Journal of Experimental Botany, Vol. 65, No. 16, pp. 4543–4559, 2014
doi:10.1093/jxb/ert395 Advance Access publication 27 November, 2013

Journal of Experimental Botany
www.jxb.oxfordjournals.org

REVIEW PAPER

Berry ripening: recently heard through the grapevine

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Received 22 August 2013; Revised 29 October 2013; Accepted 29 October 2013

Abstract

Grapevine (*Vitis vinifera* L.) is a non-climacteric fruit species used as table fruit, dried raisins, and for vinification (wines) and distillation (liquors). In recent years, our knowledge of the molecular basis of ripening regulation has improved. Water status, light conditions, and temperature may hasten, delay, or enhance ripening. Hormones seem to play a central role, as their concentrations change prior to and during ripening and in response to several environmental cues. The review summarizes recent data related to the molecular and hormonal control of grape berry development and ripening, with special emphasis on secondary metabolism and its response to the environment, and pinpoints some experimental limitations.

Key words: Aromas, flavonoids, grapevine, hormone, ripening, sugar.

Introduction

The grape berry is an organ with great economic importance. It is a non-climacteric fleshy fruit (Coombe and Hale, 1973), made up of the seeds and three tissue layers: the exocarp or skin, the mesocarp known as the pulp, and the endocarp, which is the tissue surrounding the seed (Pratt, 1971; Hardie *et al.*, 1996). Berry development is a complex process displaying a double sigmoid growth curve with three distinct phases, namely two periods of growth separated by a lag phase during which expansion slows and seeds mature (Conde *et al.*, 2007). During the first phase, organic acids accumulate in the vacuoles, and tannins, hydroxycinnamates, and several phenolic compound precursors are synthesized. At the end of the lag phase, the short period known as veraison is characterized by the initiation of sugar accumulation, and the rapid pigmentation of berries by anthocyanins in red grape varieties. High concentrations of glucose and fructose accumulate after veraison while organic acid levels decrease, and the berry softens (Zoccatelli *et al.*, 2013). The acid to sugar ratio at harvest is important for the taste of table grapes and for the sensory characteristics derived from wine grapes (Conde

Abbreviations: ABA, abscisic acid; ADH, alcohol dehydrogenase; 2-CEPA, 2-chloroethylphosphonic acid; CHI, chalcone isomerase, CHS, chalcone synthase; DAV, days after veraison; F3′H, flavonoid 3′-hydroxylase; F3′5′H, flavonoid 3′,5′-hydroxylase; F6P, fructose 6-phosphate; FLS, flavonol synthase; GIN1, vacuolar invertase 1; G1P, glucose 1-phosphate; G6P, glucose 6-phosphate; LDOX, leucoanthocyanidin dioxygenase; 1-MCP, 1-methylcyclopropene; MDH, malate dehydrogenase; NAA, naphthalene acetic acid; PEPC, phosphoenolpyruvate carboxylase; UFGT, UDPglucose:flavonoid 3-*O*-glucosyltransferase; XET, xyloglucan endo-transglcosylase.

et al., 2007). Towards the end of the third phase of berry development, many precursors of aroma and many aroma compounds (terpenes, norisoprenoids, esters, and thiols) are synthesized (Lund and Bohlmann, 2006). The changes occurring from veraison onwards are accompanied by an increase in the content of abscisic acid (ABA), a hormone that promotes berry ripening (Wheeler *et al.*, 2009). Other hormones and signals have also been shown to be involved in the control of berry development and ripening (ethylene, Chervin *et al.*, 2004, 2008; steroids, Symons *et al.*, 2006; ethylene and ABA, Sun *et al.*, 2010).

The grape berry is probably one of the fruits whose composition is the most sensitive to the natural (micro)environment. For example, grape berries may adapt to light radiation by increasing the content of secondary metabolites such as flavonols and anthocyanins (Koyama and Goto-Yamamoto, 2008; Matus *et al.*, 2009; Azuma *et al.*, 2012), which protect against oxidative damage and may alter the colour and sensory attributes of fruits and wines. Global warming is changing grapevine development and phenology, which results in earlier harvest dates (Webb *et al.*, 2007) and may affect the viticultural suitability in Mediterranean regions (Hannah *et al.*, 2013). Seasonal drought and high temperatures may significantly impact yield and the final composition of primary and secondary metabolites at harvest. Yet, many viticultural practices may be used to modulate the environmental effects (van Leeuwen *et al.*, 2013), and more and more studies deal with the interactions between environment and berry metabolism and composition (Conde *et al.*, 2007; Dai *et al.*, 2011; Geros *et al.*, 2012).

Several recent reviews and books have been devoted to berry development and ripening, and the factors affecting berry composition (Conde *et al.*, 2007; Dai *et al.*, 2011, 2013; Geros *et al.*, 2012). In this context, and because of limited space, the present paper will review only some aspects of berry ripening in which significant progress has been made through the publication of recent articles.

Grape berry composition and metabolism

Sugars

Sugar composition is mainly determined by genotype, and sugar concentration is strongly affected by environment and cultural management (reviewed in Dai *et al.*, 2011). For example, Shiraishi *et al.* (2010) conducted a detailed analysis of table grape berry composition (sugars, organic acids, and amino acids) for 129 cultivars from Europe, North America, and Japan, that included 57 *Vitis vinifera*, and 72 *V. labruscana*×*V. vinifera* hybrids. They identified two types of grapes based on sugar composition: hexose accumulators for which the glucose/(fructose+sucrose) ratio was >0.8, and sucrose accumulators for which this ratio was <0.8. The hexose- and sucrose-accumulating traits were related to genetic or regional differences (Shiraishi *et al.*, 2010). *Vitis vinifera* cultivars only accumulate trace amounts of sucrose, while *V. labrusca*, *V. rotundifolia*, and interspecific hybrids contain non-negligible amounts of sucrose. Most *V. vinifera* cultivars have a glucose/fructose ratio of 1 at maturity, while this ratio varies from 0.47 to 1.12 in wild species, with only a few species (*V. champinii* and *V. doaniana*) accumulating more glucose than fructose (Dai *et al.*, 2011). Xin *et al.* (2013) resequenced the genomes of 13 wild *Vitis* species and 14 cultivated *V. vinifera* accessions to investigate the genetic variation of 138 genes involved in sugar biosynthesis and transport. Among 132 genes analysed in detail, the cultivated *V. vinifera* had 1.65-fold fewer single nucleotide polymorphisms (SNPs)/InDels than wild *Vitis* species, probably as a result of domestication. Furthermore, eight genes involved in sugar metabolism and compartmentation, including three monosaccharide transporters (HT8/HT1, HT15, and PMT3), one hexokinase (HT4), one sucrose synthase, and six phosphofructokinases exhibited a much lower allelic diversity in the cultivated species than in the wild-type species, suggesting that they may be involved in the higher sugar content of the cultivated species. This approach looks promising for investigating other important traits.

Acidity

The acidity of grape berry at harvest depends on the ratio of concentrations between free organic acids and their potassium salt forms, which increases throughout ripening, and is an important quality trait (Dai *et al.*, 2011). *Vitis vinifera* grapes contain higher total acid and malic concentrations than hybrids between *V. labrusca* and *V. vinifera*, and within *V. vinifera*, wine grapes are more acid than table grapes (Liu *et al.*, 2006). In pre-veraison grape berries, sucrose can enter glycolysis and enable malate synthesis. The malic acid concentration peaks before veraison and then decreases throughout the second half of the growing season (Sweetman *et al.*, 2009). This decrease has been mainly associated with mitochondrial malate oxidation. High-throughput RNA sequencing suggests that the post-veraison decrease of malate results both from decreased expression of cytoplasmic malate dehydrogenase (MDH) and mitochondrial malic enzyme (biosynthesis), and altered expression of phosphoenolpyruvate carboxylase (PEPC) and PEPC kinase (PEPCK) which would shift the function of MDH enzymes towards malate catabolism. Malate degradation may also be promoted by the increased expression of dicarboxylate transporters (Sweetman *et al.*, 2012). Regalado *et al.* (2013) recently characterized three *V. vinifera* mitochondrial dicarboxylate/tricarboxylate carriers (VvDTC1–VvDTC3) putatively involved in the transport of mitochondrial malate, citrate, and other di/tricarboxylates. The three VvDTCs exhibited a developmental pattern of expression during berry ripening. VvDTC2 and VvDTC3 are strongly up-regulated in the mesocarp at the onset of ripening, which suggests that they may be involved in the transport of malate into mitochondria.

The response of malate content to increased atmospheric CO_2 and temperature is important in the frame of different strategies that will allow the adaptation of vineyards to climate change without relocating them (Sweetman *et al.*, 2009). In this context, Duchêne *et al*. (2013) proposed a simple mathematical model describing the kinetics of malic acid

evolution and evaluated the genetic variability of the organic acid contents and of the pH of the grapes in progeny from a Riesling×Gewurztraminer cross. Genotypes with a higher malic or tartaric acid content than the parental varieties could be found, but genotypes with a higher total acidity than Riesling were rare.

Anthocyanins

Anthocyanins are a class of flavonoids responsible for the colour of grape berries. Berry coloration is an important factor for market acceptance of table grape cultivars and of the red wines produced. Anthocyanin accumulation, which commences at veraison and continues throughout ripening, is frequently used as a fingerprint for cultivar recognition. Two clones of the same variety may present different anthocyanin contents (van Leeuwen *et al.*, 2012). There are only 3-monoglucosides of five anthocyanidins, delphinidin, cyanidin, petunidin, peonidin, and malvidin, in *V. vinifera*, whereas there are also much more abundant 3, 5-diglucosides of the five anthocyanidins in the other *Vitis* species and hybrids (Liang *et al.*, 2008). Malvidin derivatives are the main anthocyanins in most *V. vinifera* cultivars, with the exception that cv. Graciano primarily has cyanidin derivatives (Núñez *et al.*, 2004; Liang *et al.*, 2008). Recently, trace amounts of pelargonidin derivatives were detected in *Vitis* (Castillo-Muñoz *et al.*, 2009; He *et al.*, 2010; Guan *et al.*, 2012). Most anthocyanins do not combine with organic acids in *V. vinifera*. For example, Pinot Noir produces only non-acylated anthocyanins (Mazza *et al.*, 1999). For those anthocyanins that combine with organic acids, *p*-coumaroyl derivatives are dominant (Núñez *et al.*, 2004; Liang *et al.*, 2008).

For most cultivars, berry skin is the main/unique organ to accumulate anthocyanins. However, teinturier grape cultivars (also called dyers) synthesize anthocyanins not only in skin but also in pulp. Besides berry skin and pulp, some teinturier cultivars accumulate anthocyanins in other organs, such as pedicels, rachis, leaves, and stem epidermis (Jeong *et al.*, 2006; Guan *et al.*, 2012). Various teinturier grapes have been identified and their genetic relationship explored (Santiago *et al.*, 2008). Most recognized teinturier cultivars are *V. vinifera* L. cv. Gamay Fréaux, cv. Alicante Bouschet, and cv. Yan 73, and *Vitis* hybrid Bailey Alicant A. Anthocyanin content (Castillo-Muñoz *et al.*, 2009), accumulation (Guan *et al.*, 2012), and the expression of anthocyanin biosynthesis genes (Castellarin *et al.*, 2011) in berry skin and pulp have been investigated in some of these varieties. Teinturier cultivars generally have a much higher anthocyanin concentration per unit juice volume or fresh mass than non-teinturier cultivars (Ageorges *et al.*, 2006). Malvidin predominates in berry skin while peonidin predominates in the pulp, and both malvidin and peonidin were found in similar concentrations in pedicels, rachis, leaf lamina, vein, and petioles, and living bark of a teinturier grape cultivar 'Yan73' (Guan *et al.*, 2012). In Alicante Bouschet, colour development, sugar accumulation, and acid loss begin in the flesh at the stylar end of the fruit, indicating that ripening originates in this region (Castellarin *et al.*, 2011). Currently, cell suspension lines derived from Gamay Fréaux and Bailey Alicant A are used to gain a better understanding of the regulation of anthocyanin biosynthesis in response to abiotic and biotic factors (Ananga *et al.*, 2013).

In grape cells, anthocyanins are synthesized at the cytosolic surface of the endoplasmic reticulum (ER) by a multienzyme complex via the flavonoid pathway (Boss *et al.*, 1996). Most of the genes encoding enzymes of the anthocyanin biosynthesis pathway have been well identified in grapevine (Fig. 1). Genes encoding the early step enzymes of the biosynthetic pathway: chalcone synthase (CHS), chalcone isomerase (CHI), and flavanone-3-hydroxylase (F3H), belong to multicopy families. Different gene copies may have different temporal and spatial partitioned expression profiles that sometimes coincide with the biosynthesis of a particular flavonoid (Jeong *et al.*, 2008; Harris *et al.*, 2013). The expression of *F3′5′H* and *F3′H* affects the anthocyanin composition (Castellarin *et al.*, 2006; Jeong *et al.*, 2006). The prevalence of *F3′5′H* over *F3′H* would lead to more delphinidin, the precursor of petunidin and malvidin, and in contrast it would yield less cyanidin, the precursor of peonidin. The relative proportion of the two types of anthocyanins determines the colour variation among red/purple/blue in berry grape varieties and their corresponding wines and juices (Castellarin *et al.*, 2006). *F3′5′H* genes are present in highly redundant copy numbers and their spatio-temporal expression increases the complexity and diversification of the fruit colour among red grape varieties. In contrast, only two copies of the *F3′H* genes are present in the grape genome, with one copy being expressed and the other being transcriptionally silent (Falginella *et al.*, 2010, 2012). The expression of UFGT (UDPglucose:flavonoid 3-*O*-glucosyltransferase) is critical for anthocyanin biosynthesis (Boss *et al.*, 1996; Zheng *et al.*, 2013).

The regulatory complexes controlling the expression of the structural genes of the anthocyanin biosynthesis pathway include DNA-binding R2R3-MYB proteins, bHLH (basic helix–loop–helix, also known as MYC) proteins, and WDR (tryptophan–aspartic acid repeat) proteins (Fig. 1). Matus *et al.* (2008) described and classified 108 members of the grape *R2R3 MYB* gene subfamily. There are positive transcriptional regulators involved in the general flavonoid pathway (VvMYB5a and VvMYB5b; Deluc *et al.*, 2006, 2008) and synthesis of tannins (VvMYBPA1; Bogs *et al.*, 2007), flavonols (VvMYBF1; Czemmel *et al.*, 2009), and anthocyanins (VlMYBA1 and VlMYBA2; Kobayashi *et al.*, 2004). The presence of *Gret1*, a Ty3-gypsy-type retrotransposon, in the promoter region of *MYBA1* is associated with white-fruited cultivars when present in a homozygous state (Kobayashi *et al.*, 2004). Among >200 accessions of *V. vinifera*, the insertion of *Gret1* in the promoter region of *VvMYBA1* is tightly correlated with the white-skinned phenotype, and additional polymorphisms in the gene are also strongly associated with a red- or pink-fruited phenotype (This *et al.*, 2007). *VvWDR1* promotes anthocyanin accumulation when it is ectopically expressed in *Arabidopsis thaliana* (Matus *et al.*, 2010). *VvMYC1* is involved in the regulation of anthocyanin and tannin synthesis, and in its own feedback inhibition (Hichri *et al.*, 2010).

In parallel with their biosynthesis in the cytosol, anthocyanins are rapidly transported into the vacuole for storage,

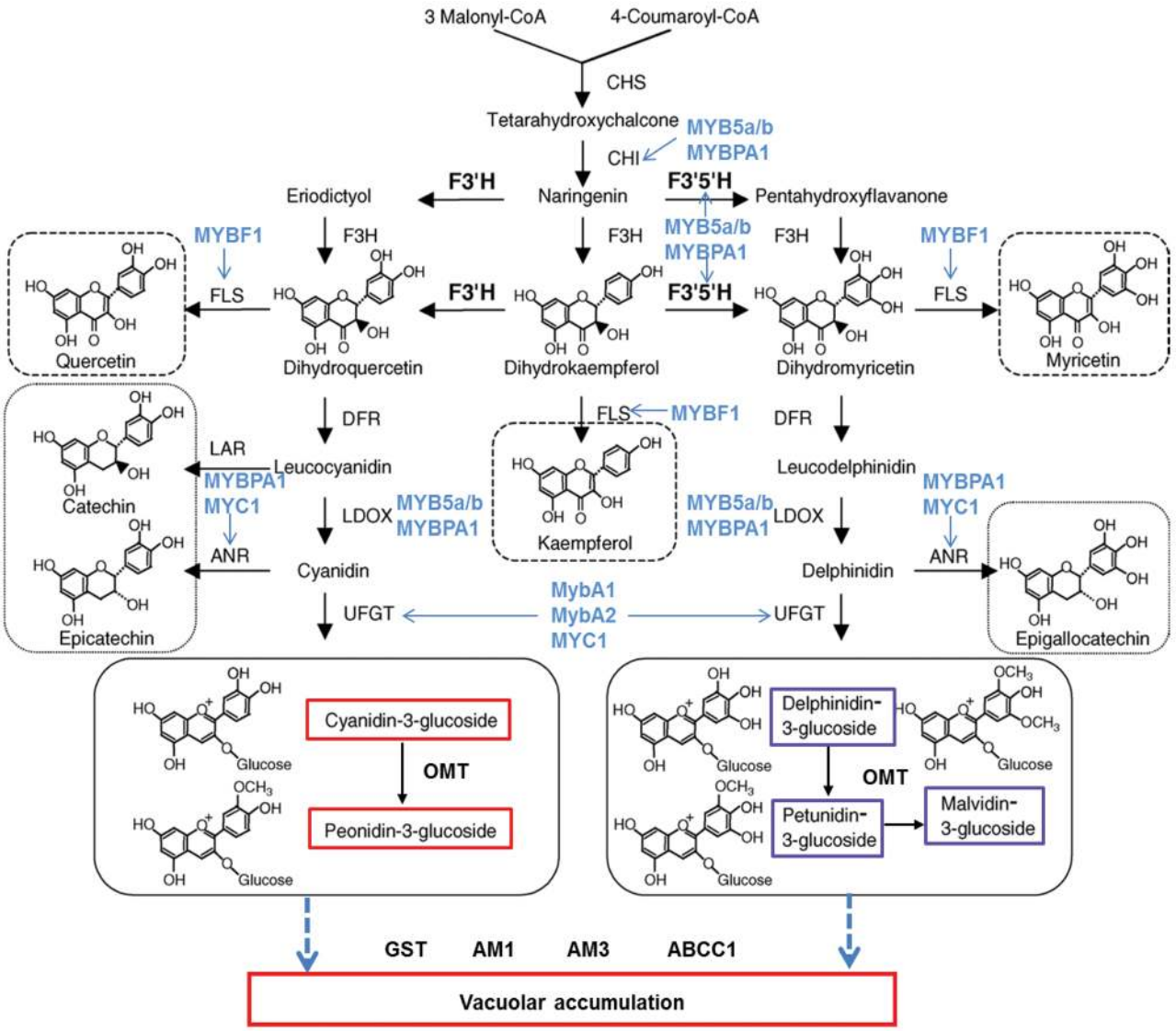

Fig. 1. Simplified pathways of flavonoid biosynthesis and its regulation in grape. CHS, chalcone synthase; CHI, chalcone isomerase; F3H, flavanone 3-hydroxylase; F3′H, flavonoid 3′-hydroxylase; F3′5′H, flavonoid 3′5′-hydroxylase; DFR, dihydroflavonol 4-reductase; LDOX, leucoanthocyanin dioxygenase; UFGT, UDPglucose:flavonoid 3-*O*-glucosyltransferase; OMT, *O*-methyltransferase; FLS, flavonol synthase; GST glutathione *S*-transferase; AM1/AM3, anthocyanin multidrug and toxic extrusion transporters; ABCC1, ATP-binding cassette transporter. Dotted arrows indicate anthocyanin transport by the transporter-mediated model. (This figure is available in colour at *JXB* online.)

which reduces feedback inhibition of cytosolic biosynthetic enzymes. Two major transport models have been proposed: membrane vesicle- and membrane transporter-mediated transport (Zhao *et al.*, 2010). Spherical pigmented inclusions on the cytosolic side of the tonoplast and in the vacuole have been identified as anthocyanin transport and storage complexes (Conn *et al.*, 2010). The cytosolic membrane-covered anthocyanin bodies are called anthocyanoplasts. Anthocyanic vacuolar inclusions (AVIs) are non-membrane-surrounded structures concentrating anthocyanins by intravacuolar coalescence (Zhao *et al.*, 2010). A strong correlation between AVI abundance and anthocyanin content was found in *V. vinifera* cell suspension cultures (Conn *et al.*, 2010). AVIs purified from this material are enriched in long-chain proanthocyanidins and show a high affinity for acylated anthocyanins (Conn *et al.*, 2008). Concerning the tonoplast transporter, two mechanisms have been identified in grapevine: primary transport mediated by ATP-binding cassette (ABC) transporters (Francisco *et al.*, 2013) and secondary transport depending on the H^+ gradient (Gomez *et al.*, 2009, 2011). ABC transporters, in particular from the ABCC subfamily, are involved in vacuolar flavonoid sequestration (Klein *et al.*, 2006). ABCC1 mediates the transport of glucosylated anthocyanidins, and the transport depends on GSH (tripeptide glutathione) without the formation of an anthocyanin–GSH conjugate in grapevine (Francisco *et al.*, 2013). Multidrug and toxic extrusion (MATE) transporters have been identified as candidate secondary transporters for flavonoid/H^+ exchange (Yazaki, 2005). Two grapevine MATEs, AM1 and AM3, mediate the specific transport of acylated anthocyanins in the presence of MgATP. Glutathione *S*-trasferases (GSTs) might act as escort proteins/ligandins of

anthocyanins that deliver these compounds to the transporter (Mueller *et al.*, 2000). The transcriptional profile of *VvGST* genes is positively correlated with key anthocyanin biosynthesis genes and anthocyanin accumulation in grape cell suspension (Conn *et al.*, 2008; Cutanda-Perez *et al.*, 2009). Complementation experiments in maize Bronze-2-deficient corn kernels provided evidence for the function of VvGSTs as anthocyanin transporters (Conn *et al.*, 2008).

Aroma and aroma precursors

Wine flavour is the result of a complex mixture of volatile compounds. More than 800 volatile molecules have been formally identified in wines, in concentrations ranging from hundreds of milligrams per litre down to a few picograms per litre (Ebeler and Thorngate, 2009; Styger *et al.*, 2011). Among them, a relatively limited number of compounds, called varietal (or primary) aromas, play a crucial role in wine flavour and typicality. These aromas, which are related to the grape variety, belong to a limited number of chemical families, including monoterpenes and sesquiterpenes, C13 norisoprenoids, volatile sulphur compounds, and methoxypyrazines (Ebeler and Thorngate, 2009). These compounds are often present as non-volatile, odourless, bound forms that can be released by chemical and enzymatic reactions occurring during the wine making and wine ageing processes, but can also exist as free molecules in the berries (Styger *et al.*, 2011). Knowledge about their biosynthetic pathway is rapidly expanding with the 'omics' approaches (see, for example, Agudelo-Romero *et al.*, 2013), and we will focus on three major classes of aromas present in berries: the methoxypyrazines (MPs), the volatile thiol precursors, and the monoterpenes.

MPs are strongly odorant volatile nitrogen-containing heterocycles, with vegetable-like fragrances ('green' notes), that are widely occurring in the plant kingdom (Maga, 1982). Three MPs can be found in grapevine berries: 2-methoxy-3-isobutylpyrazine (IBMP, associated with bell pepper scent), which is the most abundant, and two others, 2-methoxy-3-isopropylpyrazine (IPMP) and 2-methoxy-3-sec-butylpyrazine (SBMP) (Ebeler and Thorngate, 2009). Both IBMP and IPMP display very low sensory detection thresholds in the wine matrix, ranging from 1 ng l^{-1} to 16 ng l^{-1}, and their concentration in wine is highly correlated with the grape berry content at harvest (Roujou de Boubée *et al.*, 2002). MP concentrations increase in grape berries during the herbaceous growing phase, peak before veraison, and decline throughout the ripening phase (Dunlevy *et al.*, 2010; Guillaumie *et al.*, 2013). The extent of MP accumulation is strongly genotype dependent, and some cultivars such as Carmenère, Cabernet Sauvignon, Cabernet Franc or Sauvignon Blanc produce large amounts; whereas other such as Pinot Noir or Petit Verdot seldom produce detectable amounts (Belancic and Agosin, 2007; Koch *et al.*, 2010). MPs are considered as part of the typicality of white wines made from Sauvignon Blanc (Dubourdieu *et al.*, 2006; Lund *et al.*, 2009) when present in moderate amounts, but excessive concentrations reduce consumer acceptance (Parr *et al.*, 2007). In red wine, MPs are considered as off-flavour, and red wines can be depreciated by concentrations >10 ng l^{-1} (Allen *et al.*, 1991; Belancic and Agosin, 2007).

In spite of their importance either as typical varietal aromas (i.e. in Sauvignon Blanc wines) or as detrimental off-flavour in red wines, until recently little was known about the MP biosynthetic pathway or the corresponding biosynthetic genes. A hypothetical pathway was proposed 25 years ago by Gallois *et al.* (1988), starting from the branched amino acids (leucine for IBMP, valine for IPMP) to form a 2-hydroxy-3-alkylpyrazine, which is later methylated into the corresponding MP. In 2001, an *S*-adenosyl-L-methionine (SAM)-dependent *O*-methyltransferase (OMT) was partially purified from young grapevine shoots, and shown to be able to convert 2-hydroxy-3-isobutylpyrazine (IBHP) and 2-hydroxy-3-isopropylpyrazine (IPHP) into IBMP and IPM, respectively, *in vitro* (Hashizume *et al.*, 2001*a*, *b*). However, the grapevine genes coding for SAM-dependent OMTs exhibiting IBHP and IPHP methylation activities were only recently identified. Two cDNAs, corresponding to the protein partially sequenced by Hashizume *et al.* (2001*a*, *b*), were cloned by Dunlevy *et al.* (2010) and named *VvOMT1* and *VvOMT2*. The transcripts of both genes were detected in berries, shoots, and roots. When expressed as recombinant proteins in *Escherichia coli*, VvOMT1 and VvOMT2 are able to convert IBHP into IPHP into their corresponding MPs *in vitro*, albeit with high apparent K_m values, ranging from 539 µM to 1264 µM. Considering the very low abundance of IBHP and IPHP in grape berry tissues (<1 pg g^{-1} fresh weight according to Harris *et al.*, 2012), such K_m values called into question the actual role of VvOMT1 and VvOMT2 in IBMP and IPMP production *in vivo*. Indeed, very recently, a third gene, named *VvOMT3*, was independently identified by two research groups, through a combination of quantitative trait locus (QTL) mapping and transcriptomic analysis, and shown to encode a SAM-dependent OMT with a much lower apparent K_m, in the micromolar range (Dunlevy *et al.*, 2013; Guillaumie *et al.*, 2013). Recombinant proteins produced by expressing *VvOMT3* alleles from high and low methoxypyrazine-producing cultivars in *E. coli* did not differ in their catalytic properties. However, large differences in transcript levels were observed in these contrasting genotypes. Altogether, these results support the role of VvOMT3 as a key molecular determinant for MP accumulation in grapevine berries. Future studies should now concentrate on the genes encoding the enzymes acting upstream of VvOMT3 in the MP biosynthetic pathway.

Volatile thiols are associated with various flavours and aromas in both red and white wines (blackcurrant bud, citrus fruit, coffee notes, meaty notes, etc.) and contribute strongly to the sensory profile of wines (for a recent review, see Roland *et al.*, 2011). The current paradigm is that these molecules exist as bound *S*-conjugate precursors in the berry and are released as free volatile forms by *Saccharomyces cerevisiae* β-lyase activity during wine making (Howell *et al.*, 2005). The precursors for volatile thiols are accumulated during the ripening phase of the berry growth, after veraison, and reach their maximum level 16–18 weeks post-flowering (Kobayashi *et al.*, 2010). Several precursors have been

isolated from grapevine berries and formally identified as cysteine *S*-conjugates: *S*-3-(hexan-1-ol)-L-cysteine (P-3SH), a precursor of 3-mercaptohexan-1-ol (3MH, grapefruit); *S*-4-(4-methylpentan-2-one)-L-cysteine, a precursor of 4-mercapto-4-methylpentan-2-one (box tree); and *S*-4-(4-methylpentan-2-ol)-L-cysteine, a precursor of 4-mercapto-4-methylpentan-2-ol (lemon peel) are a few examples of such molecules (Tominaga *et al.*, 1998). Beside these cysteine *S*-conjugates, a glutathione *S*-conjugate precursor of 3-mercaptohexan-1-ol [*S*-3-(hexan-1-ol)-glutathione, or P-GSH] has also been found in white grape berries such as Sauvignon Blanc or Semillon (Peyrot des Gachons *et al.*, 2002). P-GSH acts as a pro-precursor for 3MH release, being first converted in P-3SH and then to 3MH during the wine-making process (Thibon *et al.*, 2011). This phenomenon is enhanced in the case of noble rot, when the berries are colonized by *Botrytis cinerea* for dessert wine making, suggesting that the fungus somehow enhances the conjugation of 2-hexenal on glutathione, thus increasing P-GSH levels in the ripening fruits. The genes coding for the enzyme responsible for P-3SH and P-GSH synthesis in ripening berries have not been formally identified. However, Kobayashi *et al.* (2011) recently reported that two grapevine GSTs, VvGST3 and VvGST4, are able to produce P-GSH from reduced glutathione and trans-2-hexenal *in vitro*. Their potential role for P-GSH synthesis during berry development remains to be shown.

Monoterpenes are molecules based on a skeleton of two five-carbons isoprene units. Over 70 monoterpenes have been formally identified in grapes and wines (Mateo and Jiménez, 2000). Among them, five monoterpene alcohols (monoterpenols) are the most frequent monoterpenes in grape must and strongly contribute to wine and table grape aroma profiles: linalool, geraniol, nerol, α-terpineol, and citronellol. These compounds are responsible for the floral notes ('rose' or 'lily of the valley') that are particularly noticeable in the 'Muscat' varieties or the Gewurztraminer, but also contribute significantly to the aroma of several other varieties (Mateo and Jiménez, 2000). They can be found in grapes as free, volatile molecules or glycoside-bound, odourless compounds (Williams *et al.*, 1989). The first step of their biosynthetic pathway is the production of isopentenyl diphosphate (IPP), which can be produced in plant cells though two pathways (Lange and Ghassemian, 2003): the mevalonate pathway (cytosolic) and the 1-deoxy-D-xylulose-5-phosphate pathway (DXP). In grape, there is strong evidence that the DXP pathway is the dominant route for monoterpenol biosynthesis (Luan and Wüst, 2002); and genetic analysis of F_1 cross progeny from grapevine contrasted for their aromatic potential have shown that a major QTL for berry total monoterpenol content co-localizes with a deoxy-D-xylulose-phosphate synthase gene (Battilana *et al.*, 2009; Duchêne *et al.*, 2009). The following step of the pathway is the synthesis of geranyl diphosphate (GPP) through the condensation of IPP and its isomer dimethyl allyl diphosphate. Once GPP is synthesized, terpene synthases (TPS) such as linalool synthases or geraniol synthases can compete for this substrate. The grapevine genome displays a surprising expansion of the TPS gene family: >80 putative TPS have been identified, twice as many as in *Arabidopsis*, rice, or poplar (Martin *et al.*, 2010). Around a quarter of these TPS have been characterized to date *in vitro* (Lucker *et al.*, 2004; Martin and Bohlmann, 2004), and, in a recent study, Martin *et al.* (2012) reported that the transcript profiles of linalool synthase and neridol synthase, two TPS, parallel monoterpenol glycoside accumulation in Gewürztraminer grapes, supporting their role in terpene biosynthesis in grape.

Metabolite markers defining grape typicity

Grapes are cultivated as wine grapes and as table grapes, whose expected properties may differ significantly. Table grapes are usually more vigorous than wine grapes and grow in areas with soils high in nutrients. High yields up to 15 kg per vine are desired for table grapes, whereas it is often considered that the yield of wine grapes should be limited to ~5 kg per vine, depending on the variety and the region, to reach the best quality for wine making. Table grapes are bigger, with small seeds or seedless, with thicker pulp and thinner skins. The skin of wine grapes should be easily extracted, while the skin of table grapes must resist mechanical handling and allow extended shelf life. Wine grapes are often picked much riper than table grapes and deteriorate faster when picked. Seedless berries are preferred for table grapes while the seed tannins play an important role for the organoleptic properties and the ageing ability of red wines. At harvest, berries from wine grapes are sweeter than table grapes, with total soluble solid levels comprised between 24–26 °Brix and 17–19 °Brix, respectively. Wine grapes are more acid than table grapes, which favours wine stability and ageing.

The characteristic features of one grape cultivar may be largely determined by a unique mixture of all the above-mentioned metabolites. To illustrate the usefulness of the metabolite markers, an example of multivariate analysis that was conducted in table and wine grapes based on their chemical composition is shown in Fig. 2. To do this, we first gathered published chemical analysis at maturity for sugars and organic acids in 98 cultivars (Liu *et al.*, 2006) and for anthocyanins in 110 cultivars (Liang *et al.*, 2008), of which there were 45 cultivars in common from the same germplasm collection. These 45 cultivars comprised eight wine grapes and 37 table grapes, among which there are 19 *V. vinifera*, 25 hybrids of *V. labrusca*×*V. vinifera*, and one hybrid of *V. vinifera*×*V. amurensis*. The sugar, organic acid, and anthocyanin data were extracted from these common cultivars and used for a principal component analysis (PCA). The PCA readily discriminates the table and wine grapes, with the first two principal components (PC_1 and PC_2) explaining 43.2% of the total variance. Most table and wine grapes, with several exceptions, were discriminated mainly based on their organic acid and anthocyanin compositions, while sugars play a minor role.

The effect of environment on berry ripening

In recent years, our knowledge about the connection between the environment and grapevine berry ripening has increased

considerably (Fig. 3). The most studied environmental cues influencing ripening are light conditions (Downey *et al.*, 2004; Matus *et al.*, 2009; Berli *et al.*, 2010), water status (Deluc *et al.*, 2009; Deis *et al.*, 2011), temperature (Spayd *et al.*, 2002; Yamane *et al.*, 2006; Tarara *et al.*, 2008; Cohen *et al.*, 2012; Pillet *et al.*, 2012), and pathogens (Singh Brar *et al.*, 2008; Vega *et al.*, 2011; Lorrain *et al.*, 2012). Moderate water deficit, UV-B radiation, and low temperatures positively affect ripening by increasing the content of total soluble solids and anthocyanins (Mori *et al.*, 2005; Castellarin *et al.*, 2007; Berli *et al.*, 2010), while high temperatures, shade, and pathogens impair ripening-associated processes (Jeong *et al.*, 2004; Mori *et al.*, 2007; Greer and Weston, 2010; Vega *et al.*, 2011; Lorrain *et al.*, 2012; Pillet *et al.*, 2012; Carbonell-Bejerano *et al.*, 2013), as summarized in Table 1. Some of these signals are associated with changes in hormone contents, which in turn affect ripening (Table 2). For example, ABA levels increase in response to water deficit and low temperatures (Yamane *et al.*, 2006; Deluc *et al.*, 2009; Zarrouk *et al.*, 2012), with both environmental cues positively affecting berry ripening. The fact that the results on environmental and hormonal effects may concern either whole berries

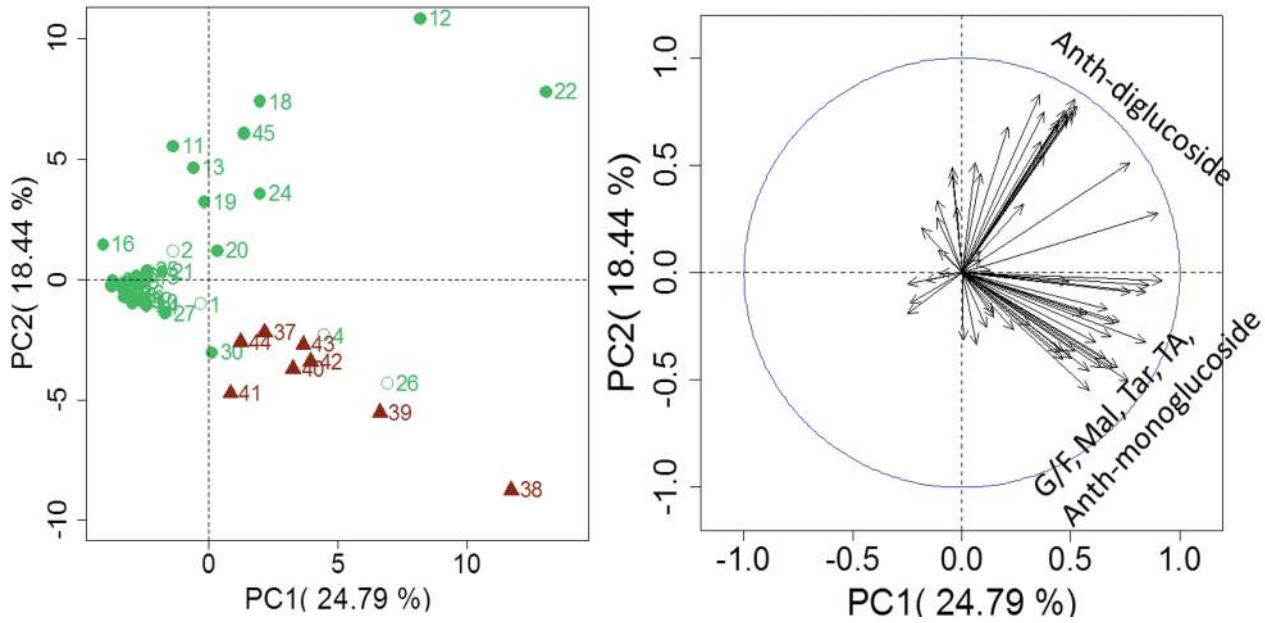

Fig. 2. PCA of grape primary and secondary metabolites for discriminating table (cycle) and wine (triangle) grapes. Table and wine grapes were discriminated mainly based on their organic acid and anthocyanin compositions, while sugars play a minor role. Published data of 45 grape cultivars (numbered from 1 to 45) were used (Liu *et al.*, 2006; Liang *et al.*, 2008). (This figure is available in colour at *JXB* online.)

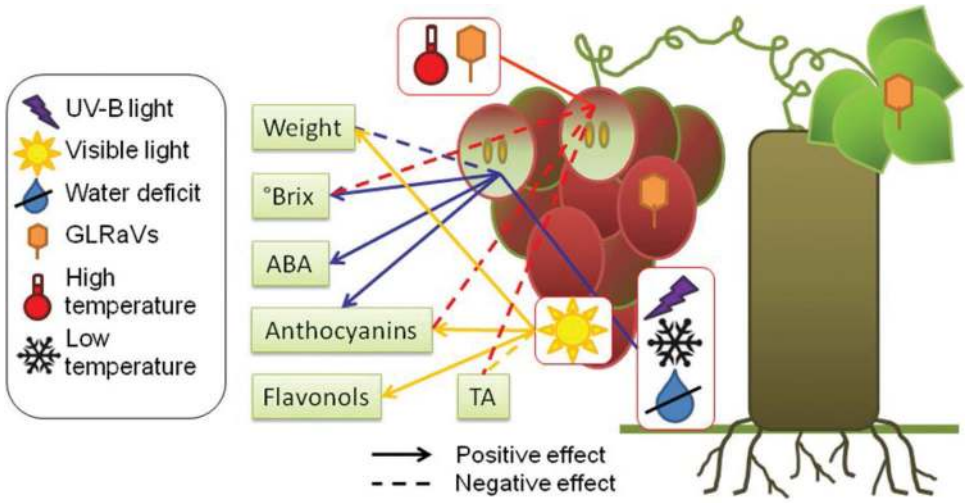

Fig. 3. Environmental cues affecting typical biochemical parameters associated with berry ripening in coloured grapes. Boxes with a red outline enclose environmental signals with similar effects on the indicated parameters, with the exception of UV-B light, which does not enhance ABA content in berries. Arrows and dashed lines indicate positive and negative effects on the indicated ripening parameters, respectively. TA, titrable acidity.

Table 1. *Effect of some environmental cues on ripening-associated biochemical parameters of coloured berries*

Environmental cues having similar effects on anthocyanin content are grouped together. Berry weight (BW) or volume (BV), percentage soluble solids (%SS) or °Brix, titrable acidity (TA), anthocyanins (As), flavonols (Fs), and abscisic acid (ABA).

	BW/BV	%SS/°Brix	TA	As	Fs	ABA	References
Water deficit[a]	↓[1,3] ↑[4] =[6]	↑[1,3,4] =[6]	=[3,6]	↑[1,3,4] =[2] ↓[6]	=[1,3] ↓[6]	↑[3,6]	[1]Castellarin *et al.* (2007); [2]Deis *et al.* (2011); [3]Deluc *et al.* (2009); [4]Koundouras *et al.* (2009); [6]Zarrouk *et al.* (2012)
UV-B radiation[b]	↓[7]	=[7]	–	↑[7]	↑[7]	=[7]	[7]Berli *et al.* (2011)
Low temperature[c]	–	↑[8]	↓[8]	↑[8,9] ↓[2]	–	↑[9]	[2]Deis *et al.* (2011); [8]Mori *et al.* (2005); [9]Yamane *et al.* (2006)
High temperature[d]	↓[12]	↓[11,12]	↓[11]	↓[10,11,13,14]	↓[10] =[14]	↓[10] ↑[11]	[10]Azuma *et al.* (2012); [11]Carbonell-Bejerano *et al.* (2013); [12]Greer and Weston (2010); [13]Mori *et al.* (2007); [14]Tarara *et al.* (2008)
GLRaV infection[e]	↑[15,17]	=[15] ↓[16]	=[15] ↓[17]	↓[15,16,17]	=[15,17] ↑[16]	–	[15]Lee and Martin (2009); [16]Vega *et al.* (2011); [17]Singh Brar *et al.* (2008)
ESCA disease[e]	–	↓[18]	↑[18]	↓[18]	–	–	[18]Lorrain *et al.* (2012)
Light exposure[f]	=[19] ↑[20]	=[19,21] ↓[20]	↓[20]	=[19,23] ↑[10, 20,21,22]	↑[10,19,20,21, 23]	=[10]	[10]Azuma *et al.* (2012); [19]Downey *et al.* (2004); [20]Koyama and Goto-Yamamoto (2008); [21]Matus *et al.* (2009); [22]Niu *et al.* (2013); [23]Tarara *et al.* (2008)

–, not assessed; ↑, increase; ↓, decrease; =, no significant change.
[a] Vines not irrigated during a given period of berry development in relation to irrigated plants.
[b] Vines under UV radiation (normal sunlight; +UV-B) in relation to plants under reduced UV radiation (filter exclusion; –UV-B).
[c] Vines under night low temperature (T) and daytime high T in relation to plants grown under continuous high T (Mori *et al.*, 2005). Vines under low T compared with plants grown under high T (Yamane *et al.*, 2006). Detached berries under low T compared with control (Deis *et al.*, 2011).
[d] Clusters treated with heated air (Tarara *et al.*, 2008). Vines grown in a phytotron under high daytime T in relation to vines grown under room T (Mori *et al.*, 2007; Carbonell-Bejerano *et al.*, 2013). Detached grape berries under high T compared with berries under low T (Azuma *et al.*, 2012).
[e] GLRaV-infected plants or ESCA-affected vines in relation to healthy plants.
[f] Detached grape berries under fluorescent light compared with those under darkness (Azuma *et al.*, 2012). Bunches exposed to incident light in relation to shaded bunches (Tarara *et al.*, 2008; Matus *et al.*, 2009). Light-proof boxes, light-proof bags, or black shadeclothes were also used (Downey *et al.*, 2004; Koyama and Goto-Yamamoto, 2008; Niu *et al.*, 2013, respectively).

Table 2. *Effect of the hormones abscisic acid (ABA), brassinosteroids (BRs), ethylene, auxin, and cytokinins (CKs) on ripening-associated biochemical parameters of coloured berries*

Berry weight (BW) or volume (BV), percentage coloured berries (%CB), percentage soluble solids (%SS) or °Brix, titrable acidity (TA), anthocyanins (As) and abscisic acid (ABA).

	BW/BV	%CB	%SS/°Brix	TA	As	ABA	References
ABA[a]	↓[1] ↑[6,8]	↑[8]	↓[1] =[6,8]	↓[6]	↑[1,2, 4,5,6,8]	↑[1,3,7,8]	[1]Berli *et al.* (2011); [2]Koyama *et al.* (2010); [3]Giribaldi *et al.* (2010); [4]Hiratsuka *et al.* (2001); [5]Jeong *et al.* (2004); [6]Peppi and Fidelibus (2008); [7]Sun *et al.* (2010); [8]Wheeler *et al.* (2009)
BRs	–	↑[9]	↑[9]	–	–	–	[9]Symons *et al.* (2006)
Ethylene	=[10]	–	–	–	↑[10]	↑[7]	[10]El-Kereamy *et al.* (2003); [7]Sun *et al.* (2010)
Auxin	↓[11,12,13]	–	↓[11,12,13]	↑[13]	↓[5, 11,12,13]	↓[12]	[11]Bötcher *et al.* (2011a); [12]Davies *et al.* (1997); [5]Jeong *et al.* (2004); [13]Ziliotto *et al.* (2012)
CKs	↑[6]	–	↓[6]	=[6]	↓[6]	–	[6]Peppi and Fidelibus (2008)

–, not assessed; ↑, increase; ↓, decrease; =, no significant change.
[a] Detached grape berries were cultured *in vitro* in Hiratsuka *et al.* (2001).

or berry compartments (skin, pulp) may explain some of the discrepancies sometimes observed.

Light enhances flavonoid metabolism

Light increases the content of flavonoids (especially the flavonols and anthocyanins) that act as sunscreens. Flavonols are especially sensitive to light. Downey *et al.* (2004) found a decrease in total flavonols and an extremely low expression of *VvFLS1* in Shiraz bunches maintained inside light-proof boxes, compared with control bunches. Similarly, both flavonol contents and the transcript abundance of FLS (flavonol synthase) decline in the berries of shaded bunches of Cabernet Sauvignon and Merlot cultivars (Fujita *et al.*, 2006;

Koyama and Goto-Yamamoto, 2008; Matus *et al.*, 2009). However, the response of anthocyanins to light is more complicated. Total anthocyanins were significantly reduced by light exclusion in one out of three seasons in Shiraz berries while they not affected in the two remaining years (Downey *et al.*, 2004). Conversely, light exclusion or leaf shading consistently and significantly decreases the berry anthocyanin content in the cultivar Cabernet Sauvignon (Koyama and Goto-Yamamoto, 2008; Matus *et al.*, 2009), in parallel with down-regulation of genes associated with anthocyanin synthesis, such as *VvUFGT*, *VvMybA1*, and *VvMybA2*. In detached cv. Pione grape berries maintained under light, anthocyanin content increased compared with those kept under darkness (Azuma *et al.*, 2012). These different response patterns may reflect genotypic sensitivity to light. For example, Zheng *et al.* (2013) compared two grape cultivars (Jingyan and Jingxiu), with the former accumulating considerable anthocyanins (although reduced compared with light-exposed berries) under light exclusion conditions while the later remained colourless. This difference is mainly related to *UFGT*, which is expressed in light-excluded Jingyan berries but not in Jingxiu berries (Zheng *et al.*, 2013). A further proteomic analysis conducted with skins of shaded Jingxiu berries showed that the amount of UFGT protein declined in shaded conditions, while proteins related to energy production, glycolysis, and the tricarboxylic acid cycle were more abundant (Niu *et al.*, 2013). All these results confirm that light is an important cue in the control of the flavonoid pathway, which is a key part of the ripening process (Fig. 3, Table 1).

Water deficit, UV-B radiation, and temperature affect ripening-related processes

In general, water deficit results in an increase in total anthocyanins, stilbenoids, and sugar contents in berries compared with normal or full irrigation, although berry weight under water stress is lower compared with control (Castellarin *et al.*, 2007; Deluc *et al.*, 2009, 2011). Flavonol content is either not affected (Deluc *et al.*, 2009) or decreased (Zarrouk *et al.*, 2012) by water stress. Genes encoding leucoanthocyanidin dioxygenase (LDOX), dihydroflavonol reductase (DFR), UFGT, and VvMybA1 are up-regulated and the anthocyanin content is augmented under water deficit (Castellarin *et al.*, 2007).

Several metabolites and transcripts are affected in a different way by water deficit in Cabernet Sauvignon (red-wine grape) and Chardonnay (white-wine grape) (Deluc *et al.*, 2009). For instance, an *ADH* gene was up-regulated by water stress in Cabernet Sauvignon berries at veraison and ripening stages, while it did not change in Chardonnay berries. The same was true for the sugars (sucrose, glucose, and fructose) and transcripts encoding a cell wall invertase, *UFGT*, and homologues to *VvMybA1* and *VvMybA2*. In contrast, the flavonol content and flavonol synthase transcripts were higher in Chardonnay than in Cabernet Sauvignon under water deficit at veraison and ripening stages. Interestingly, under water stress, ABA was higher in Cabernet Sauvignon than in Chardonnay berries (Deluc *et al.*, 2009), and this could explain in part their different responses to water stress.

The effects of water stress depend on the time the stress is applied. Castellarin *et al.* (2007) compared the effects of early water deficit established from fruit set to veraison and late water deficit applied after veraison in Cabernet Sauvignon berries. Early water stress accelerated both sugar and anthocyanin accumulation, and both early and late water deficit increased anthocyanin accumulation after veraison. Okamoto *et al.* (2004) compared the effects of water deficits starting 14 d after veraison (early deficit) and 24 d after veraison (late deficit) in Chardonnay berries. Interestingly, a steep increase in berry ABA level was observed under early deficit, while this increase was gradual under late deficit or control conditions. Total soluble solids, fructose, glucose, and malate were lower under early deficit than in control and late deficit conditions. Thus, early deficit caused a more negative effect on ripening than late stress. The effects of water deficit on Chardonnay seem to differ from those observed in Cabernet Sauvignon. The reported effect of water deficit on berry traits has been observed in whole berries (Deluc *et al.*, 2009) as well as in berry skins (Castellarin *et al.*, 2007) in Cabernet Sauvignon, hence processes occurring in the skin may account for the changes observed in the pericarp.

UV-B also significantly affects grape berry ripening. Malbec vines under UV-B radiation (normal sunlight; +UV-B) and vines under reduced UV-B radiation (filter exclusion; –UV-B) were compared in order to assess the effects of UV-B on ripening (Berli *et al.*, 2010, 2011). Although berry weight was lower in +UV-B berries, anthocyanin content was higher. This is similar to what was observed in water-stressed berries as described above. However, in contrast to grapes under water deficit, in which flavonol content was not affected, UV-B radiation increased the content of several flavonols (quercetin, myricetin, and kaempferol 3-glucoside). Berry ABA content was not affected by UV-B radiation, although it is well known that ABA leaf levels increase under this stimulus (Berli *et al.*, 2010).

Low temperature is another environmental cue associated with changes in berry ripening. Total soluble solid and anthocyanin content increases and total acidity decreases under low temperatures, as shown by comparing vines grown under low night/high daytime temperatures with vines grown under continuous high temperature (Mori *et al.*, 2005). In another study, ABA content in berries was shown to increase under low temperatures (Yamane *et al.*, 2006). Use of a forced-air delivery system to reduce the diurnal temperature fluctuations caused a more marked effect than only applying cold temperatures (Cohen *et al.*, 2008, 2012). So, cool day temperatures and warm night temperature increased berry weight and sugar content, and caused more changes in flavonoid composition, showing that the temperature effect on ripening is more complex than expected.

Also, high temperatures negatively impact ripening, causing a reduction in berry weight (Greer and Weston, 2010), total soluble solids (Greer and Weston, 2010; Carbonell-Bejerano *et al.*, 2013), anthocyanins (Mori *et al.*, 2007; Tarara *et al.*, 2008; Azuma *et al.*, 2012; Carbonell-Bejerano *et al.*, 2013), and flavonol contents (Azuma *et al.*, 2012). Interestingly, ABA levels in berries were also reduced, in

accordance with changes in flavonoid content (Azuma *et al.*, 2012). In contrast, in Muscat Hamburg berries, high temperatures increased ABA concentration while they reduced anthocyanin content (Carbonell-Bejerano *et al.*, 2013). These apparent inconsistencies might be explained by the fact that high temperatures can uncouple some ripening-related traits, such as sugars and anthocyanins (Sadras and Moran, 2012; Sadras *et al.*, 2013). Although anthocyanin content increases linearly with sugar content, from certain sugar concentration values onwards, high temperatures uncouple these traits by delaying the onset of anthocyanin accumulation rather than the rate (Sadras and Moran, 2012).

The effect of hormones on berry ripening

Several hormones may participate in the control of ripening in grape berry, which is a non-climacteric fruit since the increase of ethylene production is slight and the typical respiration peak does not occur (Coombe and Hale, 1973).

Several studies have pinpointed ABA as the signal triggering berry ripening, since a strong increase in berry ABA content is recorded at the end of the colour turning period and during the initial stages of ripening (Wheeler *et al.*, 2009; Giribaldi *et al.*, 2010; Lacampagne *et al.*, 2010; Sun *et al.*, 2010). The effect of ABA treatment on anthocyanin content is observed in whole berries (Wheeler *et al.*, 2009) as well as in berry skins (Koyama *et al.*, 2010). In Cabernet Sauvignon berries, the highest ABA content has been observed at veraison (Giribaldi *et al.*, 2010; Lacampagne *et al.*, 2010) or 2 weeks after veraison during three consecutive seasons (Wheeler *et al.*, 2009). The abrupt increase in ABA content coincides with the rise in total anthocyanin content, but anthocyanins keep increasing throughout the ripening period, while ABA levels start to decrease at the same time (Wheeler *et al.*, 2009), suggesting that ABA triggers but does not necessarily sustain colour acquisition. Treatments by exogenous ABA cause an increase in berry weight (Peppi *et al.*, 2008; Wheeler *et al.*, 2009), a decrease in titrable acidity (Peppi and Fidelibus, 2008), and an increase in total anthocyanin content (Hiratsuka *et al.*, 2001; Jeong *et al.*, 2004; Wheeler *et al.*, 2009; Berli *et al.*, 2010; Gambetta *et al.*, 2010). The abrupt change in ABA content might be sustained by an amplification mechanism, since ABA treatments increase ABA content (Wheeler *et al.*, 2009; Giribaldi *et al.*, 2010; Sun *et al.*, 2010; Berli *et al.*, 2011). The sugar increase in the berry correlates with a rise in ABA content (Wheeler *et al.*, 2009), but ABA treatment does not affect sugar content (Peppi and Fidelibus, 2008; Wheeler *et al.*, 2009). An ABA treatment performed 3 weeks before veraison even decreases the sugar content per berry (Berli *et al.*, 2011).

ABA up-regulates genes associated with the ripening process. For instance, in Cabernet Sauvignon skins treated with ABA at veraison, genes encoding phenylalanine ammonia-lyase, CHS, naringenin 2-oxoglutarate 3-dioxygenase, F3H, and a Myb-related transcription factor, among others, are up-regulated, in parallel with an increase in total anthocyanins (Koyama *et al.*, 2010). Interestingly, ABA treatments up-regulate genes related to ethylene biosynthesis and repress putative auxin response genes, suggesting that ABA effects are mediated in concert with other hormones. Proteomic analysis of Cabernet Sauvignon berries showed that ABA treatment up-regulates the expression of several ripening-associated proteins such as vacuolar invertase 1 (GIN1), chalcone isomerase (CHI), alcohol dehydrogenase 2 (Adh), xyloglucan endo-transglycosylase (XET), and several other proteins associated with stress response and general metabolism (Giribaldi *et al.*, 2010).

Ethylene may be involved in the trigger of berry ripening

Treating berries at veraison with 2-chloroethylphosphonic acid (2-CEPA), an ethylene-releasing compound, produces an increase in the concentration of several anthocyanin derivatives in Cabernet Sauvignon berries (El-Kereamy *et al.*, 2003). In Cabernet Sauvignon berries, an ethylene peak occurs just before veraison (Chervin *et al.*, 2004). In Muscat Hamburg berries, an ethylene peak has also been observed which precedes the ABA peak (Sun *et al.*, 2010). 1-Methylcyclopropene (1-MCP), a specific inhibitor of ethylene receptors, decreases berry diameter and anthocyanin content and increases acidity in Cabernet Sauvignon berries (Chervin *et al.*, 2004). Additionally, ethylene treatment induces an increase in berry size which is related to changes in the transcripts of various genes encoding aquaporins, polygalacturonases, XET, cellulose synthases, and expansins (Chervin *et al.*, 2008). Interactions between ABA and ethylene were recently investigated. MCP treatment prior to veraison reduced ABA content in Muscat Hamburg berries (Sun *et al.*, 2010), suggesting that the effect of ethylene over-ripening may be mediated in part by ABA.

Auxin delays grapevine berry ripening

The negative role of auxin in berry size, sugar accumulation, and anthocyanin content is well documented (Davies *et al.*, 1997; Böttcher *et al.*, 2011*a*; Ziliotto *et al.*, 2012). The auxin level, which is high in the green phase of rapid berry growth, decreases to very low levels at veraison and throughout the ripening period (Böttcher *et al.*, 2010). Auxin levels might be controlled by conjugation. Indeed, when the free auxin content is low, auxin conjugated to aspartate is high (Böttcher *et al.*, 2010).

Auxin delays the ripening-related processes (Böttcher *et al.*, 2011*b*) and, interestingly, treatments of Shiraz berries with benzothiazole-2-oxyacetic acid (BTOA), an artificial auxin, caused a 2 week delay in the ABA content increase (Davies *et al.*, 1997). Thus, ABA-triggered processes may be delayed by auxin treatment. Additionally, auxin-treated bunches contain a higher number of berries having the same Brix degree, which suggests that auxin delay is associated with a synchronization of sugar accumulation (Böttcher *et al.*, 2011*b*). Transcriptomic analyses of berries treated with naphthalene acetic acid (NAA) 1 week before veraison showed that the delay observed for anthocyanins and sugar accumulation is

paralleled by changes in the transcripts involved in ABA and ethylene pathways (Ziliotto *et al.*, 2012). Specifically, genes associated with ABA biosynthesis and perception were down-regulated, while genes associated with ethylene biosynthesis were induced, suggesting that auxin negatively regulates ABA-induced ripening processes. On the other hand, *CEB1*, a gene strongly induced during ripening and associated with cell expansion induction, is induced by auxin (Nicolas *et al.*, 2013). It is possible that low levels of auxin may induce this gene, thus allowing cell expansion and berry growth. In this context, auxin could be modulating ripening rather than only impairing it. Therefore, negative effects could be associated with auxin antagonism with ABA and positive effects could perhaps be achieved through ethylene biosynthesis promotion and induction of other ripening-promoting genes, such as *CEB1*.

The levels of endogenous brassinosteroids dramatically increase at the onset of grapevine berry ripening, and treatments with exogenous epi-brassinolide induce ripening, while application of brassinazole, an inhibitor of brassinosteroid biosynthesis, delays it (Symons *et al.*, 2006). Brassinosteroids have been suggested to be the first signal for ripening, perhaps through modulation of ethylene content (Ziliotto *et al.*, 2012).

Jasmonic acid, which is mainly associated with defence against pathogens, increases anthocyanin production in grape cell suspensions (Belhadj *et al.*, 2008). On the other hand, cytokinins increase berry weight but decrease sugar and anthocyanin content (Peppi and Fidelibus, 2008).

In summary, the information available to date suggest that ABA, brassinosteroids, and ethylene promote ripening through complex interactions, while auxin delays some ripening-associated processes and also interacts with other hormones such as ABA and ethylene.

Hormones as environmental mediators

ABA promotes grapevine berry ripening but is also a stress-related signal. Under water deficit and cold, ABA content increases in the berry (Yamane *et al.*, 2006; Deluc *et al.*, 2009), while under UV-B it increases in the leaves (Berli *et al.*, 2010, 2011). The observed changes in ABA could reflect its participation in the response to these environmental cues. There is an increase in the transcript abundance of ABA signalling genes (Gambetta *et al.*, 2010) and ABA biosynthetic genes (Deluc *et al.*, 2009) in response to water deficit. Under high temperatures, ABA content decreases (Azuma *et al.*, 2012), although an increase in the transcript abundance of *NCED2*, a gene involved in ABA biosynthesis, was recently reported (Carbonell-Bejerano *et al.*, 2013), possibly due to a compensation mechanism. ABA is thus involved in the perception of several environmental cues in grapevine berries. Brassinosteroids, also considered as ripening-promoting hormones (Symons *et al.*, 2006), are also sensitive to the external signals. The transcript abundance of several genes related to brassinosteroid biosynthesis and perception is altered by water deficit (Gambetta *et al.*, 2010). *BR6OX1* encodes a brassinosteroid biosynthetic enzyme that is under a negative feedback regulation by brassinosteroids, and the putative receptor *BRI1* is down-regulated under water deficit, which normally occurs during ripening. Hence, a transcriptional induction of ripening-related processes mediated by brassinosteroids under water stress is possible. Concerning ethylene, the transcripts of *ERF083*, a gene encoding a transcription factor, are up-regulated by high temperatures (Carbonell-Bejerano *et al.*, 2013). Regarding auxin, NAA treatment produces effects similar to shade conditions: both treatments decrease the flavonol content and the transcript abundance of *VvFLS4* (Fujita *et al.*, 2006). Both treatments also decrease the anthocyanin content (Jeong *et al.*, 2004), and the *LDOX*, *UFGT*, and *MybA1* transcripts (Jeong *et al.*, 2004). It would be interesting to test whether auxin signalling is involved in the light effect.

Concluding remarks

Grapevine might be considered as a model species to study the ripening of non-climacteric species. However, several difficulties slow down the progress made in the understanding of grape berry ripening, including the lack of easy tools for functional genomics, the heterogeneity of berry ripening in the bunches, and the lack of reliable experimental systems to control and study the effects of nutrients and hormones on ripening. High-throughput approaches have confirmed the basic biochemical and molecular trends that were already known to occur during ripening, but, with a few exceptions, have failed to identify new major structural or regulatory genes. The most recent and impressive work using these tools confirms vintage effects known for several centuries, and provides some clues about possible candidate genes responsible for berry plasticity (Dal Santo *et al.*, 2013). Yet, the demonstration of the role and activity of genes expressed in the berries needs a long and difficult functional analysis impaired by the almost total lack of grapevine mutants, the low efficiency of grape transformation, and the time needed to grow fruiting plants. Improved bioinformatic tools are still needed to better understand gene–gene and gene–metabolite networks, and to cluster and analyse gene promoter sequences. In the context of climate change, much effort is underway to better understand how endogenous and external signals are perceived by the grapevine plant, thus modulating ripening, metabolic fluxes, and hence berry composition. A further layer of complexity through epigenomic regulation of ripening also deserves more attention (Zhong *et al.*, 2013).

Acknowledgments

The authors thank the ECOS Sud programme (no. C11B01) for its support of FG, and the Eiffel Fellowship programme for supporting LG. Work at ISVV is supported by INRA, the University of Bordeaux, Bordeaux Sciences Agro, the Regional Council of Aquitaine, and the Conseil Interprofessionnel du Vin de Bordeaux. This work was prepared under the auspice of COST Action FA 1106.

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