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

(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