Jasmonate-induced transcriptional changes suggest a negative interference with the ripening syndrome in peach fruit

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Received 14 September 2007; Revised 9 November 2007; Accepted 27 November 2007

Abstract

Peach (Prunus persica L. Batsch) was chosen as a model to shed light on the physiological role of jasmonates (JAs) during fruit ripening. To this aim, the effects of methyl jasmonate (MJ, 0.40 mM) and propyl dihydrojasmonate (PDJ, 0.22 mM), applied in planta at different fruit developmental stages, on the time-course of ethylene production and fruit quality traits were evaluated. MJ-induced changes in fruit transcriptome at harvest and the expression profiling of relevant JA-responsive genes were analysed in control and JA-treated fruit. Exogenously applied JAs affected the onset of ripening depending upon the fruit developmental stage, with PDJ being more active than MJ. Both compounds enhanced the transcription of allene oxide synthase (PpAOS1), the first specific enzyme in the biosynthesis of jasmonic acid, and altered the pattern of jasmonic acid accumulation. Microarray transcriptome profiling showed that MJ down-regulated some ripening-related genes, such as 1-aminocyclopropane-1-carboxylic acid oxidase (PpACO1) and polygalacturonase (PG), and the transcriptional modulator IAA7. MJ also altered the expression of cell wall-related genes, namely pectate lyase (PL) and expansins (EXPs), and up-regulated several stress-related genes, including some of those involved in JA biosynthesis. Time-course expression profiles of PpACO1, PL, PG, PpExp1, and the transcription factor LIM confirmed the array results. Thus, in peach fruit, exogenous JAs led to a ripening delay due to an interference with ripening- and stress/defence-related genes, as reflected in the transcriptome of treated fruit at harvest.

Key words: Allene oxide synthase, ethylene, fruit ripening, jasmonic acid, methyl jasmonate, microarray, propyl dihydrojasmonate, Prunus persica.

Introduction

Jasmonic acid, its volatile ester methyl jasmonate (MJ), and other derivatives, collectively known as jasmonates (JAs), are ubiquitous signalling molecules which mediate plant responses to environmental stress such as wounding, and insect and pathogen attack (Wasternack, 2007). JAs are synthesized via a series of steps starting from ω-linolenic acid in the octadecanoid pathway. Allene oxide synthase (AOS) is the first specific enzyme and the major control point of their biosynthetic pathway. AOS genes have been cloned in several plant species, and their expression pattern analysed in response to wounding, and the major control point of their biosynthetic pathway. AOS genes have been cloned in several plant species, and their expression pattern analysed in response to wounding, biotic stress, and JA treatments (Maucher et al., 2000; Ziegler et al., 2001). Indeed, exogenous JAs provoke dramatic transcriptional responses in most plant tissues; this has led to the identification of JA-responsive genes (JRGs), coding for JA-induced proteins (JIPs), such as JA
biosynthetic enzymes, enzymes of secondary metabolism, and pathogenesis-related and cell wall-related proteins (Jung et al., 2007; Walia et al., 2007).

JAs also play a role during developmental processes, including root growth, seed germination, pollen development, and fruit development and ripening (Peña-Cortés et al., 2005; Wasternack, 2007). Ripening is a complex, genetically programmed process; in climacteric fruit, progressive physiochemical and physiological changes involving colour, texture, flavour, 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). Studies on the role of ethylene during ripening of climacteric fruit have been carried out mainly in tomato (Alba et al., 2005); however, recent advances in genomics technologies have extended research to other fruit species (da Silva et al., 2005; Newcomb et al., 2006). In this context, peach fruit is becoming a very promising climacteric drupe model as genes involved in ethylene biosynthetic and signal transduction pathways have been extensively characterized. As far as ethylene biosynthesis is concerned, members of the 1-aminocyclopropane-1-carboxylic acid (ACC) synthase (ACS) and oxidase (ACO) families strictly related to ripening have been identified (Ruperti et al., 2001). The transcripitional profile of some orthologues of Arabidopsis genes involved in ethylene perception and signal transduction (ETR1, ERS1, ETR2, EIN4, and CTR1) point to their different expression pattern and ethylene sensitivity during peach fruit growth and ripening (Rasori et al., 2002; Dal Cin et al., 2006; Trainotti et al., 2006).

Multiple interferences and intersections between the JA and ethylene signalling pathways were inferred by analysing Arabidopsis mutants (Devoto and Turner, 2005). In climacteric fruit, such as apple and tomato, JA levels increase at ripening, suggesting that they could be involved in the modulation of this process (Fan et al., 1998a; Kondo et al., 2000). However, the JA–ethylene relationship in fruit is still largely unclear: in tomato, ethylene production is stimulated by MJ application independently of fruit growth stage (Saniewsky et al., 1987), while, in apple and pear, this molecule enhances ethylene emission in pre-climacteric fruit, but inhibits it in climacteric and post-climacteric fruit (Fan et al., 1997, 1998a; Kondo et al., 2007). In apple, the impact of MJ application on ethylene production was even cultivar dependent (Kondo et al., 2005).

In the present work, peach was chosen as a model to shed some light on the physiological role of JAs during fruit ripening and the reciprocal relationship between ethylene and JAs. The natural derivative of jasmonic acid, MJ, and its synthetic analogue n-propyl dihydrojasmonate (PDJ) were applied to peach fruit at different developmental stages under field conditions, and the following were analysed: (i) time-course of ethylene production and fruit quality; (ii) transcription of PpAOS1 and changes in JA levels; (iii) comparative transcriptome profiling of MJ-treated versus control fruit at harvest; and (iv) expression patterns of some JA-responsive genes following treatments.

Materials and methods

Plant material and experimental design

Trials were carried out at the experimental farm of the University of Bologna, Italy, on 8-year-old peach (Prunus persica L. Batsch, ‘Stark Red Gold’ nectarine) trees (seven plants per treatment) grafted on seedling rootstock and trained to a Y shape. Four branches per plant, homogeneous for size and fruit load (3–4 fruit per branch), were selected for the experiments. For each treatment, 28 branches were sprayed with 50 ppm (0.22 mM) or 100 ppm (0.44 mM) MJ, or 50 ppm (0.20 mM) PDJ (Nippon Zeon Co., Tokyo, Japan). Both compounds were applied as an aqueous solution (150 ml per branch), which was prepared by diluting a 5% MJ or PDJ stock solution containing 30% (v/v) surfactant (Rheodor460, Nippon Zeon Co.) and 32.5% (v/v) ethanol, following the manufacturer’s instructions. Control branches only received an aqueous solution containing the same concentration of surfactant and ethanol.

The double sigmoid growth pattern of nectarines was established as previously described (Bregoli et al., 2002; Ziosi et al., 2006) in order to discriminate the four growth stages S1, S2, S3, and S4 from 30 d after full bloom (dAFB) up to 130 dAFB (Fig. 1). MJ and PDJ were applied at the S3 stage (102 dAFB, 21 d before harvest, i.e. early application), the S3/S4 transition (109 dAFB, 14 d before harvest, i.e. mid-period application), and the S4 stage (116 dAFB, 7 d before harvest, i.e. late application). Following each MJ and PDJ treatment, 20 control and 20 treated fruits were sampled at time intervals up to first harvest (123 dAFB; tree-ripe control fruit). On the same day, a sample of 20 control and 20 treated fruit was set apart and stored at 4 °C for 7 d to monitor the progression of ripening. A second harvest was performed 7 d later (130 dAFB; over-ripe control fruit). For each concentration and time of application, ethylene production and fruit quality traits were determined on whole fruit at each sampling time, while, for
molecular analyses, mesocarp tissues from fruit which had received an early application of 100 ppm MJ and 50 ppm PDJ were stored separately at –80 °C until use.

Ethylene and fruit quality traits determination
Ethylene production was measured by placing the whole detached fruit in a 1.0 l jar sealed 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) packed-gas chromatograph (Bregoli et al., 2002). Flesh firmness (FF) was measured using a pressure tester (EFFEGI, Ravenna, Italy), and the soluble solids concentration (SSC) was determined with an Atoo digital refractometer (Optolab, Modena, Italy), as previously described by Bregoli et al. (2002). Fruit ground and blush colour were measured in the CIE L* (light to dark) a* (green to red) b* (blue to yellow) colour space by using a colorimeter (AvaMouse, Avantes, Boulder, CO, USA). According to McGuire (1992), values of a* and b* were converted to hue angle (h°, tan⁻¹ b/a), which quantifies colour (0°=red, 90°=yellow, and 180°=green).

Isolation of a peach AOS fragment
Total RNA from fruit mesocarp was extracted following the protocol described by Bonghi et al. (1998). A 2 µg aliquot of total RNA from mesocarp of nectarines at S3 stage was treated with 2 U of DNase I Amplification Grade (Invitrogen, Carlsbad, CA, USA). The first-strand cDNA was obtained from 1 µg of the DNase-treated RNA by using the SuperScript™ III First Strand Synthesis System for RT-PCR (Invitrogen) with oligo(dT12–18) as a primer. A 1 µl aliquot of cDNA was PCR amplified with 25 pmol of degenerate primers [5'-CTC(T/G) GA(T/C) AA(A/G) AG(C/T) TT(T/C) CC-3', sense; 5'-GT(C/T)TC CG(G/A) G(A/T)CC(A/ G)TT(A/T/G) GA CCA-3', antisense] designed on the basis of the conserved amino acid regions of other plant AOSs. The PCR conditions were: 95°C for 5 min, followed by 40 cycles of 95°C for 1 min, 56°C for 1 min, 72°C for 2 min, and a final extension of 7 min at 72°C. The PCR produced a single fragment of the expected size, which was purified with the Qiaquick PCR Purification Kit (Qiagen) and cloned into the pGEM-T or Easy vector (Promega, Madison, WI, USA) for sequencing. The sequenced fragment (named PPpAOS1) was 1035 bp in length and its deduced amino acid sequence shared 76%, 71, and 70% identity with PpAOS1-AGG GGG GCC CTT TGT AC-3' (AF230371), and Cucumis melo (AF081954) AOSs, respectively.

Semi-quantitative RT-PCR
A 1 µg aliquot of DNase-I treated total RNA (DNase I Amplification Grade, Invitrogen) was reverse-transcribed as described above. Semi-quantitative RT-PCR amplification was performed by amplifying 1 µl of cDNA with 15 pmol of specific primers for PpAOS1 (5'-TCC AGC TAC ACG GAG CCT TT-3', sense; 5'-AGG GGG TCT TCG ATG AAC TT-3', antisense) or peach 18S rRNA (5'-ATG GCC GTT CTG AGT TG-3', sense; 5'-TAC CCT CCT GCC CCT TGT AC-3', antisense). These primers amplified fragments of 238 bp and 356 bp, respectively, with a melting point at ~58 °C. The PCR conditions were: 5 min at 95 °C, followed by 28 cycles at 95 °C for 45 s, 58 °C for 45 s, 72 °C for 45 s. To verify primer specificity, the PCR products were purified with the Qiaquick PCR Purification Kit (Qiagen) and sequenced. For each sample, 10 µl of the amplification reaction was size-fractionated on a 2% (w/v) agarose gel and stained with ethidium bromide. To ensure that amplifications were linear in the range, for each template and primer pair, PCRs were run every three cycles starting from cycle 15 up to cycle 40.

Extraction and analysis of jasmonates
Extraction and quantification of jasmonic acid and MJ were carried out as described previously (Kondo et al., 2005, 2007). The deuterium-labelled jasmonic acid ([^2H]jasmonic acid; ±[^2H]jasmonic acid: (±)[9,10-^2H]jasmonic acid) was prepared from adipic acid through the catalytic semi-deuteriogenation of methyl (±)-9,10-dehydrojasmonic acid. The deuterium-labelled MJ ([^2H]MJ; methyl (±)[9,10-^2H]jasmonic acid) used for the internal standard was prepared as described in Kondo et al. (2000). Freeze-dried mesocarp samples (~1 g, three replications) were homogenized with 1 µg of[^2H]jasmonic acid or[^2H]MJ of the internal standard in 50 ml of diethyl ether containing 11.3 µM butyraldehyde-d3 as an antioxidant, 20 ml of saturated NaCl solution, and 1 ml of 1 M citric acid. Homogenates were centrifuged and dried. Extracts were dissolved in chloroform/diisopropylethylamine, 1:1 (v/v), and derivatized for 60 min at 50 °C with pentafluorobenzyl bromide (PFB). Analysis of PFB-jasmonates was conducted using GC-mass spectroscopy selected ion monitoring (QP 5000; Shimadzu, Kyoto, Japan; 25 mm×25 mm i.d. column (CP-Sil 5CB; Chrompack, Middelburg, The Netherlands); column temperature step gradient, 60 °C for 2 min, 60–270 °C at 1 °C min⁻¹, and 270 °C for 35 min; linear He flow, 50.2 cm s⁻¹; electron potential, 70 eV.

Microarray hybridization
Total RNA was extracted from 7–8 g of mesocarp of MJ-treated and control fruit at first harvest following the protocol described by Bonghi et al. (1998). RNA conversion into target cDNA, microarray hybridization, and data analysis was carried out as extensively described by Trainotti et al. (2006) and in the Supplementary data available at JXB online.

Northern blot analysis
Total RNA (12 µg per track) was size-fractionated on a 1.2% (w/v) agarose–formaldehyde gel and blotted onto nylon membranes (Hybond-N, Amersham Biosciences, Buckinghamshire, UK), which were hybridized with[^32P]DNA-labelled homologous probes of PpACO1, LIM, polygalacturonase (PG), PpExp1, and pectate lyase (PL) genes, respectively. The images were then acquired with an IP-fluorescent Reader FLA-3000 series (Fujifilm). Equal loading of gels was verified by ethidium bromide staining of RNA.

Statistical analysis
Data on ethylene production and quality parameters represent the means (n=20) ±SE and were analysed by analysis of variance (ANOVA) procedures using the SAS Statistical Software (SAS Institute, Cary, NC, USA). Means were separated, between controls and treatments, and among treatments, using Duncan’s multiple range test at the 5% level. Data on jasmonic acid content represent mean values ±SE of three replications; they were analysed by the SAS ANOVA procedure, and mean separation was analysed by Fisher’s least significant difference (P≤0.05).
Results

Ethylene production and fruit quality traits

To determine if JA application had an influence on the onset and progression of ripening, ethylene and fruit quality evolution were analysed. In control fruit, ethylene production was first detectable 119 dAFB (17 d after treatment), peaked at 123 dAFB (harvest), and then declined sharply (130 dAFB, second harvest; Fig. 2A).

The lower MJ concentration tested (0.22 mM) did not significantly affect ethylene production and fruit quality traits at any application time (data not shown). In contrast, early treatments with 0.44 mM MJ and 0.20 mM PDJ led to fruit with dramatically inhibited (by ~90%) ethylene production at harvest (i.e. 21 d after application; Fig. 2A); 7 d later, ethylene emission in treated fruit increased by 2–4-fold relative to the previous harvest. Similarly, mid-period application of MJ (0.44 mM) and PDJ inhibited whole fruit ethylene production at harvest (14 d after application) relative to controls, though to a lesser extent than after the early application (~60%; Fig. 2D).

At the concentrations and application times described above, MJ and PDJ also significantly affected fruit quality traits. In treated fruit at harvest, both chemicals were able to retain FF substantially up to 2.5- (early application) or 1.5-fold (mid-period application) relative to controls (Fig. 2B). After early JA treatments, inhibition of flesh softening lasted up to 130 dAFB (28 d after treatment), while after the mid-period application it did not differ from controls on the same day. At 123 dAFB, early and mid-period treated fruit showed a lower SSC (Fig. 2C, F) and higher h° values of ground and blush colour than

Fig. 2. Time-course of ethylene production (A, D), flesh firmness (B, E), and soluble solids content (SSC; C, F) in peach fruit either untreated (C) or treated with 0.44 mM MJ or 0.20 mM PDJ after the early (A, B, C) or the mid-period (D, E, F) application. Data represent the means ±SE and, for every sampling time, different letters indicate significant differences at P <0.05. Numbers along the x-axis indicate days after treatment, and in parentheses the corresponding dAFB.
controls (Table 1). The inhibitory effect of JAs on SSC and fruit colour development was later overcome (130 dAFB). Following late (7 d before harvest) MJ and PDJ applications, no significant effects on ethylene production and fruit quality traits were recorded during the considered period (data not shown). After 7 of storage at 4 °C, however, JA-treated fruit (early application) had reached the same stage of ripeness in terms of ethylene production, FF, and SSC as control fruit at harvest (data not shown).

**Changes in AOS transcription and jasmonic acid concentration**

The effects of JAs on the time-course of their own biosynthesis and endogenous levels was then evaluated. In control fruit, the *PpAOS1* message was initially low and then increased gradually to reach the highest amount at second harvest (Fig. 3A). One day after applications, both MJ and PDJ equally and strongly enhanced *PpAOS1* mRNA abundance relative to controls. Subsequently, this stimulation persisted, though attenuated, only for MJ.

In control fruit, jasmonic acid content slowly decreased up to first harvest and then rose at second harvest (twice control levels; Fig. 3B). In treated fruit, both MJ and PDJ caused an early (1 d after treatments) and transient accumulation (~30%) of jasmonic acid; thereafter, its concentration did not change until, at second harvest, it fell well below (>50%) control levels. Overall, JA-treated fruit displayed a decreasing trend in jasmonic acid concentration. Only traces of MJ were detectable in treated and control fruit samples (data not shown).

**MJ-induced transcriptional changes at harvest**

Having established that JAs induce relevant changes in ripening physiology, a transcriptome analysis was conducted in fruit at first harvest. Hybridization of μPeach1.0 with cDNA probes from mesocarp of MJ-treated and control fruit allowed the identification of 80 differentially expressed genes (see Supplementary data at JXB online), of which 33 were down-regulated and 47 up-regulated. Out of all 80 genes, those (30) relevant to fruit development and ripening are listed in Table 2. In particular, as regards ethylene biosynthesis, a down-regulation of *PpACO1* was detected. Moreover, 12 of these genes are involved in transcriptional regulation.

Among these, genes coding for a transcription factor with high similarity to the tobacco LIM1 (Kawaoka et al., 2000), a tousled-like protein kinase, and two leucine-rich repeat (LRR) proteins were up-regulated. In contrast, a gene showing similarity to *Arabidopsis* IAA7 was down-regulated; IAA7 is a repressor of IAA-induced gene expression, belonging to the AUX/IAA family of transcriptional modulators (Zenser et al., 2001).

The array analysis revealed that transcription of several genes involved in cell wall metabolism, and hence in fruit quality, was altered in MJ-treated fruit. In fact, mRNA levels of an endo-PG and of an expansin (*PpExp3*) gene homologous to *Arabidopsis* expansin 6 (EXP6) were substantially reduced; transcription of two other EXPs (*PpExp1* and *PpExp2*), homologous to *AtExp8* and *AtExp1*, and of a PL, was enhanced compared with controls. MJ also affected the transcription of some stress-related genes; up-regulation of a lipoxygenase (LOX) and a 12-oxophytodienoic acid-reductase 3 (OPR3), both

**Table 1. Effects of early and mid-period applications of 0.44 mM MJ and 0.20 mM PDJ on epicarp ground and blush colour relative to untreated fruit (controls) during ripening in peach fruit**

Fruit ground and blush colour were measured in the CIE L° (light to dark) a° (green to red) b° (blue to yellow) colour space. Values of a° and b° were converted to hue angle (h°, tan⁻¹ b/a), which quantifies colour (0°=red, 90°=yellow, and 180°=green). Letters represent significant differences at P<0.05.

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<tr>
<th>Ground colour</th>
<th>Blush colour</th>
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<td></td>
<td>119 dAFB</td>
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<td>Controls</td>
<td>81.2a</td>
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<td>Early MJ</td>
<td>82.3a</td>
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<td>Early PDJ</td>
<td>82.6a</td>
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<td>Mid-period MJ</td>
<td>80.9a</td>
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<td>Mid-period PDJ</td>
<td>83.4a</td>
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**Fig. 3. Time-course of transcript levels of *PpAOS1* in peach mesocarp from untreated fruit (C) or fruit treated early with 0.44 mM MJ (M) or 0.20 mM PDJ (P).** (A) Semi-quantitative RT-PCR analysis was performed by amplifying 1 μl of cDNA with specific primers for *PpAOS1* or peach 18S rRNA. (B) Changes in jasmonic acid concentration in untreated fruit (C) or fruit treated with MJ or PDJ. Data represent the means ±SE; LSD, least significant difference (P<0.05).
encoding enzymes of the JA biosynthetic pathway (Wasternak, 2007), was observed in treated fruit. The expression of catalase 2 (CAT2), a dehydration-responsive protein (RD22), a DNA repair protein (RAD23), and a glycine-rich RNA-binding protein (GR-RBP) was stimulated as well.

Transcript abundance of genes involved in primary metabolism was also differentially affected; in fact, MJ enhanced expression of genes encoding the large RuBisCo subunit (RbcL) and PSII 32 kDa protein (PsbA). Another evident effect was the transcriptional repression of ω-6 desaturase (ω-6-DES) and stearoyl-ACP-desaturase (SSI2), both of which are involved in fatty acid desaturation, and of a pyruvate decarboxylase 1 (PDC1).

**Time-course expression profile of relevant JA-responsive genes**

The expression pattern of a selected set of MJ-responsive genes (PpACO1, PG, PL, PpExp1, and LIM) was examined during the 28 d period following treatments in control and in MJ- and PDJ-treated fruit. In control fruit, both PpACO1 and PG transcripts became detectable at first harvest (21 d after treatment), and their amount increased dramatically at second harvest (28 d after treatment, Fig. 4). MJ and PDJ slightly inhibited PpACO1 transcription at first harvest; at second harvest, this inhibition was particularly marked with PDJ. In the case of PG, mRNA levels were dramatically reduced by both JAs only at second harvest. In controls, PL transcript was hardly detectable up to first harvest, and increased 7 d later (Fig. 4). MJ and PDJ slightly inhibited transcription at first harvest; at second harvest, this inhibition was also marked with PDJ. In the case of PpExp1 both control and in MJ- and PDJ-treated fruit. In control fruit, both PpACO1 and PG transcripts became detectable at first harvest (21 d after treatment), and their amount increased dramatically at second harvest (28 d after treatment, Fig. 4). MJ and PDJ slightly inhibited PpACO1 transcription at first harvest; at second harvest, this inhibition was particularly marked with PDJ. In the case of PG, mRNA levels were dramatically reduced by both JAs only at second harvest. In controls, PL transcript was hardly detectable up to first harvest, and increased 7 d later (Fig. 4); similarly, PpExp1 was weakly expressed later (Fig. 4); similarly, PpExp1 was weakly expressed until second harvest. LIM transcript accumulation decreased during the considered period. While MJ enhanced PL, PpExp1, and LIM mRNA accumulation throughout, PDJ did so only at first and second harvest.

**Discussion**

This work highlights that the delayed evolution of the ripening syndrome, as induced in peach fruit by early JA.
application, is associated with reduced ethylene production and relevant modifications of the fruit transcriptome.

**JAs are developmentally regulated and deeply influence ripening physiology**

Endogenous jasmonic acid and *PpAOS1* transcript accumulation during the late fruit developmental phases show that peach, like other climacteric fruit, accumulate JAs at ripening (Fan *et al.*, 1997; 1998b; Kondo *et al.*, 2005). In JA-treated fruit, the stimulation of *PpAOS1* transcription was accompanied by an increase in jasmonic acid concentration soon after application. This confirms the positive feedback regulation of JA biosynthesis (Wastermark, 2007), reported here for the first time in the case of PDJ. Subsequently, a decreasing trend in jasmonic acid accumulation was observed during the considered time span, and, differently from controls, it did not increase at ripening. Consequently, treated fruit displayed the same concentration of jasmonic acid as 1 week younger control fruit, thus supporting the notion of a JA-induced ripening delay.

Field applications of MJ and PDJ slowed down ripening to a different extent depending upon the time of application. In fact, early and mid-period treatments strongly reduced climacteric ethylene production at harvest, and counteracted fruit softening and the increase in SSC and epicarp red colour; in contrast, the late application was ineffective. Presumably, the molecular processes underlying ripening were too far advanced to be counteracted. Indeed, a dependence of the effects of JA upon the physiological stage of application is in agreement with previous findings in apple and pear (Fan *et al.*, 1997, 1998b; Kondo *et al.*, 2007). PDJ was as active as MJ at half its concentration, confirming its stronger biological activity, most probably due to its higher chemical stability (Fujisawa *et al.*, 1997; Koshiyama *et al.*, 2006; Ziosi *et al.*, 2007).

Heterogeneous results have been reported concerning JA effects on ripening-related parameters; in fact, while anthocyanin accumulation is generally stimulated in JA-treated fruit (Rudell *et al.*, 2002, 2005), other ripening-related parameters such as FF and SSC may be unaltered or differentially affected (González-Aguilar *et al.*, 2004; Kondo *et al.*, 2005). For instance, in apple, field application of MJ retained FF, as presently observed; however, in contrast to the present results, it enhanced peel red colour (Rudell *et al.*, 2005). It is also unclear whether, and to what extent, ethylene mediates the effects of exogenous JA in fruit. In apple, JA-induced degreening and anthocyanin accumulation have been reported to be ethylene independent (Fan and Mattheis, 1999; Kondo *et al.*, 2004), while the enhancement of the production of volatile compounds by JAs seemed to be ethylene mediated (Kondo *et al.*, 2005). In addition, JA responses strongly depend upon the concentration applied (Fan *et al.*, 1998a; Rudell *et al.*, 2002). In peach field-treated with a 20-fold higher MJ concentration than in the present study, fruit ripening and leaf senescence were strongly accelerated (Janoudi and Flore, 2003). In contrast, the present data show that JAs, at relatively low doses, negatively affect ripening-related metabolic events. Alterations in ripening physiology may be relevant to the observed effects of MJ treatment in prolonguing fruit shelf-life (Peña-Cortés *et al.*, 2005) and in post-harvest protection against chilling injury (Yoshikawa *et al.*, 2007) and pathogens (Yao and Tian, 2005). Pre-harvest application of MJ also enhanced fungal disease resistance in sweet cherry fruit during storage due to up-regulation of defence-related enzyme activities (Yao and Tian, 2005).

**Transcriptional effects of JAs are consistent with a delay in fruit ripening**

The microarray analysis shows that MJ down-regulates several genes that are strongly induced during ripening, and involved in ethylene biosynthesis (*PpACO1*), transcriptional regulation (*IAA7*), and cell wall metabolism/fruit softening (PG; Trainotti *et al.*, 2006). The down-regulation of *PpACO1*, also confirmed by northern analysis, may at least in part account for the dramatically reduced ethylene production, which, in JA-treated fruit,
remained at the basal levels typical of system 1 of ethylene biosynthesis (Barry et al., 2000). At second harvest, a recovery in PpACO1 transcript abundance, relative to first harvest levels, associated with a recovery in ethylene production, may possibly have marked the onset of a late ripening, suggesting a slowing down of the system 1 to system 2 transition. MJ also down-regulated a gene similar to the Arabidopsis IAA7 transcriptional modulator that is induced during ripening, and by propylene treatment in peach fruit (Begheldo et al., 2007); the same behaviour was observed for the DR12 orthologue of tomato (Jones et al., 2002), suggesting a role for some AUX/IAA genes in the regulation of fruit ripening. The reduction in IAA7 transcript levels by MJ also suggests that MJ influences, directly or via ethylene, auxin responses, and this is in accord with the hypothesis of a cross-talk between the signal transduction pathways of these hormones (Nemhauser et al., 2006).

The down-regulation by MJ of the cell wall-related gene PG is in agreement with firmness retention. In fact, fruit softening in peach is strongly correlated with PG activity, which is ethylene regulated (Trainotti et al., 2003, 2006). Thus, it is reasonable to assume that JA effects on PG are ethylene mediated. On the other hand, the expression of other cell wall-rearranging enzymes, such as PL, and three EXPs (PpExp1, 2, and 3; Hayama et al., 2006), was stimulated in MJ-treated fruit. The stimulatory effect on PL and PpExp1 expression by MJ was early (day 3) and persistent, while that of PDJ only became apparent at first and second harvest (see below). In Arabidopsis, it has been shown that PL is involved in JA-mediated defence responses (Ellis et al., 2002), for instance through the production of oligogalacturonides (Ridley et al., 2001); the latter may even stimulate AOS expression and JA synthesis (Norman et al., 1999). Equally, EXPs, besides correlating with fruit softening in peach (Trainotti et al., 2003, 2006; Hayama et al., 2006), have been reported to respond to biotic and abiotic stresses (Choi et al., 2006).

Another gene that may contribute to the regulation of cell wall structure, and whose message is up-regulated early by MJ, is the LIM transcription factor; its homologue in tobacco regulates many genes involved in the phenylpropanoid biosynthetic pathway (Kawaoka et al., 2000). In transgenic tobacco, NtLIM overexpression does not increase lignin deposition, but is associated with enhanced transcription of phenylalanine ammonia-lyase (PAL) and, particularly, of hydroxycinnamoyl-alcohol dehydrogenase (CAD) (Kawaoka et al., 2000). In strawberry, enhanced CAD gene expression is associated with firmer flesh (Salentijn et al., 2003). Thus, LIM overexpression may profoundly alter the phenylpropanoid pathway in MJ-treated fruit, possibly leading to accumulation of lignin precursors, which can represent defence compounds by contributing to cell wall strengthening.

The up-regulation by MJ of specific stress/defence-associated genes which is largely in accord with the literature (Schmidt and Baldwin, 2006; Jung et al., 2007; Walia et al., 2007) further supports this notion. Amongst them, besides PpAOS1, are LOX and OPR3, both involved in JA biosynthesis. Other genes potentially involved in improving fruit defences are those encoding the LRR proteins; these belong to a large gene family some of whose members are involved in stress responses (Dievart and Clark, 2004). A tousled-like protein kinase gene, homologue of the Arabidopsis Tousled gene, whose product in mouse cells has been shown to afford protection against UV radiation (Sen and De Benedetti, 2006), was also up-regulated. Up-regulation of stress/defence-related genes is concurrent with down-regulation of primary metabolism genes. PDC1 is involved in volatile biosynthesis, which increases during ripening (Moyano et al., 2004). The down-regulation of GS1 mRNA, which increases during senescence in Arabidopsis (Masclaux et al., 2000), further supports the notion that MJ-treated fruit were less ripe. Similarly, the up-regulation of two genes coding for the photosynthesis-related proteins RbcL and PsbA, though in contrast to the well-known chlorophyll-degrading effect of JAs (Wasternack, 2007), is probably the consequence of a delayed chloroplast– chromoplast transition. The Venn diagram in Fig. 5 shows that 19 out of 33 genes which are down-regulated in MJ-treated fruit are up-regulated during peach fruit ripening.

![Venn diagrams showing the number of genes differentially regulated by MJ of specific stress/defence-associated genes which is largely in accord with the literature](https://academic.oup.com/jxb/article-abstract/59/3/563/575690/515842536353756290)

**Fig. 5.** Venn diagrams showing the number of genes differentially regulated by MJ of specific stress/defence-associated genes which is largely in accord with the literature (Schmidt and Baldwin, 2006; Jung et al., 2007; Walia et al., 2007) further supports this notion. Amongst them, besides PpAOS1, are LOX and OPR3, both involved in JA biosynthesis. Other genes potentially involved in improving fruit defences are those encoding the LRR proteins; these belong to a large gene family some of whose members are involved in stress responses (Dievart and Clark, 2004). A tousled-like protein kinase gene, homologue of the Arabidopsis Tousled gene, whose product in mouse cells has been shown to afford protection against UV radiation (Sen and De Benedetti, 2006), was also up-regulated. Up-regulation of stress/defence-related genes is concurrent with down-regulation of primary metabolism genes. PDC1 is involved in volatile biosynthesis, which increases during ripening (Moyano et al., 2004). The down-regulation of GS1 mRNA, which increases during senescence in Arabidopsis (Masclaux et al., 2000), further supports the notion that MJ-treated fruit were less ripe. Similarly, the up-regulation of two genes coding for the photosynthesis-related proteins RbcL and PsbA, though in contrast to the well-known chlorophyll-degrading effect of JAs (Wasternack, 2007), is probably the consequence of a delayed chloroplast–chromoplast transition. The Venn diagram in Fig. 5 shows that 19 out of 33 genes which are down-regulated in MJ-treated fruit are up-regulated during peach fruit ripening.
(unripe versus ripe fruit, i.e. S3/S4 transition; Trainotti et al., 2006; and Supplementary data at JXB online). Conversely, 11 out of 47 genes up-regulated in MJ-treated fruit overlap with those that are down-regulated during ripening. These shared genes further support the notion that MJ counteracts ripening.

**MJ versus PDJ**

Although MJ and PDJ induced highly comparable effects on ethylene production, fruit quality, and jasmonic acid accumulation, their transcriptional effects only partly coincided. Most probably this difference is due to PDJ’s synthetic nature. A methyl esterase has been reported which hydrolyses MJ to jasmonic acid (Stuhfelder et al., 2004); the latter may be further metabolized to other JA-like compounds (Staswick and Tiryaki, 2004). Little is known about PDJ metabolism in plants; in grape berries, it is converted into compounds other than MJ or jasmonic acid (Koshiyama et al., 2006). In tomato, a microarray analysis showed that, while some genes respond in the same manner to all members of the JA family, others do so in a differential manner (Uppalapati et al., 2005). Thus, early on, the different transcriptional responses to MJ versus PDJ may be direct and depend upon differential gene behaviour. At ripening, the response could be indirect, due to biochemical transformation of the applied JAs, and the complex transcriptional framework resulting from the overlap of defence responses and ripening.

In conclusion, when exogenously supplied, under field conditions, and at proper concentrations and growth stages, JAs enhance their own biosynthetic pathway. Transcriptome profiling of JA-treated fruit confirms the down-regulation of crucial ripening-related genes, in agreement with the inhibition of ethylene production, and the up-regulation of stress/defence-related genes. Taken together these results may suggest that, in JA-treated fruit, resources are diverted from growth and ripening, consistent with a trade-off between development and defence (Schmidt and Baldwin, 2006). Finally, most experimental outcomes derive from studies conducted in simple and controlled environments (in vitro, growth chambers, or greenhouses), and often these result in partially overlapping, but definitely not coinciding, effects. The present information, arising from JA application in a field environment, where plants have to cope with multiple biotic and abiotic elicitors, may open up new perspectives for the use of JAs in the control of fruit ripening and defence.

**Supplementary data**

Supplementary data are available at JXB online. Supplementary Table S1 contains a full listing of all genes up-regulated by MJ at harvest in peach fruit. Supplementary Table S2 contains a full listing of all genes down-regulated by MJ at harvest in peach fruit. Moreover, a detailed description of oligonucleotide synthesis and microarray construction, microarray hybridization, transcript profiling using the μPEACH1.0 microarray, and data analysis is provided (Materials and methods).

**Acknowledgements**

This research was supported by funds PRIN 2002 (20022078818_004) from the Italian MIUR to PT.

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