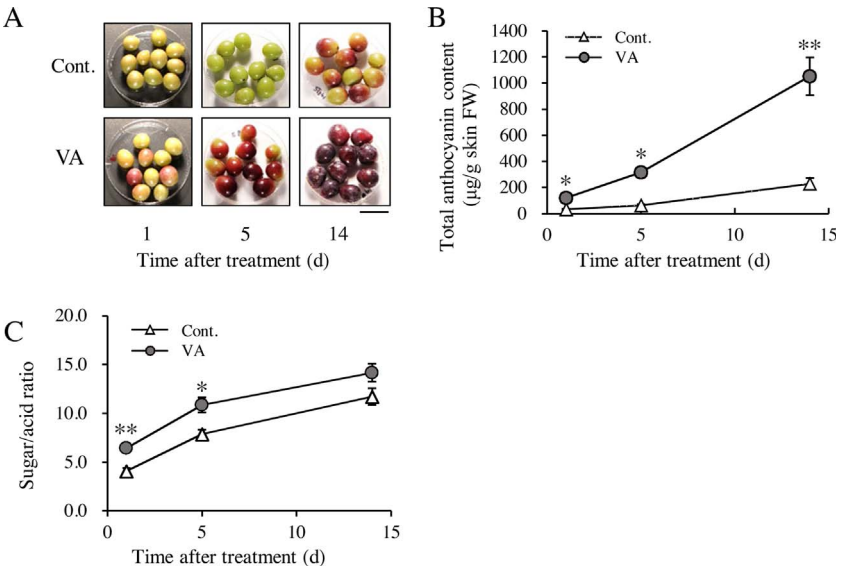


**Fig. 3.** Effects of vanillylacetone treatment of grape berries. Photograph of berries on 1, 5, and 14 d are shown. Bar = 3 cm (A). Effect of 1 mM VA on total anthocyanin content (B) and sugar/acid ratio (C). Error bars indicate S.E (n = 5). * and ** indicate significant difference compared with control treatment (without VA) for each time-point at $P < 0.05$ and 0.01, respectively (*t*-test).