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Ethylene and auxin biosynthesis and signaling are impaired by methyl jasmonate leading to a transient slowing down of ripening in peach fruit

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ABSTRACT

Peach (*Prunus persica*) was chosen as a model to further clarify the physiological role of jasmonates (JAs) during fruit ripening. To this aim, the effect of methyl jasmonate (MJ, 0.88 mM), applied at a late stage (S3) of fruit development under field conditions (in planta), on the time-course of fruit ripening over a 14-day period was evaluated. As revealed by a non-destructive device called a DA-meter, exogenously applied MJ impaired the progression of ripening leading to less ripe fruit at harvest. To better understand the molecular basis of MJ interference with ripening, the time-course changes in the expression of ethylene-, cell wall-, and auxin-related genes as well as other genes (*LOX*, *AOS* and *bZIP*) was evaluated in the fruit mesocarp. Real-time PCR analyses revealed that transcript levels of ethylene-related genes were strongly affected. In a first phase (days 2 and/or 7) of the MJ response, mRNAs of the ethylene biosynthetic genes *ACO1*, *ACS1* and the receptor gene *ETR2* were strongly but transiently down-regulated, and then returned to or above control levels in a second phase (days 11 and/or 14). Auxin biosynthetic, conjugating, transport and perception gene transcripts were also affected. While biosynthetic genes (*TRPB* and *IGPS*) were up-regulated, auxin-conjugating (*GH3*), perception (*TIR1*) and transport (*PIN1*) genes were transiently but strongly down-regulated in a first phase, but returned to control levels subsequently. Transcript levels of two JA-related genes (*LOX*, *AOS*) and a developmentally regulated transcription factor (*bZIP*) were also affected, suggesting a shift ahead of the ripening process. Thus, in peach fruit, the transient slowing down of ripening by exogenous MJ was associated with an interference not only with ethylene but also with auxin-related genes.

Introduction

Jasmonic acid, its volatile ester methyl jasmonate (MJ), and other derivatives, collectively known as jasmonates (JAs), are ubiquitous signaling molecules, synthesized from α-linolenic acid via lipoxygenase (LOX) activity, with allene oxide synthase (AOS) as the first specific enzyme and the major control point of the JA biosynthetic pathway (Haga and Iino, 2004). JAs mediate plant responses to environmental stress such as salt stress, wounding and pathogen and insect attacks (Peña-Cortés et al., 2005; Wasternack, 2007), and play a role during developmental processes, including plant growth, seed and pollen germination, and fruit development and ripening (Wasternack, 2007; Rohwer and Erwin, 2008). Indeed, exogenous JAs provoke dramatic transcriptional responses in most plant tissues; previous work led to the identification of JA-responsive genes, coding for JA-induced proteins, such as JA biosynthetic enzymes, enzymes of secondary metabolism, and pathogenesis-related and cell wall-related proteins. Genome-wide transcript profiling provided a deeper insight into the pleiotropic control exerted by JAs on plant development and survival (Memelink, 2009; Pauwels et al., 2009).

Ripening is a complex, genetically programmed process: in climacteric fruit, progressive physicochemical changes involving color, texture, flavor and aroma, which all contribute to overall fruit quality, are induced and, at least in part, co-ordinated by changes in ethylene biosynthesis and perception (Giovannoni, 2004). Peach fruit is considered a drupe model as ripening has been

Abbreviations: ACO, aminocyclopropane-1-carboxylate oxidase; ACS, 1-aminocyclopropane-1-carboxylate synthase; AOS, allene oxide synthase, jasmonates; bZIP, basic leucine zipper; ERF, ethylene response factor; ETR, ethylene receptor; EXP, expansin; FF, flesh firmness; I_{AD}, index of absorbance difference; GH3, IAA-amino acid synthase; IAA, indole-3-acetic acid; IGPS, indole-3-glycerol phosphate synthase; JAs, jasmonates; LOX, lipoxygenase; MJ, methyl jasmonate; PDJ, propyldihydrojasmonate; PG, polygalacturonase; PIN, PIN-FORMED1; qRT-PCR, quantitative reverse transcription-polymerase chain reaction; SSC, soluble solids concentration; TIR, transport inhibitor response; TRPB, tryptophan synthase β subunit.

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extensively characterized (Ruperti et al., 2001; Rasori et al., 2002; Dal Cin et al., 2006; Ziliotto et al., 2008); in particular, a microarray transcriptome analysis performed during the transition from the pre-climacteric to climacteric stage has shown a dramatic up-regulation of genes encoding transcription factors and involved in ethylene biosynthesis, perception and signaling (Trainotti et al., 2006, 2007).

Hormones other than ethylene are involved in the control of the ripening process (Gillaspy et al., 1993). Amongst these, auxin seems to play a major role. Most knowledge about IAA-regulated processes including biosynthesis, conjugation, perception and transport comes from the model plant *Arabidopsis* (Woodward and Bartel, 2005; Delker et al., 2008); less is known about the potential role of IAA in the development of fleshy fruit. In tomato, a climacteric fruit, the importance of auxin for fruit set and growth is well established and correlates with IAA levels, which peak during cell division and decline to low levels at the onset of ripening (Buta and Spaulding, 1994). In fruit, auxin can stimulate climacteric ethylene synthesis (system 2) through the up-regulation of the biosynthetic key enzyme 1-aminocyclopropane carboxylate synthase (ACS) (Abel and Theologis, 1996; Kondo et al., 2009). Indeed, Trainotti et al. (2007) identified several genes involved in auxin biosynthesis, transport and signaling whose expression substantially increased during ripening in peach mesocarp, thus demonstrating that an important cross-talk between auxin and ethylene occurs, with genes in the auxin domain regulated by ethylene, and genes in the ethylene domain regulated by auxin. Accordingly, an increase in free auxin levels has been reported in peach prior to ripening (Miller et al., 1987; Masia et al., 1992).

In climacteric fruit, such as apple, peach and tomato, JA levels and AOS gene expression increase at ripening suggesting that they are involved in the regulation of this process (Fan et al., 1998; Howe et al., 2000; Kondo et al., 2000; Ziosi et al., 2008a; Torrigiani et al., 2012). Indeed, in general, exogenous JAs positively affect fruit quality traits (Rohwer and Erwin, 2008); for instance they stimulate β-carotene synthesis (Pérez et al., 1993) and phenolic compound (anthocyanin) accumulation (Rudell and Mattheis, 2008), promote volatile emission (Kondo et al., 2005), improve fruit quality and antioxidant activity (Wang et al., 2009; Cao et al., 2009), and induce disease resistance during storage (Jin et al., 2009). However, the reciprocal relationships between JAs and ethylene are not always univocal. Exogenously applied MJ has shown to inhibit or enhance fruit ethylene production in apples and pears in relation to the fruit ripening stage (Fan et al., 1997; Kondo et al., 2007). By contrast, in nectarines, both early and late JA treatment during fruit development resulted in down-regulation of the ethylene biosynthetic and softening-related genes (Ziosi et al., 2008a; Ruiz et al., 2010).

In the present work, peach was chosen as a model to further clarify the molecular bases of the effects of JAs on fruit quality and ripening through the analysis of transcript profiles of several ripening-related genes during fruit ripening, with a special focus on those related to auxin given the positive involvement of this hormone in the process (Trainotti et al., 2007). To this aim, the natural derivative of jasmonic acid, MJ, was applied to Redhaven peach fruit at a late developmental stage (S3, pre-climacteric), under field conditions, and the following were analyzed in the mesocarp: (i) extent of ripening, fruit quality and ethylene production and (ii) transcript levels of ethylene- and cell wall-related genes, of auxin biosynthetic, conjugating, perception and signaling genes, of two JA-related genes, and of a developmentally regulated transcription factor. Results show that peach ripening physiology was profoundly, though transiently, affected by MJ.

Materials and methods

Plant material and experimental design

The trial was conducted at the S. Anna experimental field of the University of Bologna, Italy, on twenty-year-old peach trees (*Prunus persica* L. Batsch) cv. Redhaven, grafted on seedling rootstock and trained to a free open-vase. Four branches per plant (seven plants per treatment), homogeneous for size and fruit load (3–4 fruit per branch) were randomly selected for the experiments. Branches were sprayed with 0.88 mM MJ (Nippon Zeon Co., Tokyo, Japan) aqueous solution (2 L per tree); the latter was prepared by diluting a 5% MJ stock solution containing 30% (v/v) surfactant (Rheodor460, Nippon Zeon Co., Tokyo, Japan) and 32.5% (v/v) ethanol (Ziosi et al., 2008a); control branches were sprayed with an aqueous solution containing the same concentration of surfactant and ethanol. The double sigmoidal growth pattern of the peach fruit was established in order to discriminate the four growth stages S1–S4 as previously described (Bregoli et al., 2002). MJ treatments were performed at the late S3 (97 days after full bloom, dAFB) stage of fruit growth which precedes the onset of ripening and is characterized by a high growth rate (Tonutti et al., 1997). Control and treated fruit were picked 2, 7, 11 and 14 days (commercial harvest) after treatment; at harvest 80 fruits were used for non-destructive ripening assessments by the DA-meter (see below). For fruit quality and ethylene measurement 10 fruit were used and for molecular analysis mesocarp pieces from 10 fruit were pooled for each sampling time, and stored at −80 °C until use.

Fruit ripening assessment and ethylene and fruit quality trait determination

The extent of fruit ripening was non-destructively assessed for each sampling time by means of the DA-meter, a portable and non-destructive device based on visible/near infrared (vis/NIR) spectroscopy developed and patented by the University of Bologna (Costa et al., 2005). This instrument gives a fruit maturity index, called "Index of Absorbance Difference" (I_{AD}) that is based on fruit absorbance spectra acquired by a spectrometer in the 650–1200 nm wavelength range and is calculated as:

$$I_{AD} = A_{670} - A_{720}$$

where A_{670} and A_{720} were the absorbance values at the wavelengths of 670 and 720 nm, respectively. This difference in absorbance between two wavelengths near the chlorophyll-*a* peak (I_{AD}) is strictly correlated to the actual chlorophyll-*a* content in peach fruit flesh and to the time course of ethylene production during fruit ripening (Ziosi et al., 2008b). Therefore, considering that peach is a climacteric fruit whose chlorophyll content decreases during ripening (Chalmers and van den Ende, 1975), the I_{AD} allows to group peach fruit on the basis of their ripening stage in homogeneous classes. In particular, the I_{AD} continuously decreases during the progression of peach fruit ripening (Ziosi et al., 2008b).

Ethylene production was measured by placing the whole detached fruit in a 1.0 L jar with an air-tight lid equipped with a rubber stopper, and left at room temperature for 1 h. A 10 mL gas sample was taken and injected into a Dani HT 86.01 (Dani, Milan, Italy) gas chromatograph fitted with a flame ionization detector and a Porapak Q column (Supelco, Bellefonte, PA, USA). The carrier gas was nitrogen at a flow rate of 16 mL min^{-1}. The oven temperature was 80 °C for the column and 150 °C for the injector and flame ionization detector. Ethylene was identified and its concentration was calculated as described in Bregoli et al. (2002).

Flesh firmness (FF) was measured using a pressure tester (EFFE.GI, Ravenna, Italy), and soluble solids concentration (SSC)