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Carbohydrate metabolism in grape cultivars that differ in sucrose accumulation

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Summary

Sugar concentrations and sucrose-metabolism related enzyme activities in berries and leaves were investigated during berry development using grape cultivars with different sucrose concentrations. Sucrose concentration was significantly negatively related to acid invertase activity in berries. Acid invertase showed the lowest activities in berries of high-sucrose cultivars, 'Honey Juice' and 'B180', and the highest in trace-sucrose cultivars, 'Concord', 'Jingxiu', and 'Jingya'. Acid invertase activities in berries of low-sucrose cultivar 'Canadice' were between high- and trace-sucrose cultivars. There was no significant difference in glucose and fructose concentrations, the activities of neutral invertase, sucrose synthase and sucrose phosphate synthase in berries among high-, low- and trace-sucrose cultivars as acid invertase. Sugar concentrations and sucrose-metabolism related enzymes activities in leaves also did not show such difference among all cultivars. The results suggest that differences in sucrose concentration in berries among grape cultivars mainly be due to acid invertase activity. In addition, the final sucrose concentration in berries at maturity for a grape cultivar might be decided at véraison, and véraison is the key period for sucrose accumulation.

K e y w o r d s : grape, sucrose accumulation, acid invertase, sucrose phosphate synthase, sucrose synthase

Introduction

The levels of sugars are important factors in determining fruit quality. The main sugars in grape berry, glucose and fructose, are presented with similar concentrations, and sucrose is present at trace amounts (Kliewer 1965). According to some reports, glucose and fructose concentrations ranged from 45.9 to 131.0 mg·mL^{-1}, and sucrose generally accounts for less than 2.0 % of total sugars (Shiraishi 1993, Liu *et al.* 2006). However, a few cultivars were detected with high amount of sucrose, e.g. Lott and Barrett (1967) studied eight cultivars from *Vitis. Labrusca* × *V. vinifera*, and found sucrose accounted for 15.2 % ('Bath') to 32.6 % ('Sweet Blue') of total sugars; Carroll *et al.* (1971) and Shiraishi (1993) found sucrose in seven cultivars of *V. rotundifoli* accounted for 18.5 % ('Magoon') to 32.7 % ('Hunt'). In a previous report on 98 grape cultivars, including 45 table grapes from *V. labrusca* × *V. vinifera*, 30 table grapes and 18 wine grapes of *V. vinifera*, 3 wine grapes from *V. vinifera* × *V. amurensis*, 1 juice grape from *V. vinifera* × *V. thunbergii*, and 1 juice grape of *V. labrusca*, we noted that sucrose concentration was less than 1 mg·mL^{-1} or non-detectable in 74 cultivars. In another 22 cultivars sucrose concentration ranged from 1.0 to 8.0 mg·mL^{-1}, and 'Honey Juice' and 'B180' (*V. labrusca* × *V. vinifera*) had 27.1 and 49.7 mg·mL^{-1} of sucrose, accounting for 16.5 % and 25.3 % of total soluble sugars.

These observations indicate that some grape cultivars behave differently with respect to production and/or utilization of sucrose. Sucrose accumulation in grape berry depends on several factors: metabolism in leaves, transport in phloem, and metabolism in berry. Sucrose may be cleaved into glucose and fructose by acid invertase (AI, EC 3.2.1.26) and neutral invertase (NI, EC 3.2.1.26), or produce UDP-glucose and fructose by sucrose synthase (SS, EC 2.4.1.13). UDP-glucose and fructose-6-phosphate can be synthesized sucrose-6-phosphate by sucrose phosphate synthase (SPS, EC 2.4.1.14) (Fernie *et al.* 2002). Sugar accumulation and related enzymes have been widely studied in grape berry. Generally, AI activity was found to be low at flowering, but increased during berry development and was high in mature berries, which might result in low sucrose levels in mature grape berries (Hawker 1969 b, Davies and Robinson 1996, Xie *et al*. 2009). SS and SPS activity changed slightly with berry development (Xie *et al*. 2009). There seemed to be no significant correlation neither between hexose concentrations and SS activity, nor between hexose concentrations and SPS activity (Xie *et al*. 2009). These studies on sucrose metabolism in grape berries were based on plant materials with low level of sucrose, such as 'Shiraz', 'Riesling' and 'Kyoho', being lower than 1.0 mg·g^{-1} FW (Davies and Robinson 1996, Xie *et al*. 2009). Takayanagi and Yokotsuka (1997) studied sugar accumulation and sucrose-metabolizing enzyme activity in flesh of 'Muscat Bailey A' with 17.5 mg·g^{-1} FW of sucrose and 'Steuben' with 41.3 mg·g^{-1} FW of sucrose, and found that AI activity was significantly higher in low-sucrose cultivar 'Muscat Bailey A' than that in high-sucrose cultivar 'Steuben', suggesting that the higher proportion of sucrose in 'Steuben' grapes is associated with a lower AI activity. However, little is known on the effect of leaf sugar accumulation and metabolism on berry sugar concentrations. The present study was carried out to further gain insight into the

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mechanism(s) regulating sucrose accumulation in grape. Grape cultivars with different sucrose concentration were chosen: high-sucrose grape cultivars 'B180' and 'Honey Juice', with 27.1 and 49.7 mg·mL^{-1} of sucrose, respectively; low-sucrose 'Canadice' with 2.2 mg·mL^{-1} of sucrose; trace-sucrose 'Concord', 'Jingxiu', and 'Jingya' with less than 0.08 mg mL^{-1} of sucrose, according to the previous study on 98 grape cultivars (Liu *et al.* 2006). Seasonal variation in sugars concentrations and related enzyme activities (AI, NI, SPS, SS) were detected in both grape berries and leaves to investigate the biochemical background that may be responsible for differences in sucrose accumulation, and the relationship of sucrose accumulation and metabolism in leaf with those in berry.

Material and Methods

Plant material and sample preparation: High-sucrose grape cultivars 'B180' and 'Honey Juice' with 27.1 and 49.7 mg·mL^{-1} of sucrose, low-sucrose 'Canadice' with 2.2 mg·mL^{-1} of sucrose, trace-sucrose 'Concord', 'Jingxiu', and 'Jingya' with lower than 0.1 mg·mL^{-1} of sucrose were used in this study. All the cultivars were planted in the spring of 1993 in the experimental vineyard of the Institute of Botany, the Chinese Academy of Sciences located in Beijing. The vines, trained to single-hand fence trellis with 1.7 m high of hedge, were spaced 1.5 m apart within the row and 2.5 m apart between rows, with north-south row orientation. The vines are under the same cultivation conditions, such as irrigation, fertilization, soil management, pruning and disease control.

From 25 d after anthesis (DAA) onwards, 15-20 berries from each of three clusters as one replication, were harvested every two weeks during the 2004 growing season. Meanwhile, 4 leaves nearby the clusters were harvested. The berry maturity date was based on the seed color changing to dark brown without senescence of berry tissue, and on the previous maturity date record.

The berries were taken to the laboratory immediately. Some berries of each replication were squeezed into grape juice using a manual squeezer. The grape juice was centrifuged at 5,000 × g for 6 min, and the supernatant was stored at -80 °C until sugar measurement. After seeds were removed from the other berries of each replication, the tissues were frozen and powered in liquid nitrogen, and then stored at -80 °C for enzyme activities measurement. Leaves without veins were immersed in liquid nitrogen and powdered, and then stored at -80 °C until sugar and enzyme activities measurement.

The extraction of soluble sugars in grape leaves: Two g of frozen powder of leaves were mixed with 12 mL ethanol/water (4:1 v/v), homogenized and incubated at 80 °C for 10 min. After incubation, the solution was centrifuged at 15,000 × g for 6 min, and the supernatant was recovered. A second extraction was run for the residue with 5 mL ethanol/water (4:1 v/v) under the same condition, and then the supernatant from two extractions was mixed. The supernatant was dried at 80 °C, and re-dissolved in 4 mL water. The new solution was centrifuged at 5,000 × g for 6 min again, and the supernatant was stored at -40 °C for later analysis of sugars.

Sugar and acid analysis: Soluble sugars were analyzed using a Dionex P680 HPLC system, according to Liu *et al*. (2006). Soluble sugars concentrations were determined through external standard solution calibrations (sucrose, glucose, fructose, Sigma Chemical Co.), and expressed as mg·mL^{-1} of juice and mg·g^{-1} FW of leaf.

Enzyme extraction: All enzyme extraction operations were performed at 4 °C. The frozen powders of grape berry and leaf were ground in a mortar with 8 mL buffer containing 50 mmol·L^{-1} Hepes-NaOH (pH 7.5), 10 mmol·L^{-1} $MgCl_2$, 1 mmol·L^{-1} EDTA, 2.5 mmol·L^{-1} DTT, 0.05 % (w/v) Triton X-100 and 0.1 % (w/v) BSA. The solution was centrifuged at 15,000 × g for 10 min. The supernatant was dialyzed immediately against a tenfold volume of diluted extraction buffer (except for Triton X-100) for 24 h at 2 °C, and the dialyzate was changed once. The dialyzed enzyme extracts were then assayed.

Enzyme assays: SPS activity was measured according to Rufty and Huber (1983) with slight modifications. The assay mixture (140 µL) contained 50 mmol·L^{-1} Hepes-NaOH (pH 7.5), 10 mmol·L^{-1} $MgCl_2$, 1 mmol·L^{-1} EDTA, 2.5 mmol·L^{-1} DTT, 3 mmol·L^{-1} Uridine Diphosphate Glucose, 4 mmol·L^{-1} fructose-6-phosphate and 70 µL enzyme extract. Mixtures were incubated at 37 °C for 40 min, and reactions were terminated by addition of 70 µL 1 N NaOH. After boiling for 10 min, 0.25 mL of 0.1 % (v/v) resorcinol in 95 % ethanol and 0.75 mL of 30 % HCl were added to the assay mixture, and incubated at 80 °C for 8 min. Sucrose and sucrose-6-phosphate content was determined by color development at 520 nm.

SS activity was assayed by the same method as SPS, except that fructose replaced fructose-6-phosphate. SS and SPS activity were experessed by µmol sucrose-6-phosphate per gram fresh weight per hour (µmol sucrose g^{-1} FW h^{-1}).

AI and NI activity were measured according to Merlo and Passera (1991) with slight modifications. The assay mixture (1 mL) contained 0.1 mol·L^{-1} sodium acetate/acetic acid (pH 4.8), 0.1 mol·L^{-1} sucrose and 0.1 mL enzyme extract. The assay mixture for NI was the same, except that 0.1 mol·L^{-1} KH_2PO_4 aqueous/0.1 mol·L^{-1} Tri-sodium citrate-Citric Acid (pH 7.2) replaced NaAe/HAc. Mixtures were incubated at 37 °C for 40 min, followed by addition of 1 mL 3,5 - Dinitro salicylic acid to terminate reaction, and then boiled for 5 min. The reducing sugars released from sucrose were determined according to Miller (1959). AI and NI activity were expressed by liberated glucose per gram fresh weight per h (µmol glucose g^{-1} FW h^{-1}).

Statistical analysis: Glucose-to-fructose ratio and α ratio (glucose/(fructose+sucrose)) in berries were calculated, as Shiraishi *et al*. (1993) proposed they are useful descriptors for the evaluation of sugar composition in grape berries. Glucose-to-fructose ratio in leaves was also calculated. An analysis of variance was applied to study the effects of cultivar and DAA, and their two-way interactions. Moreover, an analysis of variance was applied on each of the sampling dates with cultivar as a factor. All

analyses of variance were run using the S-Plus function 'aov'.

Results

Dynamic changes in sugars in grape berries and leaves: The statistical analyses were summarized in Tab. 1. Sucrose, glucose, fructose, glucose-to-fructose ratio in both berries and leaves, and α ratio in berries were significantly influenced by cultivar and DAA. Moreover, there were significant interactions between cultivar and DAA.

Cultivar had significant effects on sucrose concentration on each harvest date. Sucrose concentrations in berries at 25 DAA and 40 DAA were very low, about 0-1.6 mg·mL^{-1} in all the six cultivars (Fig. 1). 'Honey Juice' had significantly higher sucrose concentration (1.6 mg·mL^{-1}) at 40 DAA than the other five cultivars (0-0.7 mg·mL^{-1}). Then sucrose concentration drastically increased to about 25.0 mg·mL^{-1} in berries of 'Honey Juice' and 'B180' at 55 and 68 DAA, respectively, and constantly increased to 30.3-39.0 mg·l^{-1} until berry maturity, accounting for 25.5-26.6 % of total sugars. Sucrose concentrations in berries in the other three cultivars 'Concord', 'Jingxiu', and 'Jingya' were always lower than 3.1 mg·mL^{-1}, accounting for less than 1.9 % of total sugars, and did not show significant difference among the three cultivars on each harvest date except at 25 DAA. At berry maturity, sucrose level in 'Canadice' was higher (5.0 mg·mL^{-1}) than that in the above three cultivars, accounting for 3.4 % of total sugars.

Glucose and fructose concentrations in berries had similar trends, and constantly increased with berry development from 0.2-4.7 to 42.9-76.8 mg·mL^{-1} at berry maturity, while showing a sharp increase at 40 DAA for 'Canadice' and 'Jingxiu', 55 DAA for 'Honey Juice' and 'Jingya', and 68 DAA for 'B180' and 'Concord' (Fig. 1). At berry maturity, they totally accounted for 98.2-99.3 % of total sugars in 'Concord', 'Jingxiu', and 'Jingya', for 96.7 % in 'Canadice', while for 74.7-83.5 % in 'Honey Juice' and 'B180'. Glucose-to-fructose ratio showed a few high values at early berry development, at 25 and 40 DAA in 'B180' (8.9 and 8.2), at 25 DAA in 'Concord', 'Jingya'

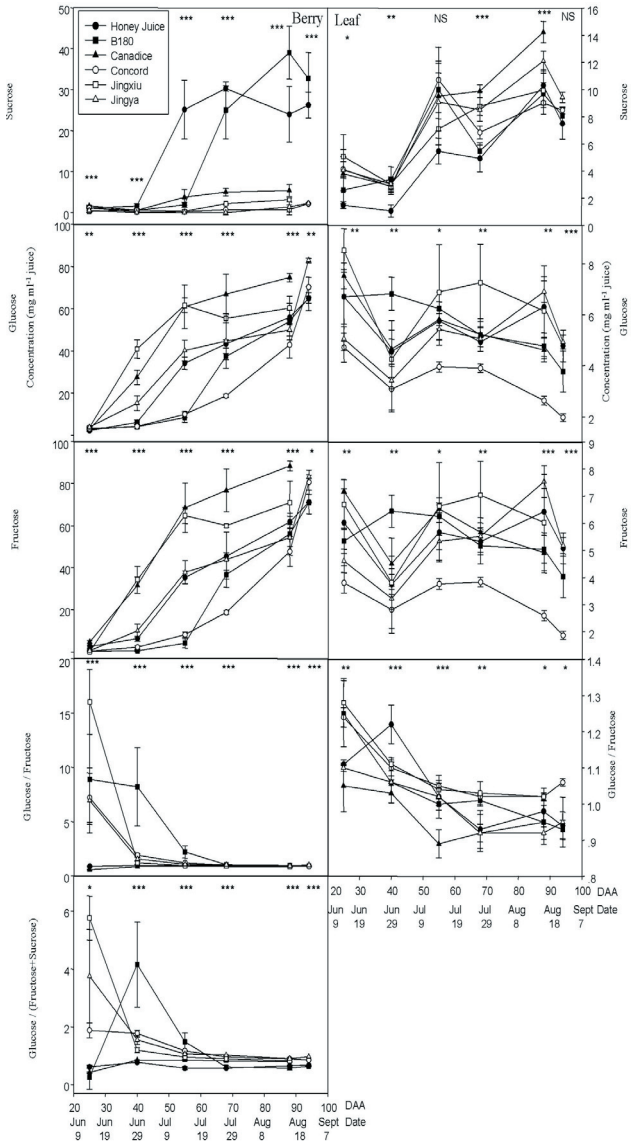

Fig. 1: Seasonal variation for sugar concentrations, glucose-to-fructose ratio and glucose/(fructose + sucrose) ratio (α ratio) in grape berries (left) and leaves (right). Bars represented standard deviation. Differences among cultivars were either non-significant (NS) or significant at $P < 0.05$ (*), $P < 0.01$ (**) or $P < 0.001$ (***) on each harvest date via analysis of variance.

Table 1

Analysis of variance for sugar concentrations, glucose-to-fructose ratio and α ratio (glucose/(fructose + sucrose)) in grape berries and leaves

Factors	Df	Sucrose	Glucose	Fructose	Glucose/ Fructose	Glucose/ (Fructose + Sucrose)
Berry						
Cultivar	5	162.7***	60.0***	67.8***	17.5***	4.1**
DAA	5	74.2***	530.9***	543.5***	54.1***	6.3***
Cultivar × DAA	23	27.1***	13.9***	12.3***	12.9***	3.4***
Leaf						
Cultivar	5	13.5***	25.6***	29.2***	23.7***	
DAA	5	133.4***	15.1***	12.1***	78.2***	
Cultivar × DAA	23	3.5***	3.3***	4.1***	4.3***	

** and *** represented that the effect of factor was significant at $P < 0.01$ and $P < 0.001$, respectively.

and 'Jingxiu' (7.2, 6.9 and 16.0). Except these cases, glucose-to-fructose ratio ranged from 0.6 to 2.2, especially at late berry development (from 68 DAA to maturity) it ranged from 0.9 to 1.0 for all the cultivars.

Except that α ratios in berries of 'B180' were higher (4.2 and 1.9, respectively) than the other five cultivars (0.8-1.8 and 0.6-1.7, respectively) at 40 DAA and 55 DAA, generally α ratios in berries of 'Honey Juice' and 'B180' were lower than in berries of the other four cultivars. At 25 DAA, α ratios in berries of 'Honey Juice', 'B180' as well as 'Canadice' (0.4-0.6) were lower than in berries of 'Concord', 'Jingxiu', and 'Jingya' (1.9-5.8). From 68 DAA to berry maturity, α ratio in berries of 'Honey Juice' and 'B180' (0.6-0.7) were always lower than in berries of 'Canadice' (0.8), followed by cultivars 'Concord', 'Jingxiu', and 'Jingya' (0.8-1.0).

Sucrose concentrations in grape leaves did not show such difference as in grape berries among cultivars. They were low in June, ranging 1.5-5.0 $mg \cdot g^{-1}$, and high in July and August, ranging 4.9-14.2 $mg \cdot g^{-1}$. In late-August, sucrose concentrations were lower than those in Aug 17 (DAA 88), which might be due to leaves senescense and reduced photosynthesis. Glucose and fructose concentrations in leaves basically share the same tendency for each cultivar. Glucose and fructose concentrations in leaves of 'Concord' generally decreased, and were lower (0.9-3.5 $mg \cdot g^{-1}$) than the other five cultivars (3.7-8.5 $mg \cdot g^{-1}$). Though cultivar had a significant effect on glucose-to-fructose ratio in leaves on each harvest date, glucose-to-fructose ratio was around 1.0 (0.9-1.3) for all the cultivars during berry development.

Sucrose-metabolizing enzyme activity in berries and leaves: Via an analysis of variance, AI and NI activities in berries, and AI activity in leaves were significantly influenced by cultivar, while cultivar had no significant effect on SPS and SS activities in both berries and leaves, and NI activity in leaves (Tab. 2). DAA significantly influenced all the enzyme activities, except SS activity in leaves. The interactions between cultivar and DAA were significant for AI and NI activities in berries, and SS activity in leaves.

AI, NI and SS are responsible for sucrose degradation (Hawker 1985, Fernie *et al.* 2002). AI activity in berries was significantly different between cultivars with berry development (Fig. 2). At 25 DAA, AI activities in berries of 'Concord' and 'Jingya' (62.3-63.2 μmol fructose·g^{-1} FW·h^{-1}) were significantly higher than those of the other cultivars. From 40 DAA, AI activities in berries of 'Jingxiu' started to increase together with 'Concord' and 'Jingya', and at berry maturity ranged from 161.5-260.3 μmol fructose·g^{-1} FW·h^{-1} in berries of these three cultivars. AI activity in berries of 'Canadice' was substantially lower than 'Concord', 'Jingya' and 'Jingxiu', and gradually increased

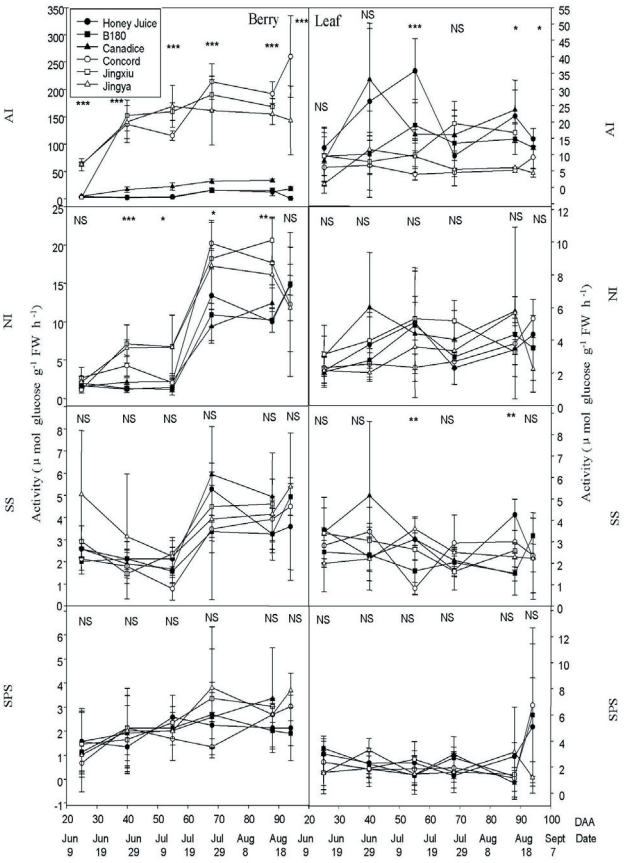

Fig. 2: Seasonal variation for activities of acid invertase (AI), neutral invertase (NI), sucrose phosphate synthase (SPS), and sucrose synthase (SS) activities in grape berries (left) and leaves (right). Bars represented standard deviation. Differences among cultivars were either non-significant (NS) or significant at $P < 0.05$ (*), $P < 0.01$ (**) or $P < 0.001$ (***) on each harvest date via analysis of variance.

Table 2

Analysis of variance for enzyme activities in grape berries and leaves

Factors	Df	AI	NI	SPS	SS
Berry					
Cultivar	5	144.4***	8.1***	0.8ns	1.4ns
DAA	5	25.6***	69.8***	4.5**	12.2***
Cultivar × DAA	23	6.0***	1.8*	0.6ns	0.8ns
Leaf					
Cultivar	5	10.5***	1.8ns	1.0ns	1.1ns
DAA	5	3.0*	2.8*	3.9**	1.5ns
Cultivar × DAA	23	1.7ns	1.0ns	0.9ns	1.8*

NS, *, ** and *** represented that the effect of factor was non-significant, significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.

from 4.6 μmol fructose·g^{-1} FW·h^{-1} at 25 DAA to 33.5 μmol fructose·g^{-1} FW·h^{-1} at berry maturity. Further, AI activity in berries of 'B180' and 'Honey Juice' always kept a much lower level, 2.5 to 14.9 μmol fructose·g^{-1} FW·h^{-1}, and was about 50 % of those of 'Canadice' during berry development. NI activity in berries tended to increase for all the six cultivars, though it showed a decrease at 88 and 94 DAA for 'Concord' and 'Jingya'. NI activities in berries of 'Canadice', 'Honey Juice' and 'B180' were lower than those of 'Concord', 'Jingya' and 'Jingxiu' at 55, 68 and 88 DAA. However, the highest level of NI activity in berries of all the cultivars was 20.6 μmol fructose·g^{-1} FW·h^{-1}, only accounting for about 12.2 % of AI at the same harvest date. SS activity in berries fluctuated with the berry development and did not show significant differences among cultivars with berry development. It was even lower than NI, ranging from 0.8-5.9 μmol fructose·g^{-1} FW·h^{-1} (Fig. 2). It was relatively high (2.0-5.0 μmol fructose g^{-1} FW h^{-1}) at 25 DAA, attained its minimum level at 55 DAA, and then maximum level again at 68 DAA, 3.4-5.9 μmol fructose·g^{-1} FW·h^{-1}.

SPS mainly synthesized sucrose (Geigenberger and Stitt 1991). During the berry development, SPS activity was also low, ranging from 0.7 to 3.8 μmol fructose·g^{-1} FW·h^{-1} (Fig. 2). In berries it had no significant difference among cultivars on each harvest date. SPS activity in berries tended to increase at early berry development for all the six cultivars, however, after véraison it kept steady for 'Honey Juice' and 'B180', and continued to slightly increase for 'Canadice', 'Concord', 'Jingya' and 'Jingxiu'.

In leaves, AI activity generally was higher than NI, SS and SPS as in berries. Except a sharp increase (33.0 and 35.6 μmol fructose·g^{-1} FW·h^{-1}) on June 29th and July 14th (40 and 55 DAA) for 'Canadice' and 'Honey Juice', it ranged from 8.2-26.3 μmol fructose·g^{-1} FW·h^{-1} during the season and 9.7-16.1 μmol fructose·g^{-1} FW·h^{-1} at berry maturity. It tended to be lower in 'Concord' and 'Jingya' in late season. However, generally AI activity in leaves fluctuated and did not show certain trends during the season. Also, there was no significant difference in AI activity in leaves among cultivars, except on July 14th and Aug 17th (55 DAA and 88 DAA). Another two enzymes in leaves for sucrose degradation varied with time in a narrow range, NI of 2.1-6.7 μmol fructose·g^{-1} FW·h^{-1}, SS of 0.8-5.1 μmol fructose·g^{-1} FW·h^{-1}, not showing significant difference among cultivars either, except SS activity on July 14th and Aug 17th (55 DAA and 88 DAA). SPS, responsible for sucrose synthesis, ranged 0.8-3.2 μmol fructose·g^{-1} FW·h^{-1} in leaves, except that it reached a relatively high level (5.2-6.2 μmol fructose·g^{-1} FW·h^{-1}) for 'Honey Juice' and 'B180', and 'Concord' at berry maturity.

Discussion

Dynamic changes of sugars in berries showed that in high-sucrose cultivars 'Honey Juice' and 'B180', increases in sucrose accumulation occurred at véraison, 55 DAA for 'Honey Juice' and 68 DAA for 'B180', respectively, and low-sucrose cultivars 'Canadice' had a slight increase at its véraison, 55 DAA. However, trace-sucrose cultivars 'Concord', 'Jingya' and 'Jingxiu' did not show obvious variations at véraison, 68 DAA for 'Concord' and 'Jingya', and 55 DAA for 'Jingxiu', and always maintained a very low level of sucrose until berry maturity. It suggested that the final sucrose concentration in berries at maturity for a grape cultivar might be decided at véraison, and véraison is the key period for sucrose accumulation. In addition, the linear relationships between glucose and fructose plus sucrose concentrations (glucose/(fructose + sucrose),α ratio) could be helpful to discriminate between high-, low-, and trace-sucrose cultivars at late berry development, *i.e.* a cultivar with high sucrose concentrations in berries generally had low α ratio, as suggested by Shiraishi *et al*. (1993).

The investigation on sucrose-metabolizing enzymes in grape berries showed that using overall measured values during berry development, AI activity had a significant negative correlation with sucrose concentrations (correlation coefficient r = -0.42***, $P < 0.001$), consistent with that of Takayanagi and Yokotsuka (1997). NI and SS are also involved in sucrose degradation, while they were much lower than AI activity. Moreover, their levels did not obviously differ among cultivars. In tomato, muskmelon, citrus, and mango fruits, it was reported that sucrose accumulation is associated with an increase in SPS activity (Hubbard *et al*. 1989, Schaffer *et al*. 1989, Miron and Schaffer 1991, Dali *et al*. 1992, Komatsu *et al.* 1996, Wei *et al*. 2009). Our study showed that SPS activity in grape berries (0.7-3.8 μmol fructose·g^{-1} FW·h^{-1}) was much lower than that in peach (3.0-3.5 μmol·g^{-1} fresh weight·h^{-1}), muskmelon (7.4-32.0 μmol g^{-1} fresh weight h^{-1}) and tomato (5.0-40.0 μmol g^{-1} fresh weight h^{-1}) that mainly accumulated sucrose (Huber *et al*. 1989, Miron and Schaffer 1991, Vizzotto *et al*. 1996), and it did not exhibit obvious differences between different grape cultivars.

Sucrose is a translocating sugar in grape. The extent of import of translocating sugars to fruits depends on their unloading mechanism which was influenced by the hydrolytic activities of sucrose- and sorbitol-related enzymes and also leaf source (Bantog *et al*. 1999). In peach, sucrose accumulation in flesh related to the import of photosynthate during the late developmental period, however, that process did not affect fruit sucrose metabolism (Chapman *et al*. 1990, Moriguchi *et al*. 1991). In our study, sugar concentrations and sucrose-metabolizing enzymes in leaves had no significant difference among cultivars with different level of sucrose. On one hand, sucrose accumulation in berries seemed not to suppress enzyme activity related to photosynthesis and sugar synthesis in leaves. On the other hand, level of sugars and its metabolism in leaves did not appear to be directly associated with sucrose concentration in berries, while it might influence the amount of sugars transported by phloem, which requires further study.

In addition, hexose did not show obvious difference among cultivars because of sucrose level. Glucose and fructose accumulation also generally occurred at véraison or before véraison (Davies and Robinson 1996), while their concentrations in berries of high-sucrose cultivars 'Honey Juice' and 'B180' did not show difference with low-sucrose cultivars, and were not influenced by less sucrose

cleavage. Lower sucrose hydrolysis rate by AI in berry cell vacuole of 'Honey Juice' and 'B180' did not enhance glucose and fructose accumulation in berries, which was in accordance with the study in lime (Echeverria and Burns 1989), indicating metabolic utilization of sucrose breakdown products, e.g by hexokinase and respiration, might be different.

These results suggest that differences in sucrose concentration among grape cultivars mainly be related to AI activity. Molecular mechanisms of sucrose accumulation have been clarified to some extent. In higher plant such as Arabidopsis, tomato and potato, sucrose-H^+ transporter cDNA were isolated and its protein, responsible for the loading of sucrose via phloem to cells, were identified (Sauer and Stolz 1994, Wiese *et al*. 2000). In grape berries, two putative sucrose transporter genes, which likely facilitate sucrose loading from apoplast to cells, were cloned and expressed (Manning *et al*. 2001). Meanwhile, two grape invertase cDNA, which involved in hexose accumulation, were cloned (Davies and Robinson 1996). However, the expression of the invertases genes and the synthesis of the enzymes were some weeks earlier than the onset of hexose accumulation (Davies and Robinson 1996), which suggests that there may exist other sucrose accumulation mechanisms. It would be interesting to further study sucrose accumulation at the molecular level using grape cultivars with different level of sucrose that we used in this study.

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