INFLUENCE OF ABSCISIC ACID IN TRIGGERING « VÉRAISON » IN GRAPE BERRY SKINS OF *VITIS VINIFERA* L. cv. CABERNET-SAUVIGNON

IMPLICATION DE L'ACIDE ABSCISSIQUE DANS LE DÉCLENCHEMENT DE LA VÉRAISON AU NIVEAU DES PELLICULES DE BAIES DE *VITIS VINIFERA* L. cv. CABERNET-SAUVIGNON

Séverine GAGNÉ, Karine ESTEVE, Christelle DEYTIEUX, SAUCIER C. and Laurence GENY

UMR 1219 Œnologie-Ampélologie, Faculté d'œnologie, Université Victor Segalen Bordeaux 2
INRA-ENITA, 351, cours de la Libération, 33405 Talence, France

Abstract : Grape berry ripening is characterised by numerous metabolic and physiological changes, placed under hormonal control. The phytohormone abscisic acid (ABA) is considered as a possible promoter of « véraison », since it accumulates at this time. In order to evaluate its impact, we assess the effect of ABA on several parameters of maturity: anthocyanins synthesis, phenylalanine ammonia-lyase activity, sugar contents, acidity and maturity index, both in untreated berry skin and in skin of berries submitted to applications of exogenous ABA. The maturation processes we focused on appear ABA-dependent, as they occured immediately after ABA accumulation in the skin. Treatment also shows a close relationship between ABA and the beginning of ripening. These results allow us to conclude that ABA content could modulate the triggering of « véraison »

Résumé : Au moment de la véraison, la baie de raisin connaît de nombreux changements physiologiques, qui sont, au moins en partie, contrôlés par les régulateurs de croissance. Plusieurs d'entre eux (éthylène, auxines) sont connus pour influencer le métabolisme de la baie et modifier l'expression des gènes liés à la maturation. Comme pour les autres fruits non climactériques, l'action de l'éthylène semble limitée par rapport à d'autres régulateurs. Parmi eux, l'acide abscissique (ABA) semble déterminant dans l'initiation de la véraison. En effet, il s'accumule jusqu'à atteindre un maximum en milieu de véraison. De plus, des expériences à base d'applications exogènes d'ABA ont montré qu'il induisait une modification du profil d'expression génique. Cependant, son rôle et son importance ne sont pas clairement définis. Dans cette étude, nous avons cherché à évaluer l'impact de l'ABA dans le déclenchement de la « véraison ». Pour cela, des baies de Cabernet-Sauvignon cultivées en plein champs ont été traitées avec une solution d'ABA au stade petit pois. Plusieurs critères impliqués dans la définition de la maturité, dont les teneurs en ABA et en anthocyanes, ainsi que l'activité phénylalanine ammonia-lyase (PAL) et les indices de maturité traditionnels, ont alors été étudiés dans la pellicule des baies traitées à l'ABA puis comparées aux données obtenues pour des baies témoins, traités avec de l'eau. Le traitement a permis de mettre en évidence une accumulation plus importante et plus rapide d'ABA dans la pellicule des baies traitées. Un déclenchement précoce de la maturation par comparaison avec les baies témoins a pu être corrélé à cette observation. Il se manifeste par une augmentation avancée dans le temps des teneurs en anthocyanes, augmentation simultanée au pic d'ABA et directement corrélée à une activation de la PAL, enzyme-clef du métabolisme des composés phénoliques. De plus, une augmentation précoce des teneurs en sucres et une diminution de l'acidité a été détectée. À maturité, tous les paramètres testés sont apparus équivalents entre baies témoins et baies traitées. Ceci suggère un rôle déclencheur de l'ABA dans le processus de maturation plutôt qu'un rôle activateur, confirmant l'importance du statut hormonal de la baie de raisin au moment de la véraison. Les mécanismes multiples d'action mis en jeu (action au niveau transcriptionnel, activation de voies de signalisation intracellulaires, activation d'enzymes) restent à préciser.

Key words: ABA, berry, skin, ripening

Mots clés : acide abscissique, baie, pellicule, véraison

corresponding author : laurence.geny@œnologie.u-bordeaux2.fr

J. Int. Sci. Vigne Vin, 2006, **40**, 7-14

INTRODUCTION

The growth of the grape berry consists in two successive sigmoid curves separated by a plateau. The first stage of development, characterized by a rapid increase in berry size, is followed by a lag phase, during which little or no growth occurs. The second growth period, called ripening, begins at « véraison », with the onset of berry softening, sugar accumulation and decrease in acid contents, while secondary products, as anthocyanins or flavour compounds, are synthesized (OLLAT *et al*., 2002).

Among them, anthocyanins are flavonoid pigments responsible for coloration and involved in many interactions with other molecules, conferring them essential biological and gustative properties for both grape berry and wine. Synthesized in the skins of red and « rosé » cultivars, and also in the pulp of « Teinturier » cultivars, they derive from phenylalanine: this aromatic amino acid is the substrate of the enzyme phenylalanine ammonia-lyase (PAL), that is considered as a key regulatory enzyme of the polyphenols biosynthesis. It is well-known that anthocyanins begin to accumulate immediately at « véraison » and enhance their concentrations throughout ripening until harvest. Environmental conditions and viticultural practices influence extensively this phenomenon. Like the other traditional indices of ripening (sugar content, acidity…), the amount of anthocyanins in berries should also be taken into account to define maturity stage.

At « véraison », the transition from a relative quiescent status of the berry to the array of different activities indicates the triggering of a new set of controls for berry metabolism, involving hormonal factors. As grape berry is a non-climacteric fruit, ethylene is thought to play a limited role in developing and maturation processes, in contrast with other regulators, like abscisic acid (ABA). This phytohormone is known to be involved in many physiological responses such as stomatal closure, adaptation to stress, flowering, seed dormancy and photosynthesis (ZEEVART and CREELMAN, 1988). Moreover, several studies suggest that ABA is a promoter of ripening. High levels of ABA has been observed in the middle of « véraison » (DURING and ALLEWELDT, 1984 ; BROQUEDIS, 1987, BAIGORRI *et al*., 2001 ; ANTOLIN *et al*., 2003). Coloration of the berry, gene expression patterns and enzyme activities can be modified by exogenous applications of ABA (HIRATSUKA *et al*., 2001 ; JIANG *et al*., 2003 ; JEONG *et al*., 2004). It has been shown that other phytohormones, like ethylene (EL-KEREAMY *et al*., 2003) and AIA (DAVIES *et al*., 1997), alter the expression of genes related with ripening and influence maturation in terms of anthocyanin contents, maturity index and hormonal status. However, the enhancing impact of ABA on the different parameters of ripening has not been yet clearly demonstarted.

In this study, we report the relation between ABA contents and evolution, in the skin of *Vitis vinifera* cv. Cabernet-Sauvignon, and ripening indices evolution as anthocyanins, PAL, sugar and acids.

MATERIALS AND METHODS

I - PLANT MATERIALS

The experiment was conducted on berries (*Vitis vinifera* L. cv. Cabernet-Sauvignon) in a Pessac-Léognan vineyard near Bordeaux (France). The vineyard was planted in 1990, oriented N/S, grafted onto 101-14 rootstock. Planting density was 6 500 plants per ha. Guyot double was the training system.

Grape clusters were collected at different stages of ripening in 2003 and 2004. During the year 2003, 6 samplings were made :

- at a green stage (GS) 32 days after anthesis (DAA) corresponding to the stage 31 (berries pea-sized) of the phenological scale of EICHORN and LORENZ (1977).

- at different points during the colour change period : 10 %, 50 %, 80 % and 100 % red ripe (RR) corresponding respectively to 52, 54, 58, 65 DAA and to the phenological stages 35, 36 and 37.

- and at maturity (M), 112 DAA, corresponding to the harvest or stage 38.

During the year 2004, 7 samplings were made :

- at two green stages respectively 30 and 48 DAA corresponding to the stages 31 and 33 (berries pea-sized and berry touch) defined by Eichorn and Lorenz.

- at different points during the colour change period: 50 % (phenological stage 36), 80 % and 100 % (phenological stage 37) RR, corresponding respectively to 63, 65 and 71 DAA.

- during fruit maturation : 10 days after the end of the colour change (I) 82 DAA.

- and at maturity (M), 110 DAA, corresponding to the harvest or stage 38.

Random samples of 5 grape clusters on ten plants were selected for each stage, immediately frozen in liquid nitrogen and berries were stored at -80 °C until analyses. Except for maturity indexes requiring entire fruit, all measurements were conducted on the berry skin after peeling freezed berries in liquid nitrogen.

II - TREATMENTS

Ten plants were treated at fruit set corresponding to stage 27 of the scale defined by Eichorn and Lorenz (22 DAA in 2003 and 20 DAA in 2004) by spraying an aqueous solution of synthetic abscisic acid (±-*cis, trans*-ABA, Sigma) at a concentration of 2.10^{-4} mol/l (HIRATSUKA *et al*., 2001). Each grape was sprayed with 10 ml of this solution and with water for the control, both containing 0.05 % Tween 20 as wetting agent.

III - ANTHOCYANINS ANALYSIS

Extraction procedure is a one-step method adapted from REVILLA *et al*. (1998). The skins of 10 berries were weighed then ground in liquid nitrogen and processed by carrying out 2 successive extractions in 40 ml of methanol containing 0,1 % of 12 N HCl for 3 hours, at room temperature and under agitation (150 rpm). After each incubation, 1 ml of medium was collected and anthocyanins content was measured by colorimetric analysis according to RIBÉREAU-GAYON and STONESTREET (1965). The data represent the means ± SD of 3 replicates.

IV - PAL EXTRACTION AND ACTIVITY ASSAY

PAL was extracted by the method of MESSNER *et al.* (1991) with slight modification and enzyme activity was measured as described by LI *et al*. (2004). 10 berry skins were ground in liquid nitrogen and mixed with 150 mg of PVP in 3 ml of 0,1M Tris-HCl buffer pH 8.0 containing 4 mM β-mercaptoethanol. The homogenate was centrifuged at 12 000 g for 10 min and the supernatant, diluted with 1 ml of buffer, was percolated through a column Sephadex G-25 (Amersham Biosciences). The recovered suspension was used for determination of enzyme activity: the reaction mixture contained 800 µl of cytosolic extract and 400 µl of L-phenylalanine 15 mM in extraction buffer and was incubated at 35 °C for 3 hours. Increase in *trans*-cinnamic concentration was monitored by spectrophotometry at 290 nm. The data represent the means ± SD of 3 replicates.

V - ABA ANALYSIS

Free ABA was determined following the method described by ANTOLIN *et al*. (2003). Free ABA in the skins of 10 berries was first extracted with methanol 80 % (v/v), followed by purification with polyvinyl poly-pyrrolidone and a final extraction with diethyl-ether. Hormonal determination was carried out by HPLC in combination with UV spectrophotometry for ABA (λ: 254 nm) and with fluorescence spectrometry for IAA (λ: emission at 280 nm / excitation at 360 nm). The assays were validated independently with purified hormone extracts by mass spectrometry. The data represent the means ± SD of 3 replicates.

VI - DETERMINATION OF INDEX MATURITY

Two samples of 25 berries per stage were randomly collected. After weighing, they were wrapped in a double-layer of cheesecloth and handly crushed. Aliquots were used for the immediate analysis of pH, titrable acidity (TA) by titration with NaOH (GUYMON and OUGH, 1962) and soluble solids by refractometry (AMERINE and OUGH, 1980). The index maturity was based on the ratio of sugars to TA ($g.l^{-1}$). The data represent the means ± SD of 2 replicates.

RESULTS

The results presented here are expressed per gram of fresh weight (g^{-1} FW). The expression per berry was also done (data not shown). Since the increase in skin weight is limited, differences in quantity or activity can not be due to dilution or concentration effects.

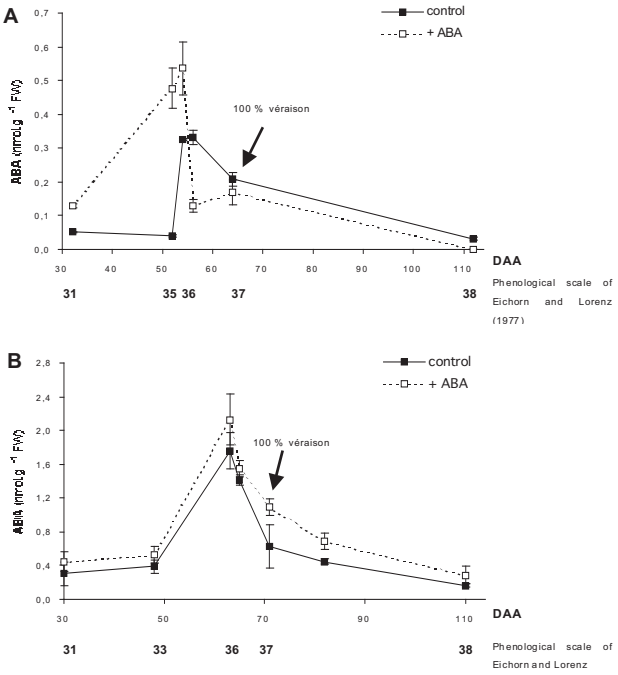

Figure 1 – Évolution of ABA levels (nmol.g^{-1} FW) in the skins of control berries (■) and berries treated with ABA (❑) harvested in 2003 (A) and in 2004 (B). The phenological stages according to EICHORN and LORENZ (1977) are indicated under the DAA axis. The data represent the means ± SD of 3 replicates.

Évolution des teneurs en ABA (nmol.g^{-1} MF) dans la pellicule des baies témoins (■) et traitées à l'ABA (❑) prélevées en 2003 (A) et en 2004 (B). Les stades phénologiques définis par Eichorn et Lorenz (1977) sont indiqués en dessous de l'axe des abscisses. Les résultats correspondent à 3 répétitions ± écart-type.

J. Int. Sci. Vigne Vin, 2006, **40**, 7-14

I - ABA EVOLUTION IN BERRY SKIN

Evolution of free ABA levels in berry skin during fruit development is shown at figures 1A for the year 2003 and 1B for 2004. For untreated berries, profiles appear similar within years. At berry set (stages 31 and 33), ABA concentration is quite low and dramatically increases during the colour change period, with a maximal concentration at 50 % red ripe (54 DAA in 2003 and 63 DAA in 2004). It subsequently decreases up to harvest.

Treatment modified ABA profile with accumulation of ABA during « véraison » but the effect is quite greater in 2003 than in 2004. In 2004, ABA evolution in treated berries parallels the evolution observed for the control with greater amounts of hormone. The previous vintage shows an accelerated accumulation of ABA in berry skin : at the stage of 10 % « véraison » (52 DAA, stage 35) : ABA levels are ten-fold those of control berries, which accumulate ABA only from 50 % « véraison » (54 DAA, stage 36). As a probable consequence, ABA dropped suddenly after the 50 % « véraison » peak, in contrast with the soft decrease occurring in control berries.

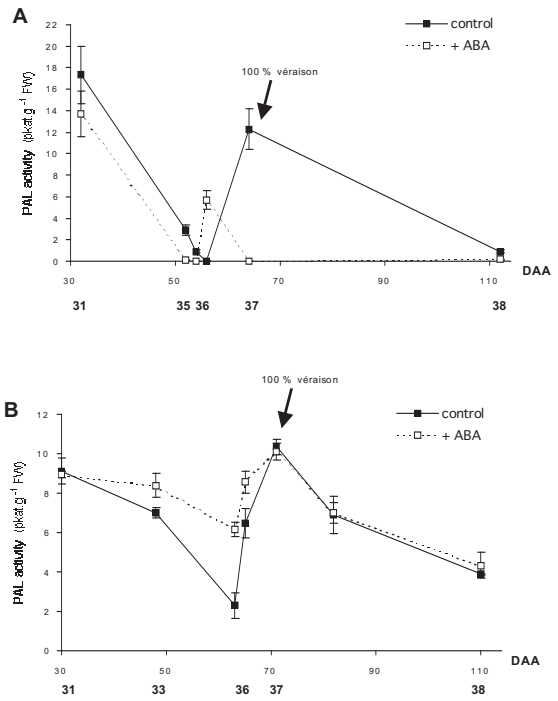

Figure 2 – Evolution of PAL activity (pkat.g^{-1} FW) in the skins of control berries (■) and berries treated with ABA (❑) harvested in 2003 (A) and in 2004 (B). The phenological stages according to EICHORN and LORENZ (1977) are indicated under the DAA axis. The data represent the means ± SDof 3 replicates.

Évolution de l'activité PAL (pkat.g^{-1} MF) dans la pellicule des baies témoins (■) et traitées à l'ABA (❑) prélevées en 2003 (A) et en 2004 (B). Les stades phénologiques définis par Eichorn et Lorenz sont indiqués en dessous de l'axe des abscisses. Les résultats correspondent à 3 répétitions ± écart-type.

II - PAL ACTIVITY

PAL activity patterns, shown in figure 2, are similar in 2003 (2A) and 2004 (2B) and present two peaks. The first one is detected in green berry and is therefore unrelated to anthocyanin accumulation. A markedly activation occurs during ripening, leading to a second activity peak at 100 % « véraison » (65 DAA and 71 DAA respectively, phenological stage 37), which matched directly with the pigment accumulation. Then, activity declines gradually until harvest.

As regards treated berries, the second peak of PAL activity is observed seven days earlier in 2003. In 2004, it also occurs precociously while, at stage 36 (63 DAA), PAL activity is increased three-fold when compared with control.

III - ANALYSIS OF RIPENING PARAMETERS

a) Anthocyanin evolution

Evolution in anthocyanin contents appear to be similar comparing years and in both control and treated berries. During the first growth period, no anthocyanin was detected. From the inception of « véraison » to harvest, contents continuously increase, thereby increasing skin coloration. At maturity, a decrease or a stabilization is observed.

Considering the berries treated with ABA, an earlier accumulation of anthocyanins is performed, when ripening begins. In 2003, accumulation is noticed as soon as stage 35 and anthocyanin contents remain superior to those measured in control berries up to 80 % « véraison ». In 2004, synthesis seems to be faster in treated berries as levels are twice those measured in control berries at stage 36 (63 DAA, 50 % « véraison »). Besides, this discrepancy is still observed at the end of « véraison ». Then, accumulation remains stable whereas control berries continue to increase their anthocyanin levels. However, contents become nearly identical at latter stage of maturation. As mentioned above, the growth of berry skin has no influence on anthocyanin concentration : the evolution of anthocyanins contents are the same between 2003 and 2004 or when expressed per berry. This supports a positive effect of ABA on anthocyanins biosynthesis rather than a side effect of concentration linked with skin enlargement or environmental conditions.

b) Maturity indices

As observed for results above, untreated and treated berries are characterised by nearly identical parameters

Table I – Maturity parameters of control berries and berries treated with ABA harvested in 2003 and 2004. The data represent the means ± SD of 2 replicates for 2 distinct samples.

Indices de maturité des baies témoins et traitées à l'ABA prélevées en 2003 et en 2004. Les résultats correspondent à 2 répétitions ± écart-type d'analyse pour deux prélèvements.

Year	DAA	Treatment	Weight of 25 berries	Sugar concentration $g.1^{-1}$	Titrable acidity $g.1^{-1}$	Index maturity
2003	74	control	34,96 ± 0,1	161 ± 1,4	8,55 ± 0,9	18,8 ± 1,2
		ABA	33,20 ± 0,7	172 ± 1,7	7,55 ± 0,2	22,8 ± 1,0
	112	control	40,49 ± 0,7	225 ± 1,4	3,56 ± 2,7	63,2 ± 2,1
		ABA	36,99 ± 2,0	213 ± 7,9	3,56 ± 0,3	59,8 ± 4,1
2004	82	control	29,20 ± 1,5	182 ± 1,0	9,53 ± 1,0	20,0 ± 1,5
		ABA	30,38 ± 1,0	190 ± 3,8	8,96 ± 0,3	28,3 ± 2,1
	110	control	33,27 ± 0,4	208 ± 2,0	5,33 ± 0,1	36,6 ± 0,0
		ABA	32,80 ± 1,2	201 ± 1,9	5,33 ± 0,2	35,5 ± 0,0

at maturity (table I). On the contrary there is a significant shift, considering all the values presented here, at the end of the colour change period (respectively 74 DAA in 2003 and 82 DAA in 2004). Maturity indices are 21 % (2003) and 41,5 % (2004) higher in treated berries than in control ones. This can be linked with a greater sugar concentration and especially with a lower content of titrable acidity (88 % of the control value in 2003).

DISCUSSION

This study presents the influence of hormonal status on different maturity parameters in the skin of grape berry. The ABA treatment carried out at nouaison was performed to monitor the ripening process in order to show the impact of ABA on triggering « véraison ». As shown in table I, ABA treatment impacts neither on berry weight nor on skin weight. Increases in contents or activation measured in our study can not be related to berry growth but to an effect of ABA application or content.

Grape berry is a non-climacteric fruit, that implies the involvement of different growth regulators along growth. For instance, auxins (DAVIES *et al*., 1997) or salicylic acid (KRAEVA *et al*., 1998) delay ripening whereas ABA hastens ripening. According to several recent studies (HIRATSUKA *et al*., 2001; BAN *et al*., 2003, JEONG *et al*., 2004), ABA is thought to be predominant in the promotion of ripening since it positively affect both coloration and expression of genes involved in anthocyanin biosynthesis when exogenously applied.

Our results confirm the accumulation of ABA in the middle of « véraison », as previously described (BAIGORRI *et al*., 2001 ; ANTOLIN *et al*., 2003). This peak is clearly related to the coloration of berry skin, since anthocyanins continuously enhance their concentrations at this time.

ABA treatment modified ABA contents and evolution in the skin of grape berry. Not only are amounts greater in treated berries compared to control, but it also induces an earlier accumulation of ABA. As no ABA synthesis takes place in berry skin after « véraison » (ANTOLIN *et al*., 2003), importation from leafs seems to be enhanced. It is worth to notice that this shift is observed in anthocyanins synthesis and in PAL activation. Such a trend has also been shown by JIANG *et al*. (2003) in strawberry treated with ABA and by HIRASTUKA *et al*. (2001) in Kyoho grape berries. At the end of berry growth, levels of hormone, pigments and enzyme activity are identical between control and treated berries, indicating that ABA acts at the beginning, and not throughout the maturation process : metabolic changes arise earlier but remain the same in the two sets of berries. This enforces our hypothesis on the crucial role of ABA in triggering « véraison ».

Berry pigmentation implies activation of biosynthetic enzymes, especially PAL, which can therefore be considered as one of the criteria defining ripening. A peak of PAL activity is detected during « véraison » and is closely related to coloration as anthocyanins derive from the phenylpropanoid pathway, starting with PAL. It has also been reported that white cultivars present a profile of ABA evolution similar to the profile described here (DEYTIEUX *et al*., 2003). In this case too, ABA triggers accumulation of uncoloured molecules and yellow pigments, as flavonols, that are stored in berry skin. Other studies we made on white cultivars show an activation of PAL in the same way as in red ones (data not shown). This is also in favour of an action of ABA on the beginning of « véraison ». However, the first peak of PAL activity occurs in the green berry where no anthocyanin is synthesised. Enzyme activation at this time aims at producing other precursors : PAL is indeed the first step of different metabolic pathways, as tannins, coumarins, stilbenes

or lignins pathway for instance. Comparing years, results appears different as no activity is detected in 2003 at the onset of « véraison » and after ripening. In contrast, a basal activity is still detected in 2004, which is in accordance with results from other cultivars (HRAZDINA *et al*, 1984 ; HIRATSUKA *et al*., 2001). This doesn't allow us to precise the exact role of ABA. JEONG *et al*. (2004) demonstrate an increased expression of genes encoding biosynthetic enzyme, including PAL, in berries treated with exogenous ABA. It follows the accumulation of a common transcriptional regulator, suggesting a direct and unique activation of anthocyanin biosynthesis by ABA at genomic level. Further studies are on the way to elucidate that point. On the other hand, a direct effect on PAL enzyme can be considered (HIRATSUKA *et al*., 2001) as ABA plays a central role in activating many intracellular signaling pathways. Moreover, PAL activity maxima are not significantly different in 2003 and 2004, while ABA levels in 2004 are twice the levels of the previous year : activation would happen once an ABA threshold would be reached.

The evolution of anthocyanin contents determined here is consistent with data previously reported, as this aspect of ripening is well-documented. The synthesis that begins at « véraison » and lasts along maturation has been described in the skins of different cultivars like Shiraz (BOSS *et al*., 1996), Tempranillo (DELGADO *et al*., 2004) and Cabernet-Sauvignon (HRAZDINA *et al*., 1984 ; JEONG *et al*., 2004), as well as in other organs of the plant. The decrease in the later stages of growth has also been shown and is generally attributed to degradation of anthocyanins by glycosidases and peroxidases. Concentrations measured in 2004 samples are higher compared with the results of the previous year. This is probably due to significative climatological differences between the two vintages : the very hot 2003 summer firstly prevented pigments from accumulating and, next, enhanced early oxydative degradation of anthocyanins. It points to the influence of climate on the anthocyanins metabolism and their contents in berry skin. Tannins and anthocyanins exhibit an opposite and simultaneous evolution during ripening (data not shown). The decrease in tannins contents is accompanied with coloration and anthocyanin accumulation, suggesting a relationship between their biosynthesis : a part of the tannins pool present in the skin could be converted in anthocyanins (DARNÉ, 1991).

Our results demonstrate a positive effect of ABA on anthocyanins accumulation. This can be explained by an activation of biosynthetic enzymes like PAL (HIRATSUKA *et al*., 2001) or by an increase in enzymes expression (BAN *et al*., 2003 ; JEONG *et al*., 2004).

Moreover, this accumulation can be explained by the activation of the enzyme PAL, which is considered as one of the criteria defining « véraison » : enzyme activity is significantly detected immediately after ABA level reached its maximum.

On the contrary, a first peak of PAL activity was observed in the green berry, as HIRATSUKA *et al*. (2001) did in the skin of *Vitis labruscana* Bailey cv. Olympia grapes. At this stage of development, there is no synthesis of anthocyanins. But PAL is a key enzyme as it is the first of different metabolic pathway. Anthocyanins and tannins derive from the phenylpropanoid pathway, from which other compounds are synthesised. PAL activity in green berry is probably necessary to tannins accumulation or production of precursors, such as hydroxycinnamic acids.

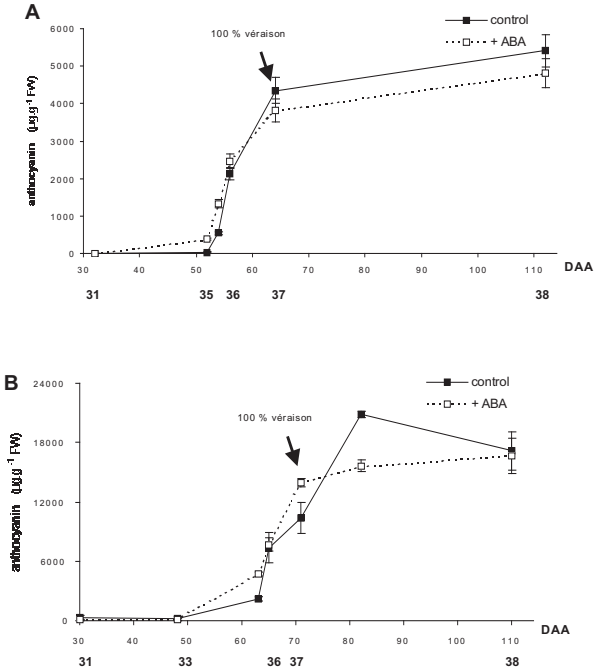

Figure 3 – Evolution of anthocyanin contents (µg.g^{-1} FW) in the skins of control berries (■) and berries treated with ABA (❑) harvested in 2003 (A) and in 2004 (B). The phenological stages according to EICHORN and LORENZ (1977) are indicated under the DAA axis. The data represent the means ± SD of 3 replicates.

Évolution des concentrations en anthocyanes (µg.g^{-1} MF) dans la pellicule des baies témoins (■)et traitées à l'ABA (❑) prélevées en 2003 (A) et en 2004 (B). Les stades phénologiques définis par Eichorn et Lorenz (1977) sont indiqués en dessous de l'axe des abscisses. Les résultats correspondent à 3 répétitions ± écart-type.

In addition, our study points out the influence of environmental conditions on the maturation process. Even if global trends are the same, amounts are quite different between 2003 and 2004, not to mention that profiles are more accentuated in 2003 than in 2004, especially for treated berries. The vintage 2003 was characterized by a long time of high temperatures and very rare rains. Such a warm and dry weather can explain the abundant levels of ABA accumulated in a precociously way (figure 1A) : since the onset of ripening, ABA was nearly at his maximum concentration in the skin. This is concordant with the well-known role of ABA in response to drought stress and can explain the 9 days delay observed at the stage 36 (50 % « véraison ») in 2004. The timing of ABA accumulation is completely correlated with anthocyanin synthesis, even if PAL activity appears to be delayed. In berries treated with ABA, the increase of ABA levels is followed by a dramatic drop even before the end colour change period, which is surprising. No explanation appears undisputable. We can suggest that ABA is, firstly, accumulated in order to respond to the unusual water and temperature stress that occured in 2003. Once a threshold is reached and when weather conditions became softer, ABA was then used and largely metabolised to promote the set of maturation processes. In 2004, climate was more likely to the warm but rainy climate of the Bordeaux region. Consequently, ABA profile is in accordance with previous results and no discrepancy is noticed between control and treated berries, except that levels of treated ones are higher.

CONCLUSIONS

Our results show that ABA accumulation in the berry skin is followed by an increase in coloration, owing to anthocyanin accumulation and PAL activation. Secondly, ABA treatment enhances ABA contents in the berry skin and accelerates the beginning of « véraison », without influencing maturity indices at harvest. Thus, ABA appears as a major promoter of the triggering of ripening.

We are now planning to examine the expression of the gene PAL in the same plant materials, so as to precise the way ABA stimulates anthocyanins accumulation.

Acknowledgements : We gratefully acknowledge the CIVB (Conseil interprofessionnel du vin de Bordeaux) for financial support.

REFERENCES

AMERINE M.A., OUGH C.S., 1980. Methods for analysis of musts and wines. *John Wiley and Sons Ed*., New York. pp 341.

ANTOLIN C., BAIGORRI H., DE LUIS I., AGUIRREZABAL F., GENY L., BROQUEDIS M., SANCHEZ-DIAZ M., 2003. ABA during reproductive development in non-irrigated grapevines (*Vitis vinifera* L. cv. Tempranillo). *Austr. J. Grape Wine Res*., **9**, 169-176.

BAIGORRI H., ANTOLIN C., DE LUIS I., GENY L., BROQUEDIS M., AQUIRREZABAL F., SANCHEZ-DIAZ M., 2001. Influence of training system on the reproductive development and hormonal levels of *Vitis vinifera* L. cv. Tempranillo. *Am. J. Enol. Vitic*., **52**, 4,357-363.

BAN T., ISHIMURA M., KOBAYASHI S., SHIOZAKI S., GOTO-YAMAMOTO N., HORIUCHI S., 2003. Abscisic acid and 2,4-dichlorohenoxyacetic acid affect the expression of anthocyanin biosynthetic pathway genes in « Kyoho » grape berries. *J. Hort. Sci. Biotechnol.*, **78**, 586-589.

BOSS P.K., DAVIES C., ROBINSON S.P., 1996. Analysis of the expression of anthocyanin pathway genes in developing *Vitis vinifera* L. cv Shiraz grape berries and the implications for pathway regulation. *Plant physiol*., **111**, 1059-1066.

BROQUEDIS M., 1987. Utilisation de la dialyse et de la chromatographie ultra-rapide pour le dosage de l'acide abscissique : application à la vigne. *Vitis*, **26**, 19-26.

DAVIES C., BOSS P.K., ROBINSON S.P., 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.

DARNÉ G., 1991. Recherches sur la composition en anthocyanes des grappes et des feuilles de vigne. *Thèse de Doctorat d'Etat*, Université de Bordeaux I.

DELGADO R., MARTIN P., DEL ALAMO M., GONZALEZ M.R., 2004. Changes in the phenolic composition of grape berries during ripening in relation to vineyard nitrogen and potassium fertilisation rates. *J. Sci. Food Agric.*, **84**, 623-630.

DEYTIEUX C, GENY L., L'HYVERNAY A., DONECHE B., 2003. Evolution de régulateurs de croissance au cours de la maturation de la baie de raisin : relation avec l'activité de dégradation de la paroi. *In 7e symposium international d'œnologie. Faculté d'œnologie,* Université Victor Segalen Bordeaux II, Bordeaux, 2003, 114-116.

DURING H., ALLEWELDT G., 1984. Zur moglichen Bedeutung Abscisinsaure bei der Zuchereinlagerung in die Weinbeere. *Ber. Deustch. Bot. Ges*., **97**, 101-113.

EICHORN K.W., LORENZ D.H., 1977. Phänologische Enwicklungsstadien der Rebe. *Nachrbl. Dtsch. Pflanzenschutzd.* (Braunschweig), **29**, 119-120.

EL KEREAMY A., CHERVIN C., ROUSTAN J.P., CHEYNIER V., SOUQUET J.M., MOUTOUNET M., RAYNAL J., FORD C., LATCHÉ A., PECH J.C., BOUZAYEN M., 2003. Exogenous ethylene stimulates the long-term expression of genes related to anthocyanin biosynthesis in grape berries. *Physiologia Plantarum*, **119**, 175-182.

GUYMON J.F., OUGH C.S., 1962. A uniform method for total acid determination in wines. *Amer. J. Enol. Vitic*., **13**, 40-45.

HIRATSUKA S., ONODERA H., KAWAI Y., KUBO T., ITOH H., WADA R., 2001. ABA and sugar effects on anthocya-

nin formation in grape berry cultured *in vitro*. *Scientia Horticulturae*, **90**, 121-130.

HIRATSUKA S., ONODERA H., KAWAI Y., KUBO T., ITOH H., WADA R., 2001. Enzyme activity changes during anthocyanin synthesis in « Olympia » grape berries. *Scientia Horticulturae*, **90**, 255-264.

HRAZDINA G., PARSONS G.F., MATTICK L.R., 1984. Physiological and biochemical events during development and maturation of grape berries. *Am. J. Enol. Vitic.*, **35,** 4,, 220-227.

JEONG S.T., GOTO-YAMAMOTO O 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 scienc*e, **167**, 247-252.

JIANG Y., JOYCE D.C., 2003. ABA effects on ethylene production, PAL activity, anthocyanin and phenolic contents of strawberry fruit. *Plant Growth Regul*., **39**, 171-174.

KRAEVA E., ANDARY C., CARBONNEAU A., DELOIRE A., 1998. Salicylic acid treatment of grape berries retards ripening. *Vitis,* **37**, 3, 143-144.

LI X.J., HOU J.H., ZHANG G.L., YANG Y.G., HU Y.X., LIN J.X., 2004. Comparison of anthocyanin accumulation and morpho-anatomical features in apple skin during color formation at two habitats. *Scientia Horticulturae*, **99**, 1, 41-53.

MESSNER B., BOLL M., BERNDT J., 1991. L-phénylalanine ammonia-lyase in suspension culture cells of spruce (Picea abies). *Plant cell. Tissue and organ culture*, **27**, 267-274.

OLLAT N, DIAKOU-VERDIN P, CARDE J.P., BARRIEU F., GAUDILLÈRE J.P., MOING A., 2002. Grape berry development : a review. *Int. J. Vine and Wine Sci*, **36**, 3, 109-131.

REVILLA E., RYAN J.M., MARTIN-ORTEGA G., 1998. Comparison of several procedures used for the extraction of anthocyanins from red grapes. *J. Agric. Food Chem*., **46**, 4592-4597.

RIBÉREAU-GAYON P., STONESTREET É., 1965. Le dosage des anthocyanes dans le vin rouge. *Bull. Soc. Chim.*, **9**, 2649-2652.

ZEEVAART J.A.D., CREELMAN R.A., 1988. Metabolism and physiology of abscisic acid. *Annual Revue of Plant Physiology and Plant Molecular Biology*, **39**, 439-473.

Manuscrit reçu le 28 juillet 2005 ; accepté pour publication, après modifications le 26 janvier 2006