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REVIEW PAPER

An update on sugar transport and signalling in grapevine

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Abstract

In addition to their role as a source of reduced carbon, sugars may directly or indirectly control a wide range of activities in plant cells, through transcriptional and post-translational regulation. This control has been studied in detail using *Arabidopsis thaliana*, where genetic analysis offers many possibilities. Much less is known about perennial woody species. For several years, various aspects of sugar sensing and signalling have been investigated in the grape (*Vitis vinifera* L.) berry, an organ that accumulates high concentrations of hexoses in the vacuoles of flesh cells. Here we review various aspects of this topic: the molecular basis of sugar transport and its regulation by sugars in grapevine; the functional analysis of several sugar-induced genes; the effects of some biotic and abiotic stresses on the sugar content of the berry; and finally the effects of exogenous sugar supply on the ripening process in field conditions. A picture of complex feedback and multiprocess regulation emerges from these data.

Key words: Fruit biology, grapevine, signalling, stress, sugar, transport.

Introduction

Sugar transport and allocation of assimilates between sources and sinks are major parameters controlling crop productivity (Gifford *et al.*, 1984). In addition to their role as a supply of energy and carbon backbone for growth and development in plants, sugars directly or indirectly control a wide range of processes, including photosynthesis, sugar transport itself, nitrogen uptake, defence reactions, secondary metabolism, and hormonal balance (Smeekens *et al.*, 2010). This control is mainly based on the regulation of gene expression (Koch, 1996), but it may also affect mRNA stability, and protein translation and stability (Wiese *et al.*, 2004). Monosaccharides (glucose, fructose, mannose, or galactinol) and disaccharides (sucrose, trehalose, and sucrose analogues) may affect gene expression. The genes affected encode sugar transporters, components of the photosystems such as chlorophyll *a*/*b*-binding protein and plastocyanin, enzymes of carbohydrate metabolism (small subunit of RubisCO, α-amylase, invertase, sucrose synthase, and ADPG-prophosphorylase), enzymes of secondary metabolism (phenylalanine ammonia lyase and other enzymes of the flavonoid biosynthesis pathway), and transcription factors (Koch, 1996; Saigne, 2008; Agasse *et al.*, 2009; Roitsch, 1999; Smeekens, 2000, Smeekens *et al.*, 2010; Nicolas et al., 2012; Tognetti *et al.*, 2013). The perception of sugars or sugar fluxes by membrane or cytosolic sensors is called sugar sensing, and the pathways that mediate sugar-controlled responses constitute sugar signalling. Components of sugar sensing and signalling provide the molecular basis through which plant cells adapt their activity as a function of their sugar status. In plants, these processes have been described for different sugars and are intimately interconnected with hormonal signalling pathways [abscisic acid (ABA), ethylene, and cytokinin] (Gibson, 2004; Rolland *et al.*,

2002, 2006; Grigston *et al.*, 2008; Ramon *et al.*, 2008; Hanson and Smeekens, 2009). Several additional sugar-derived signalling systems were recently identified and described as important players in plant development because they integrate sugar availability and developmental programmes (Smeekens *et al.*, 2010). These systems include trehalose-6 phosphate (T6P) (Ponnu *et al.*, 2011; Dai *et al.*, 2013), sucrose non-fermenting-related kinase-1 (SnRK1), and the target of rapamycin (TOR) kinase complex (John *et al.*, 2011). All these processes are interconnected and were recently shown to play key roles in plant responses to abiotic stresses by maintaining the homeostasis between sugar availability and plant metabolism (Smeekens *et al.*, 2010; Robaglia *et al.*, 2012).

Grapevine (*Vitis vinifera* L.) is a woody perennial plant providing fruit species used as fresh fruit, dried raisins, and for wine making and distillation of liquors. In 2011, vineyards occupied ~7585 thousands hectares throughout the world, and total grape production reached 69.2 Mt (OIV report 2012; http://www.oiv.int). Ripe grape berries contain a very high concentration of glucose and fructose (~1 M each) in the vacuole of flesh cells (Fontes *et al.*, 2011). The sink/source ratio is an important parameter for the sensory properties of the berries, and is manipulated through viticultural practices which affect the number of leaves and clusters. Grapevine is therefore a woody species particularly relevant for the study of sugar transport and sugar signalling, and the present review will summarize the data obtained on these topics. Initial work on sugar signalling in grape mainly concerned the regulation of anthocyanin biosynthesis, and of sugar transport (for reviews, see Agasse *et al.*, 2009; Davies *et al.*, 2012), but recent data indicate that other major processes such as cell growth are also controlled by sugars in the grape berry. A picture of complex feedback and multiprocess regulation emerges from these data.

Sugar transport and sugar transporters in grapevine

Physiological background

In grapevine, as in many other dicotyledons, sucrose is the form of long-distance transport in the phloem. The ripening grape berry is a strong sink for dry matter transported from current photosynthesis and wood reserves (Coombe, 1989). The sugars accumulated in the vacuoles of mesocarp cells account for 65–91% of the fresh weight in a mature berry. They start to accumulate in a linear way when the berries reach the veraison stage, which corresponds to the trigger of ripening. At this stage, the berries also change colour and begin to soften, which indicates a coordinated reprogramming of cell activities. Grapevine is a non-climacteric fruit, and the molecular signals controlling ripening are still debated (see Kühn *et al.*, 2014). However, sugar and ABA accumulation in the berries follow a similar pattern.

Since the sugars accumulated in the vacuoles of flesh cells are glucose and fructose, the accumulation of these hexoses in the berries involves the activity of sucrose-metabolizing enzymes, sucrose transporters, and monosaccharide transporters (MSTs; Agasse *et al.*, 2009). The events occurring at veraison were studied in detail in a *Vitis vinifera*×*Vitis labrusca* hybrid. Zhang *et al.* (2006) conducted electron microscope analysis, measured the activity of sucrose-degrading enzymes (invertases and sucrose synthases), and studied the movement of symplastic tracers and of companion cell-expressed green fluorescent protein (GFP)-tagged viral protein. They demonstrated that a shift from a symplasmic to an apoplasmic unloading pathway occurs at or just prior to the onset of ripening. In addition, they observed an increase in the expression and activity of cell wall invertases that was concomitant with a rise in apoplastic sugar concentration and osmotic pressure. Apoplastic phloem unloading coupled with solute accumulation in the berry apoplast may be responsible for the decline in xylem water influx into ripening grape berries (Keller *et al.*, 2006).

Sugar concentrations in the skin are generally lower than those in the flesh tissue (Coombe, 1987), and hexose concentration gradients have been detected in the flesh (low hexose concentration near the brush, high at the stylar end). Active uptake of D-glucose in the flesh is only detected after the beginning of ripening, while it can be measured at both pre- and post-veraison stage in isolated skin pieces (Coombe and Matile, 1980). However, a high proportion of the sugar uptake by skin is diffusive, whatever the sampling time, in contrast to the mesocarp tissue where the uptake is principally active.

Sucrose transporters

Analysis of the grape genome sequence suggests that sucrose transporter genes constitute a small multigenic family of four members in this species (Agasse *et al.*, 2009; Afoufa-Bastien *et al.*, 2010). Three sucrose transporter cDNAs have been cloned from Shiraz and Cabernet Sauvignon berries (*VvSUC11*; AF021808, also identified as *VvSUT1* AF182445; *VvSUC12* AF021809; and *VvSUC27* AF021810) and characterized as proton-dependent sucrose transporters by heterologous expression in *Saccharomyces cerevisiae*. VvSUC11 and VvSUC12 are intermediate affinity sucrose transporters (K_m 0.9 mM and 1.4 mM, respectively) (Ageorges *et al.*, 2000; Manning *et al.*, 2001), and VvSUC27 is a low affinity sucrose transporter (K_m of 8–10 mM; Zhang *et al.*, 2008). VvSUT2, whose sequence is close to that of VvSUC27, is weakly expressed in the berries (Afoufa-Bastien *et al.*, 2010).

The sucrose transporters expressed in the berries must fulfil two opposite functions: maintain sucrose in the conducting bundles (through uptake and/or retrieval) until it reaches the site of unloading, and, at this site, they must allow its leakage or mediate efflux. Heterologous expression in yeast indicates that these three sucrose transporters act as proton–sucrose symporters, and are thus expected to mediate sucrose uptake (in the phloem, or in the mesocarp cells). Whether one or several of these transporters may act in the reverse mode (Carpaneto *et al.*, 2005) and may play a role in sucrose efflux from the phloem has not yet been tested. Such reverse mode function may not be necessary if the transmembrane motive force that maintains constant uptake and retrieval of sucrose

along the conducting complex, against a very high concentration gradient, decreases at the site of unloading. *VvSUC11* and *VvSUC12* transcripts concomitantly increase with post-véraison sugar accumulation, in contrast to *VvSUC27* transcripts whose amounts significantly decrease at this stage (Davies *et al.*, 1999). The different expression profile and the different K_m of VvSUC27 clearly indicate a function that differs from that of the other SUC transporters. Unfortunately, no immunolocalization data are available to determine precisely the cellular and subcellular localization of these transporters.

AtSUC4 (*Arabidopsis thaliana*) and HvSUT2 (barley), which belong to group 4 of the sucrose transporters family (Sauer, 2007), have low affinity for sucrose (K_m=5–6 mM), are expressed in sink organs, and are localized in the vacuolar membrane. In *Lotus japonicus*, LjSUT4 functions in the proton-coupled uptake of sucrose and possibly other glucosides into the cytoplasm from the vacuole (Reinders *et al.*, 2008). VvSUC11 is the closest homologue to AtSUC4 (68%), but it has a higher affinity for sucrose (K_m=0.88 mM) than AtSUC4. No data on its subcellular localization are available. Although SUT2/SUC3 was initially described as a sucrose sensor located in the plasma membrane of sieve tubes in tomato (Barker *et al.*, 2000; Reinders *et al.*, 2002), this conclusion was later questioned (Sauer, 2007), and its putative sucrose-sensing function no longer seems to be valid. The closest homologue to AtSUC3 in the grape genome is VvSUC12, but it has a lower K_m (1.4 mM) than AtSUC3 (11.7 mM).

Hexose transporters

In grape, 59 putative hexose transporter homologues have been identified based on protein motif recognition (Samson *et al.*, 2004; Jaillon *et al.*, 2007; Agasse *et al.*, 2009; Afoufa-Bastien *et al.*, 2010). Six full-length cDNAs encoding MSTs and named *VvHT1–VvHT6* (*V. vinifera* hexose transporter; *VvHT1* AJ001061; *VvHT2* AY663846; *VvHT3* AY538259 and AY854146; *VvHT4* AY538260; *VvHT5* AY538261; *VvHT6* AY861386, DQ017393) have been cloned from various cultivars such as Pinot noir, Ugni blanc, Chardonnay, Cabernet Sauvignon, and Syrah (Fillion *et al.*, 1999; Vignault *et al.*, 2005, Hayes *et al.*, 2007). In spite of the high number of putative monosaccharide genes in the grape genome, no other VvHT was identified in grape berries, suggesting that the most important transporters for sugar accumulation in this organ have already been cloned. *VvHT1*, *VvHT2*, and particularly *VvHT3* are highly expressed compared with the other *VvHT* genes, at all stages of berry development. The diversity of hexose transporter genes expressed during berry development is consistent with the shift from a symplastic to an apoplastic phloem unloading pathway that occurs prior to veraison. With the exception of VvHT6, all VvHTs isolated so far from grape berries present high homologies with AtSTPs; that is, functional hexose transporters. VvHT1, the first transporter of this family identified in grape (Fillion *et al.*, 1999), and functionally characterized (Vignault *et al.*, 2005), is homologous to AtSTP1.

Plasma membrane hexose transporters

The plasma membrane localization of VvHT1, VvHT4, and VvHT5 has been demonstrated by immunofluorescence, immunolabelling, and GFP fusion proteins (Vignault *et al.*, 2005; Hayes *et al.*, 2007). Functional studies based on expression in a *hxt-null* mutant yeast EBY VW 4000 showed that *VvHT1*, *VvHT4*, and *VvHT5* encode high-affinity, H^+-dependent glucose transporters. VvHT1 has a higher affinity for glucose (K_m of 70 μM) than VvHT4 and VvHT5 (K_m ~150 μM and 100 μM, respectively) and displays broad substrate specificity, being able to transport galactose, xylose, and glucose analogues such as 3-*O*-methyl-D-glucose (3-OMeG). Uptake of radiolabelled D-[U-^{14}C]fructose in grape cells also displays Michaelis–Menten kinetics, with a V_{max} similar to that measured for glucose uptake, suggesting that both glucose and fructose are transported by the same protein, although the K_m for fructose is much higher than the K_m for glucose (Conde *et al.*, 2006). Both *VvHT1* transcripts and protein levels (Conde *et al.*, 2006) are much higher at pre-véraison stages, indicating that it is not directly responsible for the post-véraison sugar accumulation (Vignault *et al.*, 2005; Conde *et al.*, 2006). *VvHT1* transcripts are abundant in the phloem region of the conducting bundles of the leaf, petiole, and berry (Vignault *et al.*, 2005). This localization, and its high affinity for hexoses, suggest that it could be involved in the retrieval of minor amounts of hexoses leaking from the conducting complex.

While VvHT4 transport activity may be restricted to glucose, VvHT5 is able to transport both glucose and fructose (Hayes *et al.*, 2007). *VvHT5* transcripts are relatively more abundant at late ripening, but their amount remains low. *VvHT3*, whose transcript levels are reduced at véraison but high at both green and ripening stages (Hayes *et al.*, 2007), is homologous to *AtSTP7*, which has not been functionally characterized. VvHT3 is not able to transport any of the tested radiolabelled sugars in the deficient yeast model (Vignault *et al.*, 2005; Hayes *et al.*, 2007), and its transport function is also unknown. A gene (re)named *VvHT8* has a high similarity to *VvHT1* (99.4%) and was identified as a target of grape selection that has led to high sugar contents in domesticated grapes (Xin *et al.*, 2013).

Tonoplast hexose transporters

VvHT2 and VvHT6 seem to be localized in the tonoplast (C. Vignault *et al.*, unpublished), and VvHT6 has high sequence similarity to the previously described tonoplast transporter of *A. thaliana* AtTMT2 (Afoufa-Bastien *et al.*, 2010). The TMT (tonoplast monosaccharide transporter) subfamily of MFS (major facilitator superfamily) transporters contains tonoplast hexose–proton antiporters. This family is represented by three members in *V. vinifera* (Afoufa-Bastien *et al.*, 2010). VvTMT1 has been cloned, localized, and characterized as a functional MST localized in the tonoplast of grape cells (Zeng *et al.*, 2011). Its expression decreases strongly during berry development. Unlike *VvTMT1*, *VvHT6*/*TMT2* transcripts are highly accumulated at véraison (Terrier *et al.*, 2005; Deluc

et al., 2007), suggesting that this transporter may be responsible for vacuolar accumulation of hexose at the inception of ripening. *VvHT2*, whose expression is mainly associated with véraison (Terrier *et al.*, 2005; Hayes *et al.*, 2007; C. Vignault *et al.*, unpublished), is homologous to AtSTP5, but its transport activity has not yet been characterized.

Others

Chen *et al.* (2010) identified a new class of sugar transporters, named SWEETs, found in *Caenorhabditis elegans*, plants, and mammals, and mediating glucose efflux. AtSWEET1, cloned from *A. thaliana*, was co-expressed in human HEK293T cells, which have low endogenous glucose uptake activity, as assessed with FLIPglu600mD13V, a high-sensitivity fluorescence resonance energy transfer (FRET) glucose sensor. The glucose-dependent response of the sensor demonstrated that AtSWEET1 can mediate both uptake across the plasma membrane and efflux into the endoplasmic reticulum. SWEET1 seems to function as a bidirectional uniporter/facilitator, which is largely pH independent. It has a low affinity for glucose, and does not efficiently complement mannose, fructose, and galactose uptake deficiencies of yeast mutants (Chen *et al.*, 2010). Sixteen SWEET homologues may be identified in the grapevine genome (Fig. 1A) but none has yet been studied in detail. Among these homologues, only three are expressed significantly during berry development (Fig. 1B). The SWEET gene that is the most expressed in the berries is close to AtSWEET15 (Chen *et al.*, 2012).

Mechanisms involving endocytosis have been described in a species accumulating concentrations of mannitol in the fruit (olive tree; Conde *et al.*, 2007), but they have not been explored in grapevine.

Control of sugar transporters by sugars

The promoter region (2500 bp upstream of the site for initiation of transcription) of *VvHT1* contains several sugar-responsive elements (Fillion *et al.*, 1999), and this led us to investigate the possibility that the expression of this sugar transporter gene may be regulated by the sugar concentration in the cell. Different lengths of the promoter were transcriptionally fused to the β-glucuronidase (GUS) reporter gene and used to transform tobacco. The *VvHT1* promoter directed expression in sink organs. Glucose (58 mM), sucrose (58 mM), and the non-transported sucrose isomer palatinose doubled the GUS activity conferred by the *VvHT1* promoter in tobacco cells, whereas fructose did not affect it (Atanassova *et al.*, 2003). These effects were the strongest with the 2.4 kb promoter, which contains all putative sugar-responsive elements (activating and repressing), but they were

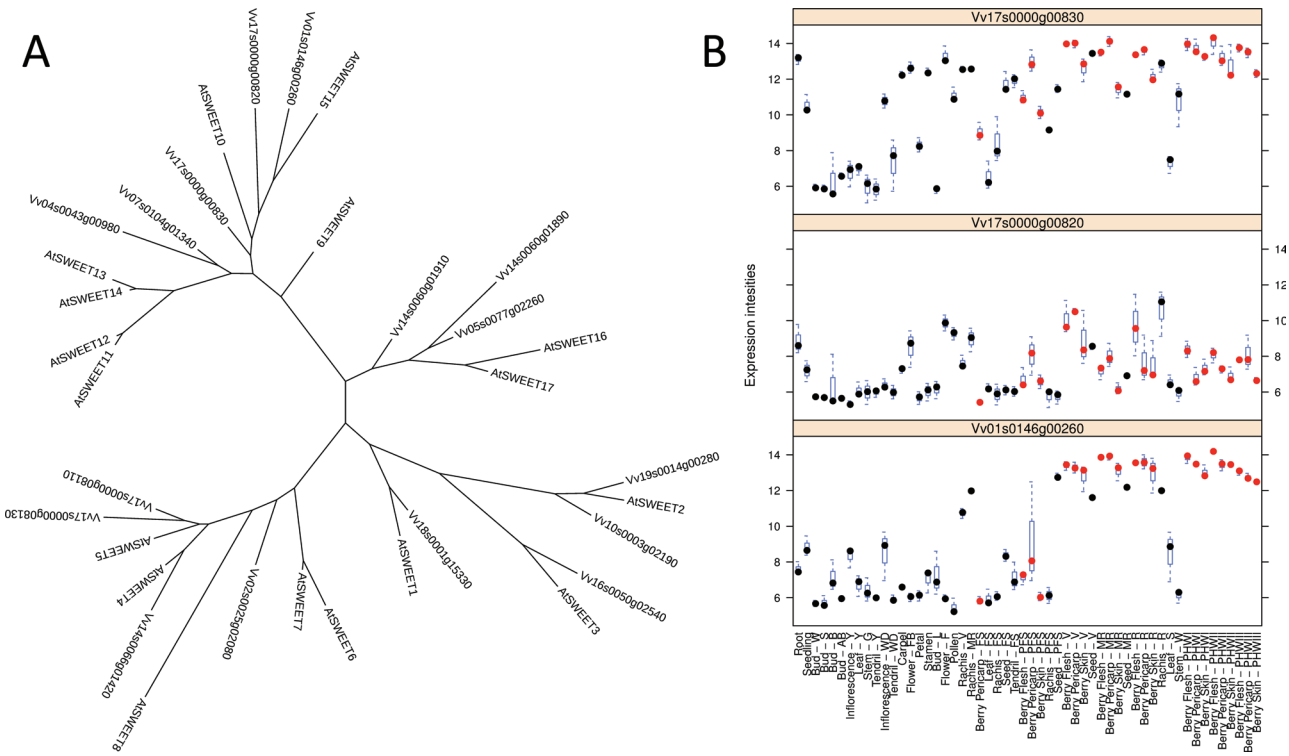

Fig. 1. Phylogenic tree and berry expression of SWEET transporter genes in grape. (A) Evolutionary tree of grapevine and *Arabidopsis* SWEET transporters inferred by using the maximum likelihood method implemented in the MEGA5 software (Tamura *et al.*, 2011). Grapevine SWEET were identified by their PFAM (Punta *et al.*, 2012) domain PF03083. (B) Boxplot of gene expression profiles over a microarray developmental series made by Fasoli *et al.* (2012) for the three most berry-specific VvSWEET genes: Vv01s0146g00260, Vv17s0000g00820, and Vv17s0000g00830. Red dots indicate median expression intensities for berry samples. Data were obtained from NCBI GEO (Barrett *et al.*, 2013; accession no. GSE36128) and RMA normalized using the R/oligo package (Carvalho and Irizarry, 2010).

also significant with the 0.3 kb promoter, which contains only activating sugar boxes. The induction of *VvHT1* expression by both sucrose and palatinose was confirmed in the homologous grape berry cell culture. These data provided the first example of a sugar transporter induced by both glucose and sucrose in higher plants. The fact that palatinose, a sucrose homologue that is only very poorly absorbed by plant cells, may up-regulate *VvHT1* expression may suggest the existence of a plasma membrane sucrose sensor and of signalling pathways that start from the plasma membrane. Although induction of *VvHT1* expression by sucrose does not require transport, the glucosyl moiety of the sucrose molecule is necessary for sugar sensing (Atanassova *et al.*, 2003).

The proximal 160 bp region of the *VvHT1* promoter upstream of the 'TATA box' was used to develop a one-hybrid approach (Cakir *et al.*, 2003). This region, which contains two positive sugar-responsive motifs, is a perfect 'sucrose box 3' encompassing an imperfect SURE1 sequence. The screening allowed the identification of a grape *ASR* (abscisic acid, stress, ripening induced gene), named *VvMSA*, interacting with these DNA elements (Cakir *et al.*, 2003). Gel shift assays confirmed specific binding of VvMSA to the two sugar-responsive elements present in the proximal region of the promoter. *VvMSA* and *VvHT1* share similar patterns of expression during the ripening of grape. Both genes are inducible by sucrose in grape berry cell culture. The positive regulation of *VvHT1* promoter activity by *VvMSA* was also shown in plants by co-expression experiments. In contrast to the data obtained in grape, the Asr1 transcripts levels were negatively correlated with glucose content and mRNA levels of the hexose transporter Ht2 in tubers of transgenic potato plants (Frankel *et al.*, 2007).

In grape cell suspension, sugar induction of *VvMSA* was strongly enhanced by ABA, suggesting that VvMSA is involved in a common transduction pathway of sugar and ABA signalling (Cakir *et al.*, 2003). Several *ASR* orthologous and paralogous genes are transcriptionally regulated by ABA and sugars (Dominguez *et al.*, 2013, and references therein). Saumonneau *et al.* (2008) described some polymorphism for grape ASRs and characterized them as chromosomal non-histone proteins. By a yeast two-hybrid approach and functional analysis, they identified a protein partner of VvMSA which was characterized as an APETALA2 domain transcription factor named VvDREB. Later, three ASR genes were isolated from two grape varieties, Cabernet Sauvignon and Ugni blanc (Saumonneau *et al.*, 2012). One was specific for Cabernet Sauvignon, one for Ugni blanc, and one was common to both varieties. Functional analysis of the promoters of these genes demonstrated the organ-preferential expression conferred on the GUS reporter gene, and the specific phloem tissue localization. These ASR may play a role in the fine tuning of hormonal and metabolic signalling which allows the integration of environmental cues (Saumonneau *et al.*, 2012).

ASRs are not only involved in the control of hexose uptake in heterotrophic organs. Recently, Dominguez *et al.* (2013) suggested that in tobacco (*Nicotiana tabacum*) ASRs were involved in the signalling cascade of interactions among glucose, ABA, and gibberellins. The fine regulation mediated by ASR through hexokinase1 and Snf1-related kinase on glucose metabolism resulted in dwarfism, reduced carbon dioxide assimilation, and accelerated leaf senescence.

Conde *et al.* (2006) used grape heterotrophic suspension-cultured cells as a model system to study glucose transport and its regulation. Glucose uptake in these cells was mediated by a high-affinity, broad-specificity H1-dependent transport system (K_m=0.05 mM) superimposed on first-order kinetics. The glucose concentration in the medium tightly regulated the transcription of *VvHT1* as well as the amount of VvHT1 protein and monosaccharide transport activity. The other MST genes expressed in grape berry (*VvHT2*, *VvHT3*, *VvHT4*, *VvHT5*, and *VvHT7*) were poorly expressed and responded weakly to glucose. Various sugars were tested for their ability to repress *VvHT1* expression: 2-DOG (2-deoxy-D-glucose), which can be phosphorylated, 3-OMeG, which cannot be phosphorylated, and mannoheptulose (MHL), a potent inhibitor of hexokinase. *VvHT1* transcription was strongly repressed by glucose and 2-DOG, but not by 3-OMeG or glucose plus MHL, indicating a hexokinase-dependent repression of transcription. Although OMeG, and glucose plus MHL did not affect the amount of *VvHT1* transcripts, they decreased the glucose uptake capacity of the cells. This was due to the reduction of VvHT1 protein in the plasma membrane. High glucose down-regulated *VvHT1* transcription and glucose uptake, whereas low glucose increased these parameters. These data provide an example showing control of plant sugar transporters by their own substrate at both transcriptional and post-transcriptional levels. In this experimental system, glucose regulated its own uptake through hexokinase-dependent repression of transcription, and through hexokinase-independent post-transcriptional regulation.

Sugar regulation of genes involved in the anthocyanin biosynthesis pathway

Flavonoids play an important role in defence against pathogens, protection from UV radiation, and coloration of flowers and fruits. The anthocyanins, which belong to the flavonoid group, are mainly accumulated in the skin cells, giving colour to the berries of the red and black varieties, and also influencing the quality of the berry. The three most abundant anthocyanins in grape are 3-monoglucoside, 3-acetylglucoside, and 3-*p*-coumarylglucoside (Boss *et al.*, 1996).

The anthocyanin content of the grape berry increases concomitantly with sugar accumulation at the post-veraison stages (Coombe, 1992; Vitrac *et al.*, 2000; Deluc *et al.*, 2009). The addition of different concentrations of sucrose (100–150 mM) at day 7 (the end of the exponential growth phase) to suspension cultures of Gamay Fréaux increases the anthocyanin production up to 12-fold compared with cells without sucrose addition (Larronde *et al.*, 1998). Addition of glucose or fructose (150 mM) also increased the anthocyanin content, although fructose is less effective than sucrose or glucose. In contrast, mannitol and sorbitol (150 mM) do not stimulate

anthocyanin accumulation in grape cells, suggesting that the effect of sucrose or glucose is not osmotic. The amount of sucrose added to the culture medium did not affect grape cell proliferation, indicating that the effect on anthocyanin content is not indirectly due to cell division (Larronde *et al.*, 1998). Similar results were observed in cell suspensions of different grape cultivars (Cabernet Sauvignon, Barbera berries, and Gamay Red) (Vitrac *et al.*, 2000; Gollop *et al.*, 2001; Belhadj *et al.*, 2008; Ferri *et al.*, 2011).

Several genes involved in the anthocyanin biosynthesis pathway have been identified, cloned, and characterized (see Kühn *et al.*, 2014). Several transcription factors regulating this pathway are known so far. Among these, the MYB transcription factor family that controls many important processes in plants (Matus *et al.*, 2008; Dubos *et al.*, 2010) is the most studied. The expression of several structural genes and transcription factors involved in the anthocyanin biosynthesis pathway is regulated by sugars in petunia (Neta-sharir *et al.*, 2000) and *Arabidopsis* (Tsukaya *et al.*, 1991; Solfanelli *et al.*, 2006).

Concerning grape, the expression of both *LDOX* (leucoanthocyanidin dioxygenase) and *DFR* (dihydroflavonol-4-reductase) was up-regulated by treatment of cv. Gamay Red cell cultures with 8.7 mM sucrose (Gollop *et al.*, 2001). When cell cultures were transformed with a DFR::GUS fusion gene, the transcripts of both GUS and endogenous DFR were more abundant after sucrose treatment (Gollop *et al.*, 2002). The transcripts and protein amounts of F3H (flavanone 3-hydroxylase) protein were also increased in grape berries incubated with different sugars. The F3H protein in berries peaked after 1 h and 2 h treatment with glucose (100 mM) and sucrose (150 mM), respectively (Zheng *et al.*, 2009). F3H transcripts peaked at 1 h (with 100 mM glucose and 100 mM fructose) or at 2 h (with 150 mM sucrose), and then decreased immediately.

Since anthocyanin biosynthesis is localized in the skin cells of the berries, except in teinturier varieties, the sugar content of the skin cells may not be directly related to the total sugar content of the berries. Also, cell suspensions provide a convenient experimental system, but the data derived from this system should be extrapolated with caution to berry physiology.

Recent field experiments tested the effects of mannose (150 mM), the C-2 epimer of glucose, on anthocyanin biosynthesis (A. Serrano *et al.*, unpublished). For many plants, mannose is toxic, because it is actively phosphorylated by hexokinases into mannose-6-phosphate, which decreases the pool of Pi. However, there are some mannose-insensitive plants which have the ability to convert mannose-6-phosphate to fructose 6-phosphate by mannose-6-phosphate isomerase, which is not toxic to the cell (Heilman *et al.*, 1997). In grape cell cultures, mannose (20–100 mM) induces a concentration-dependent accumulation of anthocyanins and is not toxic for the grape cells (Vitrac *et al.*, 2000). However, the stimulation of anthocyanin accumulation by mannose (100 mM) is only 70% of what is observed with the same concentration of sucrose. Unlike mannose, 2-deoxyglucose is toxic for the grape cells. In non-toxic concentrations, it does not affect the anthocyanin content. Thus, within the non-metabolizable sugars, mannose is a good candidate to induce the accumulation of anthocyanins in grape. To test mannose as an inductor of anthocyanin biosynthesis, it was added exogenously to cluster grape before veraison in field experiments. In parallel, other sugars that may induce anthocyanin biosynthesis were tested. Mannose had an important effect on the coloration of the berries, inducing early coloration compared with control treatment (A. Serrano *et al.*, in preparation). The addition of other sugars was less effective than that of mannose, and mannitol had no effect.

Sugars and biotic stress: effects of viral infection on sugar content of grape berries

More than 58 different viruses may affect *V. vinifera*, and many severely impact grapevine physiology and cause significant economic losses (Martelli and Boudon-Padieu, 2006; Vega *et al.*, 2011). Among them, *Grapevine leaf-roll-associated virus-3* (GLRaV-3) is one of the most widespread viruses worldwide, and one of the most important diseases affecting grapevines (Martelli and Boudon-Padieu, 2006; Vega *et al.*, 2011). It may alter primary and secondary metabolism in both the leaves and the berries. Thus, it may induce leaf deformation, deficiency in grape cluster development, and alterations in fruit quality, associated with the reduction of the sugar and anthocyanin contents in red cultivars, which dramatically affect the ripening process (Bertamini *et al.*, 2004; Vega *et al.*, 2011). In the leaves of both Carmenere and Cabernet Sauvignon grape cultivars, GLRaV-3 infection induces genes related to sugar metabolism, such as sugar transporters and glycosyl transferases (Espinoza *et al.*, 2007). The genes of the flavonoid biosynthetic pathway are up-regulated in symptomatic leaves of a red-fruited wine grape cultivar infected with GLRaV-3 (Gutha *et al.*, 2010). Among them, CHS3 (chalcone synthase 3), F3′5′H (flavonoid 3′,5′-hydroxylase), F3H1, LDOX, and LAR1 (leucoanthocyanidin reductase 1) showed a >10-fold increase and MybA1 a 19-fold increase upon virus infection. As a result, there was an increase in the flavonol, proanthocyanidin, and anthocyanin contents of the leaves of infected plants.

In the berries, global transcript profiling showed that GLRaV-3 affects a wide range of biological processes at both veraison and ripening stages (Vega *et al.*, 2011). Glucose and fructose accumulation were reduced during ripening of virus-infected vines, and, in parallel, the expression of genes implicated in sugar metabolism and transport was affected by the virus (Vega *et al.*, 2011). Among these genes, *VvHT1* and *VvMSA* were significantly repressed in virus-infected berries at most developmental stages analysed. All these results suggest that the decrease in the sugar levels in the berry of GLRaV-3-infected plants is mediated through effects on sugar transport and metabolism.

GLRaV-3 infection also decreased the anthocyanin content and the transcript levels of structural and regulatory genes of the anthocyanin biosynthesis pathway. Among these, CHS2 mRNA levels were up-regulated in immature infected berries,

but repressed at the ripening stage. The expression of the *UFGT* (UDP glucose: flavonoid-3-*O*-glucosyltransferase) gene was strongly down-regulated by the virus in infected ripening berries. In berries of virus-infected vines, the transcripts of *MYBPA1* (a transcription factor that regulates the anthocyanin biosynthesis pathway) were induced before veraison, but repressed at ripening, and those of *MYBA1* were down-regulated during ripening (Vega *et al.*, 2011). Given that anthocyanin synthesis is regulated at least in part by sugars, it is tempting to conclude that reduction in the sugar level of ripe berries by GLRaV-3 infection may down-regulate the genes related to the anthocyanin biosynthesis pathway, and consequently reduce the anthocyanin content.

Sugars and abiotic stress: effects of heat stress on galactinol synthesis

Recently, Pillet *et al.* (2012) showed that high temperature locally applied to *V. vinifera* Cabernet Sauvignon clusters induces the accumulation of galactinol in berries. This process was mediated, at least in part, through the action of VvHsfA2 (heat stress factor A2) and VvGolS1 (galactinol synthase 1). In plants, galactinol acts mainly as a galactosyl donor for biosynthesis of RFOs (raffinose family oligosaccharides). RFOs, such as raffinose and stachyose, fulfil important functions including carbon translocation and storage, and are defined as compatible solutes with a protective role during abiotic stress (Pillet *et al.*, 2012). Despite a significant galactinol accumulation, no raffinose and stachyose were detected in berries subjected to long-term heat exposure (Pillet *et al.*, 2012). Two hypotheses were proposed to explain these results. The first one suggests that the RFOs accumulated during the early stage of heat exposure were rapidly catabolized to provide metabolizable energy and carbon skeletons. The second one proposed that galactinol might not act as a galactosyl donor and RFO synthesis regulator in heat-stressed berries, but rather as an endogenous molecular signal. Galactinol was recently described as a signalling molecule involved in pathogen-induced systemic resistance (Kim *et al.*, 2008). Since both biotic and abiotic stimuli share some common features in their stress mechanisms, Valluru and Van den Ende (2011) proposed that galactinol and RFOs function as signals as well as true ROS (reactive oxygen species) scavengers. If galactinol can signal heat stress to trigger adaptive responses in grape berries, its exact function in stressed berries remains to be determined, as do the consequences of its accumulation for fruit development and fruit quality.

Sugar and berry development

A transcriptomic approach was initiated using Cabernet Sauvignon berry cells treated or not with sucrose (Lecourieux *et al.*, 2010) in order to obtain an overall idea of all the genes potentially affected by cell sugar status. Total RNA were extracted and hybridized with 70-mer oligoarrays bearing a set of 14 562 unigenes (Qiagen Operon Array-Ready Oligo Set for the Grape Genome Version 1.0). A genome ontology category enrichment analysis indicated that the most affected processes belong to signal transduction pathways, cell wall modifications, protein synthesis and degradation, abiotic and biotic stresses, secondary metabolism, lipid metabolism, and redox modifications. Here, we will describe a few molecular players that were significantly up-regulated by sucrose supply. These genes belong to the families of protein kinases and transcription factors.

Protein kinases are major components of intracellular signal transduction enabling plant cells to respond rapidly to environmental cues. VvSK1 was identified as a sugar- (and ABA-) inducible protein kinase affecting hexose transport and soluble sugar accumulation in grape cells (Lecourieux *et al.*, 2010). This protein is 468 amino acids long and belongs to clade III of the GSK3 family. *VvSK1* is only expressed in sink organs (flowers, berries, and roots). During berry development, its transcripts decreased after the green stage and increased again after the veraison stage when both sugars and ABA accumulate (Davies *et al.*, 1997; Pan *et al.*, 2005). Interestingly, both sucrose and ABA were able to increase *VvSK1* expression in grape cell suspensions, underlining the tight interaction between sugars and hormone signalling pathways (Smeekens, 2000; Finkelstein and Gibson, 2002; Léon and Sheen, 2003; Rolland *et al.*, 2006). Additionally, the use of glucose analogues (2-DOG and 3-OMeG) and of a hexokinase inhibitor (MHL) showed that the sugar control of VvSK1 is dependent on a hexokinase pathway. The production of transgenic grape cell suspensions overexpressing VvSK1 allowed a better understanding of its role in the control of sugar uptake in response to sugar supply. Indeed, in these transgenic cells, the rate of glucose uptake was increased 3- to 5-fold, and the amount of glucose and sucrose accumulation was more than doubled, while the amount of starch was not affected. This suggested that VvSK1 is involved in the control of sugar compartmentation, a hypothesis further supported by the capacity of VvSK1 to trigger transcript accumulation of four MSTs (*VvHT3*, *VvHT4*, *VvHT5*, and *VvHT6*). However, among these transporters, it is more likely that the control of *VvHT6* and *VvHT3* that accumulates parallel to the accumulation of hexoses in the berries (Deluc *et al.*, 2007; Hayes *et al.*, 2007) could mainly contribute to the *VvSK1*-mediated regulation of sugar import during berry development. Finally, this work underlines the importance of protein kinases and therefore of phosphorylation events in the control of sugar uptake and accumulation by sugar, but also provided a good tool for investigating the metabolic and physiological consequences of the deregulation of such processes.

Transcription factors are key elements that control cell metabolism reprogramming and expression of key genes involved in berry development and ripening. Different genes encoding transcription factors were identified by transcriptomic analysis of both sucrose-treated cells and developing grape berries (D. Lecourieux *et al.*, unpublished data; D. Glissant and S. Delrot, unpublished data). Among these, two showed interesting patterns. The first one belongs to the bZIP family and was linked to berry ripening processes (D. Lecourieux *et al.*, unpublished) and the second one, VvCEB1 (*V. vinifera* cell elongation bHLH 1), belongs to the basic

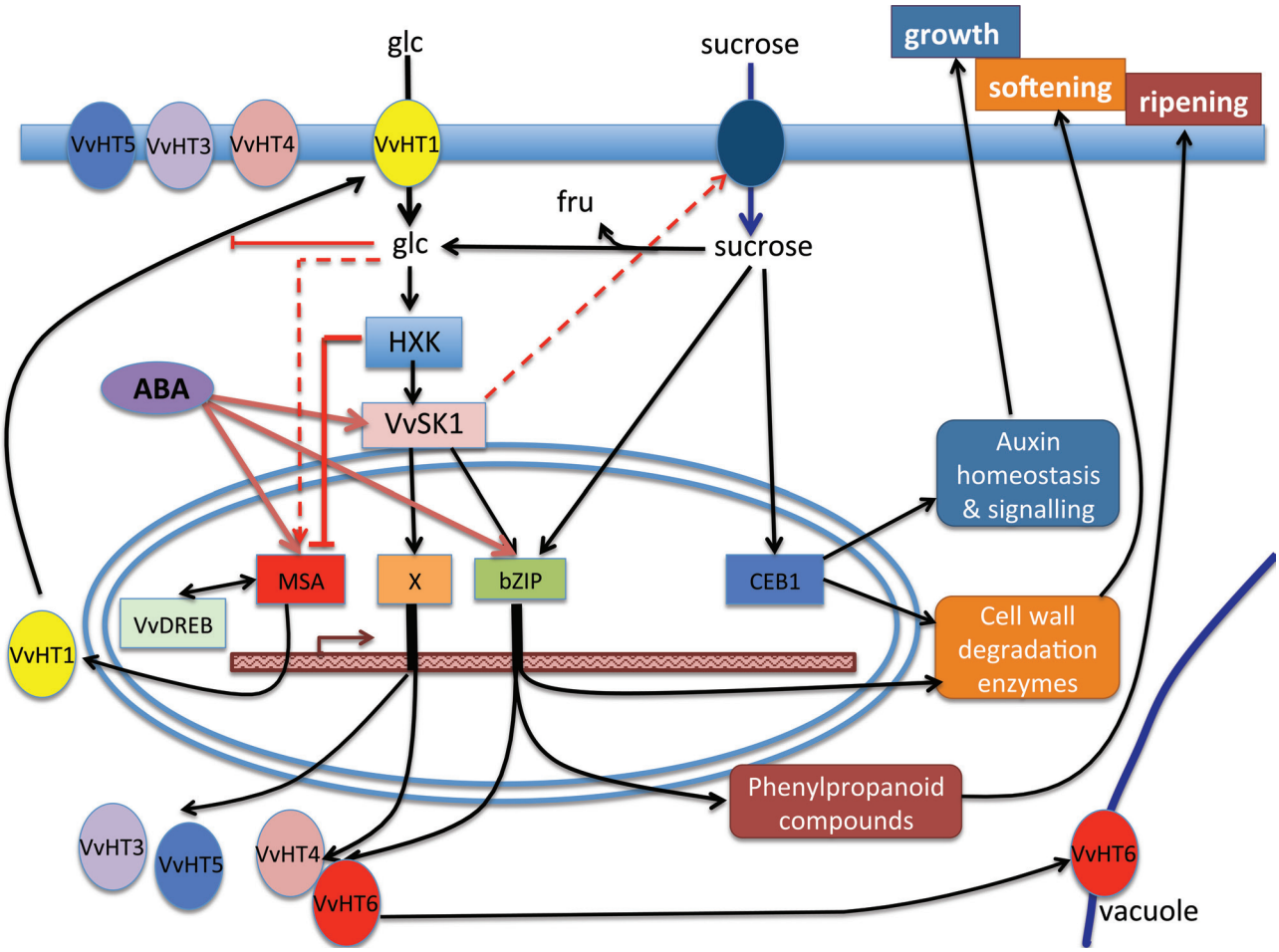

Fig. 2. Summary diagram of sugar transport and sugar-sensing processes described so far in grapevine. bZIP, basic leucine zipper; CEB1, *Vitis vinifera* cell elongation bHLH protein 1; DREB, *Vitis vinifera* drought responsive element binding protein; HXK, hexokinase; MSA, *Vitis vinifera* maturation stress abscisic acid gene (ASR family); VvHT1–VvHT6, *Vitis vinifera* hexose transporter 1–VvHT6; VvSK1: *Vitis vinifera* glycogen synthase kinase 3 protein kinase 1; X, unknown intermediate.

helix–loop–helix (bHLH) family. VvCEB1 was recently shown to affect cell size in grape. bHLH proteins belong to a vast multigenic family of transcriptional regulators present in both animals and plants, and that intervene in numerous physiological and developmental processes (Pires and Dolan, 2010). In plants, bHLH proteins function as transcriptional regulators modulating secondary metabolism pathways, fruit dehiscence, carpel and epidermal development, phytochrome signalling, and stress responses (Ramsay and Glover, 2005; Castillon *et al.*, 2007; Pires and Dolan, 2010; Feller *et al.*, 2011).

VvCEB1 belongs to the subfamily XII of the bHLH transcription factors (Pires and Dolan, 2010) containing members involved in growth regulation (Szécsi *et al.*, 2006; Kay *et al.*, 2007). *VvCEB1* expression was reduced by auxin treatment, while its transcripts predominantly accumulated in the expanding tissues of the berry (mesocarp and exocarp) with a maximum at veraison when the amounts of auxins are low. The heterologous analysis of its promoter activity in tomato confirmed that *VvCEB1* was specifically expressed in the fruit and that this expression was maintained during the fruit expansion period. The production of grape embryos overexpressing *VvCEB1* revealed that its overexpression resulted in the disruption of embryonic development and tissues organization by affecting cell size and polarity. Indeed, these embryos grew faster, and failed to acquire bilateral symmetry and to reach a more important size because of the presence of larger cells, thus suggesting a role for VvCEB1 in the stimulation of cell expansion and in the control of auxin homeostasis. This hypothesis was confirmed by the study of auxin- and cell expansion-related genes in grape embryos overexpressing *VvCEB1*. Among the *VvCEB1*-stimulated genes, we found *IAA* genes such as *VvIAA19* that was recently shown to promote growth in *Arabidopsis* (Kohno *et al.*, 2012), *SAUR* genes that accumulate in the soybean hypocotyl elongation zone (Gee *et al.*, 1991) or that simulate cell expansion (Spartz *et al.*, 2012), and *GH3* genes that were associated with longan fruit growth and ripening (Kuang *et al.*, 2011). Many genes associated with cell wall metabolism (*XET*, *PECL*, *AMY*, *AGP*, and *EXP*) and water transport (AQP) were also strongly up-regulated in *VvCEB1*-overexpressing embryos, in agreement with a role for VvCEB1 in the stimulation of cell expansion processes.

Altogether, this study highlighted the ability of sugar to act as a signalling molecule. The examples described above showed how a sugar signal could (i) regulate the fruit sugar content by modulating VvSK1 action; and (ii) regulate the final size of the fruit by controlling VvCEB1-mediated cell expansion.

Concluding remarks

The present status of our knowledge related to sugar transport and sugar sensing in grapevine is summarized in Fig. 2. This review underlines the complexity of sugar transport and sugar control as well as the gaps in our knowledge. The molecular players leading to the accumulation of high concentrations of glucose and frucose in the vacuoles of flesh cells are still loosely characterized. A lot has been learnt on the sugar control of transporters, which may be positive or negative, depending on the sugar concentration, and may involve a hexokinase-dependent or independent pathway, and transcriptional and protein regulation. The simultaneous presence of up- and down-regulation of several pathways (hexokinase dependent and independent), as well as the multilevel regulations (transcripts and proteins) described for sugar transport pinpoints the major role of these compounds as nutrients and signals, and the importance of carbon economy for the plant. Beyond transport, the sugar status directly or indirectly affects other cellular activities such as phenylpropanoid metabolism, cell wall metabolism, auxin homeostasis, and ripening, and ultimately berry growth, ripening, and adaptation to stress. Interactions between the sugar and ABA signalling pathway play a key role, also underlined by the parallel accumulation of sugars and ABA in the berry after veraison. In many cases, results have been obtained with cell suspensions rather than intact berries. Their physiological relevance must be checked systematically in the berry organ, and whole berry studies need to be developed. Although most studies on sugar sensing in grapevine have been conducted with sucrose, glucose, and fructose, recent data indicate that other sugars such as galactinol and trehalose may also play a significant role in the regulation of berry metabolism, ripening, and adaptation to stress. Quicker progress in grapevine research will obviously depend on the development of new functional genomic tools adapted to this slow growing, large sized, and not easily transformed plant.

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