

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

**Exogenous strigolactone interacts with abscisic acid-mediated accumulation of anthocyanins in grapevine berries**

**This is the author's manuscript**

*Original Citation:*

*Availability:*

This version is available <http://hdl.handle.net/2318/1661552> since 2018-09-24T12:04:00Z

*Published version:*

DOI:10.1093/jxb/ery033

*Terms of use:*

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)

# Exogenous strigolactone interacts with abscisic acid-mediated accumulation of anthocyanins in grapevine berries

Manuela Ferrero<sup>1</sup>, Chiara Pagliarani<sup>1°</sup>, Ondřej Novák<sup>2</sup>, Alessandra Ferrandino<sup>1</sup>, Francesca Cardinale<sup>1</sup>, Ivan Visentin<sup>1</sup>, Andrea Schubert<sup>1\*</sup>.

<sup>1</sup>PlantStressLab, Department of Agricultural, Forestry, and Food Sciences - University of Turin, 10095 Grugliasco, Italy

<sup>2</sup>Laboratory of Growth Regulators, Palacký University & Institute of Experimental Botany AS CR, 78371 Olomouc, The Czech Republic

\***Correspondence:** andrea.schubert@unito.it +39.11.6708654

<sup>°</sup>presently at Institute for Sustainable Plant Protection of the National Research Council (CNR), 10135 Torino, Italy.

[manuela.ferrero@unito.it](mailto:manuela.ferrero@unito.it)

[chiara.pagliarani@unito.it](mailto:chiara.pagliarani@unito.it)

[ondrej.novak@upol.cz](mailto:ondrej.novak@upol.cz)

[alessandra.ferrandino@unito.it](mailto:alessandra.ferrandino@unito.it)

[francesca.cardinale@unito.it](mailto:francesca.cardinale@unito.it)

[ivan.visentin@unito.it](mailto:ivan.visentin@unito.it)

**Date of submission:** 03-10-2017

**Number of tables:** 0

**Number of figures:** 5 (1 colour in print)

**Supplementary data:** Table S1 – Figure S1

**Word count:** 5164

**Running title:** Strigolactones affect ABA-induced anthocyanin accumulation

## 1 **Highlight**

2 The strigolactone analogue GR24 reduces ABA-induced anthocyanin accumulation in *Vitis vinifera*  
3 berries. GR24 treatment does not affect ABA biosynthesis while it activates ABA degradation and  
4 possibly ABA membrane transport.

## 5 **Abstract**

6 Besides signalling to soil organisms, strigolactones (SL) control above- and below-ground  
7 morphology, in particular shoot branching. Furthermore, SL interact with stress responses,  
8 possibly thanks to a cross-talk with the abscisic acid (ABA) signal. In grapevine (*Vitis vinifera* L.),  
9 ABA drives the accumulation of anthocyanins over the ripening season. In this study, we  
10 investigated the effects of treatment with a synthetic strigolactone analogue, GR24, on  
11 anthocyanin accumulation in grape berries, in presence or absence of exogenous ABA treatment.  
12 Experiments were performed both on severed, incubated berries, and in berries attached to the  
13 vine. Furthermore, we analysed the corresponding transcript concentrations of genes involved in  
14 anthocyanin biosynthesis, and in ABA biosynthesis, metabolism, and membrane transport.

15 During the experiment time courses, berries showed the expected increase in soluble sugars and  
16 anthocyanins. GR24 treatment had no or little effect on anthocyanin accumulation, or on gene  
17 expression levels. Exogenous ABA treatment activated soluble sugar and anthocyanin  
18 accumulation, and enhanced expression of anthocyanin and ABA biosynthetic genes, and of  
19 genes involved in ABA hydroxylation and membrane transport. Co-treatment of GR24 with ABA  
20 delayed anthocyanin accumulation, decreased expression of anthocyanin biosynthetic genes and  
21 negatively affected ABA concentration. GR24 also enhanced the ABA-induced activation of ABA  
22 hydroxylase genes while it downregulated the ABA-induced activation of ABA transport genes.

23 Our results show that GR24 affects the ABA-induced activation of anthocyanin biosynthesis in  
24 this non-climacteric fruit. We discuss possible mechanisms underlying this effect, and the  
25 potential role of SL in ripening of non-ABA treated berries.

## 26 **Key words**

27 strigolactones, GR24, abscisic acid, anthocyanin, grapevine, ripening, ABA hydroxylases, ABA  
28 transporters, ABA conjugation

## 29 **Abbreviations**

- 30 ABA: abscisic acid
- 31 ABCG: ABC Transporter G Family Protein
- 32 PYL/RCAR: PYR-like/Regulatory Component of ABA Receptor
- 33 SL: Strigolactone(s)
- 34

## 35 Introduction

36 Grapevine ranks fourth among major fruit crops worldwide, and first in Europe  
37 (<http://www.fao.org/faostat/en/#data>). Ripe berries are employed for direct consumption and for  
38 wine elaboration. At harvest, an optimal balance among berry components (sugars, acids,  
39 secondary metabolites) is an absolute requirement to guarantee consumer preference and  
40 commercial success. Grape berry secondary metabolites are represented by many polyphenols  
41 (Adams, 2006) and volatile compounds (Kalua and Boss, 2010). Overall, these molecules  
42 contribute to the colour, taste and aroma of grapes and are involved in wine stabilization and  
43 ageing. Anthocyanins are one of the major groups of polyphenols in berry skins of coloured  
44 cultivars. Their concentration and diversity controls colour intensity and stability in the fruit and in  
45 the deriving wine; furthermore, they contribute to seed dispersal and defence from oxidative  
46 stress. Anthocyanins are absent in the first stage of berry development, while they accumulate in  
47 vacuoles since the start of berry ripening (véraison) (Moskowitz and Hrazdina, 1981).

48 The molecular and physiological processes controlling ripening and anthocyanin accumulation in  
49 the non-climacteric grape berry are still poorly known, although great strides forward have been  
50 made in particular through the application of transcriptomic (Deluc *et al.*, 2007) and proteomic  
51 (Giribaldi *et al.*, 2007) approaches. Hormonal control of fruit ripening is a well-described process  
52 and several hormones were shown to interact with some aspects of ripening in grape. Auxins,  
53 brassinosteroids, and salicylic acid have an inhibitory effect on berry ripening (Davies *et al.*, 1997;  
54 Symons *et al.*, 2006). Disruption of ethylene perception negatively affects anthocyanin  
55 accumulation (Chervin *et al.*, 2004), but the relevance of ethylene in berry ripening is debated  
56 (Sun *et al.*, 2010). Methyl jasmonate treatments enhance anthocyanin accumulation in  
57 suspension cultures (Belhadj *et al.*, 2008) and in whole berries (Jia *et al.*, 2016; Symons *et al.*,  
58 2006). Besides these hormones, abscisic acid (ABA) has been long suspected to be the master  
59 controller of ripening in grapevine, as both its biosynthesis (Deluc *et al.*, 2007) and concentration  
60 in the berry (Coombe and Hale, 1973; Davies *et al.*, 1997) peak at véraison. This hypothesis is  
61 further supported by observation that exogenous ABA activates accumulation of anthocyanins  
62 and sugars in the grape berry (Coombe and Hale, 1973; Wheeler *et al.*, 2009), and expression  
63 activation of anthocyanin biosynthetic genes and of transcription factors controlling this pathway  
64 (Gambetta *et al.*, 2010; Giribaldi *et al.*, 2010; Jeong *et al.*, 2004; Villalobos-Gonzalez *et al.*, 2016).  
65 The role of ABA in the induction of anthocyanin accumulation is not limited to the grape berry,

66 indeed it has been demonstrated in other non-climacteric fruits (Kadomura-Ishikawa *et al.*, 2015)  
67 and in *Arabidopsis* and maize seed vegetative tissues (McCarty *et al.*, 1989).  
68 Strigolactones (SL) were first discovered for their ability to induce seed germination of root  
69 parasite plants when exuded in soil (Bradow and Connick, 1988). Later on, they were  
70 demonstrated to play an essential role as plant signals for other soil organisms, such as arbuscular  
71 mycorrhizal fungi (Akiyama *et al.*, 2005) and symbiotic nitrogen-fixing bacteria (Pelaez-Vico *et al.*,  
72 2016). The study of *Arabidopsis* and rice branching mutants showed however that SL also  
73 strongly repress the growth of axillary buds (Gomez-Roldan *et al.*, 2008; Umehara *et al.*, 2008).  
74 The action of SL on shoot branching may be mediated by complex interaction with other  
75 hormones, namely auxin and cytokinins (Ruyter-Spira *et al.*, 2013).  
76 SL concentration is responsive to nutrient deprivation, in particular of phosphorus and nitrogen  
77 (Yoneyama *et al.*, 2007). This is seen as an adaptive strategy to regulate interaction with  
78 arbuscular mycorrhizal fungi: plants increase SL production under nutrient starvation, in order to  
79 minimize shoot branching and promote AM colonization (Gomez-Roldan *et al.*, 2008; Umehara *et al.*  
80 *et al.*, 2008). Recent studies have demonstrated that SL are also involved in responses to other  
81 abiotic stresses, in particular drought. *Arabidopsis*, *Lotus*, and tomato genotypes with reduced SL  
82 levels are hypersensitive to drought stress (Ha *et al.*, 2014; Liu *et al.*, 2015; Lv *et al.*, 2017; Visentin  
83 *et al.*, 2016), while SL supplementation abolishes the drought-sensitive genotype. In most of  
84 these studies, SL-dependent changes in stress susceptibility were mainly linked to an ABA  
85 signalling-dependent modulation of stomatal closure, suggesting that strigolactones may  
86 interact with the ABA signal upon stress. These observations raise the question whether SL can  
87 interact with ABA also in developmentally regulated processes, such as ripening of the non-  
88 climacteric grape berries.

89 In this study, we investigated the effect of modifications of exogenous SL on ABA-induced  
90 ripening of grapevine berries. By application of the SL analogue GR24 (Besserer *et al.*, 2008) to  
91 berries at véraison in the presence and absence of exogenous ABA, we demonstrate that  
92 exogenous SL down-regulates the effects of exogenous (but not endogenous) ABA, possibly by  
93 affecting its metabolism and transport.

94

## 95 **Materials and Methods**

### 96 **Plant material and experimental setup**

97 Experiments were performed on *V. vinifera* cultivar Barbera, whose anthocyanin profile is  
98 dominated by mono- and di-methylated forms (Ferrandino *et al.*, 2012).

99 Treatments were applied in a first experiment on detached, *in vitro* incubated berries. This  
100 technique has been often used to study ripening processes in grape, however the berries at this  
101 stage are exchanging substances with the plant via the vascular system, and to take this into  
102 account, we replicated our treatments in a second experiment on intact berries attached to the  
103 plant.

104 For the *in vitro* experiment, non-coloured, field-grown berries were collected at start ripening  
105 (véraison) 2015 from vines at the Grugliasco campus vineyard (Piedmont, Italy, 45° 03'55"N  
106 7°35'35"E) by severing the apical end of their pedicel. Vines were trellised and Guyot-pruned,  
107 subjected to standard management techniques, and véraison started on July 22, 2015 (52 days  
108 after flowering). Berries were surface-sterilized with 70% ethanol followed by a 20% w/v NaClO  
109 solution, then rinsed with sterile water. Berries were laid in sterile Petri dishes (about ten berries  
110 per dish) in close contact (on the petiole side) with agar containing 8% (w/v) sucrose and the  
111 following combinations of  $\pm$ ABA (Sigma) and *rac*-GR24 (Strigolab, Turin, Italy): no hormones;  
112  $\pm$ ABA 200  $\mu$ M; *rac*-GR24  $10^{-5}$  M;  $\pm$ ABA 200  $\mu$ M and *rac*-GR24  $10^{-5}$  M. To prevent contaminations,  
113 the whole procedure was conducted under sterility conditions in a laminar hood. Sixty berries per  
114 treatment were collected 0, 24, 72 and 144 h after start of the experiment, frozen in liquid  
115 nitrogen, and stored at -80°C.

116 For the experiment on attached berries, grape bunches from ten vines were sprayed once at start  
117 véraison until runoff, at late afternoon and with the same hormone combinations, omitting  
118 sucrose (two bunches per treatment, each from a different vine). In the period of treatment,  
119 bunches were protected from direct sunlight by shading nets. Sixty berries per treatment were  
120 collected 0, 48 and 144 h after spraying, by severing the apical end of the pedicel. Berries were  
121 frozen in liquid nitrogen, and stored at -80°C.

122 Additional samples of non-treated berries were taken at different stages of development to  
123 assess expression of SL-biosynthetic genes.

124 Frozen berries were quickly peeled, and berry skins were powdered in liquid nitrogen and stored  
125 at -80°C until analysis while flesh was used for soluble solids measurement.

126 **Soluble sugars, total anthocyanin, ABA concentration**

127 Soluble sugars were assessed in triplicate with a refractometer on ten-berry flesh extracts  
128 obtained by pressing.

129 Anthocyanin content was quantified in triplicate on about 1.5 g of powdered skin tissue, diluted  
130 1:10 with acidic ethanol chloride ( $\text{CH}_3\text{CH}_2\text{OH}:\text{H}_2\text{O}:\text{HCl}$  70:30:1 v/v/v), by spectrophotometric  
131 analysis, reading absorbance at 520 nm (Ferrandino and Guidoni, 2010).

132 ABA was quantified by LC-MS (Flokova *et al.*, 2014). A 15 mg sample from powdered berry skins  
133 was extracted using 1 mL of cold extraction solvent (10% methanol). In the same tube, 10  $\mu\text{L}$  of  
134 stable isotope-labelled standard (D6-ABA  $10^{-6}$  M) were added together with ceramic beads, in  
135 order to facilitate the homogenization with a Tissue Lyser (Quiagen) for 5 min at 27 Hz. The  
136 homogenates were then sonicated for 3 min at 4°C and shaken for 30 min at 4°C. Samples were  
137 then centrifuged for 15 min at 20000 rpm (4°C). The supernatant was filtered using Oasis HLB  
138 extraction cartridges (30  $\mu\text{m}$  cutoff) previously conditioned with 2 mL of 100%  $\text{CH}_3\text{OH}$  and 1 mL of  
139 redistilled water. For the elution, 3 mL of 80%  $\text{CH}_3\text{OH}$  were used, evaporated to dryness under  
140 gentle stream of nitrogen at 30°C for about 2 h. The dried residue was resuspended in 40 mL of  
141 15% acetonitrile + 85%  $\text{HCOOH}$  and filtered using 2 mL filtration tubes 0.2  $\mu\text{m}$  and analysed with  
142 an Acquity UPLC® system (Waters, Milford, MA, USA) coupled to a quadrupole mass  
143 spectrometer Xevo™ TQ MS (Waters MS Technologies, Manchester, UK). Each sample (10  $\mu\text{L}$ )  
144 was first separated onto a RP column (Acquity® UPLC CSH™ C18; 1.7  $\mu\text{m}$ , 2.1 x 100 mm) at a flow  
145 rate of 0.4  $\text{mL min}^{-1}$ , using the following solvents: 10 mM  $\text{HCOOH}$  (A) and acetonitrile (B). The  
146 gradient elution over 35 min was as follows: 0–5 min isocratic elution (15% A; v/v); 5–15 min linear  
147 gradient to 45% A; 15–28 min, logarithmic gradient to 48.6% A; 28–29 min linear gradient to  
148 100% A. Finally, the column was washed with 100% acetonitrile and then equilibrated to the  
149 initial conditions (15% A, v/v) for 5 min. The effluent was introduced into the ESI ion source of a  
150 tandem MS analyser with a cone/desolvation gas temperature of 120/550°C at a flow of 70/650 L  
151  $\text{h}^{-1}$ , with the capillary voltage set to 3 kV; cone voltage, 23-30 V; collision energy, 12-23 eV;  
152 collision gas flow (argon), 0.21  $\text{mL min}^{-1}$ . Detection was performed by multiple reaction  
153 monitoring (MRM) in positive ion mode. Optimization of fragmentation was done with labelled  
154 standards using the MAssLynx™ software package (version 4.1 Waters, Milford, MA, USA).

155 Matrix effects were calculated as the ratio of the mean peak area of the analyte spiked post-  
156 extraction to the mean peak area of the same analyte standards multiplied by 100. The process  
157 efficiency was determined as the mean peak area of the added standards before sample



158 preparation divided by the known mean peak area of standard solutions. For assessment of the  
159 validation method, the concentration of the analyte was calculated using the standard isotope  
160 dilution method for each plant extract spiked before extraction and compared with the  
161 concentration of a proper standard solution. Each measurement was performed in quadruplicate.

#### 162 **In silico and quantitative reverse-transcriptase PCR analysis**

163 Two putative biosynthetic genes for SL, namely the *Carotenoid Cleavage Dioxygenases (CCD) 7*  
164 and *8*, were identified by BLAST searching the grapevine "PN4,0024" 12X genome draft, V1  
165 annotation, at the Grape Genome Database (<http://genomes.cribi.unipd.it/grape/>) with the  
166 *Arabidopsis* sequences.

167 Concentration changes of target transcripts were quantified on powdered berry skin samples (1.5  
168 g) by quantitative reverse-transcriptase PCR (RT-qPCR). Total RNA was extracted following a  
169 CTAB-based protocol (Carra *et al.*, 2007). RNA integrity and quantity were checked using a 2100  
170 Bioanalyzer (Agilent Technologies). RNA samples were treated with DNase I, RNase-free  
171 (Fermentas: 50 U  $\mu\text{L}^{-1}$  UAB, Vilnius, Lithuania) to avoid any risk of genomic DNA contaminations,  
172 and first-strand cDNA was synthesized starting from 5  $\mu\text{g}$  of total RNA using the High Capacity  
173 cDNA Reverse Transcription kit (Applied Biosystems, Foster City, CA, USA) following the  
174 manufacturers' instructions. cDNA integrity and primer specificity were then checked by gradient  
175 PCR and agarose gel electrophoresis. RT-qPCR was conducted in triplicate using a StepOnePlus™  
176 System (Applied Biosystems), and the SYBR Green method (Power SYBR® Green PCR Master  
177 Mix, Applied Biosystems) was used for quantifying amplification results (Giordano *et al.*, 2016;  
178 Pagliarani *et al.*, 2017). Each reaction contained 1  $\mu\text{L}$  of 5  $\mu\text{M}$  primer mix, 100 ng of template  
179 cDNA, 5  $\mu\text{L}$  of 2X SYBR Green mix and 3  $\mu\text{L}$  of diethylpyrocarbonate (DEPC)-treated water for a  
180 total reaction volume of 10  $\mu\text{L}$ . Thermal cycling conditions were as follows: 95°C for 10 min before  
181 the beginning of the amplification (holding stage), followed by 40 cycles at 95°C for 15 s and 60°C  
182 for 1 min. Specific annealing of primers was further checked on dissociation kinetics at the end of  
183 each RT-qPCR run. Expression of target transcripts was quantified after normalization to the  
184 geometric mean of the *Ubiquitin (VvUBI)* and *Actin (VvACT1)* transcripts used as endogenous  
185 controls. Expression changes were analysed for *VvMybA1* (encoding a myb transcription factor  
186 controlling anthocyanin biosynthesis in grapevine: Walker *et al.*, 2007), *VvUFGT* (terminal gene of  
187 anthocyanin biosynthesis in grapevine, encoding UDP-glucose:flavonoid 3-O-glucosyltransferase:  
188 Ford *et al.*, 1998), *VvNCED1* (rate-limiting gene of ABA biosynthesis, encoding 9-cis-  
189 epoxy-carotenoid dioxygenase: Wheeler *et al.*, 2009), two genes encoding ABA 8'-hydroxylases

190 (*VvHYD1*, *VvHYD2*; Speirs et al., 2013), a ABA-UDPG glycosyl transferase (*VvGT1*; Sun et al., 2015),  
191 a  $\beta$ -glucosidase that hydrolyses ABA-glucose ester (*VvBG1*; Sun et al., 2015), and the grapevine  
192 orthologues of the Arabidopsis ABC Transporter G Family Protein (ABCG) ABA membrane  
193 transporters *VvABCG25* (Kuromori et al., 2010) and *VvABCG40* (Kang et al., 2010). Transcript  
194 quantification of the putative grapevine *CCD7* and *CCD8* was performed on non-treated berry  
195 samples only. Gene-specific primer pairs used in RT-qPCR experiments are listed in Tab. S1.

## 196 **Statistical analyses**

197 For all measurements, three ten-berry replicates were extracted and analysed independently per  
198 each treatment and sampling time. Significant differences among treatments were statistically  
199 evaluated by applying a one-way ANOVA test using the Tukey's HSD *post-hoc* test for separating  
200 means when ANOVA results were significant ( $P < 0.05$ ). The SPSS statistical software package  
201 was used for the analysis (SPSS Inc., Cary, NC, USA, v.22).

## 202 **Results**

### 203 **Ripening and colour turning**

204 In order to investigate both specific and combined effects of GR24 and ABA on ripening of grape  
205 berries, we incubated detached berries *in vitro* on media supplied with sucrose and hormones.  
206 Furthermore, in a second experiment, the same hormone treatments were applied to intact  
207 berries at véraison, to avoid the possible interference by exogenous sucrose and to allow for  
208 transport processes to the berry via the intact vasculature. Ripening, as shown by the  
209 accumulation of soluble sugars, proceeded as expected in untreated berries, in particular in those  
210 attached to the plant that were able to import phloematic sugar. Accumulation of soluble solids  
211 was slightly (but not significantly) hampered by GR24; it was significantly enhanced by exogenous  
212 ABA; however, this effect was counteracted by GR24 co-treatment (Fig. 1 A, B). Also in both  
213 experiments, ABA induced colour turning; effects of treatment with GR24 in the absence of ABA  
214 were not visible, while GR24 administered together with ABA delayed colour accumulation  
215 compared to the samples treated with ABA alone (Fig 1 C, D).

### 216 **Anthocyanin accumulation**

217 Colour changes were reflected in anthocyanin concentrations, which increased above untreated  
218 control following ABA treatment from the first sampling time onwards in both experiments.  
219 When berries were treated with GR24 only, the anthocyanin concentration was in some cases

220 slightly lower, but never differed significantly from that measured in untreated control samples.  
221 When combined ABA and GR24 were supplied to the medium, anthocyanin accumulation was  
222 significantly lower than in the case of berries treated with ABA alone; this trend was observed in  
223 both experiments, and was particularly evident at the end of the time course (Fig. 2 A, B).  
224 The transcript concentrations of *VvMybA1* (Fig 2 B, C), and of *VvUFGT* (Fig 2 E, F) well followed  
225 the pattern of anthocyanin accumulation. In untreated controls, transcripts progressively  
226 accumulated to reach significantly higher amounts at the end of the experiment. In berries  
227 treated with GR24, transcript levels of these genes showed no difference from untreated controls  
228 at the same sampling times. In ABA-treated berries, concentration of *VvMybA1* and of *VvUFGT*  
229 transcript underwent a significant increase above untreated control since 48 (*in vitro*) or 72 hours  
230 after treatment (in intact berries), confirming that expression of these genes is induced by  
231 exogenous ABA. The combined application of ABA and GR24 negatively affected the expression  
232 of both genes compared to treatment with ABA alone, in most cases limiting transcript  
233 accumulation to the level observed in untreated berries.

#### 234 **ABA concentration and biosynthesis**

235 We explored whether GR24 could act on anthocyanin concentration by modulating ABA  
236 concentrations. ABA levels showed no significant changes over time in the untreated control  
237 samples; average concentrations across all sampling times were significantly higher in attached  
238 than in *in vitro*-incubated berries (391 vs 125 pmol g FW), consistent with ABA phloematic  
239 transport to the berry (Fig. 3 A, B). No significant effects of treatment with GR24 alone were  
240 detected. As expected, in ABA-treated berry skins, ABA concentration drastically increased at the  
241 first sampling time, and remained stable in incubated berries (Fig. 3 A) while increase was slower  
242 in attached berries (Fig. 3 B). GR24 co-treatment induced no significant effects on ABA skin  
243 concentration in the intermediate measurements, while at the end of both experiments these  
244 berries contained significantly less ABA than berries treated with ABA alone (Fig. 3A, B).

245 The expression trend of the ABA biosynthetic gene *VvNCED1* featured a decline in transcript  
246 levels over time in both experiments, and was not affected by treatment with the different  
247 hormone combinations (Fig 3 C, D).

#### 248 **ABA metabolism and transport**

249 The effect of exogenous GR24 on ABA metabolism was further explored by analysing the  
250 expression of genes involved in ABA hydroxylation (*VvHYD1*, *VvHYD2*), conjugation (*VvGT1*), and  
251 de-conjugation (*VvBG1*). Expression of *VvHYD1* increased along both time courses, and was

252 significantly higher at the end of the experiment in attached ABA-treated berries than in  
253 untreated controls, and even significantly higher following co-treatment with the two hormones  
254 in both experiments (Fig. 4 A, B). Similar transcript profiles were observed for *VvHYD2* in attached  
255 berries (Fig 4 C, D), while *in vitro* the concentration peak was anticipated at 72 h after the  
256 experiment start. Expression of *VvGT1* did not significantly differ among treatments at each  
257 sampling time (Fig 4 E, F). Transcript accumulation of *VvBG1* was enhanced by ABA only in  
258 incubated berries at 72 h from the beginning of the experiment, whereas ABA+GR24 co-  
259 treatment consistently and significantly increased expression in both experiments (Fig 4 H, G).  
260 ABA transporters tune the level of cytosolic ABA and thus the responses due to ABA recognition  
261 by PYR-like/Regulatory Component of ABA Receptor (PYL/RCAR) cytosolic receptors. Transcripts  
262 encoding the putative ABA transporters *VvABCG25* (Fig 5 A, B) and *VvABCG40* (Fig 5 C, D) were  
263 thus monitored, showing no significant concentration changes in either untreated or GR24-  
264 treated berries throughout the experiments. On the contrary, transcript levels of these genes  
265 increased significantly following ABA treatment, peaking at 72 and 48 hours in the berries treated  
266 with ABA *in vitro* and *in vivo* respectively, and decreasing afterwards. Co-treatment with GR24  
267 and ABA significantly limited this increase or hindered it completely.  
268

## 269 Discussion

### 270 Exogenous SL negatively interacts with ABA-induced anthocyanin accumulation in grape 271 berries

272 Accumulation of soluble sugars and, in coloured varieties, of anthocyanins, are main facets of  
273 grape berry ripening. Grape berries contain glucose and fructose as soluble sugars, and glucosides  
274 of cyanidin, delphinidin, peonidin, petunidin and malvidin, the latter predominant in the majority  
275 of coloured cultivars, such as Barbera (Ferrandino *et al.*, 2012). Total soluble sugar content  
276 increases from about 5°Brix at véraison (start ripening) to well above 20°Brix at end ripening;  
277 anthocyanins accumulate from véraison during 20-40 days (Hrazdina *et al.*, 1984) to reach final  
278 concentrations higher than 1.2 mg g<sup>-1</sup> skin tissue in Barbera (Ferrandino *et al.*, 2012).

279 Exogenous ABA supplemented both via the severed pedicel or sprayed on intact grape berries  
280 enhances sugar content and anthocyanin accumulation (Pirie and Mullins, 1976; Sandhu *et al.*,  
281 2011; Wheeler *et al.*, 2009). In both our experiments, ABA-treated berries followed this pattern,  
282 and reacted to exogenous ABA with an increase in soluble sugars and anthocyanins. Some  
283 molecular markers of anthocyanin accumulation are well known in grape berries: expression of  
284 the MYB transcription factor *VvMybA1*, encoding a transcriptional regulator that activates  
285 anthocyanin biosynthesis (Walker *et al.*, 2007), and of the *UDP-glucose:flavonoid 3-O-*  
286 *glucosyltransferase* (*VvUGFT*) gene, encoding the last step of the anthocyanin biosynthetic  
287 pathway (Ford *et al.*, 1998), closely follow the pattern of anthocyanin accumulation, and are  
288 correspondingly activated by exogenous ABA (Jeong *et al.*, 2004), as confirmed in our  
289 experiments.

290 The main finding of this study is that GR24 modified this pattern as it markedly inhibited the  
291 ABA-induced accumulation of both sugars and anthocyanins, and the transcriptional increase of  
292 *VvMybA1* and *VvUGFT*. GR24 is a synthetic SL analogue widely used to simulate the action of  
293 natural compounds, also due to its ability to permeate plant tissues, as shown by the fact that it  
294 efficiently reverts the effects of genetic SL depletion (Ito *et al.*, 2017; Ruyter-Spira *et al.*, 2011;  
295 Visentin *et al.*, 2016), and that it can be detected within treated tissues (Liu *et al.*, 2015). We thus  
296 assume that GR24 concentration increased in GR24-treated berries, as it was the case for ABA  
297 following ABA treatment.

298 The effects of GR24 were accompanied by a significant reduction of ABA concentration in ABA-  
299 treated berries, compared to those treated with ABA only, suggesting that the effects of GR24  
300 were mediated by changes in the ABA signal. Bi-directional hormone interactions involving ABA

301 and SL have been reported in other experimental systems. In tomato, chemically or genetically  
302 induced reduction of ABA concentration inhibits SL biosynthesis (Lopez-Raez *et al.*, 2010).  
303 Conversely, changes in SL levels or sensitivity affect ABA concentration and responses: SL-  
304 depleted or SL-insensitive *Arabidopsis* mutants in the adult stage are drought-stress  
305 hypersensitive and lack correct physiological and molecular responses to ABA (Ha *et al.*, 2014),  
306 while *max2* (SL-insensitive) mutants are hypersensitive to ABA in the seedling stage (Bu *et al.*,  
307 2014). The SL-ABA relationship seems to be organ-dependent: *Lotus japonicus* and tomato SL  
308 biosynthetic mutants show a decrease in the drought stress-induced ABA surge in leaves,  
309 suggesting a positive interaction (Liu *et al.*, 2015). On the contrary, in Lotus roots, treatment with  
310 GR24 inhibits osmotic stress-triggered increase of ABA concentration (Liu *et al.*, 2015), and  
311 drought stress decreases SL and increases ABA concentration in non-mycorrhizal roots of Lotus,  
312 tomato and lettuce (Liu *et al.*, 2015; Ruiz-Lozano *et al.*, 2016), as would be the case for a negative  
313 interaction. Clearly, the interactions at the biosynthetic, catabolic, membrane transport, and  
314 signalling levels may be intricate and diverse in the different plant organs.

315 Although our results strongly suggest that GR24 affected sugar and anthocyanin accumulation  
316 through modulation of ABA concentration, other possibilities exist. Lv *et al.* (2017) recently  
317 showed that in *Arabidopsis* leaves GR24 induces stomatal closure also in ABA-depleted mutants,  
318 and that this ABA-independent effect could be triggered by an oxidative burst. A transcriptomic  
319 study suggested that an oxidative burst takes place at véraison in grape berries (Pilati *et al.*, 2007),  
320 and this could represent an additional mechanism of action of GR24 in grape berries.

### 321 **GR24 controls the expression of ABA metabolic but not of biosynthetic genes**

322 We observed that the GR24 treatment significantly reduced ABA concentration in ABA-treated  
323 berries, compared to those treated with ABA only. The concentration of ABA is regulated by its  
324 biosynthesis, controlled by *NCED* genes, and by catabolism, which can follow both oxidation or  
325 conjugation pathways (Nambara and Marion-Poll, 2005). Oxidation reactions are catalysed by  
326 cytochrome P<sub>450</sub> monooxygenases such as ABA 8'-hydroxylase (*CYP707A* gene family (Kushiro *et al.*,  
327 2004; Saito *et al.*, 2004). In grapevine, three members of this gene family are described,  
328 among which *VvHYD1* and *VvHYD2* are most expressed in root and leaf (Speirs *et al.*, 2013). ABA  
329 oxidation to inactive compounds controls the drop in ABA concentration observed in leaves upon  
330 rehydration (Okamoto *et al.*, 2009) and in seeds upon imbibition (Okamoto *et al.*, 2006). ABA  
331 conjugation to ABA-glucose ester is performed by *ABA-GlucosylTransferase* (*AGT*) (Xu *et al.*,  
332 2002). The grapevine homologue *VvGT1* is downregulated after véraison (Sun *et al.*, 2015). In

333 Arabidopsis, ABA-glucose ester is hydrolysed by a  $\beta$ -glucosidase (*BG1*) (Lee *et al.*, 2006). The  
334 grapevine homologue of this gene (*VvBG1*) was biochemically characterized and is upregulated in  
335 berries at véraison (Sun *et al.*, 2015).

336 A straightforward hypothesis to explain the lower ABA concentration following GR24 co-  
337 treatment of ABA-treated berries is the activation of ABA catabolism. *CYP707A* genes are  
338 transcriptionally up-regulated following ABA treatment, suggesting an active contribution to  
339 homeostasis of free ABA levels (Cutler and Krochko, 1999; Saito *et al.*, 2004). We correspondingly  
340 observed a marked peak of *VvHYD1* and *VvHYD2* expression following ABA treatment. In the *in*  
341 *vitro* experiment this peak, observed 72 h after treatment, did not bring to a significant reduction  
342 of ABA concentration thereafter, probably due either to the high ABA levels induced by the  
343 treatment, or to a relatively low amount of cytosolic ABA, potential substrate of the cytosolic  
344 *CYP707A* gene products. Most interestingly, co-treatment with GR24 induced a further,  
345 significant expression increase of both hydroxylases, which could have elevated the enzyme  
346 activity to levels sufficient to observe the decrease of ABA at later sampling times. This finding,  
347 considering that GR24 application activates *CYP707A1* expression and enhances germination of  
348 *Phelipanche ramosa* seeds (Lechat *et al.*, 2012), while Arabidopsis *CYP707A3* is upregulated by  
349 gibberellin and brassinolide (Saito *et al.*, 2004), suggests that this gene family may mediate  
350 several hormone interactions in plants.

351 The effect of GR24 treatment on ABA conjugation is less clear: we observed no significant  
352 changes in expression of *VvGT1* (encoding a conjugating enzyme), and an activation of *VvBG1*  
353 (encoding a de-conjugating enzyme) transcript concentration, which could represent an  
354 homeostatic control on free ABA levels induced by the increase of ABA hydroxylation observed  
355 upon GR24 treatment. However, as *VvBG1* is two orders of magnitude less expressed than *VvGT1*,  
356 the contribution of de-conjugation to free ABA levels might be negligible.

357 Members of the *NCED* gene family are considered the main control point of ABA biosynthesis in  
358 Arabidopsis (Nambara and Marion-Poll, 2005) and are activated by ABA in some ecotypes (Cheng  
359 *et al.*, 2002). A second possible mechanism underlying the effect of GR24 on ABA-treated berries  
360 could thus be due to changes in ABA-induced ABA biosynthesis rate, which could contribute to  
361 the rise in ABA concentration, particularly in the cytosolic compartment. Two *NCED* genes were  
362 cloned from grapevine, *NCED1* being the most expressed in berries (Deluc *et al.*, 2007; Wheeler *et*  
363 *al.*, 2009; Zhang *et al.*, 2009). However, while *VvNCED1* expression decreased throughout the  
364 experiments, it was not significantly affected by ABA, as previously observed in tomato

365 (Thompson *et al.*, 2000), suggesting that GR24 does not lower free ABA concentration in ABA-  
366 treated samples by inhibiting biosynthesis at the transcription level.

### 367 **Membrane transport of ABA is regulated by GR24**

368 Besides direct effects on ABA concentration, GR24 could control the expression of ABA  
369 membrane transport genes (Boursiac *et al.*, 2013). In Arabidopsis, *ABCG40* controls ABA cellular  
370 uptake: it is expressed in leaves, roots, and seed and its downregulation dampens physiological  
371 responses to ABA (Kang *et al.*, 2010). The ABA-induced *ABCG25*, localized to the vasculature, and  
372 in the endosperm, mediates ATP-dependent extrusion of ABA (Kang *et al.*, 2015; Kuromori *et al.*,  
373 2010). Expression of these transport genes may affect the concentration of cytosolic free ABA,  
374 which interacts with the cytosolic PYL/RCAR receptors (Park *et al.*, 2009). In the grape berry, ABA  
375 transport genes have not been studied in detail yet, while *PYL/RCAR* genes been identified and  
376 are expressed in vegetative tissue and in berries (Li *et al.*, 2012). We observed an early (*viz.* 72 and  
377 48 h after treatment in the *in vitro* and *in vivo* experiments, respectively), transient induction of  
378 *VvABCG25* and *VvABCG40* transcript levels following ABA treatment, which was abolished upon  
379 GR24 co-treatment. These changes suggest that the cellular/apoplastic ABA concentration ratio  
380 may be affected upon GR24 in ABA-treated berry skins by a decrease of import coupled to an  
381 increase of export activity. Additionally, since *VvABCG25* is two orders of magnitude less  
382 expressed than *VvABCG40* with respect to the same housekeeping genes, the dampening of ABA  
383 import might contribute more than the decreased export, resulting in a lower free ABA cellular  
384 concentration in ABA and GR24-treated berry skins, compared to ABA-treated alone.

### 385 **Do natural SL play a role in grape berry ripening?**

386 SL are carotenoid derived hormones, whose core biosynthetic pathway is based on the  
387 carotenoid isomerase D27 (Dwarf27), the carotenoid cleavage dioxygenases CCD7 and CCD8, and  
388 the P450 monooxygenase MAX1 (More Axillary Growth1) (Ruyter-Spira *et al.*, 2013). They are  
389 mostly through not exclusively produced in roots, where they are detected in the nanomolar  
390 range; and are supposed to be transported to the shoots, where their concentration may be two  
391 orders of magnitude lower (Liu *et al.*, 2015) and, for most plant species, below detection  
392 threshold. Genetic evidence shows that they are active in aboveground organs at such low  
393 concentrations, controlling shoot-specific traits such as axillary bud development (Brewer *et al.*,  
394 2013). Also, reproductive defects of plants compromised in SL biosynthesis or perception suggest  
395 a largely unexplored role in flower and fruit development for certain species, besides juvenile-to-



396 reproductive phase transition (for example in tomato, kiwifruit, Lotus, tomato, petunia) (Kohlen  
397 *et al.*, 2012; Ledger *et al.*, 2010; Liu *et al.*, 2013; Snowden *et al.*, 2005).

398 In the grape berry, DNA microarray data suggest that *VvCCD7*, *VvCCD8*, and *VvMAX1* are  
399 differentially expressed in green and ripening berries (Young *et al.*, 2012), as also shown in tomato  
400 fruit for *SlCCD7* (Vogel *et al.*, 2010) and in kiwifruit for *AcCCD7* and *AcCCD8* (Ledger *et al.*, 2010). A  
401 reported attempt to quantify expression of putative *VvCCD7* and *VvCCD8* in aboveground organs  
402 of grapevine was not successful (Lashbrooke *et al.*, 2013). We assessed expression of the same  
403 two genes in berry skins during berry development by RT-qPCR and confirmed a very low relative  
404 transcript level (Fig. S1). Interestingly, expression of both *VvCCD7* and *VvCCD8* tended to increase  
405 in the late stages of ripening, in correspondence with the known decrease in ABA concentration  
406 after véraison (Wheeler *et al.*, 2009). In grapevine, no data are available on SL profiles and  
407 concentration. It must be noticed here that SL are usually undetectable in the aerial part of plants,  
408 and indeed the transcripts of the biosynthetic genes we tested are ten- or even hundredfold less  
409 concentrated than in roots, where SL are more massively produced, especially under phosphate  
410 deprivation (data not shown). These preliminary results open the possibility that changes in SL  
411 concentration at véraison may play a regulatory role in grape berry ripening.

412 While we clearly observed that GR24 limits the ripening effects of exogenous ABA, we were able  
413 to detect only very limited, and not significant, effects of GR24 treatments on non-ABA-treated  
414 berries. These observations seem contradictory, being apparently unrealistic that GR24 may have  
415 such powerful effects on the signal induced by exogenous ABA, and to be at the same time  
416 ineffective on the endogenous ABA signal. A possible reconciling hypothesis is that endogenous  
417 SL is only one of several control points of ABA concentration and/or signalling pathway, possibly  
418 cooperating at the molecular level with other effectors. In such a situation, additional, exogenous  
419 SL would not further affect the ABA signal in absence of an increase of such co-operating  
420 effectors. It is well demonstrated that ABA can reinforce its own signal by ABA-dependent  
421 upregulation of biosynthetic and signalling genes (Yang and Tan, 2014). Thus ABA treatment  
422 could entail an expression increase of SL-cooperating molecular effectors, finally allowing  
423 exogenous SL to interact with them to control the exogenous ABA concentration and signal.

424 **Acknowledgements:** The authors wish to acknowledge the Compagnia di San Paolo Foundation  
425 (project SLEPS) for financial support. The study was also partly supported by the European  
426 Union's H2020 Research and Innovation Programme under the Grant Agreement No. 727929.

427

428 **Captions to figures**

429 **Fig. 1 Accumulation of soluble solids (A, B) and colour turning (C, D)** in *V. vinifera* berries (A, C)  
430 severed from the vine and incubated at véraison in the presence of different hormones, or (B, D)  
431 attached to the vine and sprayed at véraison with the same hormone combinations. UT:  
432 untreated control (no hormones); GR24: *rac*-GR24  $10^{-5}$ M; ABA:  $\pm$ ABA 200 $\mu$ M; ABA+GR24: *rac*-  
433 GR24  $10^{-5}$  M and ABA 200  $\mu$ M. (C) and (D): pictures were taken 6 days after treatment, treatments  
434 are displayed clockwise starting from upper left panel. Values marked by the same letter do not  
435 significantly differ at  $P=0.05$ ; bars are standard errors of the means.

436 **Fig 2 Anthocyanin accumulation (A, B) and transcript accumulation of regulatory (VvMybA1:**  
437 **C, D) and biosynthetic (VvUFGT: E, F) genes of anthocyanin biosynthesis** in *V. vinifera* berry  
438 skins (A, C, E) severed from the vine and incubated at véraison in presence of different hormones,  
439 or (B, D, F) attached to the vine and sprayed at véraison with the same hormone combinations.  
440 For treatment labels and significance of differences, see caption to Fig. 1.

441 **Fig. 3 ABA concentration (A, B) and transcript accumulation of the ABA biosynthetic gene**  
442 **VvNCED1 (C, D)** in *V. vinifera* berry skins (A, C) incubated at véraison in presence of different  
443 hormones, or (B, D) attached to the vine and sprayed at véraison with the same hormone  
444 combinations. For treatment labels and significance of differences, see caption to Fig. 1.

445 **Fig. 4 Transcript accumulation of genes involved in ABA metabolism.** Relative expression of  
446 *VvHYD1* (A, B), *VvHYD2* (C, D), *VvGT1* (E, F), and of *VvBG1* (G, H) in *V. vinifera* berry skins (A, C, E,  
447 G) incubated at véraison in presence of different hormones, or (B, D, F, H) attached to the vine  
448 and sprayed at véraison with the same hormone combinations. For treatment labels and  
449 significance of differences, see caption to Fig. 1.

450 **Fig. 5 Transcript accumulation of genes involved in ABA transport.** Relative expression of  
451 *VvABC25* (A, B) and of *VvABCG40* (C, D) in *V. vinifera* berry skins (A, C) incubated at véraison in  
452 presence of different hormones, or (B, D) attached to the vine and sprayed at véraison with the  
453 same hormone combinations. For treatment labels and significance of differences, see caption to  
454 Fig. 1.

**Fig. 1**

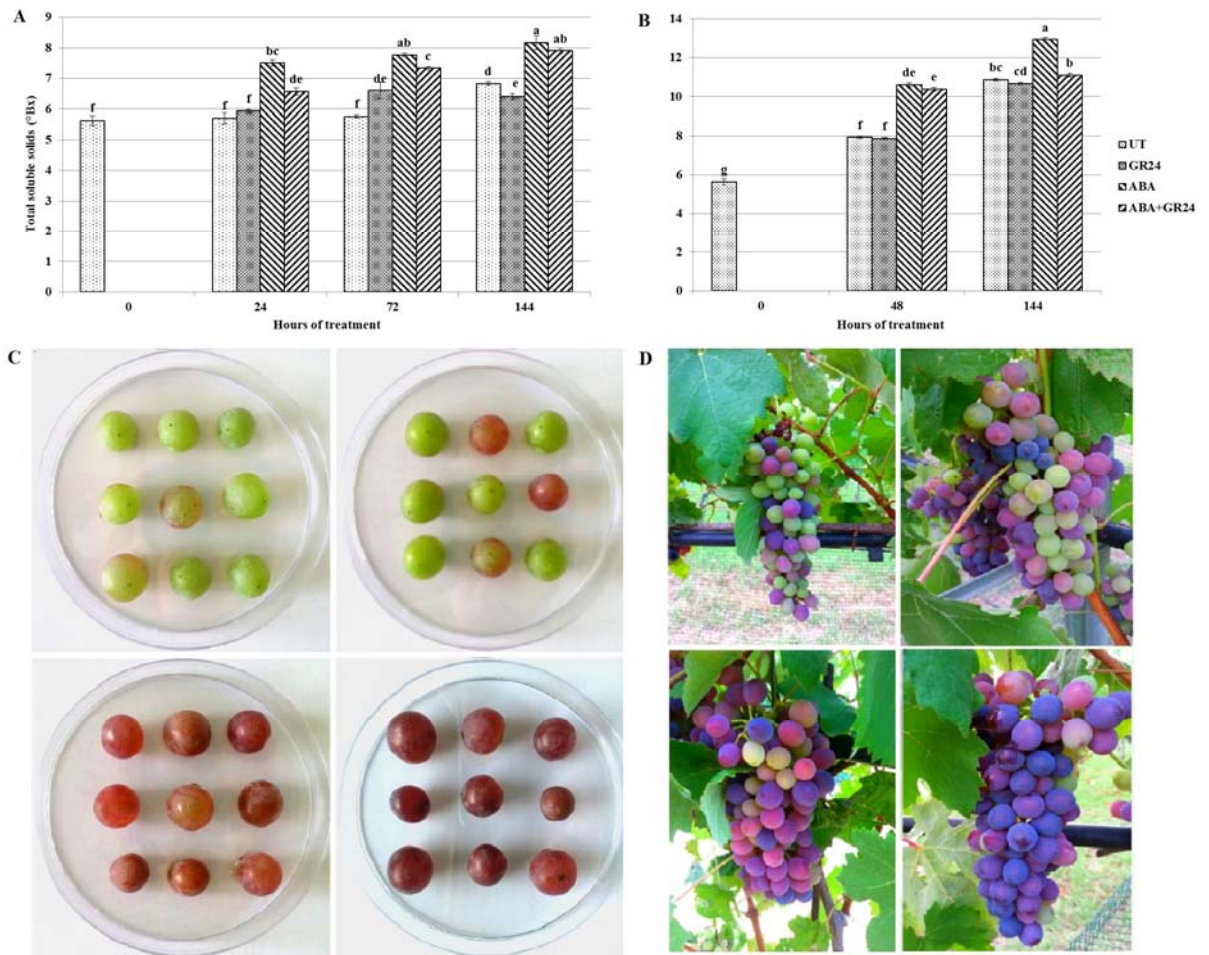


Fig. 2

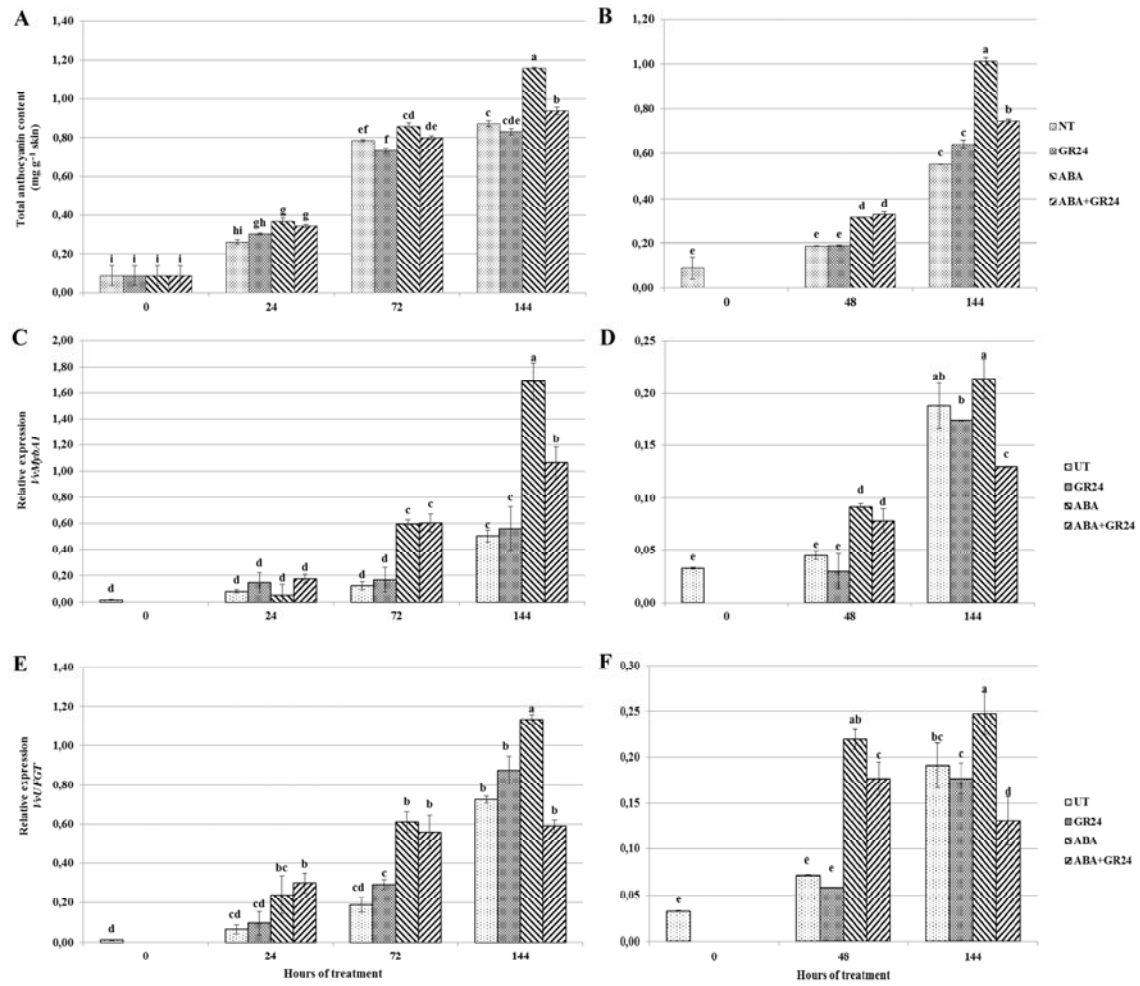
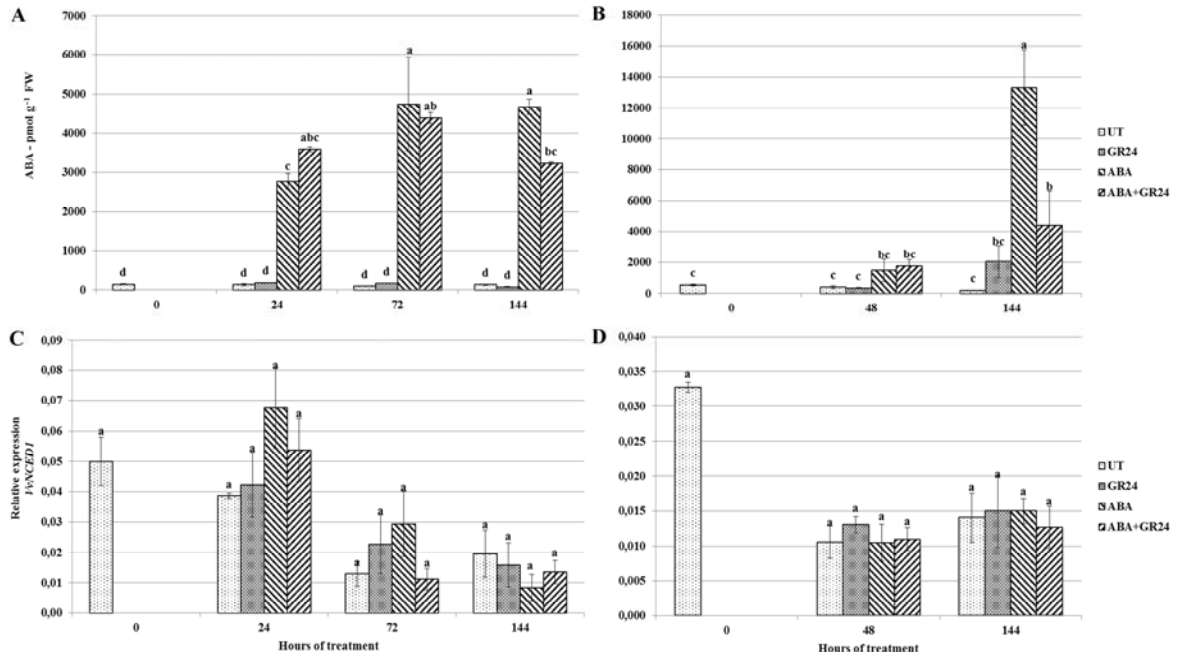


Fig. 3



**Fig. 4**

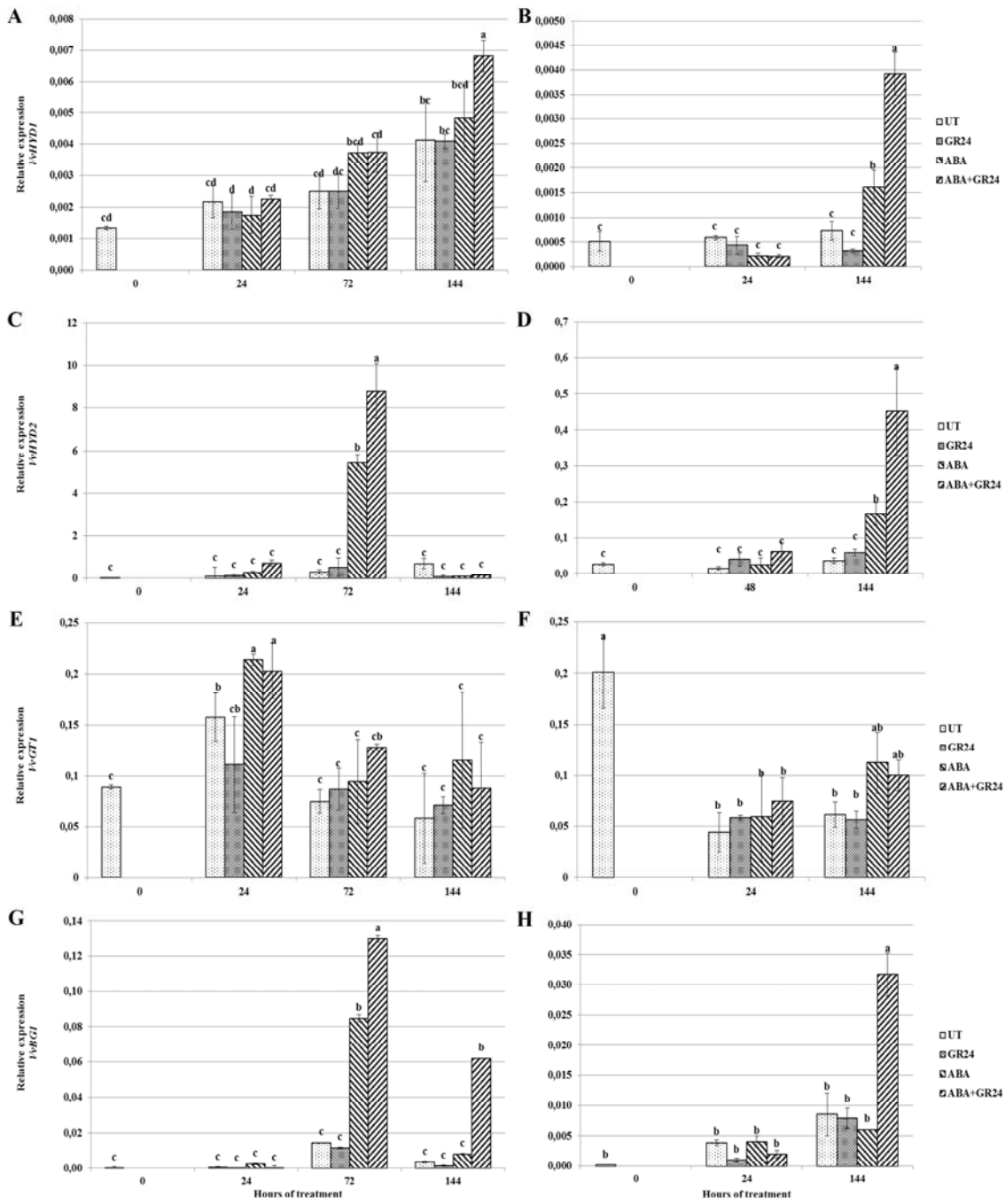
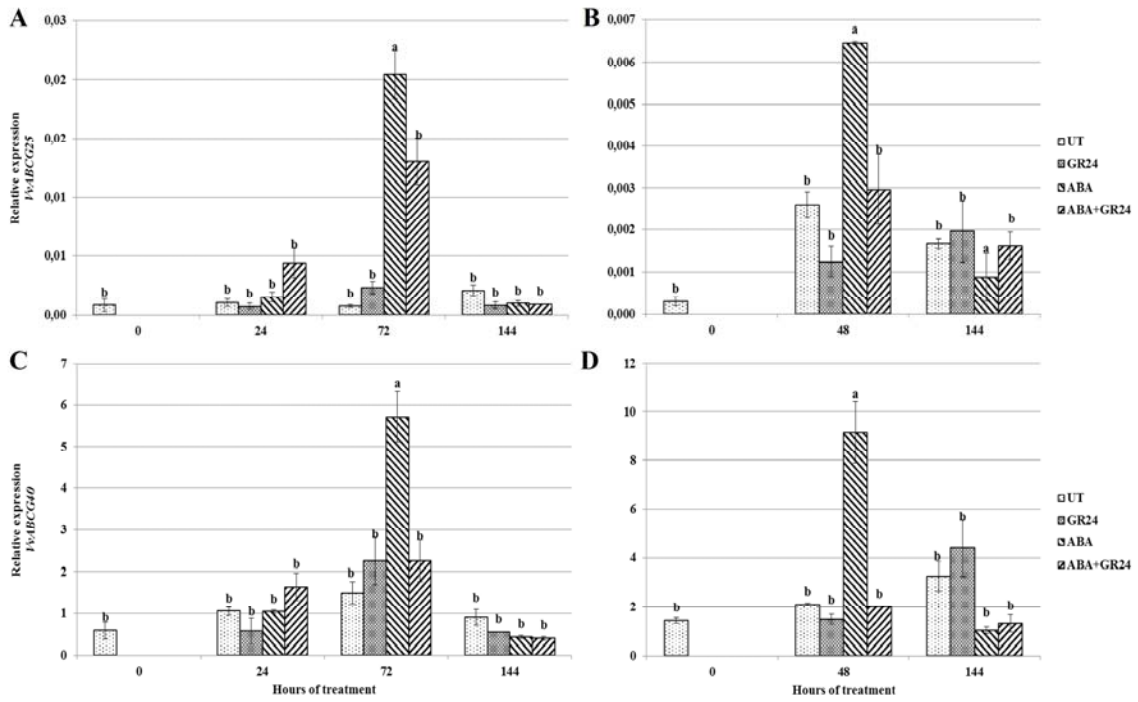


Fig. 5



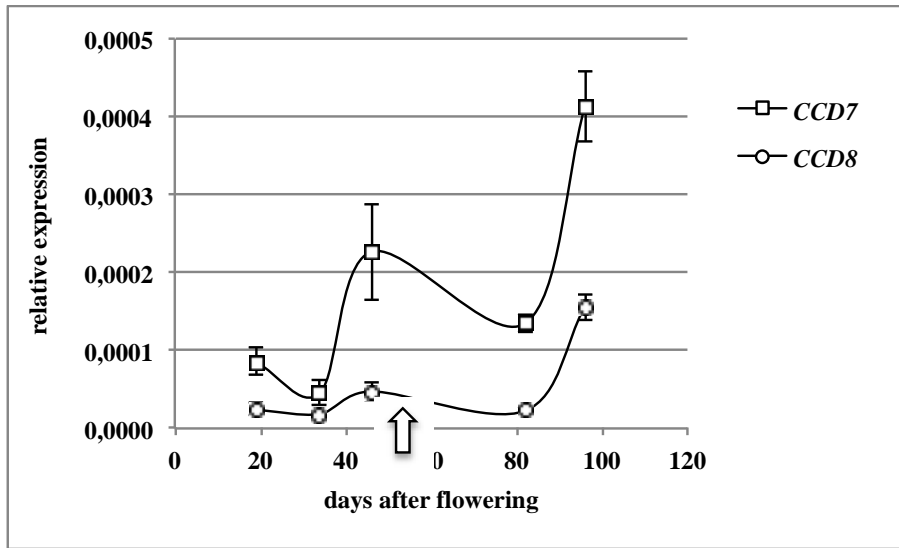
## Supplementary material

Table S1. Oligonucleotides used in this study for RT-qPCR analysis

Name	Gene accession (Grape Genome Database 12X V1)		Primer sequence (5'-3')
<i>VvACT1</i>	VIT_0450044900580	F R	GCCCCTCGTCTGTGACAATG CCTTGGCCGACCCACAATA
<i>VvABCG25</i>	VIT_1850072901220	F R	ACTCTGTATTTCGCCTTCCCC GGGCATGTCTCCAACGATTC
<i>VvABCG40</i>	VIT_0950002905600	F R	GCTAAGTTCTTCTGGTATCT TTTGATTTGGTGTGGCAGC
<i>VvBG1</i>	VIT_0150011900760	F R	TGATGGCCCCGGGAAAATAA CCTGTACCAAACCTGCTGAA
<i>VvCCD7</i>	VIT_1550021902190	F R	TGGGTATTTGAGGGCTTTTG CCACCTTCTCCCTCCTTC
<i>VvCCD8</i>	VIT_0450008903380	F R	GCTCAGGCTTCACAATCTCC TAGTGAGGGTGTGGGGAAG
<i>VvHYD1</i>	VIT_1850001910500	F R	ATGGACTTCCAGCCAGATTG GGACATCTCTCCAACCCAGA
<i>VvGT1</i>	VIT_0350063900050	F R	CAAATGGGGAAGAAGGCGTG CAGGCTGCTCATCAATGGA
<i>VvHYD2</i>	VIT_0250087900710	F R	TATTCAGTATGGCCCTTTTGCT TTGATTGGTGGCACTGAGAG
<i>VvMybA1</i>	VIT_0250033900410	F R	TAGTCACCACTTCAAAAAGG GAATGTGTTTGGGGTTTATC
<i>VvNCED1</i>	VIT_1950093900550	F R	GGTGGTGAGCCTCTGTTCCCT CTGTAAATTCGTGGCGTTCACT
<i>VvUBI</i>	VIT_1650098901190	F R	TCTGAGGCTTCGTGGTGGTA AGGCGTGCATAACATTTGCG
<i>VvUFGT</i>	VIT_1650039902230	F R	CCCGGAATGTCTAAAGTACGTTT AGCGAGTTTAGGTTTCCGAACA



Fig. S1. Expression profiles of *VvCCD7* and of *VvCCD8* in skins of untreated *V. vinifera* during berry development. Arrow shows time of ripening start (véraison). Bars are standard errors of the means.



## References

- Adams DO.** 2006. Phenolics and ripening in grape berries. *American Journal of Enology and Viticulture* **57**, 249-256.
- Akiyama K, Matsuzaki K, Hayashi H.** 2005. Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. *Nature* **435**, 824-827.
- Belhadj A, Telef N, Saigne C, Cluzet S, Barrieu F, Hamdi S, Merillon J.** 2008. Effect of methyl jasmonate in combination with carbohydrates on gene expression of PR proteins, stilbene and anthocyanin accumulation in grapevine cell cultures. *Plant Physiology and Biochemistry* **46**, 493-499.
- Besserer A, Becard G, Jauneau A, Roux C, Sejalón-Delmas N.** 2008. GR24, a synthetic analog of strigolactones, stimulates the mitosis and growth of the arbuscular mycorrhizal fungus *Gigaspora rosea* by boosting its energy metabolism. *Plant Physiology* **148**, 402-413.
- Boursiac Y, Leran S, Corratge-Faillie C, Gojon A, Krouk G, Lacombe B.** 2013. ABA transport and transporters. *Trends in Plant Science* **18**, 325-333.
- Bradow J, Connick W.** 1988. Seed-germination inhibition by volatile alcohols and other compounds associated with *Amaranthus palmeri* residues. *Journal of Chemical Ecology* **14**, 1633-1648.
- Brewer P, Koltai H, Beveridge C.** 2013. Diverse roles of strigolactones in plant development. *Molecular Plant* **6**, 18-28.
- Bu Q, Lv T, Shen H, Luong P, Wang J, Wang Z, Huang Z, Xiao L, Engineer C, Kim T, Schroeder J, Huq E.** 2014. Regulation of drought tolerance by the F-Box protein MAX2 in *Arabidopsis*. *Plant Physiology* **164**, 424-439.
- Carra A, Gambino G, Schubert A.** 2007. A cetyltrimethylammonium bromide-based method to extract low-molecular-weight RNA from polysaccharide-rich plant tissues. *Analytical Biochemistry* **360**, 318-320.
- Cheng W, Endo A, Zhou L, Penney J, Chen H, Arroyo A, Leon P, Nambara E, Asami T, Seo M, Koshiba T, Sheen J.** 2002. A unique short-chain dehydrogenase/reductase in *Arabidopsis* glucose signaling and abscisic acid biosynthesis and functions. *Plant Cell* **14**, 2723-2743.
- Chervin C, El-Kereamy A, Roustan J, Latche A, Lamon J, Bouzayen M.** 2004. Ethylene seems required for the berry development and ripening in grape, a non-climacteric fruit. *Plant Science* **167**, 1301-1305.
- Coombe B, Hale C.** 1973. The hormone content of ripening grape berries and the effects of growth substance treatments. *Plant Physiology* **51**, 629-634.
- Cutler A, Krochko J.** 1999. Formation and breakdown of ABA. *Trends in Plant Science* **4**, 472-478.
- Davies C, Boss PK, Robinson SP.** 1997. Treatment of grape berries, a nonclimacteric fruit with a synthetic auxin, retards ripening and alters the expression of developmentally regulated genes. *Plant Physiol* **115**, 1155-1161.
- Deluc LG, Grimplet J, Wheatley MD, Tillett RL, Quilici DR, Osborne C, Schooley DA, Schlauch KA, Cushman JC, Cramer GR.** 2007. Transcriptomic and metabolite analyses of Cabernet Sauvignon grape berry development. *BMC Genomics* **8**, 429.
- Ferrandino A, Carra A, Rolle L, Schneider A, Schubert A.** 2012. Profiling of hydroxycinnamoyl tartrates and acylated anthocyanins in the skin of 34 *Vitis vinifera* genotypes. *Journal of Agricultural and Food Chemistry* **60**, 4931-4945.
- Ferrandino A, Guidoni S.** 2010. Anthocyanins, flavonols and hydroxycinnamates: an attempt to use them to discriminate *Vitis vinifera* L. cv 'Barbera' clones. *European Food Research and Technology* **230**, 417-427.
- Flokova K, Tarkowska D, Miersch O, Strnad M, Wasternack C, Novak O.** 2014. UHPLC-MS/MS based target profiling of stress-induced phytohormones. *Phytochemistry* **105**, 147-157.

**Ford CM, Boss PK, Høj PB.** 1998. Cloning and characterization of *Vitis vinifera* UDP-glucose:flavonoid 3-O-glucosyltransferase, a homologue of the enzyme encoded by the maize *Bronze-1* locus that may primarily serve to glucosylate anthocyanidins *in vivo*. *Journal of Biological Chemistry* **273**, 9224-9233.

**Gambetta G, Matthews M, Shaghasi T, McElrone A, Castellarin S.** 2010. Sugar and abscisic acid signaling orthologs are activated at the onset of ripening in grape. *Planta* **232**, 219-234.

**Giordano D, Provenzano S, Ferrandino A, Vitali M, Pagliarani C, Roman F, Cardinale F, Castellarin S, Schubert A.** 2016. Characterization of a multifunctional caffeoyl-CoA O-methyltransferase activated in grape berries upon drought stress. *Plant Physiology and Biochemistry* **101**, 23-32.

**Giribaldi M, Geny L, Delrot S, Schubert A.** 2010. Proteomic analysis of the effects of ABA treatments on ripening *Vitis vinifera* berries. *Journal of Experimental Botany* **61**, 2447-2458.

**Giribaldi M, Perugini I, Sauvage FX, Schubert A.** 2007. Analysis of protein changes during grape berry ripening by 2-DE and MALDI-TOF. *Proteomics* **7**, 3154-3170.

**Gomez-Roldan V, Fermas S, Brewer P, Puech-Pages V, Dun E, Pillot J, Letisse F, Matusova R, Danoun S, Portais J, Bouwmeester H, Becard G, Beveridge C, Rameau C, Rochange S.** 2008. Strigolactone inhibition of shoot branching. *Nature* **455**, 189-194.

**Ha C, Leyva-Gonzalez M, Osakabe Y, Tran U, Nishiyama R, Watanabe Y, Tanaka M, Seki M, Yamaguchi S, Dong N, Yamaguchi-Shinozaki K, Shinozaki K, Herrera-Estrella L, Tran L.** 2014. Positive regulatory role of strigolactone in plant responses to drought and salt stress. *Proceedings of the National Academy of Sciences of the United States of America* **111**, 851-856.

**Hrazdina G, Parsons GF, Mattick LR.** 1984. Physiological and biochemical events during development and maturation of grape berries. *Journal of Enology and Viticulture* **35**, 220-227.

**Ito S, Yamagami D, Umehara M, Hanada A, Yoshida S, Sasaki Y, Yajima S, Kyozuka J, Ueguchi-Tanaka M, Matsuoka M, Shirasu K, Yamaguchi S, Asami T.** 2017. Regulation of strigolactone biosynthesis by gibberellin signaling. *Plant Physiology* **174**, 1250-1259.

**Jeong S, Goto-Yamamoto N, Kobayashi S, Esaka A.** 2004. Effects of plant hormones and shading on the accumulation of anthocyanins and the expression of anthocyanin biosynthetic genes in grape berry skins. *Plant Science* **167**, 247-252.

**H, Zhang C, Pervaiz T, Zhao P, Liu Z, Wang B, Wang C, Zhang L, Fang J, Qian J.** 2016. Jasmonic acid involves in grape fruit ripening and resistance against *Botrytis cinerea*. *Functional & Integrative Genomics* **16**, 79-94.

**Kadomura-Ishikawa Y, Miyawaki K, Takahashi A, Masuda T, Noji S.** 2015. Light and abscisic acid independently regulated *FaMYB10* in *Fragaria x ananassa* fruit. *Planta* **241**, 953-965.

**Kalua C, Boss P.** 2010. Comparison of major volatile compounds from Riesling and Cabernet Sauvignon grapes (*Vitis vinifera* L.) from fruitset to harvest. *Australian Journal of Grape and Wine Research* **16**, 337-348.

**Kang J, Hwang J, Lee M, Kim Y, Assmann S, Martinoia E, Lee Y.** 2010. PDR-type ABC transporter mediates cellular uptake of the phytohormone abscisic acid. *Proceedings of the National Academy of Sciences of the United States of America* **107**, 2355-2360.

**Kang J, Yim S, Choi H, Kim A, Lee K, Lopez-Molina L, Martinoia E, Lee Y.** 2015. Abscisic acid transporters cooperate to control seed germination. *Nature Communications* **6**, 8113.

**Kohlen W, Charnikhova T, Lammers M, Pollina T, Toth P, Haider I, Pozo M, de Maagd R, Ruyter-Spira C, Bouwmeester H, Lopez-Raez J.** 2012. The tomato CAROTENOID CLEAVAGE DIOXYGENASE8 (SlCCD8) regulates rhizosphere signaling, plant architecture and affects reproductive development through strigolactone biosynthesis. *New Phytologist* **196**, 535-547.

**Kuromori T, Miyaji T, Yabuuchi H, Shimizu H, Sugimoto E, Kamiya A, Moriyama Y, Shinozaki K.** 2010. ABC transporter AtABCG25 is involved in abscisic acid transport and responses.

Proceedings of the National Academy of Sciences of the United States of America **107**, 2361-2366.

**Kushiro T, Okamoto M, Nakabayashi K, Yamagishi K, Kitamura S, Asami T, Hirai N, Koshiba T, Kamiya Y, Nambara E.** 2004. The Arabidopsis cytochrome *P450CYP707A* encodes ABA 8'-hydroxylases: key enzymes in ABA catabolism. *EMBO Journal* **23**, 1647-1656.

**Lashbrooke J, Young P, Dockrall S, Vasanth K, Vivier M.** 2013. Functional characterisation of three members of the *Vitis vinifera* L. carotenoid cleavage dioxygenase gene family. *BMC Plant Biology* **13**, 156.

**Lechat M, Pouvreau J, Peron T, Gauthier M, Montiel G, Veronesi C, Todoroki Y, Le Bizec B, Monteau F, Macherel D, Simier P, Thoiron S, Delavault P.** 2012. *PrCYP707A1*, an ABA catabolic gene, is a key component of *Phelipanche ramosa* seed germination in response to the strigolactone analogue GR24. *Journal of Experimental Botany* **63**, 5311-5322.

**Ledger S, Janssen B, Karunairetnam S, Wang T, Snowden K.** 2010. Modified *CAROTENOID CLEAVAGE DIOXYGENASE 8* expression correlates with altered branching in kiwifruit (*Actinidia chinensis*). *New Phytologist* **188**, 803-813.

**Lee K, Piao H, Kim H, Choi S, Jiang F, Hartung W, Hwang I, Kwak J, Lee I.** 2006. Activation of glucosidase via stress-induced polymerization rapidly increases active pools of abscisic acid. *Cell* **126**, 1109-1120.

**Li G, Xin H, Zheng X, Li S, Hu Z.** 2012. Identification of the abscisic acid receptor VvPYL1 in *Vitis vinifera*. *Plant Biology* **14**, 244-248.

**Liu J, He H, Vitali M, Visentin I, Charnikhova T, Haider I, Schubert A, Ruyter-Spira C, Bouwmeester H, Lovisolo C, Cardinale F.** 2015. Osmotic stress represses strigolactone biosynthesis in *Lotus japonicus* roots: exploring the interaction between strigolactones and ABA under abiotic stress. *Planta* **241**, 1435-1451.

**Liu J, Novero M, Charnikhova T, Ferrandino A, Schubert A, Ruyter-Spira C, Bonfante P, Lovisolo C, Bouwmeester HJ, Cardinale F.** 2013. Carotenoid cleavage dioxygenase 7 modulates plant growth, reproduction, senescence, and determinate nodulation in the model legume *Lotus japonicus*. *Journal of Experimental Botany* **64**, 1967-1981.

**Lopez-Raez J, Kohlen W, Charnikhova T, Mulder P, Undas A, Sergeant M, Verstappen F, Bugg T, Thompson A, Ruyter-Spira C, Bouwmeester H.** 2010. Does abscisic acid affect strigolactone biosynthesis? *New Phytologist* **187**, 343-354.

**Lv S, Zhang Y, Li C, Liu Z, Yang N, Pan L, Wu J, Wang J, Yang J, Lv Y, Jiang W, She X, Wang G.** 2017. Strigolactone-triggered stomatal closure requires hydrogen peroxide synthesis and nitric oxide production in an abscisic acid-independent manner. *New Phytologist* **217**: 290-304.

**McCarty D, Carson C, Stinard P, Robertson D.** 1989. Molecular analysis of *viviparous-1* - an abscisic acid-insensitive mutant of maize. *Plant Cell* **1**, 523-532.

**Moskowitz AH, Hrazdina G.** 1981. Vacuolar contents of fruit subepidermal cells from *Vitis* species. *Plant Physiology* **68**, 686-692.

**Nambara E, Marion-Poll A.** 2005. Abscisic acid biosynthesis and catabolism. *Annual Review of Plant Biology* **56**, 165-185.

**Okamoto M, Kuwahara A, Seo M, Kushiro T, Asami T, Hirai N, Kamiya Y, Koshiba T, Nambara E.** 2006. *CYP707A1* and *CYP707A2*, which encode abscisic acid 8'-hydroxylases, are indispensable for proper control of seed dormancy and germination in Arabidopsis. *Plant Physiology* **141**, 97-107.

**Okamoto M, Tanaka Y, Abrams S, Kamiya Y, Seki M, Nambara E.** 2009. High humidity induces abscisic acid 8'-hydroxylase in stomata and vasculature to regulate local and systemic abscisic acid responses in Arabidopsis. *Plant Physiology* **149**, 825-834.

**Pagliarani C, Vitali M, Ferrero M, Vitulo N, Incarbone M, Lovisolo C, Valle G, Schubert A.** 2017. The accumulation of miRNAs differentially modulated by drought stress is affected by grafting in grapevine. *Plant Physiology* **173**, 2180-2195.

**Park S, Fung P, Nishimura N, Jensen D, Fujii H, Zhao Y, Lumba S, Santiago J, Rodrigues A, Chow T, Alfred S, Bonetta D, Finkelstein R, Provart N, Desveaux D, Rodriguez P, McCourt P, Zhu J, Schroeder J, Volkman B, Cutler S.** 2009. Abscisic acid inhibits type 2C protein phosphatases via the PYR/PYL family of START proteins. *Science* **324**, 1068-1071.

**Pelaez-Vico M, Bernabeu-Roda L, Kohlen W, Soto M, Lopez-Raez J.** 2016. Strigolactones in the Rhizobium-legume symbiosis: Stimulatory effect on bacterial surface motility and down-regulation of their levels in nodulated plants. *Plant Science* **245**, 119-127.

**Pilati S, Perazzolli M, Malossini A, Cestaro A, Dematte L, Fontana P, Dal Ri A, Viola R, Velasco R, Moser C.** 2007. Genome-wide transcriptional analysis of grapevine berry ripening reveals a set of genes similarly modulated during three seasons and the occurrence of an oxidative burst at veraison. *BMC Genomics* **8**, 428.

**Pirie A, Mullins MG.** 1976. Changes in anthocyanin and phenolics content of grapevine leaf and fruit tissues treated with sucrose, nitrate, and abscisic acid. *Plant Physiology* **58**, 468-472.

**Ruiz-Lozano J, Aroca R, Zamarreno A, Molina S, Andreo-Jimenez B, Porcel R, Garcia-Mina J, Ruyter-Spira C, Lopez-Raez J.** 2016. Arbuscular mycorrhizal symbiosis induces strigolactone biosynthesis under drought and improves drought tolerance in lettuce and tomato. *Plant Cell and Environment* **39**, 441-452.

**Ruyter-Spira C, Al-Babili S, van der Krol S, Bouwmeester H.** 2013. The biology of strigolactones. *Trends in Plant Science* **18**, 72-83.

**Ruyter-Spira C, Kohlen W, Charnikhova T, van Zeijl A, van Bezouwen L, de Ruijter N, Cardoso C, Lopez-Raez J, Matusova R, Bours R, Verstappen F, Bouwmeester H.** 2011. Physiological effects of the synthetic strigolactone analog GR24 on root system architecture in Arabidopsis: another belowground role for strigolactones? *Plant Physiology* **155**, 721-734.

**Saito S, Hirai N, Matsumoto C, Ohigashi H, Ohta D, Sakata K, Mizutani M.** 2004. Arabidopsis *CYP707As* encode (+)-abscisic acid 8'-hydroxylase, a key enzyme in the oxidative catabolism of abscisic acid. *Plant Physiology* **134**, 1439-1449.

**Sandhu AK, Gray DJ, Lu J, Gu L.** 2011. Effect of exogenous abscisic acid on antioxidant capacities, anthocyanin, and flavonol content of muscadine grape (*Vitis rotundifolia*) skins. *Food Chemistry* **126**, 982-988.

**Snowden K, Simkin A, Janssen B, Templeton K, Loucas H, Simons J, Karunairetnam S, Gleave A, Clark D, Klee H.** 2005. The *Decreased Apical Dominance 1* *Petunia hybrida* carotenoid cleavage dioxygenase 8 gene affects branch production and plays a role in leaf senescence, root growth, and flower development. *Plant Cell* **17**, 746-759.

**Speirs J, Binney A, Collins M, Edwards E, Loveys B.** 2013. Expression of ABA synthesis and metabolism genes under different irrigation strategies and atmospheric VPDs is associated with stomatal conductance in grapevine (*Vitis vinifera* L. cv Cabernet Sauvignon). *Journal of Experimental Botany* **64**, 1907-1916.

**Sun J, Dong Y, Li C, Shen Y.** 2015. Transcription and enzymatic analysis of beta-glucosidase VvBG1 in grape berry ripening. *Plant Growth Regulation* **75**, 67-73.

**Sun L, Zhang M, Ren J, Qi J, Zhang G, Leng P.** 2010. Reciprocity between abscisic acid and ethylene at the onset of berry ripening and after harvest. *BMC Plant Biology* **10**, 257.

**Symons G, Davies C, Shavrukov Y, Dry I, Reid J, Thomas M.** 2006. Grapes on steroids. Brassinosteroids are involved in grape berry ripening. *Plant Physiology* **140**, 150-158.

**Thompson A, Jackson A, Parker R, Morpeth D, Burbidge A, Taylor I.** 2000. Abscisic acid biosynthesis in tomato: regulation of zeaxanthin epoxidase and 9-cis-epoxycarotenoid

dioxygenase mRNAs by light/dark cycles, water stress and abscisic acid. *Plant Molecular Biology* **42**, 833-845.

**Umehara M, Hanada A, Yoshida S, Akiyama K, Arite T, Takeda-Kamiya N, Magome H, Kamiya Y, Shirasu K, Yoneyama K, Kyozuka J, Yamaguchi S.** 2008. Inhibition of shoot branching by new terpenoid plant hormones. *Nature* **455**, 195-200.

**Villalobos-Gonzalez L, Pena-Neira A, Ibanez F, Pastenes C.** 2016. Long-term effects of abscisic acid (ABA) on the grape berry phenylpropanoid pathway: Gene expression and metabolite content. *Plant Physiology and Biochemistry* **105**, 213-223.

**Visentin I, Vitali M, Ferrero M, Zhang Y, Ruyter-Spira C, Novak O, Strnad M, Lovisolo C, Schubert A, Cardinale F.** 2016. Low levels of strigolactones in roots as a component of the systemic signal of drought stress in tomato. *New Phytologist* **212**, 954-963.

**Vogel J, Walter M, Giavalisco P, Lytovchenko A, Kohlen W, Charnikhova T, Simkin A, Goulet C, Strack D, Bouwmeester H, Fernie A, Klee H.** 2010. SLCCD7 controls strigolactone biosynthesis, shoot branching and mycorrhiza-induced apocarotenoid formation in tomato. *Plant Journal* **61**, 300-311.

**Walker AR, Lee E, Bogs J, McDavid DA, Thomas MR, Robinson SP.** 2007. White grapes arose through the mutation of two similar and adjacent regulatory genes. *Plant Journal* **49**, 772-785.

**Wheeler S, Loveys B, Ford C, Davies C.** 2009. The relationship between the expression of abscisic acid biosynthesis genes, accumulation of abscisic acid and the promotion of *Vitis vinifera* L. berry ripening by abscisic acid. *Australian Journal of Grape and Wine Research* **15**, 195-204.

**Xu Z, Nakajima M, Suzuki Y, Yamaguchi I.** 2002. Cloning and characterization of the abscisic acid-specific glucosyltransferase gene from adzuki bean seedlings. *Plant Physiology* **129**, 1285-1295.

**Yang Y, Tan B.** 2014. A distal ABA responsive element in *AtNCED3* promoter is required for positive feedback regulation of ABA biosynthesis in Arabidopsis. *Plos One* **9**: e87283.

**Yoneyama K, Xie X, Kusumoto D, Sekimoto H, Sugimoto Y, Takeuchi Y.** 2007. Nitrogen deficiency as well as phosphorus deficiency in sorghum promotes the production and exudation of 5-deoxystrigol, the host recognition signal for arbuscular mycorrhizal fungi and root parasites. *Planta* **227**, 125-132.

**Young P, Lashbrooke J, Alexandersson E, Jacobson D, Moser C, Velasco R, Vivier M.** 2012. The genes and enzymes of the carotenoid metabolic pathway in *Vitis vinifera* L. *BMC Genomics* **13**: 243.

**Zhang M, Leng P, Zhang G, Li X.** 2009. Cloning and functional analysis of *g-cis-epoxycarotenoid dioxygenase (NCED)* genes encoding a key enzyme during abscisic acid biosynthesis from peach and grape fruits. *Journal of Plant Physiology* **166**, 1241-1252.