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ORIGINAL PAPER

Role of ABA and Gibberellin A_3 on gene expression pattern of sugar transporters and invertases in *Vitis vinifera* cv. Malbec during berry ripening

Germán Murcia[1] · Mariela Pontin[1,2] · Patricia Piccoli[1]

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Abstract Sugars are key constituents that affect quality of grape berries, and consequently the grape metabolic profile relevant to wine's industry. However, enzymes and transporter genes expression involved in sugar transport at different phenological stages are scarcely studied. In addition, little is known about the role of the plant hormones ABA and Gibberellin (GA_3) as endogenous regulators, over the expression pattern of the sugars transporters genes in grapevine. The aim of this study was to analyze the expression pattern of the most relevant sugar transporters and invertases in leaves and berries of grapevine plants cv. Malbec during berry ripening stages and its shift after ABA and GA_3 sprays. In leaves, *VvHT1* was the sugar transporter highly expressed, whereas *VvHT6* was the most abundant in berries throughout berry ripening. Moreover, *VvSUC12* and *VvSUC27* were expressed at veraison greater in leaves than in berries, suggesting an active phloem loading at the onset of ripening. Applications of ABA and GA_3 enhanced the expression of *VvSUC12* and *VvSUC27* in pre-veraison leaves. Furthermore, hormones increased the expression of *VvHT2, VvHT3* and *VvHT6* in berries at different stages of ripening favoring sugar unloading from phloem. In conclusion, ABA and GA_3 are involved in the long-distance sugar transport from leaves to berries in *Vitis vinifera* L. cv. Malbec, and their exogenous application could be a suitable strategy to improve the process.

Keywords ABA · GA_3 · Gene expression · Invertases · Malbec · Sugar transporters · *Vitis vinifera*

Introduction

In grape berries, sugars and the secondary metabolites derived play a crucial role in wine's quality. Photoassimilates are translocated from leaves to berries throughout the phloem, being sucrose the main sugar transported (Conde et al. 2007). Sucrose is synthesized in leaf parenchyma cells and moves via plasmodesmata towards the sieve element–companion cell complex (SE–CC), in a process called phloem loading (Dinant and Lemoine 2010). Once there, hydrostatic pressure drives the mass movement of photoassimilates from leaves to different sink organs, berries, stems and roots (Keller 2010). Even though woody plants, like grapevines, are mainly passive loaders (Slewinski et al. 2013), several evidences suggest that an active phloem loading via sugar transporters may be acting as well (Afoufa-Bastien et al. 2010; Hayes et al. 2010; Medici et al. 2014; Pastenes et al. 2014; Murcia et al. 2016).

Therefore, the phloem unloading process is a crucial step where sucrose is released from the vascular system to the sink cells. Before veraison, sucrose gets into berry mesocarp cells via plasmodesmata. Whereas from the onset of veraison onwards sucrose is hydrolyzed in the apoplast by acidic invertases yielding glucose and fructose, and then, through hexose transporters, they get into mesocarp cells (Zhang et al. 2006).

In a genome of a near-homozygous line PN40024 grapevine 59 putative hexose transporters have been identified

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✉ Germán Murcia
gmurcia@fca.uncu.edu.ar

[1] Instituto de Biología Agrícola de Mendoza, Facultad de Ciencias Agrarias, CONICET-UNCuyo, A. Brown 500, 5507 Chacras de Coria, Argentina

[2] EEA La Consulta-INTA, CC8, 5567 La Consulta, Argentina

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(Jaillon et al. 2007; Afoufa-Bastien et al. 2010), but only 6-full length cDNAs encoding monosaccharide transporters named *VvHT1-6* have been cloned from various cultivars (Fillion et al. 1999; Vignault et al. 2005; Hayes et al. 2007). Moreover, only the proteins VvHT1, VvHT4 and VvHT5 have been functionally characterized (Vignault et al. 2005; Hayes et al. 2007). The studies of Conde et al. (2006) show that the transcript level of the gene *VvHT1* was higher in pre-veraison, whereas the expression of *VvHT5*, although weak, was more related to pos-veraison (Hayes et al. 2007; Pastenes et al. 2014). On the other hand, the pattern expression of *VvHT3* is lower in veraison, but higher in pre- and pos-veraison (Hayes et al. 2007). Furthermore, the expression of *VvHT2* and *VvHT6* is higher in veraison suggesting that these transporters may be responsible for hexose accumulation at the onset of ripening (Terrier et al. 2005; Vignault et al. 2005; Deluc et al. 2007; Hayes et al. 2007).

In the grapevine genome, a small multigenic family has been identified with only 4 members encoding for putative sucrose transporters; whereas 12 and 10 putative genes encoding for neutral and acidic invertases have been identified, respectively (Jaillon et al. 2007; Velasco et al. 2007). Among the sucrose transporter genes, only 3 cDNAs (*VvSUC11, VvSUC12* and *VvSUC27*) were cloned and characterized from Syrah and Cabernet Sauvignon cultivars (Ageorges et al. 2000; Manning et al. 2001; Zhang et al. 2008). It has been shown that *VvSUC11* and *VvSUC12* were mostly expressed in berries at pos-veraison, while the gene *VvSUC27* was mainly expressed in pre-veraison (Afoufa-Bastien et al. 2010; Pastenes et al. 2014). On the other hand, only two vacuolar acidic invertases (*VvGIN1* and *VvGIN2*) and one apoplastic acidic invertase (*VvcwINV*) have been cloned (Davies and Robinson 1996; Hayes et al. 2007). Furthermore, in relation to the expression pattern of invertases, Zhang et al. (2006) and Hayes et al. (2007) demonstrated that *VvcwINV* is up-regulated at the onset of ripening in berries, whereas the expression of *VvGIN1* and *VvGIN2* is higher in pre-veraison and decrease in veraison and pos-veraison (Davies and Robinson 1996; Sarry et al. 2004; Deluc et al. 2007).

The plant hormones ABA and GA_3 are involved in carbohydrate biosynthesis and accumulation as well as sugar transport from source to sink organs in grape (Moreno et al. 2011; Xu et al. 2015; Murcia et al. 2016). In this sense, foliar applications of grape plants with ABA and GA_3 promoted carbon allocation towards roots and berries, respectively (Moreno et al. 2011). Recently, Murcia et al. (2016) found that ABA improved long-distance sugar transport by enhancing phloem area and promoting the expression of some hexose transporter genes in veraison. As well, Çakir et al. (2003) showed that ABA induced the transcription factor *VvMSA* (a member of the ABA-, Stress-, Ripening induced family), which in turn regulates the expression of the gene *VvHT1*. Also, *VvHT5* was shown to be regulated by ABA in response to a biotrophic pathogen in grapevine leaves (Hayes et al. 2010). There is only one report describing the expression of some sugar transporters and metabolic genes in the cultivar Malbec at one phenological stage (veraison) (Murcia et al. 2016). In the present work, the role of ABA and GA_3 on the expression pattern of genes involved in sugar transport (*VvHT1, VvHT2, VvHT3, VvHT5, VvHT6, VvSUC12, VvSUC27*) and metabolism (*VvGIN1, VvcwINV*) in leaves and berries of grapevine cv. Malbec at three phenological stages (pre-veraison, veraison and pos-veraison) was assessed.

Materials and methods

Plant material and experimental conditions

Cuttings of *Vitis vinifera* L. cv. Malbec were obtained from 1-year-old cane-pruned cv. Malbec shoots collected from an experimental vineyard at INTA-Mendoza (Mendoza, Argentina, 33°0′S, 68°52′W, 940 m asl). The cuttings were embedded 24 h in a solution 0.6 µM NAA (1-naphthaleneacetic acid sodium salt, S. Ando & Cía SA, Buenos Aires, Argentina). Then the bases of the cuttings were maintained at 30 °C in a sand/water bed, whereas the tops were exposed to 4 °C in a cold room. After 5 weeks, the own-rooted cuttings were planted in 10 L pots containing grape compost as substrate. Plants were watered to field capacity every 2 days. Only one shoot per plant containing one bunch was allowed to grow under field conditions. The number of leaves per shoot among treatments did not modify throughout the experiment.

The assay was set in a random design with three treatments and 5 replicates per treatment for each phenological moment, thus a total of 45 plant samples were analyzed. The samples were taken at three phenological moments: pre-veraison (30 days after anthesis, DAA), veraison (100% of colored berries, onset of ripening, 70 DAA) and pos-veraison (130 DAA), using individual plants as experimental units. The treatments consisted in the application of ABA, GA_3 and water (control) solutions with a weekly frequency from fruit set (10 DAA) until full ripeness (130 DAA). The solutions were sprayed with a hand-held sprayer onto the whole plant (leaves and bunches) until runoff, during late afternoon to minimize ABA photodegradation. The treatment doses were: 250 µg mL^{-1} ABA (±-S-*cis, trans* abscisic acid, PROTONE SL, Valent BioSciences, Libertyville, IL, USA), 500 µg mL^{-1} GA_3 (GIBERELINA KA, S. Ando & Cía. SA, Buenos Aires, Argentina) and control (water). All the solutions were supplemented with 0.05% (v/v) Triton X-100 as surfactant. All the samples were taken the day after the last application of hormones. In each phenological

moment (pre-veraison, veraison and pos-veraison), 15 plants were dissected, and berries and mature leaves (10th from the apex) were kept at −80 °C for further analysis.

Gene expression

Samples of leaves (100 mg fresh weight) and berries (400 mg fresh weight) were used for RNA extraction according to Reid et al. (2006). cDNA synthesis and experiments of qRT-PCR were performed as described by Murcia et al. (2016). For relative gene expression calculations, a previous standard quantification curve with five serial dilutions of cDNA was constructed for each gene to calculate amplification efficiency. Elongation factor 1-α (*VvEF 1-α*) was applied for normalization in all experiments, and relative transcript levels were calculated using the equation $2^{-\Delta Ct}$, where $\Delta Ct = (Ct, target - Ct, VvEF\ 1\text{-}\alpha)$. The primer sequences of the following genes: *VvHT1, VvHT2, VvHT3, VvHT5, VvHT6, VvSUC12, VvSUC27* and *VvGIN1* were obtained from Murcia et al. (2016), while the primers of the gene *VvcwINV* (Table S1) were designed using the software Beacon Designer version 7.70 (Premier Biosoft International, Palo Alto, CA, USA) over the corresponding EST available at the NCBI GenBank database. All pair of primers amplified a single product of the expected size, which was confirmed by melt-curve analysis and agarose gel electrophoresis. All experiments were performed with five biological replicates and three technical replicates.

Statistical analysis

Data were statistically analyzed by one-way ANOVA with $p < 0.05$. When significant differences were determined, treatments were compared by LSD-Fisher media analysis. Statistical analysis was carried out with InfoStat software (http://sites.google.com/site/fgstatistics).

Results and discussion

Gene expression pattern in leaves and berries of grapevine cv. Malbec

Figures 1, 2 and 3 show the overall gene pattern of sugar transporters and sucrose invertases previously characterized (Manning et al. 2001; Zhang et al. 2006; Hayes et al. 2007), from leaves and berries of cv. Malbec. In control leaves, at the stage of pre-veraison, the main expressed genes encoding a hexose transporter were *VvHT1* and *VvHT6* (Fig. 1a), whereas in veraison were *VvHT1* and *VvHT3* (Fig. 1b). In pos-veraison, the most abundant hexose transporters were *VvHT1* and *VvHT5* (Fig. 1c). According to these results, *VvHT1* shows the higher expression in

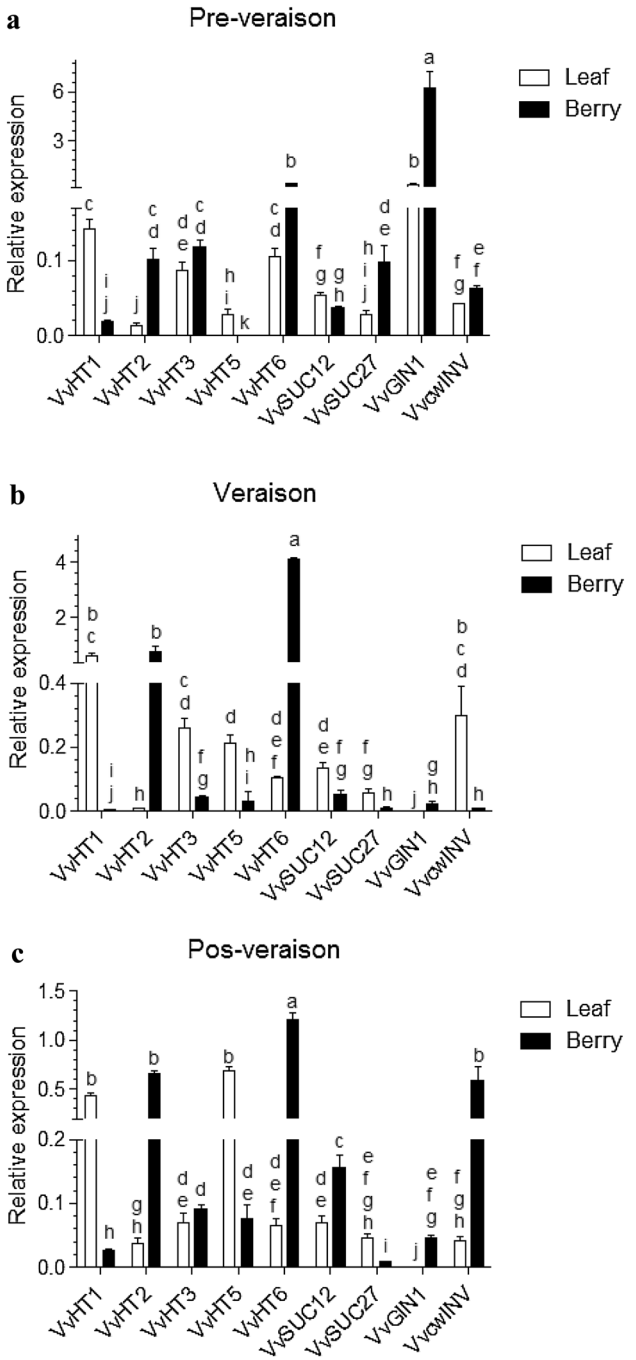

Fig. 1 Comparison of gene expression of sugar transporters and invertases between leaves and berries of *Vitis vinifera* cv. Malbec during phenological stages, pre-veraison (**a**), veraison (**b**) and pos-veraison (**c**). Values are means ± SE, n = 5. Different letters indicate statistically significant differences ($p < 0.05$). All values were normalized to the expression of *VvEF 1-α*

leaves throughout berry development. Regarding sucrose transporters, *VvSUC12* was the most expressed gene in pre-veraison and veraison (Fig. 1a, b). In relation to sucrose invertases, *VvGIN1*, which encodes a vacuolar invertase, was

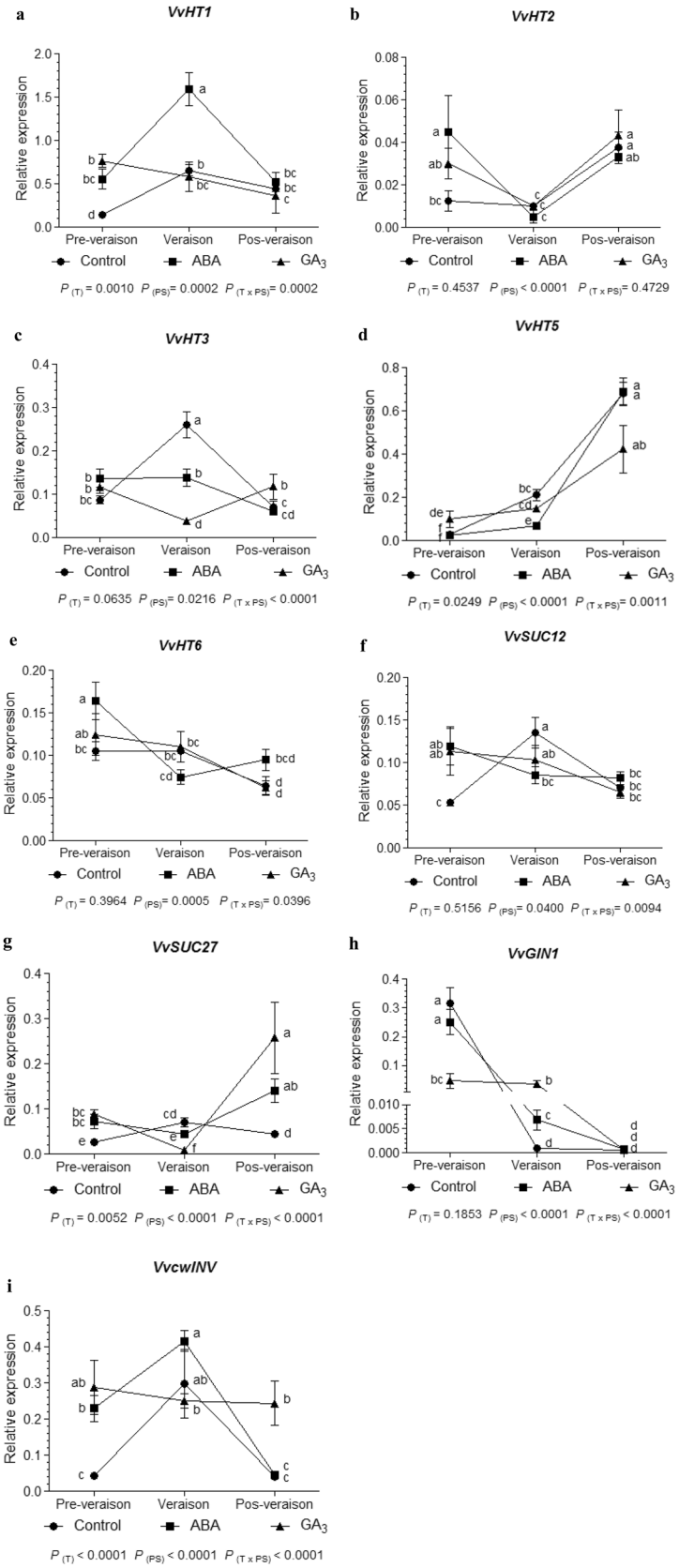

Fig. 2 Effect of ABA and GA_3 on gene expression of sugar transporters and invertases in mature leaves of *Vitis vinifera* cv. Malbec during phenological stages (pre-veraison, veraison and pos-veraison). Hexose transporters: *VvHT1* (**a**), *VvHT2* (**b**), *VvHT3* (**c**), *VvHT5* (**d**), *VvHT6* (**e**). Sucrose transporters: *VvSUC12* (**f**), *VvSUC27* (**g**). Invertases: *VvGIN1* (**h**), *VvcwINV* (**i**). Values are means ± SE, n = 5. Different letters indicate statistically significant differences ($p < 0.05$). All values were normalized to the expression of *VvEF 1-α*. $P_{(T)}$, treatment effect; $P_{(PS)}$, phenological stage effect; $P_{(T \times PS)}$, treatment x phenological stage interaction effect

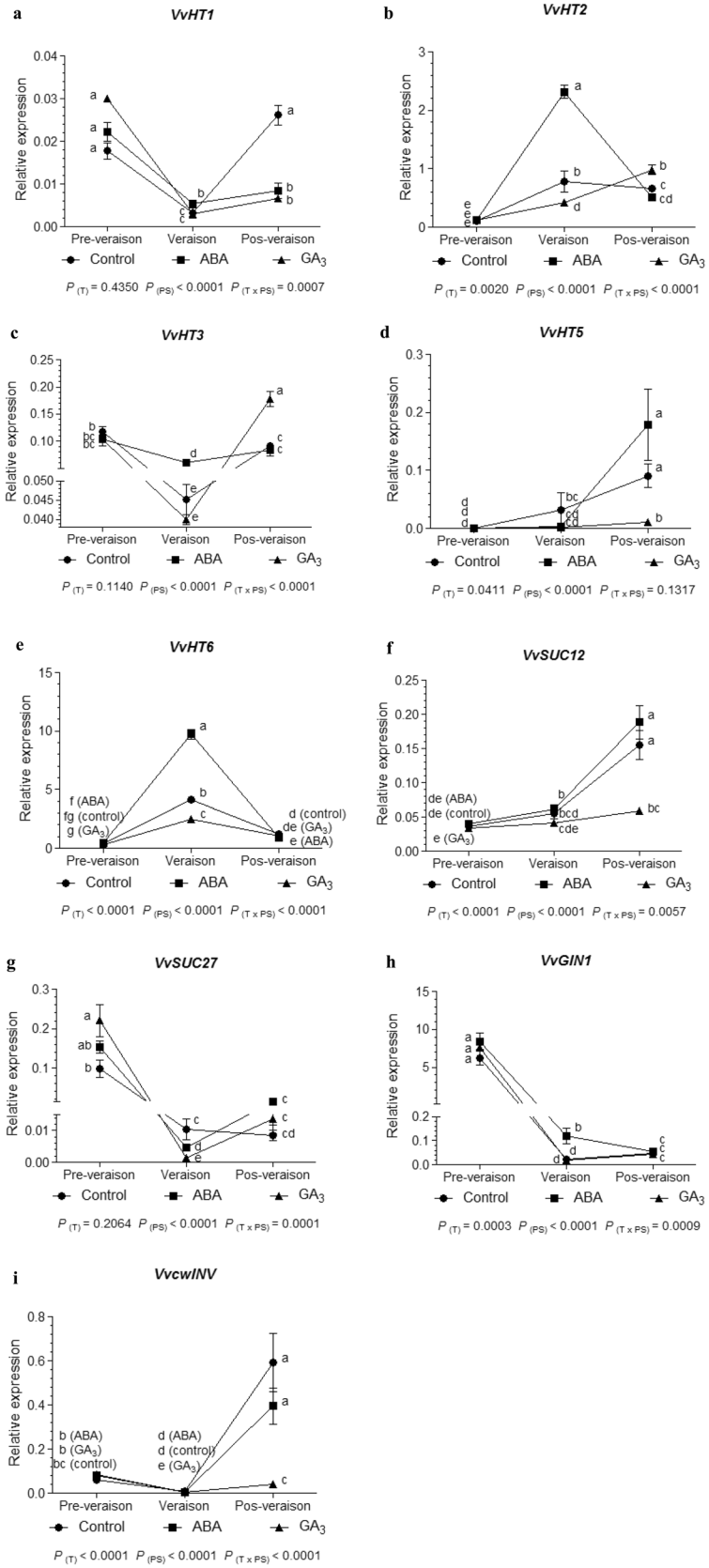

Fig. 3 Effect of ABA and GA_3 on gene expression of sugar transporters and invertases in berries of *Vitis vinifera* cv. Malbec during phenological stages (pre-veraison, veraison and pos-veraison). Hexose transporters: *VvHT1* (**a**), *VvHT2* (**b**), *VvHT3* (**c**), *VvHT5* (**d**), *VvHT6* (**e**). Sucrose transporters: *VvSUC12* (**f**), *VvSUC27* (**g**). Invertases: *VvGIN1* (**h**), *VvcwINV* (**i**). Values are means ± SE, n = 5. Different letters indicate statistically significant differences ($p < 0.05$). All values were normalized to the expression of *VvEF 1-α*. $P_{(T)}$, treatment effect; $P_{(PS)}$, phenological stage effect; $P_{(T \times PS)}$, treatment x phenological stage interaction effect

the largest abundant transcript in pre-veraison (Fig. 1a). On the other hand, *VvcwINV*, encoding for a cell wall invertase, was mainly expressed in veraison (Figs. 1b, 2i). In Cabernet Sauvignon, *VvHT1* and *VvHT3* were the most abundant hexose transporters both in young and mature leaves (Hayes et al. 2007), whereas in pot-grown Chardonnay vines were *VvHT3* and *VvHT5* (Afoufa-Bastien et al. 2010). The expression of *VvSUC12* in Malbec leaves obtained in this paper was in coincidence with that obtained by Afoufa-Bastien et al. (2010) in pot-grown Chardonnay mature leaves, even though the pot-grown Chardonnay plants had no berries (Afoufa-Bastien et al. 2010). In this regard, as far as we know this is the first report that shows the expression pattern of the main sugar transporters in mature leaves during berry development.

In control berries, the genes *VvHT2, VvHT3* and *VvHT6* were the most expressed regarding hexose transporters, being the transcript of *VvHT6* the most abundant throughout the entire berry development (Fig. 1). In relation to sucrose transporters, *VvSUC27* was mainly expressed in pre-veraison, whereas *VvSUC12* in veraison and pos-veraison (Fig. 1). Zhang et al. (2006) identified a shift from symplastic unloading (via plasmodesmata) to apoplastic unloading (via sugar transporters) in berries at the onset of ripening (veraison), showing that the activity of the VvcwINV was highly increased from that point on, providing further evidence for an apoplastic unloading at the onset of ripening. In this sense, we observed that the expression of a vacuolar invertase, *VvGIN1*, was higher in pre-veraison (Fig. 1a), whereas the transcript abundance of *VvcwINV* was higher in pos-veraison (Fig. 1c).

Comparison of gene expression pattern between leaves and berries in grapevine cv. Malbec

Figure 1 shows the comparison of gene expression of sugar transporters and invertases between leaves and berries in pre-veraison (Fig. 1a), veraison (Fig. 1b) and pos-veraison (Fig. 1c). In the first phenological stage, among the 9 genes analyzed only the transcripts *VvHT1* and *VvHT5* were more expressed in leaves than in berries. Meanwhile, the genes *VvHT2, VvHT6, VvSUC27* and *VvGIN1* were more expressed in berries than in leaves. There were no significant differences among *VvHT3, VvSUC12* and *VvcwINV* in both leaves and berries (Fig. 1a). In veraison, the genes *VvHT1, VvHT3, VvHT5, VvSUC12, VvSUC27* and *VvcwINV* were more expressed in leaves. On the other hand, the transcripts *VvHT2, VvHT6* and *VvGIN1* were more abundant in berries (Fig. 1b). In pos-veraison, the genes *VvHT1, VvHT5* and *VvSUC27* were highly expressed in leaves, and the transcripts *VvHT2, VvHT6, VvSUC12, VvGIN1* and *VvcwINV* were bigger in berries. But, there were no significant differences in the relative expression of *VvHT3* between both organs (Fig. 1c).

According to the results described above, the genes that encode for *VvHT1* and *VvHT5* are more expressed in leaves than in berries during the entire berry development (Fig. 1). In relation to this, Hayes et al. (2007) observed in Cabernet Sauvignon leaves that the transcripts *VvHT1, VvHT3* and *VvHT5* increased sharply in developing leaves (during sink/source development), finding the higher expression in mature leaves. Furthermore, the authors stated that such behavior may imply an increasing capacity in the apoplastic hexose retrieval when leaves become source organs, so allowing redistribution along the phloem. Accordingly, Vignault et al. (2005) showed that VvHT1 is localized to phloem-associated cells in grape leaves. In addition, we have observed that *VvHT3* is only highly expressed in veraison, coincident with the major bulk flow from leaves to berries.

In relation to sucrose transporters, *VvSUC12* and *VvSUC27* were more expressed in leaves only at veraison (Fig. 1), suggesting that at the onset of ripening an apoplastic phloem loading mechanism is active in addition to the symplastic phloem loading, the most important mechanism for sugar transport in woody plants (Slewinski et al. 2013). In this regard, it would help to transport a higher content of photoassimilates from leaves to berries to improve ripening. Also, the expression of a gene encoding a cell wall invertase (*VvcwINV*) is higher in leaves but only at veraison (Fig. 1). This finding is coincident with the high expression of *VvHT1* and *VvHT3* at that phenological stage (Fig. 1b), providing further evidence in the activity of hexose transporters retrieving monosaccharides for their redistribution along the phloem path.

In berries, the genes *VvHT2* and *VvHT6*, which encode for a purportedly tonoplast hexose transporters (Çakir and Giachino 2012; Lecourieux et al. 2014), seem to be the most important transporters for sugar accumulation at all stages of berry development, with higher expression at the onset of ripening (Figs. 1b, 3b, e). These results suggest that both hexose transporters are more related to phloem unloading in sink organs than to phloem loading in source organs. This may contribute to mobilize a higher content of carbohydrates from leaves to berries, reinforcing the sink strength of fruits at the onset of ripening. Moreover, high expression of the vacuolar invertase *VvGIN1* in pre-veraison (Figs. 1a, 3 h) and the significant increment of the apoplast invertase *VvcwINV* in pos-veraison (Figs. 1c, 3e) were in coincidence with the shift from symplastic to apoplastic phloem unloading in berries (Zhang et al. 2006). Additionally, *VvGIN1* is more linked to sink organs in berry development. This behavior is probably related to the high content of monosaccharides accumulated in grapevine berries from veraison onwards. Furthermore, in pre-veraison the activity of a vacuole invertase may be necessary to sustain berry growth.

In this sense, VvGIN1 may hydrolyze sucrose into hexoses to be used as source of energy and carbon skeletons. The sucrose transporters *VvSUC27* and *VvSUC12* were more abundant in berries than in leaves in pre- and pos-veraison, respectively (Fig. 1a, c). In this sense, they could be also involved in sugar uptake at these phenological moments. Pastenes et al. (2014) showed that *VvSUC12* increased its expression after grape berries reached their maximum sugar content, suggesting that it may be involved in sugar mobilization within the berry.

ABA and GA_3 treatments modify the gene expression of sugar transporters in leaves and berries of grapevine cv. Malbec

Figures 2 and 3 show the effect of ABA and GA_3 on gene expression during berry development in leaves and berries, respectively. In control leaves, as it was described above, the genes *VvHT1* (Fig. 2a), *VvHT3* (Fig. 2c), *VvSUC12* (Fig. 2f), *VvSUC27* (Fig. 2g) and *VvcwINV* (Fig. 2i) presented their maximum levels at veraison. On the other hand, the transcripts *VvHT2* (Fig. 2b) and *VvHT5* (Fig. 2d) showed their maximum level in pos-veraison, whereas *VvGIN1* (Fig. 2h) in pre-veraison. Also, at this stage in leaves, ABA sprays induced an up-regulation of *VvHT1, VvHT2, VvHT6, VvSUC12, VvSUC27* and *VvcwINV* (Fig. 2). Furthermore, ABA up-regulated *VvHT1* and *VvGIN1* expression in veraison and *VvSUC27* in pos-veraison. On the contrary, leaves of ABA-treated plants presented a down-regulation of *VvHT3, VvHT5, VvSUC12* and *VvSUC27* expression at veraison (Fig. 2). That is, in leaves, from a total of 9 genes ABA treatment up-regulated the expression of 6 in pre-veraison, but only 2 in veraison, which suggests that ABA regulates positively the apoplastic phloem loading before the onset of ripening (Fig. S1a). Moreover, we have demonstrated that ABA, and also GA_3 improve the symplastic phloem loading in veraison increasing sucrose content and phloem area in leaves (Murcia et al. 2016). In addition, we have previously shown that ABA-treated plants exhibited a transient decrease in net photosynthesis by a temporal depletion of stomatal conductance. In relation to that, we found that ABA applications reduced the content of hexoses in leaves (Murcia et al. 2016). Thus, an independent regulation by carbohydrates on gene expression instead of ABA should not be ruled out. All these results with grapevine plants of the cv. Malbec suggest that ABA may improve sugar transport (in a direct or indirect way) from source to sink organs modifying both apoplastic and symplastic phloem loading during ripening (Fig. S1). On the other hand, GA_3 applications up-regulated 5 of total 9 genes expressed in pre-veraison leaves: *VvHT1, VvHT5, VvSUC12, VvSUC27* and *VvcwINV*, whereas down-regulated the relative expression of *VvGIN1* (Fig. 2). In veraison, GA_3-treated leaves showed lower levels of *VvHT3, VvHT5* and *VvSUC27* transcripts. Moreover, in pos-veraison, GA_3 treatment increased the expression of *VvcwINV* (Fig. 2). According to these results, GA_3, as ABA, may be regulating positively the apoplastic phloem loading in pre-veraison (Fig. S1a).

In berries, the genes *VvHT1* and *VvHT3* showed their maximum expression in pre- and pos-veraison and their minimum at veraison (Fig. 3a, c). Contrary, the levels of the transcripts *VvHT2* and *VvHT6* were higher in veraison (Fig. 3b, e). Regarding the genes *VvSUC27* and *VvGIN1*, their expression was higher in pre-veraison then decreased towards pos-veraison (Fig. 3g, h). On the other hand, the levels of the transcripts *VvHT5, VvSUC12* and *VvcwINV* increased from veraison onwards (Fig. 3d, f, i). The overall expression pattern of genes encoding for hexose and sucrose transporters presented in this work, are supported by the partial results observed in Cabernet Sauvignon (Hayes et al. 2007), field-grown Chardonnay berries (Afoufa-Bastien et al. 2010) and Carmenere berries (Pastenes et al. 2014). ABA sprays increased the expression of *VvHT1, VvHT2, VvHT3, VvHT6* and *VvGIN1* at veraison (Fig. 3a–c, e, h). Likewise, ABA down-regulated the expression of *VvHT1* and *VvHT6* at pos-veraison (Fig. 3a, e). Keller (2010) stated that since veraison until full ripening, monosaccharides obtained from sucrose hydrolysis in berry apoplast get into mesocarp cells and accumulate in vacuoles via hexose transporters. Moreover, the decrease of sugars in the berry apoplast induces a decrease of osmotic and turgor pressures in berry phloem which sustains the long-distance sucrose transport from leaves to berries. In this sense, ABA treatment may improve apoplastic unloading via *VHT2, VvHT3* and *VvHT6* favoring sucrose transport and accumulation of sugars in berries at the onset of ripening (Fig. S1b). Hayes et al. (2010) and Medici et al. (2014) working with leaves of Chardonnay and Ugni blanc cultivars found out that *VvHT5* is controlled by ABA in response to biotrophic fungal infection and water-deficit stress, respectively. Also, they showed that ABA-induced expression of *VvHT5* was mediated through ABRE motifs. In the present work, it was observed that ABA did not modify the expression of *VvHT5* in berries. This difference might be due to varietal differences and/or experimental conditions. Contrary to the up-regulation of hexose transporters, we have not found an increment of the hexose content in ABA-treated berries during berry ripening (Murcia et al. 2016). This might be due to high carbohydrates consumption by an up-regulation of secondary metabolites biosynthesis in ABA-treated berries (Murcia et al. 2017).

GA_3 up-regulated the expression of *VvSUC27* at pre-veraison (Fig. 3g) and the expression of *VvHT2* and *VvHT3* at pos-veraison (Fig. 3b, c). Moreover, GA_3-treated berries showed lower expression levels of *VvHT2, VvHT6, VvSUC27* and *VvcwINV* in veraison (Fig. 3b, e, g, i). Also,

this phytohormone down-regulated the expression of *VvHT1, VvHT5, VvSUC12* and *VvcwINV* in pos-veraison (Fig. 3a, d, f, i). According to these results, GA_3 showed different patterns of expression in relation to ABA regarding the genes *VvHT2, VvHT3* and *VvHT6*. The down-regulation of *VvHT2* and *VvHT6* in veraison by GA_3 treatment may be related to a delay in ripening berries of GA_3-treated plants observed in our previous work (Murcia et al. 2016) (Fig. S1b). Likewise, the up-regulation of *VvHT2* and *VvHT3* in pos-veraison was consistent with an increase in glucose and fructose concentration observed at full ripening (Murcia et al. 2016) (Fig. S1c).

Overall, according to this work, applications with ABA and GA_3 to grapevines cv. Malbec may improve the long distance transport of photoassimilates from leaves to fruits by an up-regulation of sugar transporters at different phenological stages.

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