Postharvest Biology and Technology 139 (2018) 20–30

Contents lists available at ScienceDirect

Postharvest Biology and Technology

journal homepage: www.elsevier.com/locate/postharvbio

Ethylene regulation of sugar metabolism in climacteric and non-climacteric plums

Macarena Farcuh[a], Rosa M. Rivero[b], Avi Sadka[c], Eduardo Blumwald[a,*]

[a] Dept of Plant Sciences, University of California, Davis, CA 95616, USA
[b] CEBAS, CSIC, Murcia, Spain
[c] Dept of Fruit Tree Sciences, ARO, The Volcani Center, Rishon LeZion, Israel

ARTICLE INFO

Keywords:
Japanese plums
Sugar metabolism
Ethylene
Fruit
Non-climacteric
Ripening

ABSTRACT

We studied the effect of ethylene regulation on sugar metabolism in fruit of two Japanese plum (*Prunus salicina* Lindl.) cultivars, the climacteric Santa Rosa and its non-climacteric bud mutant Sweet Miriam, throughout ripening in postharvest storage. These cultivars share the same genetic background but due to bud mutations differ in their ripening behavior. We examined the responses to ethylene (propylene) and 1-methylcyclopropane (1-MCP) treatments on 11 key sugar metabolism-associated genes by integrating gene expression profiling and their associated sugar contents. Our results demonstrated that ethylene was a crucial factor affecting overall sugar metabolism in both ripening types. More specifically, ethylene reduced sucrose catabolism and induced sucrose biosynthesis but inversely, stimulated sorbitol breakdown and decrease sorbitol biosynthesis. Our analyses indicated that glucose and fructose contents result from sorbitol and sucrose breakdown in climacteric and non-climacteric fruit, respectively. In addition, a positive interaction was observed between ethylene and galactose metabolism; while a negative effect of ethylene was reported on galactinol, raffinose, *myo*-inositol and trehalose, which were higher in non-climacteric Sweet Miriam fruit and could contribute to increased fruit tolerance towards the stress imposed by the ripening process per se and to withstand postharvest storage.

1. Introduction

Ethylene has been considered as the key ripening-related hormone (Burg and Burg, 1965). Fleshy fruit that present an increased respiration rate and a burst of ethylene biosynthesis during ripening are classified as climacteric, whereas fruit that do not, are considered non-climacteric (Bapat et al., 2010; Biale, 1981; Brady, 1987; Giovannoni, 2001). Nevertheless, regardless of their climacteric or non-climacteric behavior, fleshy fruit undergo a complex and highly coordinated series of events comprised in the developmental process of fruit ripening (Grierson, 2013). These ripening-related changes determine the overall final quality of fruit (Bouzayen et al., 2010; Klee and Giovannoni, 2011), including the modification of properties such as color, taste, texture and aroma (Giovannoni, 2004; Kumar et al., 2014; Seymour et al., 2013). Concerning taste, sweetness is of central importance and knowledge of the mechanisms involved in sugar metabolism, which determine fruit sugar content and composition, are of crucial importance to develop cultivars that can meet consumer expectations (Borsani et al., 2009; Desnoues et al., 2014; Singh and Khan, 2010).

In the *Rosaceae* family, which includes Japanese plums, the translocation of the sugar-alcohol sorbitol (Sor) occurs in addition to sucrose (Suc) (Okie and Ramming, 1999). Suc synthesis results from the enzymatic activities of sucrose phosphate synthase (SPS) (Yamaki, 1994), while Suc cleavage reactions are catalyzed by sucrose synthase (SuSy) activity and cell wall, cytosolic and vacuolar invertases (CWINV, CytINV and VINV, respectively) (Klann et al., 1993; Li et al., 2012). Additionally, invertase inhibitors (INVINH) play roles as regulators of invertases at the posttranscriptional level (Jin et al., 2009). Sor synthesis is catalyzed by the enzyme sorbitol-6-phosphate-dehydrogenase (S6PDH) that mediates the reduction of glucose-6-phosphate (G6P) to sorbitol-6-phosphate (Suzuki, 2015; Suzuki and Dandekar, 2014; Teo et al., 2006). Sor breakdown is mediated by the activities of NAD^+-dependent sorbitol dehydrogenase (NAD^+-SDH) and sorbitol oxidase (SOX), which catabolize Sor into fructose (Fru) and glucose (Glu), respectively (Teo et al., 2006). In addition to the major sugars Suc, Sor, Glu and Fru, fruit also contain sugars that are present in significantly lower concentrations, including galactose (Gal), galactinol (Gol), raffinose (Raf), *myo*-inositol (Ino), and trehalose (Tre), among others. Gal synthesis is mediated by the activities of alpha-galactosidase (AGAL), which hydrolases Raf to yield Gal and Suc, and beta-galactosidase

* Corresponding author at: Department of Plant Sciences, University of California, 1 Shields Ave, Davis, CA 95616, USA.
E-mail addresses: mfarcuh@ucdavis.edu (M. Farcuh), rmrivero@cebas.csic.es (R.M. Rivero), vhasadka@volcani.agri.gov.il (A. Sadka), eblumwald@ucdavis.edu (E. Blumwald).

https://doi.org/10.1016/j.postharvbio.2018.01.012
Received 11 October 2017; Received in revised form 3 January 2018; Accepted 14 January 2018
Available online 03 February 2018

(BGAL), by cleaving galactosyl residues from cell wall polysaccharides; while free Gal is phosphorylated into galactose-1-phosphate by galactokinase (GALK) (Hubbard et al., 1989; Sozzi et al., 1998). The synthesis of Gol is mediated by the activity of galactinol synthase (GolS) using UDP-galactose and Ino as substrates (Nishizawa et al., 2008). Gol, together with Suc, are substrates used by raffinose synthase (RS) to synthetize Raf, releasing Ino (Pillet et al., 2012). Finally, Tre catabolism is mediated by trehalase (TRE) (Ponnu et al., 2011).

In our previous work (Farcuh et al., 2017) we identified and characterized key mechanisms associated with sugar metabolism reprogramming during ripening on-the-tree in a non-climacteric bud mutant (Sweet Miriam) of a climacteric Japanese plum cultivar (Santa Rosa). We reported higher contents of Sor and lower contents of Suc, glucose (Glu) and fructose (Fru) in the non-climacteric Sweet Miriam as compared to the climacteric Santa Rosa cultivar. In addition, the content of the minor sugars galactinol (Gol), raffinose (Raf), *myo*-inositol (Ino) and trehalose (Tre) increased in Sweet Miriam, while galactose (Gal) contents were higher in Santa Rosa. Although we were able to identify key sugar metabolism-related genes and assess their roles using a Systems Biology approach, information regarding the possible regulation of fruit sugar metabolism by hormones is poorly characterized. It has been reported that the suppression of ethylene biosynthesis or ethylene action has no effect on fruit total soluble solids (TSS) and that the accumulation of sugars in the fruit is an ethylene-independent event (Fan et al., 1999; Knee, 1976; Menniti et al., 2004). Nevertheless, some data indicated that the interaction between sugars and ethylene could be sugar-type dependent (Li et al., 2016). Furthermore, a reciprocal correlation between Sor and ethylene has been postulated. In the present work, we treated fruit with propylene, an ethylene analogue (Burg and Burg, 1967; Paul et al., 2012) and 1-methylcyclopropane (1-MCP), and inhibitor of ethylene binding to its receptors (Sisler and Serek, 1997; Watkins, 2006), and assessed gene expression profiling and fruit sugar analysis to characterize and compare the effect(s) of ethylene on the regulation of sugar metabolism in Santa Rosa and Sweet Miriam Japanese plum fruit during postharvest storage.

2. Materials and methods

2.1. Fruit material

Fruit from the Japanese plum [*Prunus salicina* L.] cultivars Santa Rosa and Sweet Miriam were harvested from a commercial orchard located in the California Central Valley production area (Parlier, CA, USA) during two seasons as described in Farcuh et al. (2017). Fruit growth and development patterns were monitored weekly (Kim et al., 2015a). Using these data, but particularly fruit firmness as the maturity index, fruit were harvested at the 'well-mature' stage (Crisosto, 1994), corresponding to a flesh fruit firmness of ~37 N. This stage was reached ~112 d after full bloom (DAFB) in Santa Rosa and ~170 DAFB in Sweet Miriam, just between the developmental stage S3/S4 (between the end of the second exponential growth phase and the onset of ripening) and S4-I (commercial harvest stage) stages described in Farcuh et al. (2017). In the case of Santa Rosa, due to its climacteric nature, the 'well-mature' stage also corresponded to the preclimacteric stage of this cultivar. Fruit with uniform size, absence of visual blemishes, bruises and/or diseases were chosen. After harvest, fruit were quickly transported to the laboratory.

2.2. Fruit postharvest storage and treatments

A total of 1260 fruit were collected from Santa Rosa and Sweet Miriam cultivars. Fruit within each cultivar were randomized and divided into 3 groups of 420 fruit each and commercially packed into cardboard boxes. Fruit from the first group were treated with 0.5 $\mu L\,L^{-1}$ 1-MCP (SmartFresh™) at 20 °C for 24 h and immediately after the treatment were left to ripen under humidified, ethylene-free air at a flow rate of 2 $L\,min^{-1}$ in 330-L aluminum tanks completely sealed and connected to a flow-through system; fruit from the second group were left to ripen under humidified, ethylene-free air containing 500 $\mu L\,L^{-1}$ of propylene (ethylene analogue, purchased from Praxair Inc., Danbury, CT, US) at a flow rate of 2 $L\,min^{-1}$ in 330-L aluminum tanks completely sealed and connected to a flow-through system; while fruit from the third group, the controls, were left to ripen under humidified, ethylene-free air at a flow rate of 2 $L\,min^{-1}$ in 330-L aluminum tanks completely sealed and connected to a flow-through system. Humidified, ethylene-free air was ensured by bubbling the gas mixture through distilled water and by filtering atmospheric air through potassium permanganate (KMnO4), respectively.

Fruit from all groups were stored at 20 °C and 90% relative humidity for a maximum of 14 d. Evaluations were carried out at harvest (0) and after 1,3,5,7,10 and 14 d of storage. For each evaluation period, six biological replications from each group were assessed. For each biological replication, 6 fruit were used for the analysis of physicochemical parameters and ripening patterns, while 4 fruit were washed, peeled, cut into small pieces, pooled together and frozen in liquid nitrogen in order to be stored at − 80 °C for further analyses.

2.3. Fruit ripening patterns and physicochemical measurements

Fruit ripening patterns and physicochemical measurements were carried out as described previously in Kim et al. (2015a) and Farcuh et al. (2017). For each cultivar (Santa Rosa and Sweet Miriam), postharvest storage stage (0,1,3,5,7,10 and 14 d of storage at 20 °C) and group/treatment assayed (1-MCP, propylene, control), fruit ethylene (C_2H_4 $ng\,kg^{-1}\,s^{-1}$) and respiration production rate (CO_2 $\mu g\,kg^{-1}\,s^{-1}$) as well as physicochemical properties including skin and flesh color, flesh firmness, soluble solids content (SSC), titratable acidity (TA), and pH were measured on six fruit from each biological replication.

2.4. Sugar concentration quantification

2.4.1. NMR analyses

Six biological replicates of Santa Rosa and Sweet Miriam plum fruit at each postharvest storage stage of evaluation (0,1,3,5,7,10 and 14 d of storage at 20 °C) and for each group/treatment assayed (1-MCP, propylene, control), were used to quantify the contents of Suc, Glu, Fru, Sor, G6P, Gal, Raf, Ino, Tre and the cofactor NAD^+. These metabolites were chosen for NMR analysis based on their biological significance, as described in our previous work (Farcuh et al., 2017). The extraction of the metabolites and subsequent quantification was carried out as described in Farcuh et al. (2017) and all the results were expressed on dry weight basis ($g\,kg^{-1}$).

2.4.2. UHPLC-QTOF-MS/MS analyses

Six biological replicates of Santa Rosa and Sweet Miriam plum fruit at each postharvest storage stage of evaluation (0,1,3,5,7,10 and 14 d of storage at 20 °C) and for each group/treatment assayed (1-MCP, propylene, control), were used to quantify the contents of Gol. The chemical extraction, sugar separation and successive quantification was carried out as described in Farcuh et al. (2017) and all the results were expressed on dry weight basis ($g\,kg^{-1}$).

2.5. Real-time quantitative RT-PCR analysis

RNA was isolated from each of the six biological replicates of Santa Rosa and Sweet Miriam plum fruit at each postharvest storage stage of evaluation (0,1,3,5,7,10 and 14 d of storage at 20 °C) and for each group/treatment assayed (1-MCP, propylene, control), using the CTAB/NaCl method (Chang et al., 1993) with some modifications (Kim et al., 2015b). First-strand complementary DNA synthesis, primer design, and quantitative PCR were performed as described before (Kim et al., 2015b). The sets of primers used for the amplification of the different

target genes were previously described (Farcuh et al., 2017). Analysis of the relative gene expression was performed according to the Comparative Cycle Threshold Method as described by Livak and Schmittgen (2001). The expression of the SAND protein-related trafficking protein (*MON*) was used as a reference (Kim et al., 2015b).

2.6. Statistical analysis

The software package JMP® (ver.10.0, SAS Institute) was used for the statistical analyses. Means of the six biological replications were submitted to three-way analysis of variance using Tukey's test to compare between cultivars (Santa Rosa and Sweet Miriam), treatments (1-MCP, propylene and control) and time in postharvest storage for significant differences at $P < 0.05$ in all cases.

3. Results

3.1. Physicochemical properties and ripening patterns of climacteric Santa Rosa and non-climacteric Sweet Miriam bud mutants throughout postharvest storage

Fruit from Santa Rosa and Sweet Miriam cultivars were harvested at the "well-mature" stage (Crisosto, 1994), as mentioned previously and were treated immediately after harvest with propylene and 1-MCP and kept in postharvest storage at 20 °C for a maximum of 14 d. Physicochemical properties of the fruit were determined and are shown on Fig. 1.

Climacteric Santa Rosa control fruit ripening in postharvest storage, maintained higher respiration rates than Sweet Miriam fruit, displaying the typical respiratory burst and increased ethylene production rates during ripening (Fig. 1A,B). Non-climacteric Sweet Miriam fruit displayed constant and low ethylene production rates throughout postharvest storage (Fig. 1B). Upon propylene treatment, Santa Rosa fruit increased their respiration and ethylene production rates after 3 d of storage, while Sweet Miriam fruit did not, supporting their non-climacteric nature (Fig. 1A,B) (Minas et al., 2015). Following 1-MCP treatment, Santa Rosa fruit dramatically decreased their respiration and ethylene production rates until 5 d of storage, reaching levels similar to those in Sweet Miriam fruit; while after 7 d of storage onwards, a restoration of the climacteric behavior in these fruit occurred (Fig. 1A,B). Skin and flesh color hue values decreased in control fruit throughout postharvest storage of both cultivars, with Santa Rosa displaying overall lower hue skin and flesh colors than Sweet Miriam (Fig. 1C,D). Following propylene treatment, skin color hue values decreased dramatically in Sweet Miriam fruit as compared to Sweet Miriam control fruit, while flesh color hue values decreased faster in Santa Rosa treated fruit (Fig. 1C,D). Upon 1-MCP treatment, Santa Rosa fruit flesh and skin color exhibited higher hue values after 5 d of storage as compared to control fruit and reached the same values as control fruit after 10 d of postharvest (Fig. 1C,D).

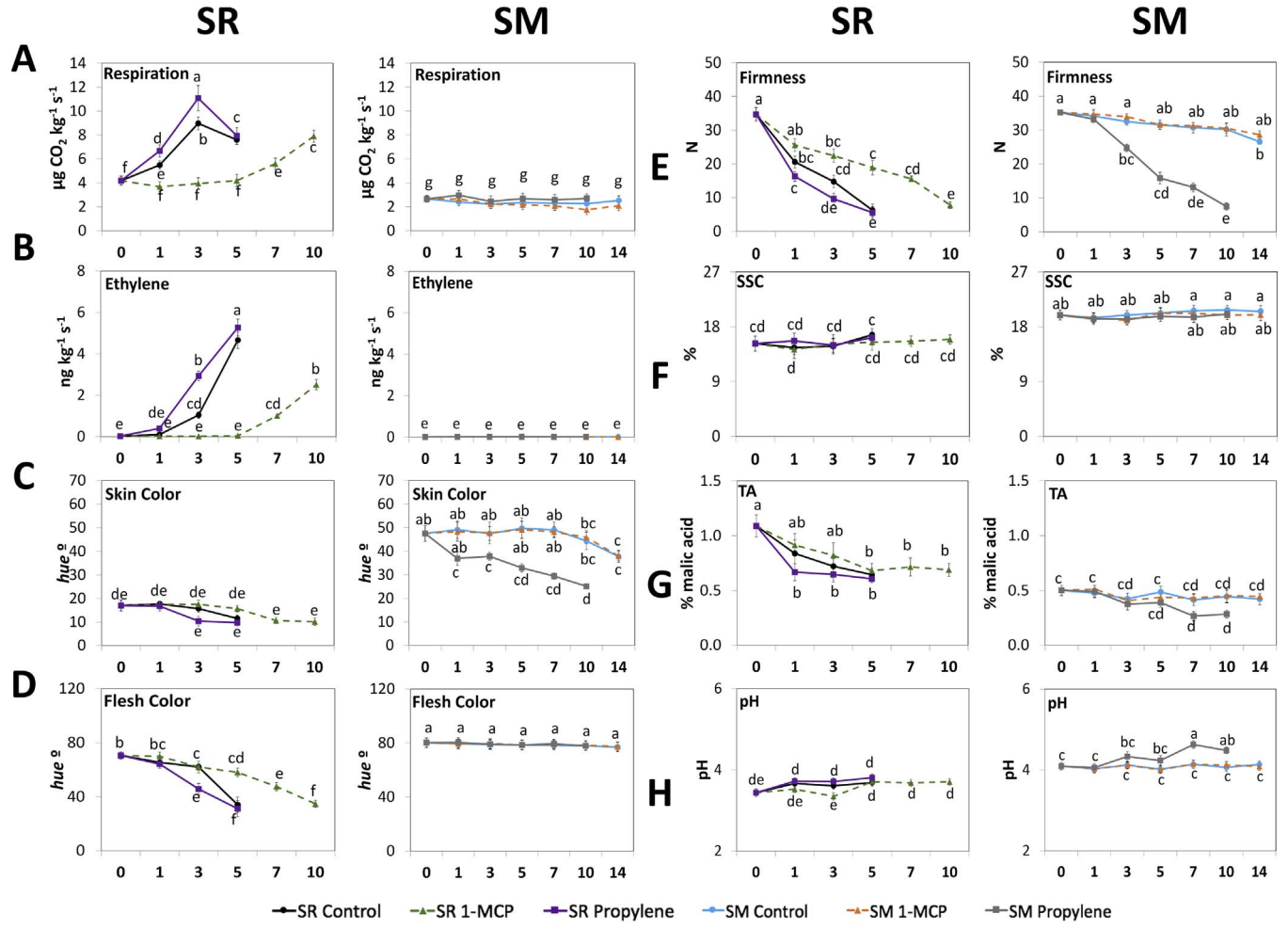

Fig. 1. Fruit respiration, ethylene production rates and physicochemical properties of Santa Rosa (SR) and Sweet Miriam (SM) Japanese plum cultivars during ripening throughout postharvest storage at 20 °C. Fruit respiration, ethylene production rates and physicochemical properties were determined in fruit from SR (left graph) and SM (right graph) cultivars submitted to no treatment (control), 1-MCP treatment, and propylene treatment after 0,1,3,5,7,10 and 14 d at 20 °C. (A) Respiration production rates; (B) ethylene production rates; (C) fruit skin color; (D) fruit flesh color; (E) fruit firmness values; (F) soluble solid contents (SSC); (G) fruit titratable acidity (TA) and (H) fruit pH. Values are means ± SE (n = 6). Different letters indicate significant differences ($p < 0.05$) according to Tukey's test.

Flesh firmness during postharvest storage of Santa Rosa control and propylene treated fruit reached the "ready-to-eat" stage (≤10 N) and thus could not be further evaluated after 5 d of postharvest; while Sweet Miriam control fruit displayed flesh firmness values of ~25 N after 14 d of storage (Fig. 1E). Nevertheless, propylene treated Sweet Miriam fruit were able to soften to the "ready-to-eat" stage after 10 d of postharvest (Fig. 1E). Following 1-MCP treatment, Santa Rosa fruit decreased their softening rate after 5 d of storage as compared to Santa Rosa control fruit and reached the "ready-to-eat" stage after 10 d of postharvest (Fig. 1E).

Sweet Miriam fruit presented higher SSC values at all stages throughout ripening on postharvest storage and this parameter did not respond to ethylene treatments (Fig. 1F). Titratable acidity values were lower in Sweet Miriam fruit and decreased in both cultivars throughout ripening (Fig. 1G). Nevertheless, during postharvest storage Sweet Miriam TA values were constant (Fig. 1G). However, propylene treatment decreased TA values in Sweet Miriam fruit, while 1-MCP treated fruit behaved as their respective controls in both cultivars (Fig. 1G). As expected, pH values were opposite to the TA values (Fig. 1H).

3.2. Effects of ethylene on key sugar metabolism-associated genes and their related metabolites in fruit of climacteric Santa Rosa and non-climacteric Sweet Miriam bud mutants throughout postharvest storage.

3.2.1. Sucrose, glucose and fructose metabolism

Suc contents in Santa Rosa control fruit increased throughout postharvest and were higher than those in Sweet Miriam control fruit, which remained constant (Fig. 2). Upon propylene treatment, Sweet Miriam fruit displayed increased Suc contents as compared to Sweet Miriam control fruit, though lower than Santa Rosa control and propylene treated fruit after 3 and 5 d of storage. 1-MCP treatment in Santa Rosa fruit decreased Suc contents with respect to control and propylene treatments, until 5 d of storage, period after which Suc concentration increased, coincident with increased rates of ethylene production; while Sweet Miriam fruit remained similar to control fruit. *SPS* (EC 2.4.1.14) transcript levels, associated with Suc synthesis (Yamaki, 1994), decreased and remained constant throughout postharvest in Santa Rosa and Sweet Miriam control fruit, respectively, although *SPS* expression levels were higher in Santa Rosa fruit, in agreement with the higher Suc contents in this cultivar (Fig. 2). Similar to what was observed for Suc, propylene treatments increased SPS transcript levels in Sweet Miriam fruit, while upon 1-MCP treatment, SPS expression levels in Santa Rosa fruit decreased until 5 d of storage, and increased thereafter (Fig. 2).

Throughout storage, Glu contents decreased and Fru contents remained constant in Santa Rosa control fruit, while both Glu and Fru contents increased and remained higher in Sweet Miriam than in Santa Rosa fruit (Fig. 2). Propylene treatments induced a decrease in Glu and Fru contents in Sweet Miriam fruit. On the other hand, Santa Rosa fruit treated with 1-MCP exhibited an increase in Glu and Fru contents as compared to Santa Rosa control fruit (Fig. 2).

Suc breakdown into UDP-Glu and Fru is mediated by the action of SuSy activity (EC 2.4.1.13) (Klann et al., 1993). Only one *Susy* transcript (*ppa017606m.g*) was identified among the 11 key sugar metabolism-associated genes that were associated with the differences in sugar composition between the Santa Rosa and Sweet Miriam cultivars (Farcuh et al., 2017). Transcript levels of SuSy decreased 5-fold in Santa Rosa control fruit throughout postharvest and were lower than Sweet Miriam control fruit, in agreement with the higher Suc contents in Santa Rosa (Fig. 2). Propylene treatment decreased SuSy transcripts accumulation in Sweet Miriam fruit as compared to control fruit. Upon 1-MCP treatments, Santa Rosa fruit displayed about a 2-fold increase in *SuSy* mRNA levels with respect to Santa Rosa control fruit, until 5 d of storage; while 1-MCP treatments had no effects on Sweet Miriam fruit. Suc catabolism into Glu and Fru is mediated by invertases (EC 3.2.1.26) (including CWINV, CytINV and VINV) (Li et al., 2012), all of which were assayed in this study. In Santa Rosa and Sweet Miriam control fruit, *CWINV* and *CytINV* transcript levels decreased throughout storage yet *VINV* expression levels decreased in Santa Rosa and increased in Sweet Miriam (Fig. 2). Sweet Miriam fruit displayed higher expression of all assayed invertases as compared to Santa Rosa control fruit. After propylene treatments, Sweet Miriam fruit displayed a decrease in invertases transcripts, while *CWINV* and *VINV* mRNA levels were higher in Sweet Miriam than in Santa Rosa propylene-treated fruit (Fig. 2). 1-MCP treatments induced an increase in mRNA levels for the three invertases in Santa Rosa fruit until 5 d of storage, with a dramatic decrease afterwards due to the decrease in 1-MCP action. 1-MCP-treated Sweet Miriam fruit performed as Sweet Miriam control fruit, yet exhibiting higher expression levels for all invertases with respect to 1-MCP-treated Santa Rosa fruit. In addition, we assayed expression levels of *INVINH* due to their well-reported role as regulators of invertases at the posttranscriptional level (Jin et al., 2009). Nevertheless, our results suggested that although both cultivars increased their *INVINH* mRNA levels throughout postharvest storage, there was no effect of ethylene on *INVINH* transcripts accumulation (Fig. 2).

3.2.2. Sorbitol metabolism

In contrast to Suc, Sor contents decreased throughout postharvest storage in Santa Rosa control fruit, but increased in Sweet Miriam fruit, with Sweet Miriam displaying a 3.5-fold higher Sor contents than Santa Rosa fruit (Fig. 3). Propylene induced a decrease in Sor contents of Sweet Miriam fruit, although Sor contents remained higher in Sweet Miriam than Santa Rosa fruit. While 1-MCP did not affect Sor contents in Sweet Miriam fruit, a 2.5-fold increased Sor contents was observed in Santa Rosa fruit. This increase in Sor contents in Santa Rosa lasted until 5 d of storage and decreased afterwards, coincident with increased rates of ethylene production (Fig. 3).

Sor synthesis is mediated by the action of S6PDH (EC 1.1.1.200), reducing G6P to Sor-6-phosphate (Suzuki and Dandekar, 2014). *S6PDH* transcripts accumulation was higher in Sweet Miriam than in Santa Rosa fruit, although remained constant during postharvest storage and was not affected either by propylene or 1-MCP treatments in either cultivar (Fig. 3). A decrease in G6P contents during postharvest storage was observed in Sweet Miriam control fruit as well as upon propylene and 1-MCP treatments, but not in Santa Rosa fruit. G6P concentrations were higher in Sweet Miriam than in Santa Rosa fruit. G6P results from Glu phosphorylation mediated by HK (EC 2.7.1.1) (Li et al., 2012). *HK* mRNA levels were not affected by ethylene and remained constant in both cultivars except after 7 d of storage in Sweet Miriam fruit, where a decrease in transcripts was observed. *HK* transcripts were more abundant in Sweet Miriam than in Santa Rosa until 7 d of postharvest storage and decreased afterwards (Fig. 3).

Sor cleavage into Fru occurs via NAD^+-SDH (EC 1.1.1.14), using NAD^+ as a cofactor (Teo et al., 2006). *NAD^+-SDH* transcript levels increased dramatically throughout postharvest in Santa Rosa control and propylene treated fruit, while increased slightly in Sweet Miriam fruit (Fig. 3). Santa Rosa fruit displayed a 2–3 fold higher *NAD^+-SDH* expression levels and lower NAD^+ contents than Sweet Miriam fruit. Nevertheless, after propylene treatment Sweet Miriam fruit increased *NAD^+-SDH* expression levels with respect to Sweet Miriam control fruit with a corresponding decrease in NAD^+ and Sor contents (Fig. 3). Following 1-MCP treatments, Santa Rosa fruit displayed significantly lower *NAD^+-SDH* transcripts accumulation as compared to Santa Rosa control and propylene treated fruit until 5 d of storage, with an increase afterwards. The opposite trend was observed with NAD^+ contents. Sweet Miriam fruit treated with 1-MCP displayed similar *NAD^+-SDH* expression levels as Sweet Miriam control fruit but lower than those of Santa Rosa 1-MCP-treated fruit (Fig. 3).

3.2.3. Minor sugars metabolism

Gal contents of Santa Rosa and Sweet Miriam control fruit increased throughout postharvest with Santa Rosa fruit displaying a 2.5-fold higher Gal content than Sweet Miriam fruit (Fig. 4). Although

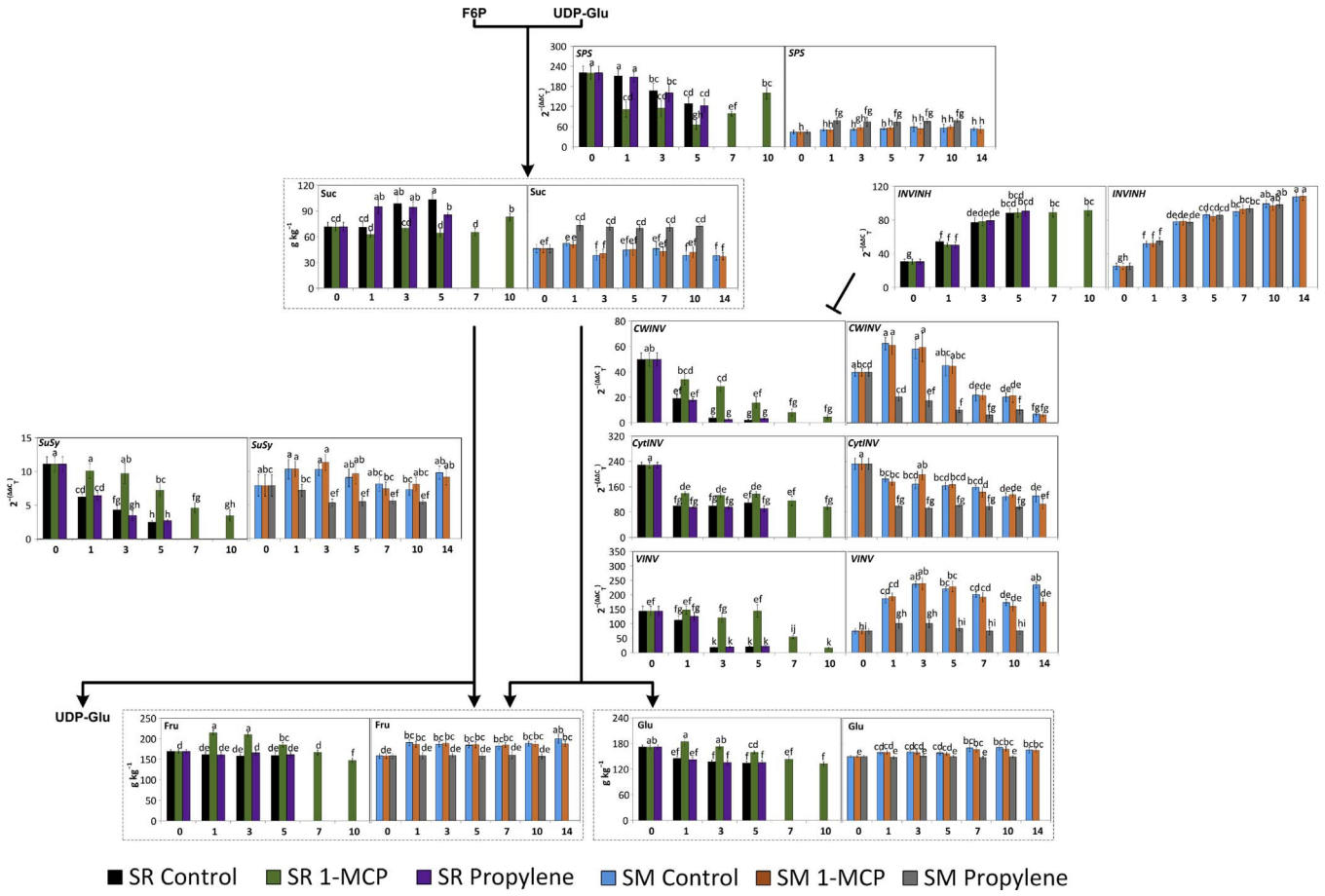

Fig. 2. Sucrose metabolism-associated pathways in fruit of Santa Rosa (SR) and Sweet Miriam (SM) Japanese plum cultivars during ripening throughout postharvest storage at 20 °C. Sucrose metabolism-associated pathways in fruit of Santa Rosa (SR; left graph) and Sweet Miriam (SM; right graph) Japanese plum cultivars submitted to no treatment (control), 1-MCP treatment, and propylene treatment after 0,1,3,5,7,10 and 14 d of storage at 20 °C. Sugar contents are presented in graphs framed by dashed lines and are expressed as g kg^{-1} on a dry weight basis. Relative gene expression levels were calculated and normalized using the SAND protein-related trafficking protein (*MON*) as reference gene. Values are means ± SE (n = 6). The data were analyzed using three-way ANOVA followed by Tukey's test. Different letters indicate significant differences ($p < 0.05$) according to Tukey's test and are comparing between both cultivars (graphs). Sucrose phosphate synthase (SPS); Sucrose phosphate synthase (*SPS*); Sucrose synthase (*SuSy*); cell wall invertase (*CWINV*); vacuolar invertase (*VINV*); cytosolic invertase (*CytINV*); Invertase inhibitor (*INVINH*); sucrose (Suc); fructose (Fru); glucose (Glu); fructose-6-phosphate (F6P); UDP glucose (UDP-Glu).

propylene treatments did not affect Gal contents in Santa Rosa, it increased dramatically in Sweet Miriam fruit after 7 and 10 d of storage. 1-MCP treatments induced a decrease in Gal contents in both Santa Rosa and Sweet Miriam fruit (Fig. 4). Gal, together with Suc, are products of Raf catabolism via the action of AGAL (α-galactosidase) (EC 3.2.1.22) (Hubbard et al., 1989). Gal synthesis is also the result of the action of BGAL (β-galactosidase) (EC 3.2.1.23), mediating the catabolism of galactosyl residues from cell wall polysaccharides (Sozzi et al., 1998). Santa Rosa fruit displayed a relatively constant *AGAL* expression during postharvest storage, while the expression of *BGAL* increased throughout storage (Fig. 5). *AGAL* and *BGAL* transcript accumulation remained constant throughout postharvest ripening in Sweet Miriam fruit (Fig. 5). *AGAL* and *BGAL* mRNA levels were 2 to 3-fold higher in Santa Rosa than Sweet Miriam control fruit, in agreement with the higher Gal contents displayed by Santa Rosa fruit. Interestingly, propylene treatments increased both *AGAL* and *BGAL* transcripts accumulation in Sweet Miriam fruit after 7 and 10 d of storage (Fig. 5), which correlated well with the increased Gal contents observed in propylene-treated Sweet Miriam fruit (Fig. 4). Following 1-MCP treatment, Santa Rosa fruit displayed decreased *AGAL* and *BGAL* mRNA levels until 5 d of storage, but increased afterwards (with the restoration of ethylene production) (Fig. 5). Gal can be phosphorylated into galactose-1-phosphate via GALK (galactokinase) EC 2.7.1.6) (Dai et al., 2006). *GALK* mRNA levels increased throughout postharvest ripening in both cultivars, while *GALK* expression levels were higher in Santa Rosa than in Sweet Miriam fruit (Fig. 5). Propylene treatment increased the *GALK* expression in Sweet Miriam fruit after 7 and 10 d of storage, while 1-MCP applications decreased *GALK* mRNA levels in Santa Rosa until 5 d, consistent with the changes in *AGAL* and *BGAL* transcripts and Gal contents (Fig. 5).

Gol contents remained constant in Santa Rosa and Sweet Miriam control fruit throughout postharvest storage, although Gol contents were higher in Sweet Miriam than in Santa Rosa fruit (Fig. 4). Propylene induced a decrease in Gol contents in Sweet Miriam fruit, while 1-MCP treatments induced a notable increase in Gol contents in Santa Rosa fruit (Fig. 4). Furthermore, Gol synthesis, using UDP-Gal and Ino as substrates, is mediated by the action of GolS (galactinol synthase) (EC 2.4.1.123) (Nishizawa et al., 2008). *GolS* transcript levels were dramatically higher (8-fold) in Sweet Miriam than in Santa Rosa fruit throughout postharvest ripening, in agreement with the increased Gol contents observed in Sweet Miriam fruit (Figs. 4 and 5). Nevertheless, upon propylene treatment, Sweet Miriam fruit displayed a 3 to 4-fold decrease in *GolS* transcript accumulation with respect to Sweet Miriam control fruit, although maintaining a higher *GolS* expression levels than Santa Rosa propylene-treated fruit (Fig. 5). Following 1-MCP treatments, Santa Rosa fruit increased their *GolS* mRNA levels with respect to Santa Rosa control and propylene-treated fruit until 5 d of postharvest, supporting the high Gol contents in this cultivar (Fig. 5).

Gol, together with Suc, are substrates used by RS (raffinose synthase) (EC 2.4.1.82) to synthetize Raf (Pillet et al., 2012). Raf contents

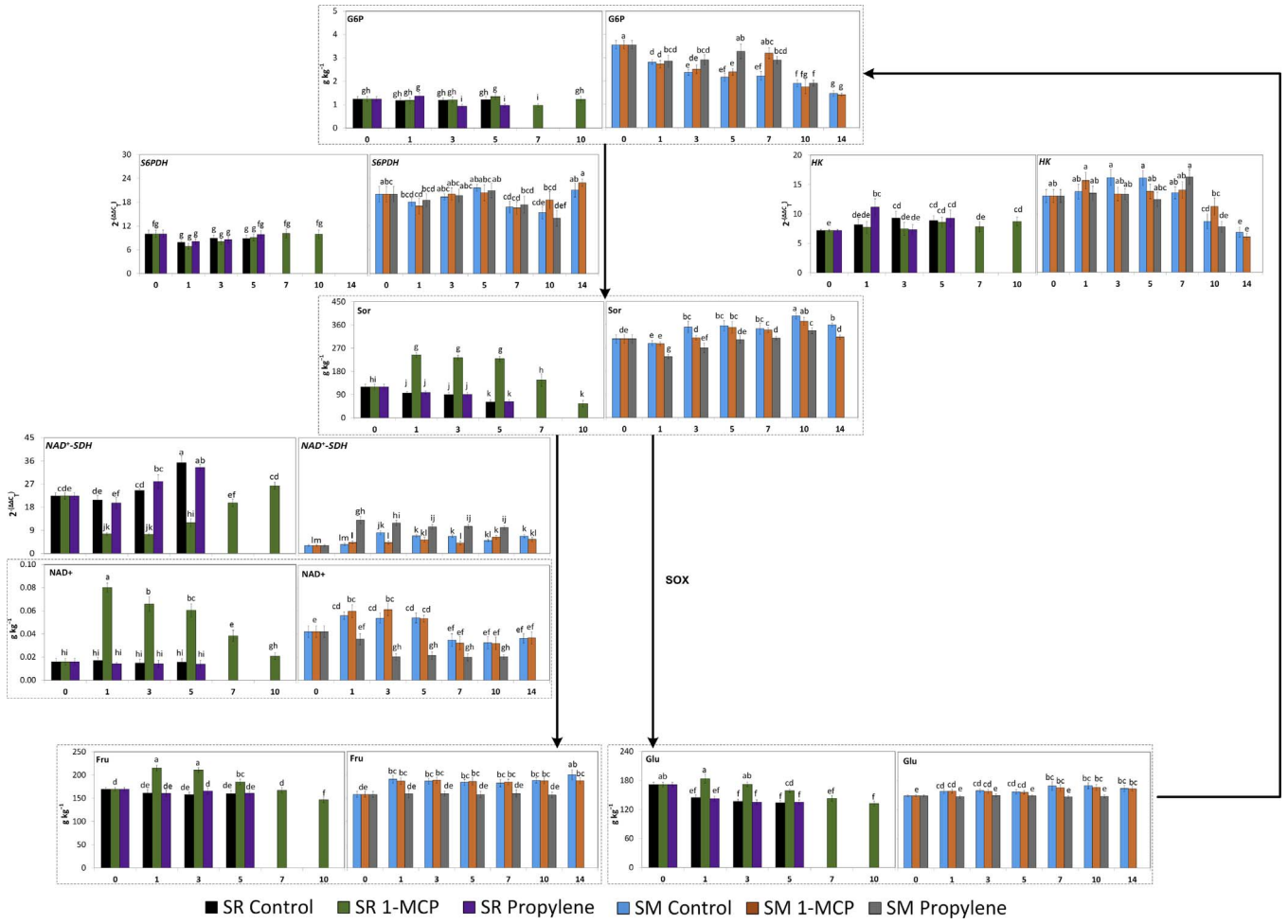

Fig. 3. Sorbitol metabolism-associated pathways in fruit of Santa Rosa (SR) and Sweet Miriam (SM) Japanese plum cultivars during ripening throughout postharvest storage at 20 °C. Sorbitol metabolism-associated pathways in fruit of Santa Rosa (SR; left graph) and Sweet Miriam (SM; right graph) Japanese plum cultivars submitted to no treatment (control), 1-MCP treatment, and propylene treatment after 0,1,3,5,7,10 and 14 d of storage at 20 °C. Sugar contents are presented in graphs framed by dashed lines and are expressed as g kg^{-1} on a dry weight basis. Relative gene expression levels were calculated and normalized using the SAND protein-related trafficking protein (*MON*) as reference gene. Values are means ± SE (n = 6). The data were analyzed using three-way ANOVA followed by Tukey's test. Different letters indicate significant differences ($p < 0.05$) according to Tukey's test and are comparing between both cultivars (graphs). Sorbitol-6-phosphate dehydrogenase (*S6PDH*); NAD^+-dependent sorbitol dehydrogenase (*NAD^+-SDH*); sorbitol oxidase (SOX); hexokinase (*HK*); glucose-6-phosphate (G6P); sorbitol (Sor); Nicotinamide adenine dinucleotide(NAD^+); fructose (Fru); glucose (Glu).

decreased throughout storage in Santa Rosa control fruit while remained constant in Sweet Miriam control fruit (Fig. 4). These results were consistent with *RS* transcript levels that were 2 to 3-fold higher in Sweet Miriam than in Santa Rosa fruit. Upon propylene treatments, Sweet Miriam fruit showed a 3 to 4-fold decreased Raf contents as well as a decrease in *RS* mRNA levels with respect to Sweet Miriam control fruit (Figs. 4 and 5). After 1-MCP treatments, Santa Rosa fruit displayed increased Raf contents as well as *RS* transcripts with respect to Santa Rosa control and propylene-treated fruit until 5 d of postharvest ripening, decreasing afterwards. Sweet Miriam 1-MCP-treated fruit behaved as Sweet Miriam control fruit (Figs. 4 and 5).

Ino contents, used by GolS and formed through the action of RS, increased throughout postharvest storage in both cultivars. Upon propylene treatment, Sweet Miriam fruit displayed lower Ino contents with respect to Sweet Miriam control fruit; while after 1-MCP treatment, Santa Rosa fruit showed 2-fold higher Ino contents as compared to Santa Rosa control and propylene-treated fruit until 3 d of storage (Fig. 4).

Regarding Tre, its breakdown occurs via TRE (trehalase) (EC 3.2.1.28) (Ponnu et al., 2011). *TRE* transcript accumulation increased throughout postharvest in both Santa Rosa and Sweet Miriam fruit, yet were higher in Santa Rosa control fruit, in agreement with the lower Tre contents in this cultivar (Figs. 4 and 5). After propylene treatment, Sweet Miriam fruit displayed 2-fold higher *TRE* mRNA levels than Sweet Miriam control fruit, consistent with the decrease in Tre contents in Sweet Miriam propylene-treated fruit. 1-MCP treatments decreased *TRE* expression levels and increased Tre contents by 2-fold in Santa Rosa fruit with respect to Santa Rosa control and propylene-treated fruit until 5 d of storage, period after which ethylene levels were restored (Figs. 4 and 5).

4. Discussion

In the present work we compared profiles of sugar metabolism-related genes and metabolites of two Japanese plum cultivars under controlled conditions and under different ethylene and 1-MCP treatments during postharvest storage. Our experimental system comprised Santa Rosa and Sweet Miriam plum cultivars, that share the same genetic background, but because of the bud-sport nature of the Sweet Miriam cultivar they display different ripening behaviors (i.e. climacteric – Santa Rosa – and non-climacteric – Sweet Miriam –) (Farcuh et al., 2017; Kim et al., 2015a; Minas et al., 2015). Although the main sugar metabolism-related enzymes and their expression levels have been identified and assayed in several climacteric and non-climacteric fleshy fruit-types (Beauvoit et al., 2014; Borsani et al., 2009; Li et al., 2012; Moriguchi et al., 1992; Yamaki, 1986), little is known about their

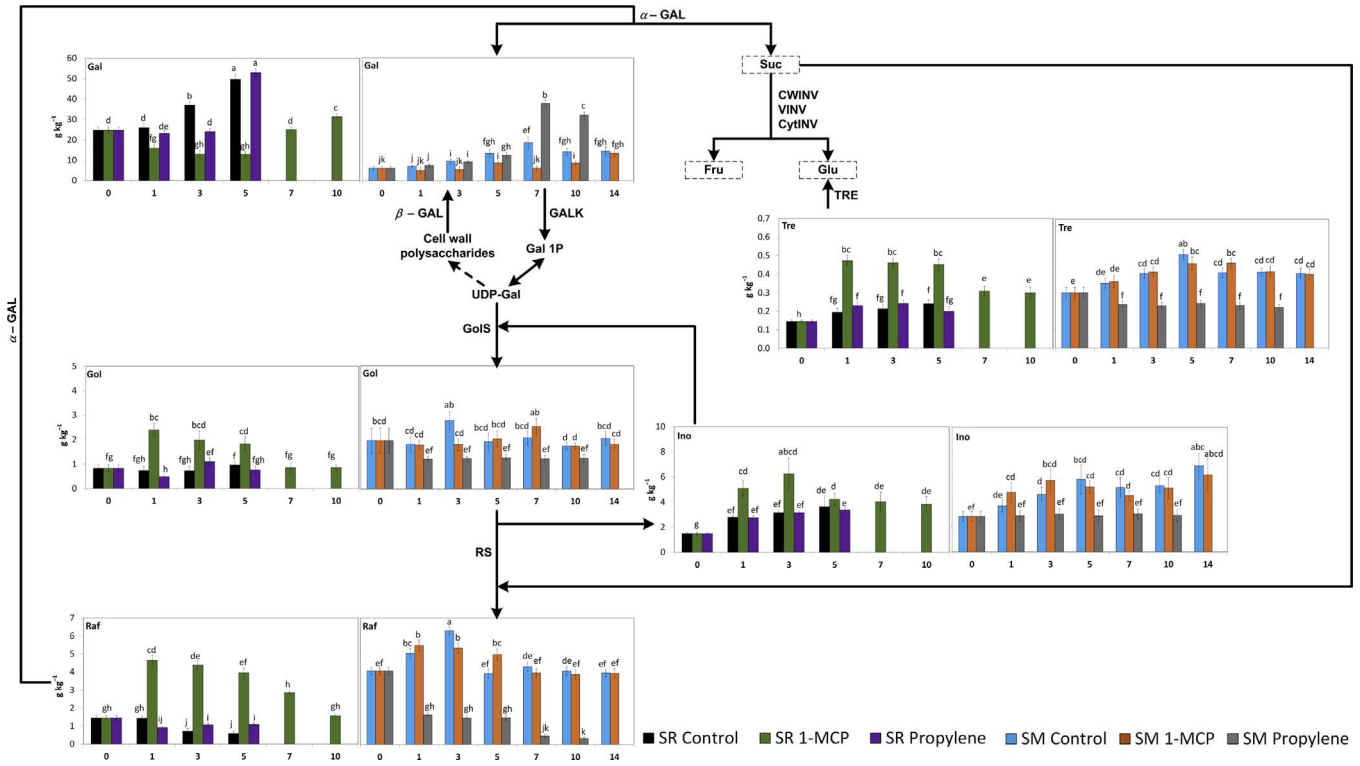

Fig. 4. Minor sugar metabolism-associated pathways in fruit of Santa Rosa (SR) and Sweet Miriam (SM) Japanese plum cultivars during ripening throughout postharvest storage at 20 °C. Galactose, galactinol, raffinose, *myo*-inositol and trehalose metabolism-associated pathways in fruit of Santa Rosa (SR; left graph) and Sweet Miriam (SM; right graph) Japanese plum cultivars submitted to no treatment (control), 1-MCP treatment, and propylene treatment after 0,1,3,5,7,10 and 14 d of storage at 20 °C. Sugar contents are expressed as g kg^{-1} on a dry weight basis. Values are means ± SE (n = 6). The data were analyzed using three-way ANOVA followed by Tukey's test. Different letters indicate significant differences ($p < 0.05$) according to Tukey's test and are comparing between both cultivars (graphs). Galatokinase (*GALK*); Alpha-Galactosidase (α-GAL); Beta-Galactosidase (*β-GAL*); Galactinol synthase (*GolS*); Raffinose synthase (*RS*); Trehalase (*TRE*); cell wall invertase (*CWINV*); vacuolar invertase (*VINV*); cytosolic invertase (*CytINV*); galactose (Gal); galactinol (Gol); raffinose (Raf); *myo*-inositol (Ino); trehalose (Tre); fructose (Fru); glucose (Glu); sucrose (Suc); galactose-1-phosphate (Gal 1P); UDP- galactose (UDP-Gal).

regulation (Génard and Souty, 1996) during postharvest storage.

Our previous study (Farcuh et al., 2017) demonstrated the reprogramming of sugar metabolism in Sweet Miriam fruit type during ripening on-the-tree, identified genes associated with the differences in sugar composition between both cultivars and suggested the possibility of a link between ethylene action and overall fruit sugar homeostasis. Here, we examined the responses to ethylene and 1-MCP treatments of these key sugar metabolism-related genes, throughout postharvest ripening. We assayed gene expression levels and their associated sugar contents and show that ethylene is a crucial factor affecting overall sugar metabolism in both ripening types. A summarized scheme of the overall results of this work is presented in Fig. 6.

4.1. Ethylene reduces sucrose catabolism but hastens sorbitol breakdown during fruit ripening in postharvest storage

Suc and Sor contents have been reported to vary dramatically among members of the Rosaceae family (Desnoues et al., 2014). For example, Suc contents are more abundant in climacteric peaches (Moriguchi et al., 1990), but Sor contents are higher in non-climacteric cherries (Gao et al., 2003). These results would support our findings on high Suc and high Sor contents in Santa Rosa and Sweet Miriam fruit, respectively (Figs. 2, 3, 6). In addition, the contrasting ethylene production rates between climacteric and non-climacteric fruit (Fig. 1B) suggest the notion of ethylene being a key player in the regulation of sugar metabolism during ripening.

In climacteric fruit such as tomatoes, Suc has been reported to act synergistically with ethylene, hastening the ripening process throughout postharvest storage (Li et al., 2016). Likewise, in transgenic apples, with a downregulation of ethylene biosynthesis, Suc contents were restored to the same concentrations as wild type apples only after exposure to exogenous ethylene throughout postharvest storage (Defilippi et al., 2004). Furthermore, in non-climacteric fruit such as strawberries, Suc contents have been shown to accelerate the ripening process (Jia et al., 2013a,b). In the case of grapes, fruit treated with 1-MCP reduced Suc accumulation with respect to control fruit (Chervin et al., 2006); while low Suc contents were reported as one of the main factors associated with the late-ripening behavior of a spontaneous sweet orange mutant as compared to its wild type (Zhang et al., 2014). Thus, these above-mentioned studies support the higher Suc contents observed in Santa Rosa with respect to Sweet Miriam control fruit and the responses of both cultivars to ethylene and 1-MCP treatments and suggest a role for ethylene positively regulating Suc contents in climacteric and non-climacteric fruit (Figs. 2 and 6). Regarding Suc metabolism, ethylene has been reported to strongly stimulate the expression of *SPS*, encoding the key Suc biosynthetic enzyme (Miron and Schaffer, 1991) in fruit such as banana, peach and kiwifruit (Choudhury et al., 2008; Langenkämper et al., 1998; Lombardo et al., 2011), in agreement with the results of this study in both cultivars (Figs. 2 and 6). On the other hand, gene expression of Suc-breakdown related enzymes, including invertases and SuSy, which catabolize Suc into hexoses (Kleczkowski et al., 2010; Li et al., 2012), are negatively affected by ethylene, as observed in tomato for invertases (Klann et al., 1996) and in the present study, supporting the higher Suc contents in Santa Rosa and propylene-treated Sweet Miriam fruit (Figs. 2 and 6).

In the case of Sor, although there is considerably less literature available as compared to Suc, there seems to be an antagonistic interaction between Sor and ethylene, as suggested in our previous work (Farcuh et al., 2017) and as shown in this study (Figs. 3 and 6). In peach, Sor contents were rapidly consumed as the fruit ripened throughout postharvest (Borsani et al., 2009; Lombardo et al., 2011); while in apples treated with 1-MCP, an increased Sor accumulation was

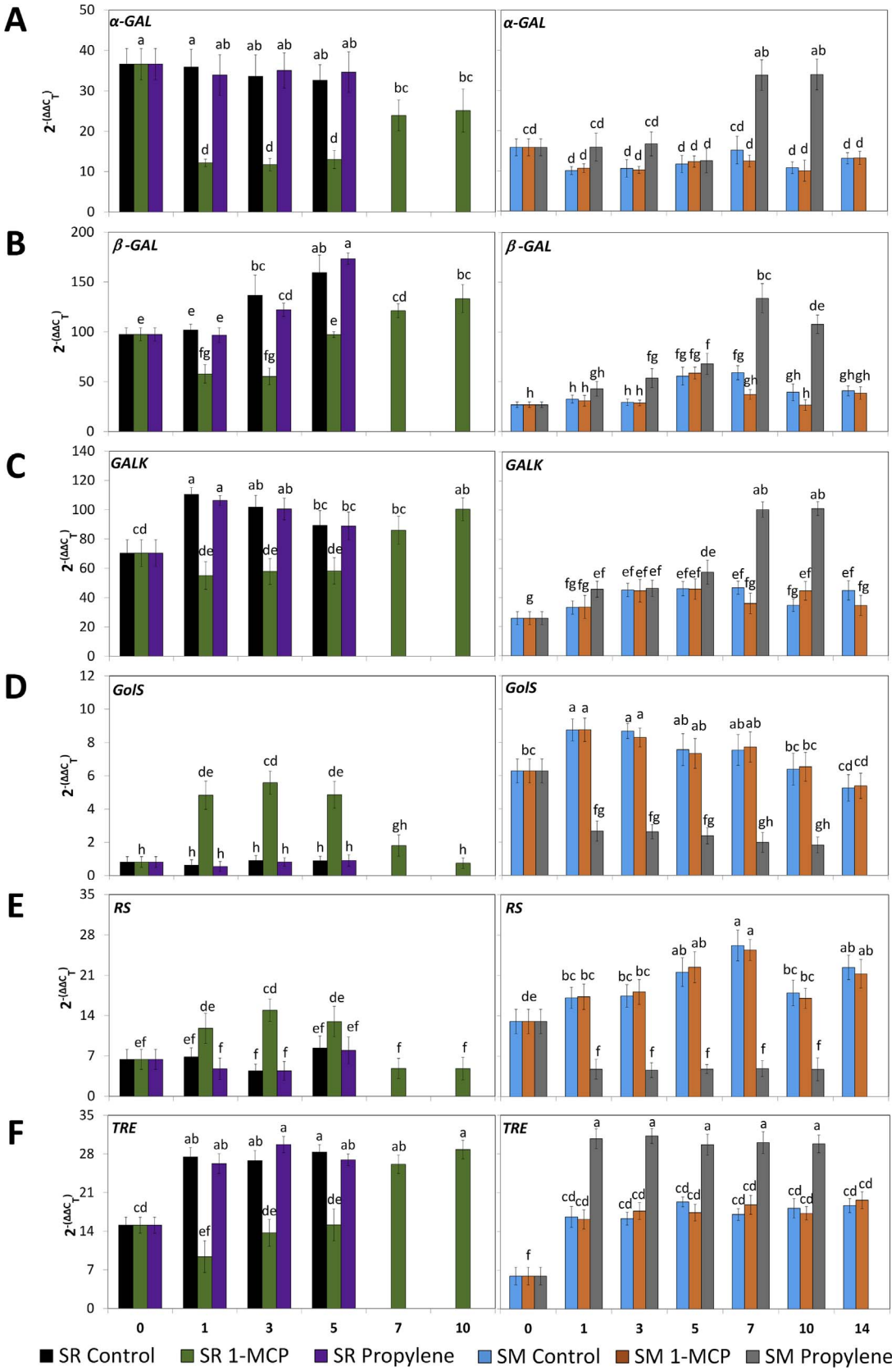

Fig. 5. Relative gene expression levels of minor sugar metabolism-associated pathways in fruit of Santa Rosa (SR) and Sweet Miriam (SM) Japanese plum cultivars during ripening throughout postharvest storage at 20 °C. Relative gene expression levels of galactose, galactinol, raffinose, and trehalose metabolism-associated pathways in fruit of Santa Rosa (SR; left graph) and Sweet Miriam (SM; right graph) Japanese plum cultivars submitted to no treatment (control), 1-MCP treatment, and propylene treatment after 0,1,3,5,7,10 and 14 d of storage at 20 °C. Relative gene expression levels were calculated and normalized using the SAND protein-related trafficking protein (*MON*) as reference gene. Values are means ± SE (n = 6). The data were analyzed using three-way ANOVA followed by Tukey's test. Different letters indicate significant differences ($p < 0.05$) according to Tukey's test and are comparing between both cultivars (graphs). (A) Alpha-Galactosidase (α-GAL); (B) Beta-Galactosidase (*β-GAL*); (C) Galatokinase (*GALK*); (D) Galactinol synthase (*GolS*); (E) Raffinose synthase (*RS*); (F) Trehalase (*TRE*).

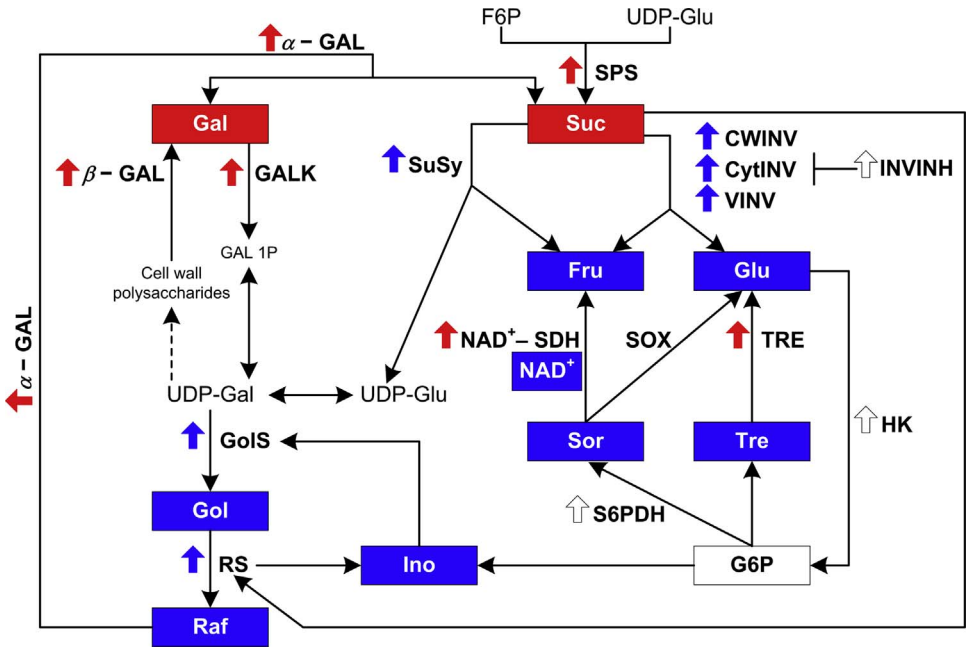

Fig. 6. Schematic summary of ethylene regulation of sugar metabolism-related pathways in climacteric and non-climacteric plum fruit during postharvest storage at 20 °C. Sugars are presented in boxes that when colored red or blue indicate that ethylene had a positive or negative effect on that specific sugar content, respectively. Thick upward red or blue arrows indicate mRNA expression levels that were induced or reduced by ethylene, respectively. Boxes and arrows colored white indicate there was no effect of ethylene. Sucrose phosphate synthase (SPS); Sucrose synthase (SuSy); Cell wall invertase (CWINV); Vacuolar invertase (VINV); Cytosolic invertase (CytINV); Invertase inhibitor (INVINH); sorbitol-6-phosphate dehydrogenase (S6PDH); NAD^+-dependent sorbitol dehydrogenase (NAD^+-SDH); sorbitol oxidase (SOX); hexokinase (HK); Galactokinase (GALK); Alpha-Galactosidase (α-GAL); Beta-Galactosidase (β-GAL); Galactinol synthase (GolS); Raffinose synthase (RS); Trehalase (TRE); sucrose (Suc); fructose (Fru); glucose (Glu); fructose-6-phosphate (F6P); UDP glucose (UDP-Glu); glucose-6-phosphate (G6P); sorbitol (Sor); Nicotinamide adenine dinucleotide (NAD^+); galactose (Gal); galactinol (Gol); raffinose (Raf); *myo*-inositol (Ino); trehalose (Tre); galactose-1-phosphate (Gal 1P); UDP- galactose (UDP-Gal). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

observed during storage (Lee et al., 2012), in agreement with our results in the climacteric Santa Rosa fruit (Figs. 3 and 6). Concerning Sor metabolism, ethylene treatments in apples during postharvest storage have been reported to decrease S6PDH protein levels, the main Sor-biosynthesis related enzyme (Zheng et al., 2013). While our results indicated higher *S6PDH* transcripts accumulation in Sweet Miriam with respect to Santa Rosa fruit, a lack of ethylene effect was observed in both cultivars (Figs. 3 and 6), what could be due to posttranscriptional modifications. Inversely, based on our results, Sor breakdown into Fru, catalyzed by NAD^+-SDH (Teo et al., 2006) is suggested to be positively regulated by ethylene in both cultivars (Figs. 3 and 6). This is in agreement with Begheldo (2008) that reported that propylene-treated peaches during storage increased their *NAD^+-SDH* mRNA levels.

Thus, our results suggested that there was an effect of ethylene on Suc and Sor metabolism and that the hexoses Glu and Fru in climacteric Santa Rosa and non-climacteric Sweet Miriam fruit resulted from Sor and Suc catabolism, respectively during ripening in postharvest storage (Fig. 6). Nevertheless, why are there overall lower Glu and Fru contents in Santa Rosa as compared to Sweet Miriam fruit? Possible explanations for this observations might be: (a) Glu and Fru have higher metabolic accessibility to respiratory loss with respect to Suc and Sor, and respiration rates are dramatically higher in Santa Rosa than Sweet Miriam fruit (Fig. 1A) and/or (b) the existence of an antagonistic interaction between Glu and ethylene that has been reported in *Arabidopsis* (Yanagisawa et al., 2003) and is supported by our results in both cultivars (Fig. 6). Furthermore, on our previous study, where we explored sugar metabolism reprogramming in Santa Rosa and Sweet Miriam cultivars during ripening on-the-tree (Farcuh et al., 2017), we observed higher Glu and Fru contents in Santa Rosa than of Sweet Miriam fruit. The inverse results obtained between on-the-tree and postharvest ripening sugar composition could be due to the effect of source-sink sugar transport occurring on-the-tree and which is not a critical factor during ripening in storage.

4.2. Ethylene positively interacts with galactose, but has negative effects on galactinol, raffinose, myo-inositol and trehalose contents throughout postharvest fruit ripening

In general, fruit ripening comprises a number of biological processes, among them the solubilization of pectic polysaccharides of the primary cell wall and a loss of Gal from the side chains of the polymers (Redgwell et al., 1997). The enzyme BGAL has been associated with the cleavage of these galactosyl residues thus contributing to the free Gal pool and to fruit softening (Ross et al., 1994; Sozzi et al., 1998). In addition, AGAL also increases Gal contents by hydrolyzing Raf to yield free Gal and Suc (Dai et al., 2006; Hubbard et al., 1989). Ethylene promotes *BGAL* and AGAL transcript accumulation in Santa Rosa and Sweet Miriam fruit, thus supports the increase in Gal contents (Figs. 4, 5A,B, 6) and contributes to the loss of fruit firmness throughout postharvest displayed in Santa Rosa and propylene-treated Sweet Miriam fruit (Fig. 1E). On the other hand, free Gal has been reported to stimulate ethylene production and promote earlier ripening in tomatoes (Gross, 1985) due to the capacity of Gal to promote the activity of 1-aminocyclopropane-1-carboxylic acid synthase (ACS), the rate-limiting step in ethylene biosynthesis (Kim et al., 1987). This possible synergistic interaction between ethylene and free Gal is currently under investigation.

Furthermore, our results suggest a negative effect of ethylene in Gol, Raf, Ino and Tre contents (Fig. 6) due to the capacity of 1-MCP treatments to increase Gol, Raf, Ino and Tre contents in Santa Rosa fruit and the inverse effect in Sweet Miriam -propylene treated fruit (Figs. 4 and 6). In addition, higher Gol, Raf, Ino and Tre contents in Sweet Miriam, as compared to Santa Rosa fruit, were also observed in our previous study during ripening on-the-tree (Farcuh et al., 2017).

Increased contents of metabolites such as Gol, Raf, Ino and Tre have been reported to be key players in mitigating overall stress effects on plants due to their roles as osmoprotectans, cell membrane stabilizers, and their high antioxidant capacities (Nishizawa et al., 2008; Sun et al., 2013; Taji et al., 2002; Valluru and Van den Ende, 2011; Xue et al., 2007). Dramatic increases in the contents of Gol and Raf were observed in peaches submitted to cold and heat stresses throughout postharvest storage, and gene expression levels of the respective biosynthetic enzymes GolS and RS paralleled this increase, rendering the fruit with better capacity to withstand storage (Lauxmann et al., 2014). Thus, it can be hypothesized that the higher contents of Gol, Raf, Ino and Tre in Sweet Miriam fruit (Figs. 4 and 6) could be priming Sweet Miriam fruit to better cope with stressful situations. This notion could support the capacity of Sweet Miriam fruit to withstand almost three times longer (14 d) in postharvest storage than Santa Rosa fruit (5 d), but needs to be further investigated.

5. Conclusion

In conclusion, we used sugar related-gene expression and sugar contents profiling in fruit of the climacteric Santa Rosa and its bud mutant, the non-climacteric Sweet Miriam cultivar, submitted to different ethylene and 1-MCP treatments throughout postharvest ripening. These treatments contributed to characterize the effect(s) of ethylene on overall sugar metabolism in both ripening types as well as to compare between them. Ethylene seems to present contrasting effects on Suc and Sor metabolism: it reduces Suc catabolism and induces Suc biosynthesis but inversely tends to stimulate Sor breakdown and decrease Sor biosynthesis. Furthermore, Glu and Fru contents are suggested to result from Sor and Suc breakdown in climacteric and non-climacteric fruit, respectively. A positive interaction is shown between ethylene and Gal metabolism, since ethylene promoted an increase in free Gal contents. Finally, the negative effect of ethylene on Gal, Raf, Ino and Tre contents, which were higher in non-climacteric Sweet Miriam fruit, could contribute to the increased capacity of this cultivar to tolerate stresses associated with the ripening process per se and the extended postharvest storage.

Acknowledgements

This research was supported by Will W. Lester Endowment of University of California, Davis. M.F. was a fellowship recipient from Programa Formacion de Capital Humano Avanzado, CONICYT, Chile

References

Bapat, V.A., Trivedi, P.K., Ghosh, A., Sane, V.A., Ganapathi, T.R., Nath, P., 2010. Ripening of fleshy fruit: molecular insight and the role of ethylene. Biotechnol. Adv. 28, 94–107.
Beauvoit, B.P., Colombié, S., Monier, A., Andrieu, M.-H., Biais, B., Bénard, C., Chéniclet, C., Dieuaide-Noubhani, M., Nazaret, C., Mazat, J.-P., 2014. Model-assisted analysis of sugar metabolism throughout tomato fruit development reveals enzyme and carrier properties in relation to vacuole expansion. Plant Cell 26, 3224–3242.
Begheldo, M., 2008. Ethylene and Peach Fruit Ripening: a Functional Genomics Approach.
Biale, J., 1981. Respiration and ripening in fruits-retrospect and prospect. Recent Adv. Biochem. Fruits Veg. 1–39.
Borsani, J., Budde, C.O., Porrini, L., Lauxmann, M.A., Lombardo, V.A., Murray, R., Andreo, C.S., Drincovich, M.F., Lara, M.V., 2009. Carbon metabolism of peach fruit after harvest: changes in enzymes involved in organic acid and sugar level modifications. J. Exp. Bot. 60, 1823–1837.
Bouzayen, M., Latché, A., Nath, P., Pech, J.-C., 2010. Mechanism of fruit ripening. Plant Developmental Biology-Biotechnological Perspectives. Springer, pp. 319–339.
Brady, C., 1987. Fruit ripening. Annu. Rev. Plant Physiol. 38, 155–178.
Burg, S.P., Burg, E.A., 1965. Ethylene action and the ripening of fruits. Science 148, 1190–1196.
Burg, S.P., Burg, E.A., 1967. Molecular requirements for the biological activity of ethylene. Plant Physiol. 42, 144–152.
Chang, S., Puryear, J., Cairney, J., 1993. A simple and efficient method for isolating RNA from pine trees. Plant Mol. Biol. Rep. 11, 113–116.
Chervin, C., Terrier, N., Ageorges, A., Ribes, F., Kuapunyakoon, T., 2006. Influence of ethylene on sucrose accumulation in grape berry. Am. J. Enol. Vitic. 57, 511–513.
Choudhury, S.R., Roy, S., Das, R., Sengupta, D.N., 2008. Differential transcriptional regulation of banana sucrose phosphate synthase gene in response to ethylene, auxin, wounding, low temperature and different photoperiods during fruit ripening and functional analysis of banana SPS gene promoter. Planta 229, 207.
Crisosto, C.H., 1994. Stone fruit maturity indices: a descriptive. Postharvest News Inf. 5, 65N–68N.
Dai, N., Petreikov, M., Portnoy, V., Katzir, N., 2006. Cloning and expression analysis of a UDP-galactose/glucose pyrophosphorylase from melon fruit provides evidence for the major metabolic pathway of galactose metabolism in raffinose oligosaccharide metabolizing plants. Plant Physiol. 142, 294.
Defilippi, B.G., Dandekar, A.M., Kader, A.A., 2004. Impact of suppression of ethylene action or biosynthesis on flavor metabolites in apple (Malus domestica Borkh) fruits. J. Agric. Food Chem. 52, 5694–5701.
Desnoues, E., Gibon, Y., Baldazzi, V., Signoret, V., Génard, M., Quilot-Turion, B., 2014. Profiling sugar metabolism during fruit development in a peach progeny with different fructose-to-glucose ratios. BMC Plant Biol. 14, 1.
Fan, X., Blankenship, S.M., Mattheis, J.P., 1999. 1-Methylcyclopropene inhibits apple ripening. J. Am. Soc. Hortic. Sci. 124, 690–695.
Farcuh, M., Li, B., Rivero, R.M., Shlizerman, L., Sadka, A., Blumwald, E., 2017. Sugar metabolism reprogramming in a non-climacteric bud mutant of a climacteric plum fruit during development on the tree. J. Exp. Bot. 68, 5813–5828.
Génard, M., Souty, M., 1996. Modeling the peach sugar contents in relation to fruit growth. J. Am. Soc. Hortic. Sci. 121, 1122–1131.
Gao, Z., Maurousset, L., Lemoine, R., Yoo, S.-D., Van Nocker, S., Loescher, W., 2003. Cloning expression, and characterization of sorbitol transporters from developing sour cherry fruit and leaf sink tissues. Plant Physiol. 131, 1566–1575.
Giovannoni, J., 2001. Molecular biology of fruit maturation and ripening. Annu. Rev. Plant Biol. 52, 725–749.
Giovannoni, J.J., 2004. Genetic regulation of fruit development and ripening. Plant Cell 16, S170–S180.
Grierson, D., 2013. Ethylene and the control of fruit ripening. Mol. Biol. Biochem. Fruit Ripening 43–73.
Gross, K.C., 1985. Promotion of ethylene evolution and ripening of tomato fruit by galactose. Plant Physiol. 79, 306–307.
Hubbard, N.L., Huber, S.C., Pharr, D.M., 1989. Sucrose phosphate synthase and acid invertase as determinants of sucrose concentration in developing muskmelon (Cucumis melo L.) fruits. Plant Physiol. 91, 1527–1534.
Jia, H., Li, C., Chai, Y., Xing, Y., Shen, Y., 2013a. Sucrose promotes strawberry fruit ripening by stimulation of abscisic acid biosynthesis. Pak. J. Bot. 45, 169–176.
Jia, H., Wang, Y., Sun, M., Li, B., Han, Y., Zhao, Y., Li, X., Ding, N., Li, C., Ji, W., 2013b. Sucrose functions as a signal involved in the regulation of strawberry fruit development and ripening. New Phytol. 198, 453–465.
Jin, Y., Ni, D.-A., Ruan, Y.-L., 2009. Posttranslational elevation of cell wall invertase activity by silencing its inhibitor in tomato delays leaf senescence and increases seed weight and fruit hexose level. Plant Cell 21, 2072–2089.
Kim, J., Gross, K.C., Solomos, T., 1987. Characterization of the stimulation of ethylene production by galactose in tomato (Lycopersicon esculentum Mill.) fruit. Plant Physiol. 85, 804–807.
Kim, H.-Y., Farcuh, M., Cohen, Y., Crisosto, C., Sadka, A., Blumwald, E., 2015a. Non-climacteric ripening and sorbitol homeostasis in plum fruits. Plant Sci. 231, 30–39.
Kim, H.-Y., Saha, P., Farcuh, M., Li, B., Sadka, A., Blumwald, E., 2015b. RNA-Seq analysis of spatiotemporal gene expression patterns during fruit development revealed reference genes for transcript normalization in plums. Plant Mol. Biol. Rep. 33, 1634–1649.
Klann, E.M., Chetelat, R.T., Bennett, A.B., 1993. Expression of acid invertase gene controls sugar composition in tomato (Lycopersicon) fruit. Plant Physiol. 103, 863–870.
Klann, E.M., Hall, B., Bennett, A.B., 1996. Antisense acid invertase (TIV1) gene alters soluble sugar composition and size in transgenic tomato fruit. Plant Physiol. 112, 1321–1330.
Kleczkowski, L.A., Kunz, S., Wilczynska, M., 2010. Mechanisms of UDP-glucose synthesis in plants. Crit. Rev. Plant Sci. 29, 191–203.
Klee, H.J., Giovannoni, J.J., 2011. Genetics and control of tomato fruit ripening and quality attributes. Annu. Rev. Genet. 45, 41–59.
Knee, M., 1976. Influence of ethylene on the ripening of stored apples. J. Sci. Food Agric. 27, 383–392.
Kumar, R., Khurana, A., Sharma, A.K., 2014. Role of plant hormones and their interplay in development and ripening of fleshy fruits. J. Exp. Bot. 65, 4561–4575.
Langenkämper, G., McHale, R., Gardner, R.C., MacRae, E., 1998. Sucrose-phosphate synthase steady-state mRNA increases in ripening kiwifruit. Plant Mol. Biol. 36, 857–869.
Lauxmann, M.A., Borsani, J., Osorio, S., Lombardo, V.A., Budde, C.O., Bustamante, C.A., Monti, L.L., Andreo, C.S., Fernie, A.R., Drincovich, M.F., 2014. Deciphering the metabolic pathways influencing heat and cold responses during post-harvest physiology of peach fruit. Plant Cell Environ. 37, 601–616.
Lee, J., Rudell, D.R., Davies, P.J., Watkins, C.B., 2012. Metabolic changes in 1-methylcyclopropene (1-MCP)-treated 'Empire' apple fruit during storage. Metabolomics 8, 742–753.
Li, M., Feng, F., Cheng, L., 2012. Expression patterns of genes involved in sugar metabolism and accumulation during apple fruit development. PLoS One 7, e33055.
Li, D., Mou, W., Wang, Y., Li, L., Mao, L., Ying, T., Luo, Z., 2016. Exogenous sucrose treatment accelerates postharvest tomato fruit ripening through the influence on its metabolism and enhancing ethylene biosynthesis and signaling. Acta Physiol. Plant. 38, 225.
Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2-\Delta\Delta CT$ method. Methods 25, 402–408.
Lombardo, V.A., Osorio, S., Borsani, J., Lauxmann, M.A., Bustamante, C.A., Budde, C.O., Andreo, C.S., Lara, M.V., Fernie, A.R., Drincovich, M.F., 2011. Metabolic profiling during peach fruit development and ripening reveals the metabolic networks that underpin each developmental stage. Plant Physiol. 157, 1696–1710.
Menniti, A., Gregori, R., Donati, I., 2004. 1-Methylcyclopropene retards postharvest softening of plums. Postharvest Biol. Technol. 31, 269–275.
Minas, I.S., Font i Forcada, C., Dangl, G.S., Gradziel, T.M., Dandekar, A.M., Crisosto, C.H., 2015. Discovery of non-climacteric and suppressed climacteric bud sport mutations originating from a climacteric Japanese plum cultivar (Prunus salicina Lindl.). Front. Plant Sci. 6, 316.
Miron, D., Schaffer, A.A., 1991. Sucrose phosphate synthase, sucrose synthase, and invertase activities in developing fruit of Lycopersicon esculentum Mill. and the sucrose accumulating Lycopersicon hirsutum Humb. and Bonpl. Plant Physiol. 95, 623–627.
Moriguchi, T., Ishizawa, Y., Sanada, T., 1990. Differences in sugar composition in Prunus persica fruit and the classification by the principal component analysis. J. Jpn. Soc. Hortic. Sci. 59, 307–312.
Moriguchi, T., Abe, K., Sanada, T., Yamaki, S., 1992. Levels and role of sucrose synthase, sucrose-phosphate synthase, and acid invertase in sucrose accumulation in fruit of Asian pear. J. Am. Soc. Hortic. Sci. 117, 274–278.
Nishizawa, A., Yabuta, Y., Shigeoka, S., 2008. Galactinol and raffinose constitute a novel function to protect plants from oxidative damage. Plant Physiol. 147, 1251–1263.
Okie, W., Ramming, D., 1999. Plum breeding worldwide. Horttechnology 9, 162–176.
Paul, V., Pandey, R., Srivastava, G.C., 2012. The fading distinctions between classical

patterns of ripening in climacteric and non-climacteric fruit and the ubiquity of ethylene—an overview. J. Food Sci. Technol. 49, 1–21.
Pillet, J., Egert, A., Pieri, P., Lecourieux, F., Kappel, C., Charon, J., Gomès, E., Keller, F., Delrot, S., Lecourieux, D., 2012. VvGOLS1 and VvHsfA2 are involved in the heat stress responses in grapevine berries. Plant Cell Physiol. 53, 1776–1792.
Ponnu, J., Wahl, V., Schmid, M., 2011. Trehalose-6-phosphate: connecting plant metabolism and development. Arabidopsis 2010 and beyond–big science with a small weed 9, 28.
Redgwell, R.J., Fischer, M., Kendal, E., MacRae, E.A., 1997. Galactose loss and fruit ripening: high-molecular-weight arabinogalactans in the pectic polysaccharides of fruit cell walls. Planta 203, 174–181.
Ross, G.S., Wegrzyn, T., MacRae, E.A., Redgwell, R.J., 1994. Apple [beta]-galactosidase (activity against cell wall polysaccharides and characterization of a related cDNA clone). Plant Physiol. 106, 521–528.
Seymour, G.B., Østergaard, L., Chapman, N.H., Knapp, S., Martin, C., 2013. Fruit development and ripening. Annu. Rev. Plant Biol. 64, 219–241.
Singh, Z., Khan, A.S., 2010. Physiology of plum fruit ripening. Stewart Postharvest Rev. 6, 1–10.
Sisler, E.C., Serek, M., 1997. Inhibitors of ethylene responses in plants at the receptor level: recent developments. Physiol. Plant. 100, 577–582.
Sozzi, G., Camperi, S., Cascone, O., Fraschina, A., 1998. Galactosidases in tomato fruit ontogeny: decreased galactosidase activities in antisense ACC synthase fruit during ripening and reversal with exogenous ethylene. Funct. Plant Biol. 25, 237–244.
Sun, Z., Qi, X., Wang, Z., Li, P., Wu, C., Zhang, H., Zhao, Y., 2013. Overexpression of TsGOLS2, a galactinol synthase, in Arabidopsis thaliana enhances tolerance to high salinity and osmotic stresses. Plant Physiol. Biochem. 69, 82–89.
Suzuki, Y., Dandekar, A.M., 2014. Sucrose induces expression of the sorbitol-6-phosphate dehydrogenase gene in source leaves of loquat. Physiol. Plant. 150, 355–362.
Suzuki, Y., 2015. Polyol metabolism and stress tolerance in horticultural plants. Abiotic Stress Biology in Horticultural Plants. Springer, pp. 59–73.
Taji, T., Ohsumi, C., Iuchi, S., Seki, M., Kasuga, M., Kobayashi, M., Yamaguchi-Shinozaki, K., Shinozaki, K., 2002. Important roles of drought-and cold-inducible genes for galactinol synthase in stress tolerance in Arabidopsis thaliana. Plant J. 29, 417–426.
Teo, G., Suzuki, Y., Uratsu, S.L., Lampinen, B., Ormonde, N., Hu, W.K., DeJong, T.M., Dandekar, A.M., 2006. Silencing leaf sorbitol synthesis alters long-distance partitioning and apple fruit quality. Proc. Natl. Acad. Sci. 103, 18842–18847.
Valluru, R., Van den Ende, W., 2011. Myo-inositol and beyond–emerging networks under stress. Plant Sci. 181, 387–400.
Watkins, C.B., 2006. The use of 1-methylcyclopropene (1-MCP) on fruits and vegetables. Biotechnol. Adv. 24, 389–409.
Xue, H., Chen, X., Li, G., 2007. Involvement of phospholipid signaling in plant growth and hormone effects. Curr. Opin. Plant Biol. 10, 483–489.
Yamaki, S., 1986. Roles of four sorbitol related enzymes and invertase in the seasonal alteration of sugar metabolism in apple tissue. J. Am. Soc. Hortic. Sci. 111, 134–137.
Yamaki, S., 1994. Physiology and metabolism of fruit development-biochemistry of sugar metabolism and compartmentation in fruits. Postharvest Physiol. Fruits 398, 109–120.
Yanagisawa, S., Yoo, S.-D., Sheen, J., 2003. Differential regulation of EIN3 stability by glucose and ethylene signalling in plants. Nature 425, 521–525.
Zhang, Y.-J., Wang, X.-J., Wu, J.-X., Chen, S.-Y., Chen, H., Chai, L.-J., Yi, H.-L., 2014. Comparative transcriptome analyses between a spontaneous late-ripening sweet orange mutant and its wild type suggest the functions of ABA, sucrose and JA during citrus fruit ripening. PLoS One 9, e116056.
Zheng, Q., Song, J., Campbell-Palmer, L., Thompson, K., Li, L., Walker, B., Cui, Y., Li, X., 2013. A proteomic investigation of apple fruit during ripening and in response to ethylene treatment. J. Proteomics 93, 276–294.