Influence of ethylene on sucrose accumulation in grape berry

Christian Chervin[1*], Nancy Terrier[2], Agnès Ageorges[2], Fabien Ribes[1] and Tira Kuapunyakoon[1]

[1]UMR 990, Génomique et Biotechnologie des Fruits, Centre INRA de Toulouse, BP 107, 31326 Castanet, France.

[2]UMR 1083, Science Pour l'Oenologie, Centre INRA/Agro- M, 2 Place Viala, 34060 Montpellier cedex 01, France.

*Corresponding author: Tel/Fax +33 5 6219 35753; email: chervin@ensat.fr

Abstract: Grape ripening is thought to be ethylene independent. However, Cabernet Sauvignon berries that were treated with 1-methylcyclopropene (1-MCP), a specific inhibitor of ethylene receptors, accumulated less sucrose over the following three weeks than did controls. This was associated with a decreased RNA accumulation of two sucrose transporters (SUC11 and SUC12), whose expression is triggered at the veraison stage when grape berries start to accumulate sugars. These observations were performed over two consecutive years. This preliminary study suggests that the role of ethylene in grape ripening needs to be reconsidered and that it could be related to sugar accumulation.

Key words: grapevine, *Vitis vinifera*, ethylene signaling, sucrose transport, ripening

Acknowledgement: We gratefully acknowledge the Domaine de Candie (Régie Agricole de la Ville de Toulouse) for the grapes, and INRA and INPT for financial support. We also thank Dr C. Romieu for participating in the set-up of the micro-array facility at UMR 1083, and Dr G. Albagnac (UMR 1083), Prs. M. Bouzayen and J.P. Roustan (UMR 990) for their support.

Introduction

Grape ripening is thought to be ethylene independent since its classification as a non-climacteric fruit (Coombe and Hale 1973, Abeles et al. 1992). However, the grape industry has been using the 2-chloroethylphosphonic acid, an ethylene precursor also named ethephon, with some success to enhance berry anthocyanin accumulation and acidity drop (Weaver and Montgomery 1974, Shulman et al. 1985). Moreover, it is now well established that ethylene is present during the ripening of non climacteric fruits such as grape or strawberry (Alleweldt and Koch 1977; Trainotti et al. 2005). Recently, it was shown that ethylene synthesis is active just before the inception of berry ripening and that treatments with 1-MCP (1-methylcyclopropene), a specific inhibitor of ethylene receptors (Blankenship and Dole 2003), partially blocked berry growth, acidity drop and anthocyanin accumulation (Chervin et al. 2004). Beyond these results, nothing is known either on the potent role of ethylene during berry ripening or on what could be the molecular target of ethylene in the grape berries.

On other plant species, there are some indications in the literature that sugar transport could be one of the targets of ethylene action (Ishizawa and Esashi 1988 on rice; Saftner 1986 on sugar beet). Interestingly, it was recently shown that sucrose, (and neither glucose nor fructose), was a key signal responsible for the triggering of anthocyanin synthesis in plant tissues (Solfanelli et al., 2006). These results can be combined to formulate the hypothesis that ethylene could alter anthocyanin accumulation in grape berries through an effect on sucrose accumulation and/or transport. In line with this hypothesis, we found the presence of four ethylene cis-elements in the promoter of the *VvSUT1* (Chervin et al, unpublished), which is a functionally validated sucrose transporter of the grape berry (Ageorges et al. 2000).

In this report, we focused on the role of 1-MCP on the sucrose accumulation as well as on the relative abundance of transcripts coding for two sucrose transporters: *VvSUC11* (another name given to *VvSUT1*) and *VvSUC12* that show increased expression around the veraison (Davies et al. 1999) and have been functionally validated as sucrose transporters (Manning et al., 2001).

In a previous report (Chervin *et al*. 2004), we showed that 1-MCP was very unlikely to have unspecific toxic effect since 1-MCP treatment before the inception of ripening did not produce any effect on several variables such as berry diameter, skin anthocyanin accumulation and decrease of juice acidity decrease.

Material and Methods

The grapevines, cv. Cabernet Sauvignon (*Vitis vinifera*, L.) were grown in a local vineyard in Toulouse (Domaine de Candie) and 1-MCP treatments were performed as described previously (Chervin et al. 2004). Briefly, the clusters were wrapped in polyethylene bags for 24 hours in which 1-MCP (4 $\mu l.l^{-1}$) was injected or not. This treatment was performed around veraison, approximately 9 weeks after full bloom, and when 50% berries per cluster had switched from green to purple, started to soften and accumulate sugars (Terrier *et al.*, 2005). The bags were removed after 24 hours and the clusters were either sampled for RNA extraction or left on the vines until sampling for sugar concentrations (5 and 19 days after treatment). The experiments were run for two consecutive years. For each extraction process, 20 berries were randomly selected on each cluster before processing.

The sugar contents were assayed enzymatically with an EnzyPlus kit (Diffchamb, Västra Frölunda, Sweden), using clear juice samples obtained from freshly harvested berries. Briefly,

whole berries (skin, pulp and seeds) were blended using a blender (Model 854501; SEB, Ecully, France), then 10 mL sub-samples were centrifuged twice at 3,000 *g* for 3 min, pumping the juice under the flocculating slurry before the second centrifugation. The determination of NADH content was performed with a spectrophotometer at 340 nm.

RNA extraction was performed according to Boss *et al.* (1996) on berry tissues frozen immediately after the 24 h period of 1-MCP treatment using liquid nitrogen. RNAs were treated with DNase and checked for DNA contamination, using primers for *UFGT* genomic DNA (forward 5'-CTGCAGGGCCTAACTCACTC-3' and reverse 5'-TAGGTAGCACTTGGCCCATC-3'). The accumulation of RNAs of specific genes was then performed by semi-quantitative RT-PCR. The primers for *SUC11,* were forward 5'-TGCCTTGATATCCACACGAA-3' and reverse 5'- GGACCCTGGATTTATCAGCA-3', for *SUC12,* forward 5'-CCTCTCAGCTGCTACAAGAACA-3' and reverse 5'-AAAGCACAAGGCATCAAAGC-3' and for β-tubulin used as standard for result normalization forward, 5'-TGCCACCTTTCAGATGAGTG.-3' and reverse 5'-TTTTCAATACAAGCCCATTATGA-3'. The reactions were performed with 6 replicates from 3 different clusters, each picked on a different vine x 2 different years. The PCR reactions were stopped after 25 cycles, as the signal intensities were approximately at 50% of the saturation. The signal intensities were obtained by scanning images of gels stained with ethydium bromide, and the scans were analyzed using SigmaScan Image (SPSS Inc., Chicago, IL).

In order to determine the LSDs at the 0.05 level, analyses of variance were performed with SigmaStat (SPSS Inc., Chicago, IL). For each parameter, we checked there was no statistical difference between the data of year one and year two (checked with t-tests; $P > 0.05$).

Results and Discussion

The 1-MCP, an inhibitor of ethylene receptors, reduced the sucrose content in grape berries in comparison to control grapes at both sampling dates, 5 and 19 days after the treatment (Fig. 1). At the same time, glucose and fructose accumulated at much higher levels than sucrose (around 60 $g.L^{-1}$ and 80 $g.L^{-1}$, 5 and 19 days after veraison, respectively), similar levels were reported in another study (Diakou et al., 1997), and both hexose levels were not affected by 1-MCP. It can be thus concluded that either 1-MCP specifically affected sucrose accumulation and transport, or it stimulated the conversion of sucrose to glucose and fructose. However this accelerated conversion could not be visible as the hexose concentrations are much higher than sucrose concentrations. The difference in sucrose accumulation was not due to a difference in berry size as no significant difference was found between berry sizes at both dates, although 1-MCP was shown to have a longer term effect on berry size (Chervin *et al*. 2004).

The relative reduction of sucrose accumulation by 1-MCP was stronger by day 5 ($P = 0.002$) than by day 19 after treatment ($P = 0.079$). This could be due to *de novo* synthesis of ethylene receptors, counter-acting the blocking of ethylene receptors by 1-MCP that is nearly irreversible (Blankenship and Dole 2003). This could also be due to a less active role of ethylene on sucrose transport as the grapes reach full ripeness. In general the more ripe the berries, the less the applications of ethephon in commercial vineyards are effective in triggering reactions in grapes (T. Desordons, pers. comm. 2005). Further experiments with 1-MCP applications at different dates are needed to clarify this point, particularly at earlier dates than mid-veraison.

Preliminary observations using micro-array (data not shown) had suggested that 1-MCP treatments reduced the expression of a sucrose transporter (*SUC11*) that contains few *cis*-elements linked with ethylene signaling in its promoter region and that is up-regulated at veraison (Davies et al., 1999; Ageorges *et al*., 2000). Figure 2 shows that the RNA accumulation for both *SUC11* and *SUC12* was down-regulated by 1-MCP although the reduction was significant only in the case of *SUC12*. These results therefore partly suggest that ethylene mediated sugar accumulation could be triggered by one or both sucrose transporters. In line with this hypothesis, it will be interesting to check for the presence of ethylene response cis-element in the promoter region of *VvSUC12*, not yet sequenced (C. Davies pers. comm., 2005).

Beyond a possible role of ethylene on sugar transporters, it is also possible that other mechanisms are involved, such as those linked to vascular fluxes, that vary over berry development (Tyerman et al. 2004 ; Rogiers et al. 2001) and are likely to modulate sugar transport.

Conclusion

The present work is preliminary but it provides some arguments in favor of a possible role of ethylene during the ripening of grape berries through a modulation of sucrose accumulation and transport. Further work is needed to address the temporal pattern of ethylene responsiveness as well as the relationship between ethylene mediated sugar accumulation and anthocyanin accumulation.

Literature Cited

1 Abeles, F.B., P.W. Morgan, and M.E. Salveit. 1992. Ethylene in Plant Biology. Academic Press,
2 New York.
3 Ageorges, A., N. Issaly, S. Picaud, S. Delrot, and C. Romieu. 2000. Identification and functional
4 expression in yeast of a grape berry sucrose carrier. Plant Physiol. Biochem. 38:177-185.
5 Alleweldt, G., and R. Koch. 1977. Ethylene content in ripening grape berries. Vitis 16:263-271.
6 Blankenship, S.M., and J.M. Dole. 2003. 1-Methylcyclopropene: a review. Postharvest Biol.
7 Technol. 28:1-25.
8 Boss, P.K., C. Davies, and S.P. Robinson. 1996. Analysis of the expression of anthocyanin
9 pathway genes in developing *Vitis vinifera* L. cv. Shiraz grape berries and the implication for
10 pathway regulation. Plant Physiol. 111:1059-1066.
11 Chervin, C., A. El-Kereamy, J.P. Roustan, A. Latche, J. Lamon, and M. Bouzayen. 2004.
12 Ethylene seems required for the berry development and ripening in grape, a non-climacteric
13 fruit. Plant Sci. 167:1301-1305.
14 Coombe, B.G., and C.R. Hale. 1973. The hormone content of ripening grape berries and the
15 effect of growth substance treatments. Plant Physiol. 51:629-634.
16 Davies, C., T. Wolf, and S. Robinson. 1999. Three putative sucrose transporters are differentially
17 expressed in grapevine tissues. Plant Sci. 147:93-100.
18 Diakou, P., A. Moing, L. Svanella, N. Ollat, D.B. Rolin, M. Gaudillère and J.P. Gaudillère. 1997.
19 Biochemical comparison of two grape varieties differing in juice acidity. Aust. J. Grape Wine
20 Res., 3:117-126.
21 Ishizawa, K., and Y. Esashi. 1988. Action mechanism of ethylene in the control of sugar
22 translocation in relation to rice coleoptile growth. I. Sucrose metabolism. Plant Cell Physiol.
23 29:131-141.

Kühn, C. 2003. Identification and functional expression in yeast of a grape berry sucrose carrier. Plant Biol. 5:215-232.

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

Manning, K., C. Davies, H.C. Bowen, and P.J. White 2001. Functional characterization of two ripening-related sucrose transporters from grape berries. Ann. Bot. 87:125-129.

Rogiers, S.Y., J.A. Smith, R. White, M. Keller, B. Holzapfel, and J.M. Virgona 2001. Vascular function in berries of Vitis vinifera (L) cv. Shiraz. Aust. J. Grape Wine Res. 7:47-51.

Saftner, R.A. 1986. Effect of ethylene on sucrose uptake in root discs of sugar beet. Plant Cell Physiol. 27:853-860.

Shulman, Y., S. Cohen, and C. Loinger. 1985. Improved maturation and wine quality of Carignane grapes by ethephon treatment. Amer. J. Enol. Vitic. 36:264-267.

Solfanelli, C., A. Poggi, E. Loreti, A. Alpi, and P. Perata. 2006. Sucrose-specific induction of the anthocyanin biosynthetic pathway in Arabidopsis. Plant Physiol. 140:637-646.

Terrier, N., D.Glissant, J. Grimplet, F. Barrieu, P. Abbal, C. Couture, A. Ageorges, R. Atanassova, C. Léon, J.P. Renaudin, F. Dédaldéchamp, C. Romieu, S. Delrot and S. Hamdi. 2005. Isogene specific oligo arrays reveal multifaceted changes in gene expression during grape berry (*Vitis vinifera* L.) development. Planta.222:832-847.

Trainotti, L., A. Pavanello and G. Casadoro. 2005. Different ethylene receptors show an increased expression during the ripening of strawberries: does such an increment imply a role for ethylene in the ripening of these non-climacteric fruits? J. Exp. Bot. 56:2037-2046.

Tyerman, S.D., J. Tilbrook, C. Pardo, L. Kotula, W. Sullivan, and E. Steudle. 2004. Direct measurement of hydraulic properties in developing berries of *Vitis vinifera* L. cv Shiraz and Chardonnay. Aust. J. Grape Wine Res. 10:170-187.

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

Figure legends

Figure 1: Sucrose accumulation in the juice of Cabernet Sauvignon berries sampled 5 and 19 days following 1-MCP treatment, this latter being performed for 24h on whole bunches with 50% colored berries wrapped in plastic bags ± 1-MCP; n = 6, error bars show SE, the *P* value is the probability that both means are equal (t-test).

.

Figure 2: Transcript accumulation of two sucrose transporters, *SUC11* and *SUC12* (named as in Davies et al. 1999), in Cabernet Sauvignon berries after wrapping the clusters in plastic bags for 24h ± 1-MCP; at the time of the treatment the clusters had 50% colored berries; n = 6, error bars show SE, data collected over two seasons, normalized on control means for each gene data set. The percentages under the arrows represent the drop of signal means, the *P* value is the probability that both means are equal (t-test).

1

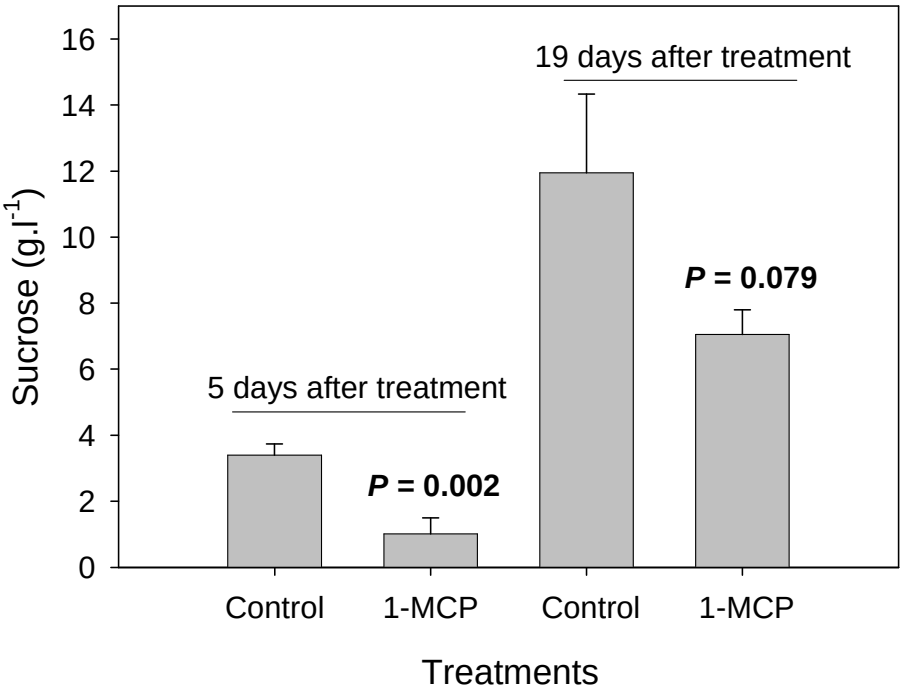
16
14
12
10
8
6
4
2
0
Sucrose (g.l-1)
19 days after treatment
P = 0.079
5 days after treatment
P = 0.002
Control
1-MCP
Control
1-MCP
Treatments

Figure 1

1

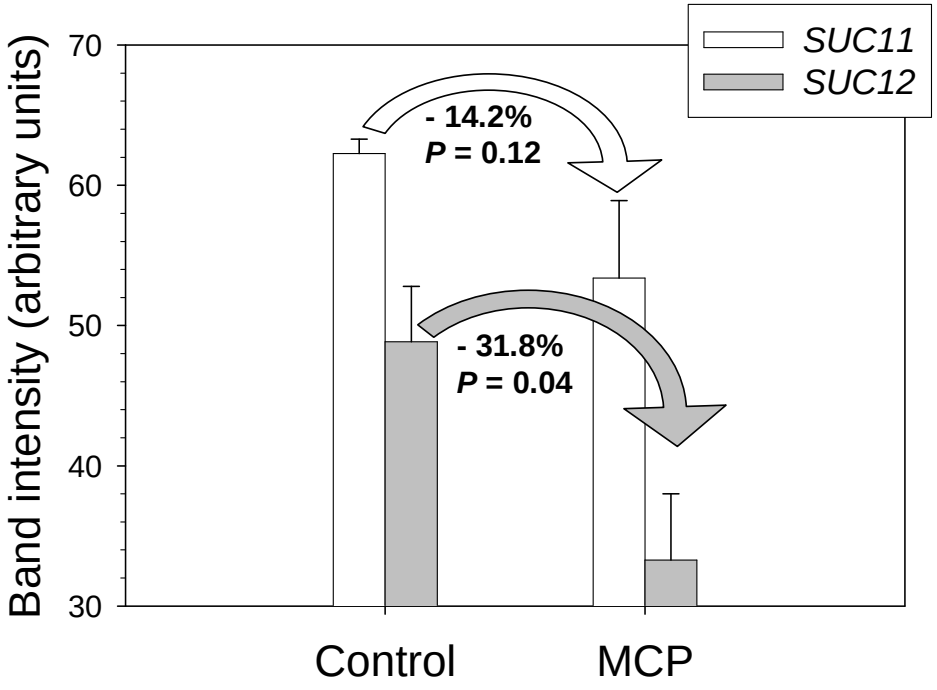
SUC11
SUC12
- 14.2%
P = 0.12
- 31.8%
P = 0.04
Band intensity (arbitrary units)
70
60
50
40
30
Control
MCP

Figure 2