Using the genetic diversity of plum to explore the complexity of fruit ripening

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Abstract

Japanese plums, which include most of the fresh-market plums commercialized globally, represent the most abundant and variable group among tree species. We characterized and compared two Japanese plum cultivars, 'Santa Rosa' (SR) and its bud-sport mutant 'Sweet Miriam' (SM). These cultivars share the same genetic background but display contrasting ripening behaviors (SR, climacteric and SM, non-climacteric). Both cultivars differ in their ripening-related changes, what influences properties related to fruit quality, including color, taste, texture and aroma. The main objective of this research was to compare SR and SM in relation to ethylene levels as well as ripening-related changes during postharvest. Fruits from each cultivar were harvested at the mature stage (flesh firmness of ~35 N which coincided with the preclimacteric stage in SR), stored at 20°C and 90% relative humidity and evaluated at harvest and after 7 and 14 days of storage. Targeted gene expression and metabolite concentrations were assessed. Our results show that ethylene biosynthesis-related genes had significantly higher transcript accumulation levels in SR than SM throughout postharvest. Concerning ripening-related changes, flesh and skin color differences between cultivars were supported by contrasting anthocyanin and carotenoid biosynthesis-related gene expression. Sugar composition was also altered between both cultivars (SR presented higher sucrose concentrations, though SM had higher sorbitol abundance); titratable acidity values were significantly lower in SM; while fruit softening rates as well as cell wall polysaccharide-related expression profiles were higher in SR as compared to SM. Overall, this experimental system could provide excellent opportunities for unraveling the complex process(es) associated with climacteric/non-climacteric fruit ripening.

Keywords: *Prunus salicina*, climacteric behavior, bud-sport mutant, fruit quality, ethylene

INTRODUCTION

Japanese plums [*Prunus salicina* Lindl.], which include most of the fresh market plums supplied globally, belong to the *Rosaceae* family and the *Prunus* genus. They are characterized by their high abundance and variability as compared to other tree crops (Blazek, 2007) and previous research described several differences in fruit ripening behavior among Japanese plum cultivars (Abdi et al., 1997; Kim et al., 2015a, b).

Fruit ripening behavior has been typically defined as either climacteric or non-climacteric based on the presence or absence of an increase in respiration rate and burst of autocatalytic ethylene biosynthesis, respectively (Klee and Giovannoni, 2011; Grierson, 2013). In the case of Japanese plums, although they are classified as climacteric, there are differences between cultivars regarding dates and ripening patterns (Abdi et al., 1998; Kim et al., 2015a). For example, cultivars such as 'Santa Rosa' (SR) behave as climacteric (Zuzunaga et al., 2001), while 'Shiro' or 'Ruby Red' displayed suppressed-climacteric ripening patterns (Abdi et al., 1997). In the latter, the cultivars are unable to produce enough ethylene to control fruit ripening; nevertheless when ethylene is applied exogenously, the climacteric behavior is reestablished (Abdi et al., 1998). We reported previously the existence of a non-climacteric behavior in Japanese plums, in the 'Sweet Miriam' (SM)

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cultivar as it ripened on the tree (Kim et al., 2015a). SM, which is a bud-sport mutant of SR, shares the same genetic background as SR, but shows contrasting ripening patterns. All this variability is associated, among others, to differences in ethylene metabolism among these cultivars (El-Sharkawy et al., 2007; Kim et al., 2015a).

Nevertheless, whether climacteric or non-climacteric, fleshy fruit ripening involves changes altering fruit physiology and biochemistry that allow fruits to become edible (Giovannoni, 2004). These ripening-related changes will influence quality-related properties, including color, taste, texture and aroma (Kumar et al., 2013). Within color, chlorophyll degradation and accumulation of non-photosynthetic pigments, such as anthocyanins and total carotenoids, increase in fruit flesh and skin throughout ripening (Singh and Khan, 2010). Regarding taste, sugars and organic acids are important determinants of sweetness and acidity, respectively. In the *Rosaceae* family, the sugar-alcohol sorbitol, as well as sucrose, are major sink-translocated forms of sugar (Moing et al., 1997); while fruit acidity is determined by malic acid contents (Osorio et al., 2013). Concerning texture, fruit softening, resulting from cell wall polysaccharide modifications, among others, are crucial factors determining postharvest fruit potential (Bennett and Labavitch, 2008). Finally, aroma is related with production of volatile compounds (Giovannoni, 2004).

The aim of this study is to characterize and compare, throughout postharvest, SR and its bud-sport mutant SM, which share the same genetic background but display contrasting ripening behaviors (SR, climacteric and SM, non-climacteric), in relation to ethylene levels as well as ripening-related changes.

MATERIALS AND METHODS

Fruit material

Fruits from SR and SM cultivars were harvested at the mature stage, following commercial standards (Crisosto, 1994) at an orchard located in Parlier, CA, USA. The maturity index corresponded to a flesh firmness of ~35 N which coincided with the preclimacteric stage of SR (~110 DAFB for SR and ~180 DAFB for SM). After harvest, fruits were commercially packed in cardboard boxes and delivered, on the same day, to be analyzed at the laboratory at UC Davis.

Postharvest storage

A completely randomized design with 3 biological replications (6 fruits each) was established for each evaluation period per cultivar. Fruit physico-chemical parameters were evaluated at harvest and after 7 and 14 days of postharvest storage at 20°C, 90% relative humidity (RH) in ethylene-free air. Immediately after these measurements, each of the six fruits of each biological replication was peeled, cut into small pieces, pooled, and frozen in liquid nitrogen and stored at -80°C for further analysis. Flesh and skin samples were frozen separately. SR could only be stored for 7 days (instead of 14 days) due to loss of tissue firmness that made fruit handling difficult after this period of time.

Respiration and ethylene production rates

A static system was used to measure fruit respiration and ethylene production rates following the same procedures as described previously (Kim et al., 2015a). Respiration and ethylene production rates were calculated by the concentration of carbon dioxide (CO_2) and ethylene (C_2H_4) in the gas phase of the jars, respectively. Ethylene production rate was expressed as C_2H_4 μL kg^{-1} h^{-1} and respiration rate as CO_2 mL kg^{-1} h^{-1}.

Fruit physico-chemical attributes

Fruit weight, color (skin and flesh), flesh firmness, soluble solids content (SSC), titratable acidity (TA) and pH were measured for each cultivar and evaluation period, as described in Kim et al. (2015a). In each case, 6 fruits were evaluated per biological replication.

Sugar analysis

The concentrations of sugars (sucrose and sorbitol) were quantified as described in Kim et al. (2015a) at harvest and after 14 days of postharvest storage (7 days in the case of SR). Values were expressed as mg g^{-1} of dry weight.

Primer design, RNA isolation, cDNA synthesis and qRT-PCR

Primer design, RNA isolation and cDNA synthesis were carried out following the methods described by Kim et al. (2015b). First strand cDNA synthesis was carried out from 500 ng of total RNA using the QuantiTect reverse transcription kit (Qiagen) following the manufacturer's instructions. Quantitative real-time PCR (qRT-PCR) was performed following the procedure described by Saha and Blumwald (2014). An endogenous control gene, *MON*, was used to normalize gene expression and the fold expression changes of target mRNAs were determined by the $2^{-\Delta\Delta CT}$ method (Kim et al., 2015b).

Statistical analysis

Data were subjected to analysis of variance and significant differences between means were determined by HSD-Tukey test at a probability level of 5% using JMP (SAS Institute Inc., Cary, NC, USA).

RESULTS AND DISCUSSION

Ripening behavior and ethylene production rates

SR displayed a burst in ethylene production together with an increased respiration rate after 7 days of postharvest storage (a typical climacteric behavior) (Abdi et al., 1997), while SM fruits displayed minimal ethylene evolution and respiration rates throughout postharvest (Figure 1). These results correlated well with ethylene biosynthesis-related gene expression levels. The biosynthesis of ethylene results from the conversion of S-adenosyl-L-methionine (SAM) to ACC (1-aminocyclopropane-1-carboxylic acid) through the action of ACC synthase (*ACS*) followed by the oxidation of ACC to ethylene, reaction catalyzed by ACC oxidase (*ACO*) (Yang and Hoffman, 1984). When measuring gene expression levels of *ACO1*, although both cultivars displayed low *ACO1* transcript accumulation at harvest, after 7 days of storage SR increased the expression levels dramatically in comparison to SM, which maintained low constant values (Table 1). This pattern was similar to that reported in the same cultivars assayed at the fully ripe stage 'on' the tree by Kim et al. (2015a), supporting the non-climacteric behavior of SM, also during postharvest.

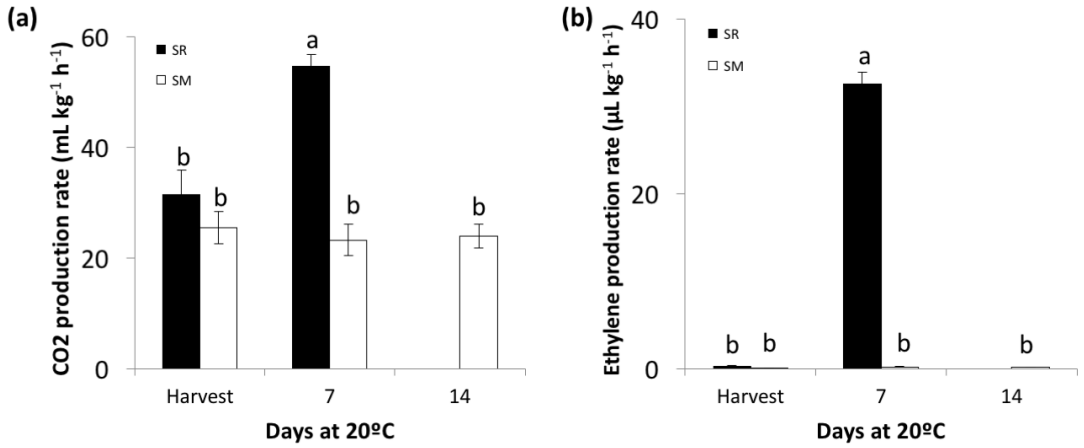

Figure 1. CO_2 (a) and ethylene (b) production rates throughout postharvest storage. SR, 'Santa Rosa' (black bars); SM, 'Sweet Miriam' (white bars). Values are mean±SE (n=3). Different letters indicate significant differences ($p<0.05$).

Table 1. Gene expression associated with ethylene biosynthesis and ripening-related changes throughout postharvest storage.

Gene expression values ($2^{-\Delta\Delta CT}$)	Cultivars	Postharvest storage at 20°C		
		Harvest	7 days	14 days
ACO1 (flesh)	SR	1.44±0.23b	170.53±20.03a	N/A[1]
	SM	0.34±0.03b	3.50±0.26b	4.6±0.18b
PAL (skin)	SR	25.71±1.93ab	29.33±3.23b	N/A[1]
	SM	0.94±0.04b	0.57±0.05b	1.88±0.20b
PSY (flesh)	SR	13.81±1.41b	28.99±2.98a	N/A[1]
	SM	7.90±1.02b	12.11±1.26b	20.02±3.24ab
PG (flesh)	SR	0.01±0.0c	1.34±0.19a	N/A[1]
	SM	0.02±0.0c	0.01±0.00c	0.10±0.01b

[1]Not available.
Values are mean ± SE (*n*=3). Different letters indicate significant differences (p<0.05).

Ripening-related changes

1. Flesh and skin color.

Fruit color changes during postharvest were expressed as hue values. In both cultivars, skin and flesh hue values decreased as postharvest storage time increased, although SR presented lower skin and flesh values as compared to SM (Table 2). These color changes are due to chlorophyll degradation and accumulation of non-photosynthetic pigments, such as anthocyanins and carotenoids (Singh and Khan, 2010; Kumar et al., 2013). Regarding expression of genes associated with anthocyanin and carotenoid pathways, *PAL* (phenylalanine ammonia-lyase) and *PSY* (phytoene synthase) were assayed in both cultivars. *PAL* transcripts accumulation in the skin was significantly higher in SR compared to SM for all periods; while *PSY* expression in the flesh was also higher in SR as compared to SM (Table 1). This could be supported by the fact that ethylene has been shown to be involved in upregulation of genes related with carotenoid (*PSY*) and anthocyanin (*PAL*) pathways in climacteric (McAtee et al., 2013) and non-climacteric (Chervin et al., 2004) fruit types, thus driving the differences observed among cultivars.

Table 2. Fruit ripening-related changes including flesh and skin color, SSC, TA, pH and firmness for each cultivar throughout postharvest storage.

Ripening-related changes	Cultivars	Postharvest storage at 20°C		
		Harvest	7 days	14 days
Flesh color (hue)	SR	81.7±0.4 d	56.9±0.7 e	N/A[1]
	SM	98.7±0.3 a	95.7±0.7 b	88.3±0.4 c
Skin color (hue)	SR	25.2±0.7 c	13.4±0.3 d	N/A[1]
	SM	60.3±1.5 a	58.4±2.2 a	42.9±2.3 b
SSC (%)	SR	12.3±0.1 b	13.7±0.3 b	N/A[1]
	SM	17.2±0.1 a	18.3±0.8 a	18.9±0.4 a
TA (% malic acid)	SR	1.1±0.0 b	0.7±0.0 b	N/A[1]
	SM	0.6±0.0 a	0.6±0.0 a	0.6±0.0 a
pH	SR	3.4±0.0 b	3.6±0.1 b	N/A[1]
	SM	3.9±0.0 a	3.8±0.0 a	3.8±0.0 a
Firmness (N)	SR	35.6±0.1 a	9.7±0.2 c	N/A[1]
	SM	34.7±0.2 a	29.9±0.1 b	27.0±0.1 b

[1]Not available.
Values are mean ± SE (*n*=3). Different letters indicate significant differences (p<0.05).

2. Sugars and titratable acidity.

SSC were significantly higher in SM throughout postharvest ripening as compared to SR, and these differences were maintained constant in both cultivars throughout storage (Table 2). Higher values for SSC were also observed by Kim et al. (2015a) in SM during fruit ripening on the tree and could be mainly due to the longer time that 'SM' fruits stay on the tree before harvest.

Regarding sugar composition, both cultivars presented opposite Sor and Suc metabolic trends. While SR presented the highest Suc concentrations, SM showed higher Sor levels (Table 3). The lack of Suc degradation in SR during postharvest could be due to the occurrence of a 'Suc futile cycling' reported for several fruit species, where there is a balance between the occurrence of Suc synthesis and degradation (Lombardo et al., 2011). The higher levels of Sor in SM could be a consequence of higher synthesis or lower degradation of Sor in SM during postharvest. It has been suggested that the presence of ethylene can increase Sor catabolism-related gene expression (Begheldo, 2008), supporting the decrease in Sor in SR throughout storage.

Table 3. Fruit sugar composition for each cultivar throughout postharvest storage.

Sugar composition	Cultivars	Postharvest storage at 20°C		
		Harvest	7 days	14 days
Sucrose (mg g^{-1} DW)	SR	75.8±1.8a	76.3±3.6a	N/A[1]
	SM	60.8±3.3b	N/A[1]	45.4±1.4c
Sorbitol (mg g^{-1} DW)	SR	41.4±1.9b	23.4±1.2c	N/A[1]
	SM	150.7±2.6a	N/A[1]	146.7±0.8a

[1]Not available.

Values are mean ± SE (*n*=3). Different letters indicate significant differences ($p<0.05$).

TA is generally characterized by a decrease in organic acids concentration throughout ripening. This was observed in SR, which presented the highest TA values at harvest that were significantly reduced during storage, paralleled by the increases in ethylene and respiration rates (Table 2; Figure 1). Guis et al. (1997) reported a strong positive association between organic acid metabolism and respiratory metabolism, thus supporting the decreased TA in SR. In the case of SM, TA values were constant throughout ripening (Table 2) and lower at all evaluation periods. Defilippi et al. (2004), working with apples, reported that organic acids were under ethylene regulation; while Salvador et al. (2003) observed that exogenous application of 1-MCP (1-methylcyclopropene) to SR plums reduced TA loss throughout postharvest ripening. Regarding pH, it showed an opposite behavior to TA, and SM values were significantly higher throughout postharvest as compared to SR (Table 2), as expected.

3. Texture.

Fruit softening is highly desirable from a consumer perspective in order to achieve the maximum sensory quality. On the other hand, fruit softening strongly limits fruit postharvest life (Usenik et al., 2008). Fruit firmness decreased in both cultivars as postharvest storage time increased (Table 2). There were no significant differences between cultivars at harvest because firmness was used as a maturity index (~35 N). Nevertheless, after 7 days of storage, SR presented significantly lower firmness values in comparison with SM. SR fruits presented a high softening rate, which did not allow the fruit to last 14 days in storage; while SM fruits softened very slowly (Table 2).

Fruit softening is a result of fruit cell wall polysaccharide modifications, therefore the expression profile of polygalacturonase (*PG*), a pectin solubilization-related gene (Bennett and Labavitch, 2008) was assayed in both cultivars throughout postharvest. *PG* showed significantly higher expression levels after 7 days of storage in SR as compared to SM (Table 1). This increased *PG* expression correlated well with the highest ethylene production rate observed in SR (Figure 1). Regarding ethylene, it has been shown that both ethylene

dependent and independent pathways of cell wall related gene regulation coexist in climacteric fruits (Bennett and Labavitch, 2008). Thus, the occurrence of fruit softening in the non-climacteric SM, reinforce the notion of ethylene-independent cell wall modification processes.

CONCLUSIONS

In conclusion, we characterized and compared throughout postharvest storage, two genetically related Japanese plum cultivars, SR and SM, which show contrasting ripening behaviors. Ethylene production rates and biosynthesis-related gene expression levels differed between both cultivars, as well as ripening-related changes including color, sugar composition, acidity and texture. Overall, this experimental system could provide excellent opportunities for the study of the complex process(es) associated with climacteric/non-climacteric fruit ripening.

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