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Ethylene and other stimuli affect expression of the UDP glucose-flavonoid 3-*O*-glucosyltransferase in a non-climacteric fruit

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Summary

The UDP glucose-flavonoid 3-O-glucoslyltransferase (UFGT) is a key enzyme for biosynthesis and stability of anthocyanin pigments of red grapes. Understanding factors affecting expression of this enzyme is thus important for the control of grape colour. A 1640 bp promoter region of the grapevine *ufgt* gene was cloned and sequenced. Sequence analysis revealed seven putative ethylene-responsive cis-elements and others related to three major signals known to induce anthocyanin accumulation in plant tissues: light, sugar, and abscisic acid. In order to evaluate the ability of ethylene and other signals to drive expression from the *ufgt* promoter, we ran transient expression experiments using an anthocyanin-rich grape cell culture, with very low green auto-fluorescence. After biolistic bombardment, the cells were treated with various combinations of the four signals on *gfp* expression (green fluorescent protein). The comparison of fluorescent light intensity in cells subjected to the various treatments showed that ethylene better stimulates expression of the *ufgt* promoter in the dark than under light. In addition, results showed that there may be a positive interaction between ethylene and abscisic acid. This system, a promoter of interest driving the *gfp* expression in cells with low auto-fluorescence, may be a good tool for studies about synergistic or antagonist roles of transcription factors. Moreover, treatment of grape berries with a specific inhibitor of ethylene receptors (1-methylcyclopropene) inhibited *ufgt* mRNA accumulation. This confirms that the ethylene signal is likely a regulator of grape UFGT expression in a non-climacteric fruit.

K e y w o r d s : anthocyanins, cis-elements, ethylene, ethylene receptors, grape, promoter, UDP glucose-flavonoid 3-*O*-glucosyltransferase (UFGT).

Introduction

Ripening is an essential step of fruit development that conditions the qualities of both whole fruit and transformed products. There are two classes of fruits with regard to their ripening behaviour: climacteric fruits for which ripening steps are controlled by a relatively strong ethylene evolution, and non-climacteric fruits in which ethylene evolution is very low and for which the ripening process seems to occur independently of ethylene production (Giovannoni 2001). Grapes are regarded as non-climacteric fruits (Coombe and Hale 1973), however a recent study has shown that grape berry tissues have a fully functional pathway for ethylene synthesis, and that this pathway is activated just before véraison (Chervin *et al*. 2004), when berries start to soften, accumulate sugars and, in red cultivars, accumulate anthocyanins. Furthermore this study showed that ethylene perception is critical for some berry changes associated with ripening, including anthocyanin accumulation (Chervin *et al*. 2004). This conclusion was supported by the fact that anthocyanin biosynthesis in berry skins was inhibited when a specific inhibitor of ethylene receptors, 1-methylcyclopropene or 1-MCP (Blankenship and Dole 2003), was applied to berries just before veraison, at the same time as ethylene was being produced in the berry (Chervin *et al*. 2004).

The control of anthocyanin accumulation during the ripening phase in red grape berries is thought to be greatly dependent on UFGT activity (Boss *et al*. 1996, Kobayashi *et al.* 2002, Kobayashi *et al*. 2004). This enzyme plays a key role in stabilising the aglycone moiety of anthocyanins (Piffaut *et al*. 1994) and may be essential for their transport to the vacuole. To investigate which factors induce grape *ufgt* expression, we cloned and sequenced the grape *ufgt* promoter, which revealed different cis-element motifs including ethylene responsive elements (ERE). The *ufgt* promoter was then fused to the *gfp* reporter gene and various transient expression studies in grape cell suspensions were conducted in order to test the importance of ethylene signals and other stimuli in the control of transcription from this promoter. We thus complemented previous results indicating that exogenous ethylene could induce grape *ufgt* expression (El-Kereamy *et al*. 2003), and confirming observations about the use of ethylene precursor to enhance grape skin colour (Weaver and Montgomery 1974). We then evaluated *in planta* whether the ethylene signal was acting in grape berries on *ufgt* expression directly via ethylene receptors or indirectly through alternate pathways. These results confirm the role of ethylene signals in the control of the *ufgt* expression in a non-climacteric fruit and present a method that could be a good tool to study steric hindrance of transcription factors on a promoter.

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Material and Methods

A subclone obtained from the 5'-end of a grapevine *ufgt* cDNA (Ford *et al*.1998) was used to probe a 400 000 plaque aliquot of a genomic library made from the cultivar 'Shiraz'. Two positive plaques were obtained and one was used to isolate a genomic fragment consisting of 736 bp of *ufgt* coding sequence and 1647 bp of promoter sequence immediately upstream of the ATG start codon. For expression analysis, the grapevine *ufgt* promoter region was amplified using the primers UTPPF (5'-TCCCCCGGGCTTTTCGGTATCATGCGTCC-3') and UTPP2 (5'-TCCCCCGGGTTGGAATGGGGGATGTT-3') and cloned into a promoterless-*gfp* shuttle vector (pART7napx+GFP) developed from pART7 (Gleave 1992) and pBINm-gfp5-ER (Haseloff *et al*. 1997). The entire expression cassette was then cloned into the binary vector pART27 (Gleave 1992) and the final construct was called pVvufgt::gfp. The cis-elements were estimated by homology search using PLACE database, http://www.dna.affrc.go.jp/PLACE/signalscan.html (Higo *et al.* 1998) or PlantCARE database, http://intra.psb.ugent.be:8080/PlantCARE/ (Lescot *et al.* 2002). Comparisons were made between results obtained before and after the randomisation of the promoter sequence using the same databases. When the number of repetitions of one cis-element was equal or higher in the randomised sequence than in the original one (indicating a highly probable unspecificity), we kept it in the figure, but showed its name in italics.

Suspensions of purple grape berry cells, 'Gamay' were grown as described previously (Triantaphylidès *et al.* 1993). Biolistic experiments were performed using a helium-driven gun with an initial propelling He pressure of 3.1 bars and a 30-mbar vacuum in the chamber at shooting time. The previously described protocol (Torregrosa *et al.* 2002) was used with slight modifications. Briefly, cells were vacuum-filtered onto sterile Whatman n°1 filter paper and set upon MS-based cell culture medium containing 60 mM sucrose and solidified with 3 $g \cdot l^{-1}$ Phytagel in small Petri dishes. Gold microprojectiles (1.0 µm, Biorad) were coated with the pVvufgt::gfp plasmid to achieve 1 µg DNA delivery per shot. Experiments were performed twice (two different dates), each time with three 55 mm-diameter Petri dishes (one dish per shot) for each treatment or treatment combination in each instance. After shooting, cells were sprayed with sterile water (control) or one ml sterile solutions of either 7 mM 2-chloroethylphosphonic acid (2-CEPA), a generator of ethylene, or 150 mM sucrose or 500 µM abscisic acid (ABA), each compound added alone or in all combinations. The dark treatment was achieved by wrapping the Petri dishes in aluminium foil. The incubation time to reach optimal GFP signal was around 40 h in a growth chamber with a 16:8 Light:Dark illumination cycle at room temperature. The observations were made on the second day after shooting, 2 h after light resumption. Image acquisition and analysis were performed according to Cormeau *et al.* (2002), with the following modifications: samples were examined with an epifluorescence microscope (DMIRB-E, Leica, Germany) equipped with suitable excitation and emission filters (*i.e.* blue range excitation BP 450-490 nm, DM 510 nm, LP 515 nm). Images were acquired using a Color CoolView 3-chip on chip CCD camera (Photonic Science, Millham, UK). Camera settings were identical for all experiments. For each image, the fluorescence intensity within the cell and in the background was measured from the green channel, using Image Pro-Plus 4.5 (Media Cybernetics, Silver Spring, MD, USA). The fluorescing cells were spotted by examining the Petri dishes with a 10X magnification. The images of all observable cells per Petri dish were captured. A total of six Petri dishes were observed per treatment (three replicates x two bombardment series). There was no indication of the number of plasmids inserted per cell, but this is likely to vary similarly between treatments, as these were performed after the bombardment. The quantification was performed using SigmaScan (SPSS Inc., Chicago, IL). The signal intensities were normalised so the mean of controls under light was equal to 100. The LSD value was calculated at the 5% level using a one way ANOVA (SigmaStat, SPSS, Chicago, IL).

The grape berries used for the 1-MCP experiments were from 'Cabernet Sauvignon' grapevines grafted on 110 Richter rootstocks and grown in Toulouse, South-West of France, in a non-irrigated vineyard. Full bloom occurred around mid-June. The 1-MCP was applied using a polyethylene bag wrapped around the cluster, for 24 h, once a week, at various times following full bloom. The initial 1-MCP concentration was 4 $\mu l \cdot l^{-1}$. Control clusters were wrapped in plastic bags for 24 h. For these experiments, only clusters growing in a shaded area of the vines were chosen to avoid direct exposure to sunlight and overheating associated with such a treatment. A total of 5 clusters (each from a different plant) per treatment were used, with ten berries (2 from each cluster) pooled for analysis (three biological repetitions). After treatments, the clusters were stored at -80 °C pending further analyses. Northern and Western blots were performed as previously described (El-Kereamy *et al*. 2003).

Results and Discussion

In order to investigate the effects of ethylene and other possible stimuli on *ufgt* expression, a genomic clone encoding the promoter of grapevine *ufgt* was isolated and a 1647 bp region upstream of the putative ATG translational start codon cloned. The 'Shiraz' *ufgt* promoter sequence isolated in this study (GenBank AY955269), displayed 97 to 99 % homology to the grapevine *ufgt* promoter sequences isolated from Kyoho, Italia, Ruby Oku, Muscat of Alexandria and Flame Muscat cultivars (Kobayashi *et al.* 2001), and 95 % to 'Cabernet Sauvignon' (GenBank AY919624). The alignment of various grape ufgt promoter sequences has already been shown (Kobayashi *et al.* 2001). From all these promoter sequences, one ethylene cis-element, initially reported by Itzhaki *et al.* (1994), attracted our attention. This ERE sequence, ATTTCAAA is located at -365 bp (Fig. 1). This element was also present with a sequence differing by a single nucleotide, ATTTTAAA, at three other sites that could thus be also potential EREs. Indeed, this latter sequence is recognised as a potential eth-

Fig. 1: Sequence of the *ufgt* promoter from grapevine 'Shiraz'. The numbering odd nucleotide is relative to the start codon. Cis-elements estimated by homology search using PLACE database (Higo *et al.* 1998) and PlantCARE (Lescot *et al.* 2002) are indicated in bold letters (ABRE: ABA responsive element; ERE: ethylene responsive element; LRE: light responsive element; SURE: sugar responsive element). Cis-elements in italics were found with the same abundance or greater in a randomized sequence.

ylene cis-element using the online-database, PlantCARE (Lescot *et al.* 2002). Furthermore we found a promoter region with sequence homology to another class of ethylene cis-elements at -160 bp (sequence GCCGCC) previously described by Fujimoto *et al.* (2000). The presence of several putative ethylene cis-elements in the grapevine *ufgt* promoters is evidence to support our observations about the potential role for ethylene in inducing *ufgt* expression in grape berries after véraison. The EREs mentioned above are also present in the 'Cabernet Sauvignon' *ufgt* promoter. Submitting this promoter to PLACE analysis (Higo *et al.* 1998), we found many sequence matches to other known cis-elements (Fig. 1), with several corresponding to three other stimuli (light, sugar and abscisic acid) known to affect plant development or metabolism and in particular to induce anthocyanin accumulation in grapes and other plant tissues (Mol *et al.* 1996).

Using the 'Shiraz' *ufgt* promoter transcriptionally fused to the *gfp* gene, we carried out transient expression experiments using purple grape cell suspension cultures established from berries of the Gamay grape cultivar. As these cells have been shown to produce glycosylated anthocyanins (Afifi *et al.* 2003), we thus expected that all the components necessary to activate the glycosylation of anthocyanins via the *ufgt* gene were present in these cells. These purple cells also present the advantage of having very low auto-fluorescence at the wavelength used to track GFP activity. The aim of this transient expression study was to check the importance of ethylene signalling in inducing *ufgt* expression along with the three other potential regulators of *ufgt* expression mentioned above. We tested the effects of darkness or light alone on reporter gene expression, the addition of single effectors (ABA, ethylene or sucrose) on GFP fluorescence, and then the influence of all double and triple combinations of the stimuli on GFP expression. Fig. 2 shows that ethylene stimulated GFP production in the transient assays in darkness (a significant increase of the GFP signal by 25 % compared to the dark control alone),

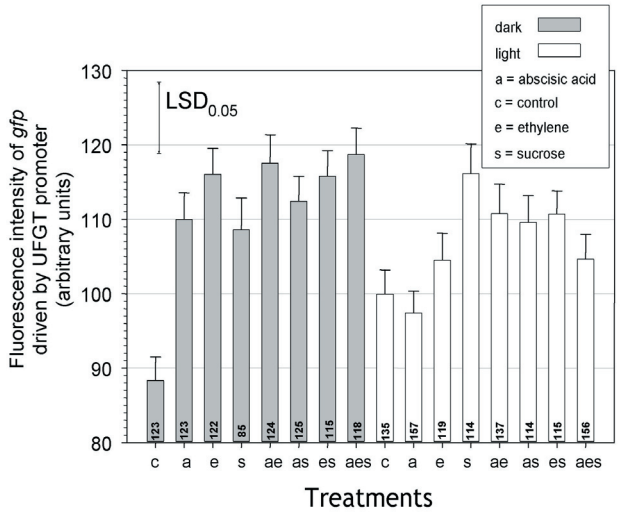

Fig. 2: Activity of the *ufgt* promoter as a function of various stimuli. Fluorescence of the GFP, driven by an *ufgt* promoter, after bombardment of grape cells 'Gamay' and spraying with water (control), or solutions of abscisic acid, or chloroethylphosphonic acid, an ethylene generator, or sucrose, and combinations of these stimuli. The cells were incubated in dark or light conditions. The bars represent the mean values of x individual fluorescing cells, x is given at the bottom of each bars. The numbers correspond to the means of 6 cell batches (3 replicates x 2 dates). The normalization was performed by giving the "100" value to the mean value of "light controls". The error bars represent SE, and the LSD bar was calculated at the 5 % level.

but ethylene did not stimulate GFP expression from the *ufgt* promoter in light (a non-significant increase by 5 %). This absence of stimulation in light could be due to overlapping or proximal cis-elements (one LRE and one ERE) such as those at -366 bp or -420 bp or -766 bp or -936 bp (Fig. 1). This is the case of nearly 60 % of the EREs located on this promoter. This type of inhibition may be due to steric hindrance of transcription factors as discussed by Hahn *et al.* (2003). It is also possible that the timing of the initiation of each stimulus, leading to presence of transcription factors in a set order, may also lead to either synergistic or antagonist effects regarding transcription. In the dark, there is no additive effect due to ethylene in combination to other effectors (Fig. 2). The signal intensity reached a maximum as soon as one effector was present. In the light, ethylene and ABA may present some additive effect, as the signal intensity was significantly increased by + 11% in comparison to control (= light alone), when none of the single effectors (ethylene or ABA) led to an increased signal. As in other experiments of transient expression published in various plant systems, one can question their biological significance. In answer to this question, we can propose this set of results as a first description of the ethylene role in a complex combination of stimuli, controlling the expression of one key protein (UFGT) involved in the production of the anthocyanins. ABA also had a stimulatory effect on GFP expression in the transient assays carried out in the dark, but no significant change was seen when the cells were maintained in the light (Fig. 2). Such an ABA effect on *ufgt* expression in grape berries has been reported recently (Peppi *et al*. 2008). Sucrose treatments increased the levels of GFP fluorescence in both dark and light grown cells (Fig. 2). In the context of berry ripening, these results are most interesting: *ufgt* expression is induced at véraison and continues throughout the rest of berry ripening (Boss *et al*. 1996). The induction of UFGT coincides with the initiation of an influx of sugars into berries and a peak of ABA levels in berries (Boss *et al*. 1996, Davies *et al*. 1997). Thus, it is possible that either of these stimuli may promote expression from the *ufgt* promoter *in vivo*. Light was also found to stimulate GFP expression from the Shiraz *ufgt* promoter in the transient assays (Fig. 2). This matches results obtained with 'Cabernet Sauvignon', in which shading of bunches has been shown to reduce the accumulation of anthocyanins (Jeong *et al*. 2004).

The application of 1-MCP significantly inhibited the accumulation of *ufgt* mRNA in 'Cabernet Sauvignon' berries when applied 10 weeks after full bloom (Fig. 3 a). Western blot analysis was run whenever *ufgt* mRNAs were detected. No significant difference was seen in UFGT protein levels between control and 1-MCP treatments at each

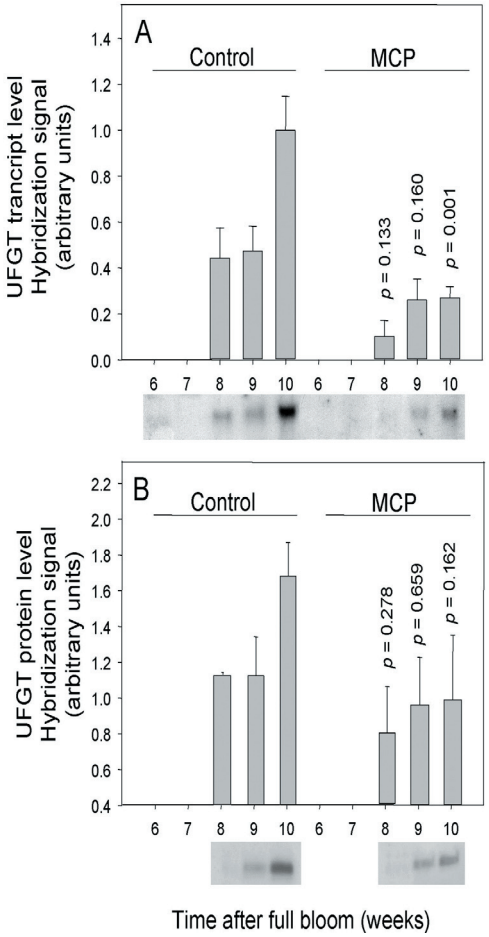

Fig. 3: Effects of 1-MCP (methylcyclopropene), inhibitor of ethylene receptors, on the expression of the *ufgt* gene in grape berries, 'Cabernet Sauvignon', as a function of the time of application after full bloom. **A**: Northern blots, results normalised with the 18S signals, the picture shows one of the three blots; **B**: Western blots, results normalised with the red Ponceau signals, the picture represents one of the three blots; n = 3 biological replicates, error bars represent SE, *p* is the probability that there was no difference between the control and the MCP means at the same sampling time (t-test).

time point analysed; however 1-MCP treatment attenuated UFGT protein accumulation levels over the three sampling times (Fig. 3 b). The fact that the 1-MCP effect was significant only on mRNA and not on proteins may be due to the higher stability of UFGT protein compared to mRNAs. To be able to observe changes in protein levels we may need to sample berries later than 24 h after treatment to allow for the reduction in *ufgt* mRNA levels to be reflected in reduced UFGT protein level. Nevertheless, the difference seen in *ufgt* mRNA levels upon 1-MCP application in the 10 weeks post flowering sample confirms the previous observation that the induction of UFGT by exogenous ethylene is probably due to ethylene signal transduction and not to indirect stimulation via other metabolisms activated by ethylene or side-effects of the relatively high dose of the ethylene precursor that was sprayed onto the grapes (El-Kereamy *et al*. 2003). The inhibition of the UFGT protein accumulation by 1-MCP (Fig. 3 b), without being significant by Western analysis, may lead to the significant decrease of anthocyanin accumulation previously observed (Chervin *et al*. 2004), as for week 10 there was a drop by almost 50 % in the protein accumulation after treatment with 1-MCP (Fig. 3 b). The application of exogenous ethylene led to significant increases of both *ufgt* mRNAs and proteins (El-Kereamy *et al*. 2003), and this is consistent with the decrease provoked in both messengers and proteins content by an ethylene inhibitor (1-MCP) as presented in this paper. The control of the anthocyanin biosynthesis pathway in plants has been shown to occur at the level of gene transcription (Martin and Gerats 1993). Studies into the promoters of anthocyanin biosynthesis genes in both *Antirrhinum* (Sablowski *et al*. 1994) and maize (Roth *et al*. 1991) have shown that transcriptional controlling elements are usually present upstream of the transcription start site. In grapevine, the *ufgt* expression correlates with anthocyanin biosynthesis, which suggests that important promoter elements upstream of this gene control anthocyanin production in this species (Boss *et al*. 1996). The results of the 1-MCP treatments above and experiments from previous work (El-Kereamy *et al*. 2003) suggest that the *ufgt* promoter can indeed respond to ethylene signalling.

In conclusion, the 1647 bp upstream region of the Shiraz grapevine *ufgt* promoter has been shown to contain cis-elements that respond to the correct stimuli required for UFGT and thus anthocyanin production in berry skins. Transient expression analyses show that light, ABA, sugar and ethylene can all stimulate expression from the *ufgt* promoter under certain conditions (Fig. 2). This is consistent with known changes in berry composition during berry development and in experiments designed to alter these variables (Boss *et al*.1996, Davies *et al*. 1997, Downey *et al*. 2004, Jeong *et al*. 2004). The trend observed here, that ethylene better enhance *ufgt* expression in the dark than in the light, matches well the trend observed in the vineyards that spraying ethylene precursors has a more perceptible effect on clusters growing in shaded areas or during years with a low total sunlight hours over the ripening period (unpublished results). From this work and previous studies (Chervin *et al*. 2004, El-Kereamy *et al*. 2003, Weaver and Montgomery 1974), it is quite clear that ethylene is involved in the signal mix leading to the *ufgt* expression in grape cells, and particularly in berry skin tissues that accumulate high concentrations of anthocyanins.

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References

Afifi, M.; El-Kereamy, A.; Legrand, V.; Chervin, C.; Monje, M. C., Nepveu, F.; Roustan, J. P.; 2003: Control of anthocyanin biosynthesis pathways by eutipin, a toxin from *Eutypa lata*, in grape cell tissue cultures. J. Plant Physiol. **160**, 971-975.

Blankenship, S. M.; Dole, J. M.; 2003: 1-Methylcyclopropene: A review. Postharvest Biol. Technol. **28**, 1-25.

Boss, P. K.; Davies, C.; Robinson, S. P.; 1996: Expression of anthocyanin biosynthesis pathway genes in red and white grapes. Plant Mol. Biol. **32**, 565-569.

Chervin, C.; El-Kereamy, A.; Roustan, J. P.; Latche, A.; Lamon, J.; Bouzayen, M.; 2004: Ethylene seems required for the ripening of grape, a non-climacteric fruit. Plant Sci. **167**, 1301-1305.

Coombe, B. G.; Hale, C. R.; 1973: The hormone content of ripening grape berries and the effect of growth substance treatments. Plant Physiol. **51**, 629-634.

Cormeau, J.; Barthou, H.; Jauneau, A.; Petitprez, M.; Pont-Lezica, R.; Galaud, J. P.; 2002: Cellular import of synthetic peptide using a cell-permeable sequence in plant protoplasts. Plant Physiol. Biochem. **40**, 1081-1086.

Davies, C.; Boss, P. K.; Robinson, S. P.; 1997: Treatment of grape berries, a non-climacteric fruit with a synthetic auxin, retards ripening and alters the expression of developmentally regulated genes. Plant Physiol. **115**, 1155-1161.

Downey, M. O.; Harvey J. S.; Robinson, S. P.; 2004: The effect of bunch shading on berry development and flavonoid accumulation in Shiraz grapes. Aust. J. Grape Wine Res. **10**, 55-73.

El-Kereamy, A.; Chervin, C.; Roustan, J. P.; Cheynier, V.; Souquet, J. M.; Moutounet, M.; Raynal, J.; Ford, C. M.; Latche, A.; Pech, J. C.; Bouzayen, M.; 2003: Exogenous ethylene stimulates the long-term expression of genes related to anthocyanin biosynthesis in grape berries. Physiol. Plant. **119**, 175-182.

Ford, C. M.; Boss, P. K.; Høj, P. B.; 1998: Cloning and characterization of *Vitis vinifera* UDP-glucose: Flavonoid 3-O-glycosyltransferase, a homologue of the enzyme encoded by the maize bronze-1 *locus* which may primarily serve to glucosylate anthocyanidins *in vivo*. J. Biol. Chem. **273**, 9224-9233.

Fujimoto, S. Y.; Ohta, M.; Usui, A.; Shinshi, H.; Ohme-Takagi, M.; 2000: *Arabidopsis* ethylene-responsive element binding factors act as transcriptional activators or repressors of GCC box-mediated gene expression. Plant Cell **12,** 393-404.

Giovannoni, J.; 2001: Molecular biology of fruit maturation and ripening, Ann. Rev. Plant Physiol. Plant Mol. Biol. **52**, 725-749.

Gleave, A. P.; 1992: A versatile binary vector system with a T-DNA organisational structure conducive to efficient integration of cloned DNA into the plant genome. Plant Mol. Biol. **20**, 1203-1207.

Hahn, M. W.; Stajich, J. E.; Wray, G. A.; 2003: The effects of selection against spurious transcription factor binding sites. Mol. Biol. Evol. **20,** 901-906.

Haseloff, J.; Siemering, K. R.; Prasher, D.; Hodge, S.; 1997: Removal of a cryptic intron and subcellular localisation of green fluorescent protein are required to mark transgenic Arabidopsis plants brightly. Proc. Nat. Acad. Sci. USA **94**, 2122-2127.

Higo, K.; Ugawa, Y.; Iwamoto, M.; Higo, H.; 1998: PLACE: A database of plant cis-acting regulatory DNA elements, Nucl. Acids Res. **26**, 1358-1359.

Itzhaki, H.; Maxson, J. M.; Woodson, W. R.; 1994: An ethylene-responsive enhancer element is involved in the senescence-related expression of the carnation glutathione-S-transferase (GST1) gene. Proc. Nat. Acad. Sci. USA **91**, 8925-8929.

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

Kobayashi, S.; Goto-Yamamoto, N.; Hirochika, H.; 2004: Retrotransposon-induced mutations in grape skin color. Science **304**, 982.

Kobayashi, S.; Ishimaru, M.; Ding, C.K.; Yakushiji, H.; Goto, N.; 2001: Comparison of UDP-glucose:flavonoid 3-O-glucosyltransferase (*ufgt*) gene sequences between white grapes (*Vitis vinifera*) and their sports with red skin. Plant Sci. **160**, 543-550.

Kobayashi, S.; Ishimaru, M.; Hiraoka, K.; Honda, C.; 2002: Myb-related genes of the Kyoho (*Vitis labruscana*) regulate anthocyanin biosynthesis. Planta **215**, 924-933.

Lescot, M.; Déhais, P.; Thijs, G.; Marchal, K.; Moreau, Y.; Van de Peer, Y.; Rouzé, P.; Rombauts, S.; 2002: PlantCARE, a database of plant cis-acting regulatory elements and a portal to tools for *in silico* analysis of promoter sequences. Nucl. Acids Res. **30**, 325-327.

Martin, C.; Gerats, T.; 1993: Control of pigment biosynthesis genes during petal development. Plant Cell **5**, 1253-1264.

Mol, J.; Jenkins, G.; Schaefer, E.; Weiss, D.; 1996: Signal perception, transduction, and gene expression involved in anthocyanin biosynthesis. Crit. Rev. Plant Sci. **15**, 525-558.

Peppi, M. C.; Walker, M. A.; Fidelibus, M. W.; 2008: Application of abscisic acid rapidly upregulated UFGT gene expression and improved color of grape berries. Vitis, **47**,11-14.

Piffaut, B.; Kader, F.; Girardin, M.; Metche, M.; 1994: Comparative degradation pathways of malvidin 3,5-diglucoside after enzymatic and thermal treatments. Food Chem. **50**, 115-120.

Roth, B. A.; Goff, S. A.; Klein, T. M.; Fromm, M. E.; 1991: C1 and R-dependent expression of the maize Bz1 gene requires sequences with homology to mammalian myb and myc binding sites. Plant Cell **3**, 317-325.

Sablowski, R. W. M.; Moyano, E.; Culianez-Macia, A.; Schuch, W.; Martin, C.; Bevan, M.; 1994: A flower specific Myb protein activates transcription of phenylpropanoid biosynthesis genes. EMBO J. **13** 128-137.

Torregrosa, L.; Verries, C.; Tesniere, C.; 2002: Grapevine (*Vitis vinifera* L.) promoter analysis by biolistic-mediated transient transformation of cell suspensions. Vitis **41**, 27-32.

Triantaphylidès, C.; Nespoulous, L.; Chervin, C.; 1993: Ammonium requirement for radiation-induced accumulation of polyamines in suspension cultured grape cells. Physiol. Plant. **87**, 389-395.

Weaver, R. J.; Montgomery, R.; 1974: Effect of ethephon on coloration and maturation of wine grapes. Am. J. Enol. Vitic. **25**, 39-41.

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