Vitis **46** (4), 210–211 (2007)

**Research Note**

# The stimulation by ethylene of the UDP glucose-flavonoid 3-*O*-glucosyltransferase (UFGT) in grape tissues is independent from the MybA transcription factors

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K e y w o r d s : $C_2H_4$, glycosyltransferase, grapevine, anthocyanin.

**Introduction:** Grapes are regarded as non-climacteric fruit (Chervin *et al*. 2004 and references herein), in which ethylene evolution is very low and for which the ripening process seems to occur independently of ethylene production. However, a recent study has shown that grape berry tissues have a fully functional pathway for ethylene synthesis, that this pathway is activated just before veraison when red berries start to accumulate anthocyanins, and that ethylene perception is critical for some berry changes associated with ripening, including anthocyanin accumulation (Chervin *et al*. 2004). This last conclusion was supported by the fact that anthocyanin accumulation in the berry skins was inhibited when a specific inhibitor of ethylene receptors, 1-methylcyclopropene was applied to berries just before veraison, at the same time as ethylene production was activated in the berry.

The control of anthocyanin accumulation during the ripening phase in red grape berries is thought to be greatly dependent on UFGT activity (Kobayashi *et al*. 2001, Yakushiji *et al*. 2006). This enzyme plays a key role in stabilising the aglycone moiety of the anthocyanins (Piffaut *et al*. 1994) and may be essential for their transport to the vacuole. We have shown previously that exogenous ethylene could induce grape *ufgt* expression (El-Kereamy *et al*. 2003), confirming observations about the commercial use of ethylene precursor to boost grape skin colour.

The anthocyanin biosynthesis in model plants for the anthocyanin pathway such as petunia and maize is controlled by Myc and Myb transcription factors, and indeed, one transcription factor of this family, MybA1, was shown to be critical for the regulation of *ufgt* expression (Yakushiji *et al*. 2006 and refs therein) and anthocyanin accumulation in grape tissues. Walker *et al.* (2007) showed that two isoforms of this transcription factor, MybA1 and MybA2 are involved in anthocyanin accumulation in grapevine.

The question remains whether the ethylene signal is acting on grape *ufgt* expression through the activation of *mybA* expression or via ethylene responsive transcription factors.

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**Materials and Methods:** 'Cabernet Sauvignon' grapevines used for the ethylene experiments are 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 ethylene was applied for 24 h at different times after full bloom, at an initial concentration of 40 $\mu l \cdot l^{-1}$, three biological replicates were then collected and stored at -80 °C. All other sampling processes and the RNA extractions were performed as described in El-Kereamy *et al*. (2003). Suspensions of purple grape cells of 'Gamay' were grown as described previously (Triantaphylides *et al*. 1993). Cells were vacuum-filtered onto Whatman n°1 filter paper and set upon 5 ml fresh MS-based cell culture medium in small Petri dishes. Cells were then sprayed with one ml of a sterile solution of 7 mM 2-chloroethylphosphonic acid (2-CEPA), a precursor of ethylene, the controls were sprayed with sterile water. Incubation time was 6 h before freezing samples for RNA extraction, performed as described above.

The qRT-PCR analyses were performed according to El-Sharkawy *et al*. (2005), with the following modifications. Oligonucletide sequences of *mybA* primers were *VvmybA1c*(F), 5'- GCAAGCCTCAGGACAGAA -3' and *VvmybA1c*(R), 5'- AAGCCCACATCAAATGGAAAA-3'. They would amplify both *VvmybA1* (AB097923) and *VvmybA2* (AB097924) isoforms. Oligonucletide sequences for the *ufgt* primers were *Vvufgt*(F) 5'-GGCTTTT-GTCACACATTGCG-3' and *Vvufgt*(R) 5'-AAAAAG-GGCCTGCAAATCAA-3'. The *myb* et *ufgt* mRNA levels were expressed as transcript accumulation indexes relative to a control gene *ef1α,* as previously shown (Terrier *et al*. 2005). The LSD value between treatments was calculated at the 5 % level using a one way ANOVA (SigmaStat, Systat Software Inc., San Jose, CA).

The ethylene cis-elements were estimated by homology search using PLACE database, http://www.dna.affrc.go.jp/PLACE/signalscan.html or PlantCARE database, http://bioinformatics.psb.ugent.be/webtools/plantcare/html/

**Results and Discussion:** The application of gaseous ethylene significantly stimulated the accumulation of *ufgt* mRNA in berries when applied 8 and 9 weeks after full bloom (Fig. 1 a). These observations made in 'Cabernet Sauvignon' berry tissues were confirmed in another cultivar, under different experimental conditions, the 'Gamay' cell suspension sprayed with the 2-CEPA, an ethylene precursor (Fig. 1c). These results confirmed previous observations, either the enhancement of anthocyanin accumulation after spraying 2-CEPA onto the grape clusters (El-Kereamy *et al*. 2003), or the inhibition of anthocyanin accumulation following a treatment of the clusters with the ethylene inhibitor, 1-methylcyclopropene (Chervin *et al*. 2004).

However no significant difference was seen for the accumulation *mybA* mRNAs in the same experimental conditions (Fig. 1 b and c). This absence of responsiveness to an ethylene signal was further confirmed by the analysis

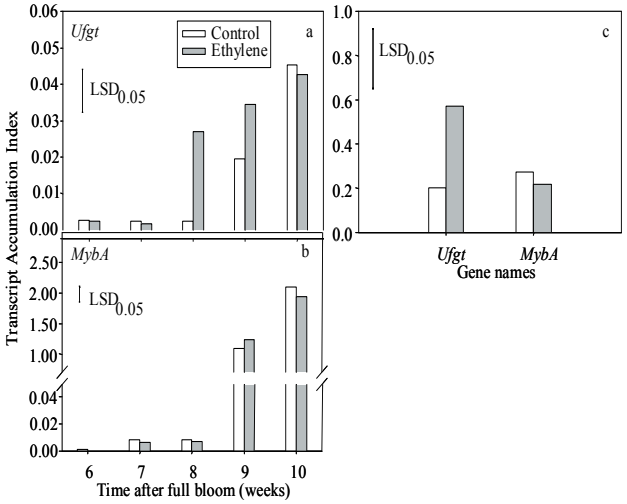


Fig. 1: Effects of exogenous ethylene to **a**) the *ufgt* mRNA levels, to **b**) the *mybA1* mRNA levels in 'Cabernet Sauvignon' berries according to time after full bloom, and to **c**) mRNAs of both genes in 'Gamay' cell suspensions. The mRNA levels were determined by qRT- PCR analyses and are expressed as the transcript accumulation index relative to *ef1*-α. Each bar represents the mean of three biological replicates, and LSD bars were calculated at the 0.05 level for differences between the treatments (control or ethylene).

of the *VvmybA1* promoter, GenBank AB242302 (the sequence of the *VvmybA2* promoter was not available when we wrote this article), which showed three ethylene cis-elements (ethylene response elements, ERE) in the -2 kb upstream region (Fig. 2 a). The closest GCCGCC box, one of the most potent EREs (FUJIMOTO *et al*. 2000), locates around -2100 bp. The closest ATTTnAAA box, the other common ERE, locates at -850 bp from *mybA1* start codon (Fig. 2). In comparison there are seven EREs in the *ufgt* promoter, GenBank AY955269, (Fig. 2). The GCCGCC sequence was found at -160 bp from the *ufgt* start codon, a good place to be active (Dr Ohme-Takagi, pers. comm. and analyses of ethylene responsive promoters, data not shown). Two other ERE sequences, ATTTnAAA are reasonably close to the gene start codon, located between -450 and -350 bp. In another ethylene responsive promoter, they were shown to be active when placed at -500 bp (ITZHAKI *et al.* 1994). Here we used the sequence of the Shiraz *ufgt* promoter, which has 97 to 99 % homology to the grapevine *ufgt* promoter sequences isolated from the '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 *ufgt* promoter sequences has already been shown (KOBAYASHI *et al*. 2001).

So we conclude that the stimulation of *ufgt* expression by ethylene is independent from *mybA* expression. But it does not preclude the importance of MybA in controlling the *ufgt* expression. Indeed, Dr. GOTO-YAMAMOTO found that the MybA protein binds to a region of the *ufgt* promoter around -428 to -303 bp from the start codon (pers. comm.) and recent works confirm the role of MybA in stimulating anthocyanin accumulation (YAKUSHIJI *et al*. 2006, WALKER *et al*. 2007). To our knowledge this is the first report addressing the responsiveness of *myb* expression to an ethylene signal.

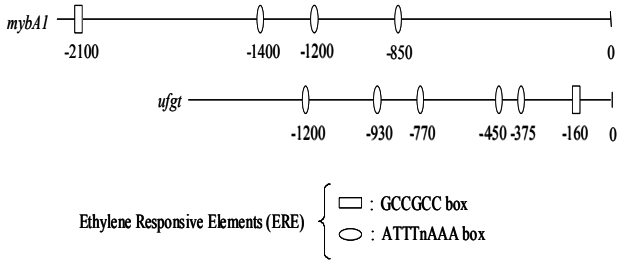


Fig. 2: Locations of ethylene responsive elements (cis-acting) on the promoter of *mybA1* (AB242302) and on the promoter of *ufgt* (AY955269) of *Vitis vinifera*. The nucleotide numbering is relative to the start codon (0). Cis-elements were estimated by homology search using PLACE and PlantCARE databases.

The authors wish to acknowledge the INRA-INP/ENSAT, and the Midi-Pyrénées regional council for a partial funding of this collaborative work, the Domaine de Candie (Régie Agricole de la Ville de Toulouse) for the grapes and the team of CRGS platform of Genopole Toulouse where the qRT-PCR analyses were performed.

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*Received March 9, 2007*