Plant Science xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Plant Science

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

# Non-climacteric ripening and sorbitol homeostasis in plum fruits

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ARTICLE INFO

*Article history:*
Received 20 August 2014
Received in revised form 3 November 2014
Accepted 6 November 2014
Available online xxx

*Keywords:*
Bud sport mutant
Delayed ripening
Ethylene
Non-climacteric ripening
Plum fruit development
Sorbitol metabolism

ABSTRACT

During ripening fruits undergo several physiological and biochemical modifications that influence quality-related properties, such as texture, color, aroma and taste. We studied the differences in ethylene and sugar metabolism between two genetically related Japanese plum cultivars with contrasting ripening behaviors. 'Santa Rosa' (SR) behaved as a typical climacteric fruit, while the bud sport mutant 'Sweet Miriam' (SM) displayed a non-climacteric ripening pattern.

SM fruit displayed a delayed ripening that lasted 120 days longer than that of the climacteric fruit. At the full-ripe stage, both cultivars reached similar final size and weight but the non-climacteric fruits were firmer than the climacteric fruits. Fully ripe non-climacteric plum fruits, showed an accumulation of sorbitol that was 2.5 times higher than that of climacteric fruits, and the increase in sorbitol were also paralleled to an increase in sucrose catabolism. These changes were highly correlated with decreased activity and expression of $NAD^+$-dependent sorbitol dehydrogenase and sorbitol oxidase and increased sorbitol-6-phosphate dehydrogenase activity, suggesting an enhanced sorbitol synthesis in non-climacteric fruits.



## 1. Introduction

Japanese plums [*Prunus salicina* Lindl.] belong to the genus *Prunus* of the family *Rosaceae* and include most commercial fresh-market plums worldwide [1]. In addition to their wide environmental adaptation, there is also a high variability of Japanese plum cultivars with respect to their fruit developmental and ripening patterns [2].

Traditionally, fruit ripening has been defined as either climacteric or non-climacteric. Climacteric fruits are characterized by increased levels of autocatalytic ethylene production and respiration rates during ripening. Once ripening is initiated, fruits continue to ripen past harvest. Non-climacteric fruits show no increase or autocatalytic ethylene production or respiration rates during ripening [3–5]. Although Japanese plums have been classified as climacteric fruits, there are differences in ripening patterns among cultivars [2,6]. While some cultivars, such as 'Santa Rosa' are climacteric [7]; other cultivars, such as 'Shiro' or 'RubyRed' have suppressed-climacteric ripening [8]. Lower ethylene production during ripening and a reduced respiratory peak characterize the latter. Nevertheless, when these cultivars are exposed to exogenous ethylene, typical climacteric ripening is restored [9]. These variations in ethylene production rates among plum cultivars result from differences in their capacity to synthetize ethylene as well as to respond to ethylene, due to differences in ethylene perception and signal transduction pathways [6].

Ethylene biosynthesis requires the activities of two key enzymes: 1-aminocyclopropane-1-carboxylic acid synthase (EC 4.4.1.14, ACS), which mediates the synthesis of 1-amino-cyclopropane-1-carboxylic acid (ACC), and 1-amino-cyclopropane-1-carboxylic acid oxidase (EC 4.4.17.4, ACO), which produces ethylene from ACC [10,11]. Once synthesized, ethylene interacts with a family of membrane-bound receptors (ETR and ERS) [12] that in the absence of the hormone, actively suppress ethylene responses [13]. Upon ethylene binding, the response's suppression is removed, and the signal is transmitted into the nucleus and consequently amplified by a transcription factor cascade, which

*Abbreviations:* ACO, 1-amino-cyclopropane-1-carboxylic acid oxidase; CA, control air; CP, control propylene; CWI, CI, VI, cell wall,, cytosolic and vacuolar invertase, respectively; DAFB, day after full bloom; EOL, ethylene-overproducer-like; ERF, ethylene response factor; ETR, ethylene receptor; MA, 1-MCP air; MP, 1-MCP propylene; $NAD^+$-SDH, $NAD^+$-dependent sorbitol dehydrogenase; SM, Sweet Miriam; SOT, sorbitol transporter; SOX, sorbitol oxidase; S6PDH, sorbitol-6-phosphate dehydrogenase; SPS, sucrose phosphate synthase; SR, Santa Rosa; Susy, sucrose synthase.

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http://dx.doi.org/10.1016/j.plantsci.2014.11.002
0168-9452/

includes Ethylene Insensitive (EIN) and EIN-like-proteins (EILs) [6,10,14]. Finally, members of the AP2/ERF transcription factor family, which include ERFs (Ethylene Response Factors), are involved in a feedback loop that stimulates autocatalytic ethylene synthesis [13] and bind *cis*-elements found in the promoters of target genes, modulating their transcription [15] and thereby inducing downstream ethylene responses that lead to fruit ripening [10].

Japanese plum ripening, like that of other fleshy fruits, is a complex and highly coordinated developmental process [13]. During ripening, fruits undergo several physiological and biochemical modifications that influence properties associated with fruit quality, such as texture (fruit softening), color (chlorophyll degradation and accumulation of non-photosynthetic pigments), aroma (production of volatile compounds) and taste (increase in sugars and decline in organic acids) [5,16,17]. Within taste, sugars are important determinants of sweetness and thus of fruit quality due to their direct association with palatability. In Japanese plums, as in other *Rosaceae* family members, the sugar-alcohol sorbitol is translocated to the fruit along with sucrose. Differences in sugar metabolism with concomitant variations in sucrose, sorbitol, glucose and fructose have been reported [18]. Sucrose synthesis can occur through the enzymatic activity of sucrose phosphate synthase (SPS) or sucrose synthase (SuSy). The first enzyme uses UDP–glucose and fructose-6-phosphate as substrates [19,20], while the second uses UDP–glucose and fructose [21]. Sucrose degradation results from the reversible activity of SuSy and from the activity of cell wall, vacuolar and cytosolic invertases (CWI, VI and CI, respectively), which break down sucrose into glucose and fructose [22]. The sugar-alcohol sorbitol is synthesized by the enzyme sorbitol-6-phosphate dehydrogenase (S6PDH), which reduces glucose-6-phosphate to sorbitol-6-phosphate [23]. Sorbitol is taken up into parenchyma cells via sorbitol transporter (SOT), and once in the cytosol, sorbitol catabolism results from the activity of $NAD^+$-dependent sorbitol dehydrogenase ($NAD^+$-SDH) and sorbitol oxidase (SOX), which convert sorbitol to fructose and glucose, respectively [24].

Here, we study the differences in ethylene and sugar metabolism between two Japanese plum cultivars with contrasting ripening behaviors, but sharing the same genetic background (Farcuh et al., unpublished). 'Santa Rosa' (SR) is a typical climacteric fruit, while 'Sweet Miriam' (SM) has a non-climacteric ripening pattern [25]. The existence of these cultivars provides a very attractive system to study fruit ripening and the interactions between ethylene biosynthesis, signaling and sugar metabolism during ripening. Gene expression, enzymatic activity and metabolite concentrations of the cultivars were compared at an early (end of pit hardening) and late (fully ripe fruit) stage of development. Our aim is to study the relationship between ethylene production and fruit sugar homeostasis during fruit development. The comparison between climacteric and non-climacteric behavior in two genetically related plum varieties offers an ideal model system for the study of the relation between ethylene and fruit ripening and senescence, and could provide valuable insight that would aid breeders in the improvement of fruit quality.

## 2. Materials and methods

### 2.1. Plant material

Japanese plum fruits (*Prunus salicina* cv. Lindl.) were collected during two seasons from a commercial orchard located in the California Central Valley production area (Parlier, CA, USA). Two different cultivars were used: 'Santa Rosa' (SR) and 'Sweet Miriam' (SM). Twenty fruits per each of six biological replications were harvested and immediately transported to the laboratory. Six fruits were used for the evaluation of fruit quality and ripening patterns for each replication and the remaining fruits were peeled, cut into small pieces, frozen in liquid nitrogen and stored at −80 °C until further use.

### 2.2. Fruit growth and development

Fruit sampling started immediately after the natural fruit drop occurring approximately 80–85 days after full bloom (DAFB). Fruit growth patterns were monitored weekly by measuring fruit diameter (size), skin color and firmness. To assess diameter, two dots were initially labeled on a total of 20 fruits and the distance between these was measured weekly. Based on this data, we collected samples of SR and SM plums at specific developmental stages (Table 1). Stages S2, S3, and Stage S4-1 were defined based on fruit size and skin color changes [6,26]. In SR fruits, stage S4-2 was defined based on the high production of ethylene and high respiration rates according to El-Sharkawy et al. [6,26]. Since SM fruits did not display a repiratory burst or a burst in ethylene synthesis, stage S4-2 in these fruits was determined based on the fruit firmness, since firmness is also the parameter of ripening index [27].

For fruit quality and ripening pattern evaluations, fruits from all the stages were harvested. For sugar analysis, relative gene expression and enzymatic assays fruits from S2 and S4-2 were used (Table 1 and Fig. 1A).

### 2.3. Fruit quality evaluations

For each cultivar and sampling date, fruit weight, diameter, flesh firmness, skin and flesh color, soluble solids content (SSC), titratable acidity (TA), and pH were measured on six fruits from each biological replication. Fruit weight and diameter were quantified using an electronic balance (Sartorius, AG Gottingen, Germany) and a digital caliper (Manostat Co., NY, USA). Skin was removed on two opposite sides of each fruit along the equatorial axes and then the chroma ($c^*$), lightness ($L$) and $a^*$ and $b^*$ values were measured using a colorimeter (Konica Minolta CR400 Chroma Meter, Konica Minolta Sensing, Inc., Osaka, Japan). The hue angle ($H^\circ$) that represented changes in primary colors was calculated [28]. Flesh firmness was measured using a Güss FTA Penetrometer with an 8 mm tip (Güss, Strand, Western Cape, South Africa). A wedge from each fruit was removed and pooled to create a composite sample of each replication. Juice was extracted from these composite samples with a hand press, filtered through cheesecloth, and the soluble solids content (SSC), pH and titratable acidity (TA) were determined. SSC was measured using a digital refractometer (AR6 Series Reichert Technologies, Reichert, Inc., NY, USA) and expressed as %, while pH and TA were computed by automatic titration (TIM 850 TitraLab, Radiometer Analytical SAS, Lyon, France) with 0.1 N sodium hydroxide solution to an end point of pH 8.2, and was expressed as % malic acid.

### 2.4. Ripening patterns

At each collection date, fruit ethylene ($C_2H_4$) and carbon dioxide ($CO_2$) evolution were measured using a static system. Each fruit was sealed in a 1 l airtight container and at 20 °C, and ethylene production was calculated by measuring ethylene concentration in the gas phase of the containers, determined by withdrawing a 10 ml headspace gas sample from each container and injecting into a 2 ml fixed sample volume valve of a gas chromatograph (model Carle AGC-211, EG&G Chandler Engineering, Tulsa, OK, USA) equipped with two stainless steel columns (1.22 m and 0.305 m) packed with 8% NaCl on Alumina F-1 80/100 DV (EG&G Chandler Engineering, Tulsa, OK, USA) and a flame ionization detector. Nitrogen ($N_2$) was used as the carrier gas at a flow rate 30 ml $min^{-1}$ while $O_2$ and $H_2$ were used to create the flame of the detector at flow



Please cite this article in press as: H.-Y. Kim, et al., Non-climacteric ripening and sorbitol homeostasis in plum fruits, Plant Sci. (2014), http://dx.doi.org/10.1016/j.plantsci.2014.11.002