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Abscisic acid and pyrabactin improve vitamin C contents in raspberries

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ABSTRACT

Abscisic acid (ABA) is a plant growth regulator with roles in senescence, fruit ripening and environmental stress responses. ABA and pyrabactin (a non-photosensitive ABA agonist) effects on red raspberry (*Rubus idaeus* L.) fruit development (including ripening) were studied, with a focus on vitamin and antioxidant composition. Application of ABA and/or pyrabactin just after fruit set did not affect the temporal pattern of fruit development and ripening; neither provitamin A (carotenoids) nor vitamin E contents were modified. In contrast, ABA and pyrabactin altered the vitamin C redox state at early stages of fruit development and more than doubled vitamin C contents at the end of fruit ripening. These were partially explained by changes in ascorbate oxidation and recycling. Therefore, ABA and pyrabactin applications may be used to increase vitamin C content of ripe fruits, increasing fruit quality and value. However, treatments containing pyrabactin—combined with ABA or alone—diminished protein content, thus partially limiting its potential applicability.

1. Introduction

Climacteric fruits are well characterized in physiological terms, showing a peak in ethylene production and a respiratory burst at the onset of ripening (Seymour, Østergaard, Chapman, Knapp, & Martin, 2013) regulating the development of the traits associated with ripe fleshy fruits: (i) change of color due to alteration of chlorophyll, carotenoid, and/or anthocyanin contents; (ii) modification of texture via alteration of cell turgor and cell wall structure and/or metabolism; (iii) import, accumulation and modification of sugars, acids, and volatiles that determine nutritional quality, flavor, and aroma; and (iv) enhanced susceptibility to opportunistic pathogens and herbivores (Seymour et al., 2013; Symons et al., 2012). Less is known about ripening regulation in non-climacteric fruits and its association with ripening traits. These fleshy fruits sometimes present a rise in ethylene levels, but do not exhibit a respiratory peak (Perkins-Veazie & Nonnecke, 1992).

Without ethylene as the key signal regulating fruit ripening, evidence has accumulated that the balance between abscisic acid (ABA) and indole-3-acetic acid (IAA) plays this role in non-climacteric fruits (Seymour et al., 2013). IAA levels are high in the early growth and expansion phases of the developing fruit but decrease in later ripening stages, antagonistically to ABA, accumulated from the onset of ripening to later stages (Seymour et al., 2013; Symons et al., 2012). ABA contents and signaling are strictly regulated all through fruit development and ripening (Romero, Lafuente, & Rodrigo, 2012; Symons et al., 2012), with very low ABA levels at the start of fruit development that sharply rise as fruit ripens, especially after the onset of fruit ripening. Conserving the capacity to synthesize and perceive ABA is essential for a proper fruit ripening (Chai, Jia, Li, Dong, & Shen, 2011; Jia et al., 2011). And so forth, it is widely suggested that ABA plays an important role in the regulation of the rate of fruit ripening in non-climacteric fruits, with disturbs on ABA metabolism or perception representing a great impact on the associated fruit characteristics (Chai et al., 2011; Romero et al., 2012; Setha, 2012; Seymour et al., 2013; Zhang, Yuan, & Leng, 2009).

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Vitamins are a group of organic compounds that are absolutely required in the human diet and the major source in most diets are vegetables and fruits. Notably, vitamin C (ascorbate) contents in fruits are high but very variable and especially sensitive to pre- and post-harvest conditions (Lee & Kader, 2000). Due to its importance in health and fruit quality, it is important to understand the evolution of vitamin C contents through its production and losses during fruit development and ripening. The study of vitamin C dynamics throughout fruit developing and ripening may help in establishing harvest dates in order to maximize the amount of vitamin C in fruits, thus improving its quality and value. To the best of our knowledge there are no previous studies of vitamin C contents throughout the development and ripening of raspberry fruits.

Red raspberry (*Rubus idaeus* L.) is an important commercial crop due to its rich nutritional value and organoleptic characteristics. Despite the fact that ethylene partially promotes fruit softening and abscission, raspberries are non-climacteric (Perkins-Veazie & Nonnecke, 1992). Raspberries are a valuable source of phytochemicals, notably antioxidants (Beekwilder, Hall, & Ric Vos, 2005; Rao & Snyder, 2010), with important contents of two classes of health-promoting polyphenols—anthocyanins and ellagitannins (Beekwilder et al., 2005)—vitamin C (De Ancos, Gonzalez, & Cano, 2000), carotenoids (Beekwilder et al., 2008), vitamin E (Beekwilder et al., 2005) and various secondary metabolites (Beekwilder et al., 2005). The content of different antioxidant compounds in red raspberry fruits is influenced by the stage of ripeness at the time of the harvest. Raspberries are usually harvested once fully ripe and the berry starts to detach from the receptacle, and they can only be stored for a few days and at low temperatures (Rao & Snyder, 2010). This limited shelf-life stresses the importance of understanding its physiology and the dynamics of their valued phytochemicals throughout its development and ripening to select the best harvest date.

Variations in phenolic contents are well described in a number of raspberry varieties and growth conditions; only in the late stages when the red fruit fully matures, anthocyanins sharply increase (Beekwilder et al., 2005). However, little has been described about the evolution of carotenoids (provitamin A), vitamin C and vitamin E contents throughout ripening in raspberries (Beekwilder et al., 2008; Krüger, Dietrich, Schöpplein, Rasim, & Kürbel, 2011); and even less if these might be associated or not with plant growth regulators (Miret et al., 2014). The efforts to manipulate antioxidant and vitamin levels of raspberry fruits—almost always focused in flavonoids and antioxidant activity—have been concentrated in postharvest and storage practices (De Ancos et al., 2000; Krüger et al., 2011); a number of studies have analyzed the possibilities of manipulating growth conditions (Anttonen & Karjalainen, 2005; Neocleous & Vasilakakis, 2007); while only a few explore the potential of plant growth regulators, i.e.: with the application of methyl jasmonate before harvest (Wang & Zheng, 2005) or manipulating auxins biosynthesis (Mezzetti, Landi, Pandolfini, & Spena, 2004).

Understanding the physiology and biochemical basis of vitamin and antioxidant contents throughout fruit development and ripening is necessary to help in selecting the best harvest time and to prevent loss of nutritional and commercial value at harvest and postharvest. The aim of the present study was the evaluation of the potential effects of ABA applications just after fruit set on fruit quality, with a focus on antioxidants and vitamins. We extended this approach to pyrabactin, a non-photosensitive ABA agonist with great agronomic potential. Pyrabactin is a non-photosensitive partial ABA agonist and antagonist (Park et al., 2009). Apart from mimicking and blocking ABA signals, it has been proven to be effective in modulating stomatal closure (Puli & Raghavendra, 2012) as well as leaf and flower senescence (Arrom & Munné-Bosch, 2012a, 2012b) but its potential effects in fruit ripening are still unknown.

2. Material and methods

2.1. Plant material and treatments

Fruits from 2-year-old plants of cv. 'Heritage' raspberries were harvested between the 22th and 27th of June 2012. Twenty raspberry plants were purchased from a nursery (Ejea de los Caballeros, Spain) and grown in pots containing a mixture of peat:perlite:vermiculite (1:1:1, v/v/v) in a greenhouse at the experimental fields of the Faculty of Biology at the University of Barcelona (NE Spain) with controlled temperature (24/18 °C, day/night) and irrigated with half Hoagland solution every day. Raspberry bushes were sprayed monthly with recommended fungicides and acaricides on a preventive schedule. Green fruits (one or two days after fruit set) were treated with 10^{-5} M ABA (dissolved in 0.5% dimethyl sulfoxide and 0.1% Tween-20 (v/v)), 10^{-5} M pyrabactin, 10^{-5} M pyrabactin + 10^{-5} M ABA, and a control treatment (with 0.5% dimethyl sulfoxide 0.1% Tween-20 only). The different treatments were applied once to fruits at the start of the green stage (small green fruits, after fruit-set) with a brush until the solution ran off (*ca.* 200 μl of solution was applied to each berry). Application was done at dawn to limit ABA photo-destruction (Zaharia, Walker-Simmon, Rodríguez, & Abrams, 2005).

Fruits were collected at different ripening stages, defined according to a subjective assessment of berry color as green, white, pink, red and dark red (abbreviated as G, W, P, R and DR, adapted from Perkins-Veazie & Nonnecke, 1992). Sampling always took place between 8 a.m. and 10 a.m. (UTC/GMT +1). Fruits were cut with intact receptacle and pedicel to minimize wounding effects, weighed, immediately frozen in liquid nitrogen and subsequently stored at −80 °C until biochemical analyses. Only the drupelets (the edible part of the fruit, with neither the pedicel nor the receptacle) were considered for all biochemical analyses. Unless sated otherwise, 5 independent biological replicates where used for all biochemical analyses. The ripening time pattern was monitored by the daily register of the ripening stage of 85–100 fruits per treatment. Further, a subset of these was also harvested at each stage to determine whole fruit fresh and dry weight, as well as the maximum efficiency of photosystem II photochemistry (F_v/F_m ratio, an indicator of chloroplast functionality).

Unless stated otherwise, all chemicals and reagents were purchased from Sigma-Aldrich (Germany) and were of HPLC grade or superior.

2.2. Vitamin E and pigment determination

The extraction of vitamin E compounds, total carotenoids, chlorophyll and anthocyanins was performed after Miret et al. (2014), as follows. In brief, 100 mg of tissue was ground with the mixer mill MM400 (Retsch GmbH, Germany) and extracted with cold methanol by repeated vortexing and ultrasonication (Branson 2510 ultrasonic cleaner; Bransonic, USA). The procedure was repeated several times until the pellet was colorless. After centrifugation, the collected supernatants were merged. Vitamin E was determined by HPLC with fluorescence detection using a calibration curve prepared with authentic standards for each tocopherol and tocotrienol compound (Miret et al., 2014). Chlorophyll, carotenoids and anthocyanin levels (as cyanidin-3-glucoside equivalents) were determined spectrophotometrically (Lichtenthaler & Wellburn, 1983; Siegelman & Hendricks, 1958).