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RESEARCH PAPER

# Hormonal and transcriptional profiles highlight common and differential host responses to arbuscular mycorrhizal fungi and the regulation of the oxylipin pathway

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## Abstract

**Arbuscular mycorrhizal (AM) symbioses are mutualistic associations between soil fungi and most vascular plants. The symbiosis significantly affects the host physiology in terms of nutrition and stress resistance. Despite the lack of host range specificity of the interaction, functional diversity between AM fungal species exists. The interaction is finely regulated according to plant and fungal characters, and plant hormones are believed to orchestrate the modifications in the host plant. Using tomato as a model, an integrative analysis of the host response to different mycorrhizal fungi was performed combining multiple hormone determination and transcriptional profiling. Analysis of ethylene-, abscisic acid-, salicylic acid-, and jasmonate-related compounds evidenced common and divergent responses of tomato roots to *Glomus mosseae* and *Glomus intraradices*, two fungi differing in their colonization abilities and impact on the host. Both hormonal and transcriptional analyses revealed, among others, regulation of the oxylipin pathway during the AM symbiosis and point to a key regulatory role for jasmonates. In addition, the results suggest that specific responses to particular fungi underlie the differential impact of individual AM fungi on plant physiology, and particularly on its ability to cope with biotic stresses.**

**Key words:** Arbuscular mycorrhiza, hormones, jasmonates, LC-MS/MS analysis, microarrays, oxylipins.

## Introduction

About 80% of all terrestrial plants, including most agricultural and horticultural crop species, are able to establish mutualistic associations with soil fungi from the phylum Glomeromycota (Smith and Read, 2008). The resulting symbiosis is known as arbuscular mycorrhiza (AM) and is widely distributed throughout the world. This association is considered to be older than 400 million years and a key step in the evolution of terrestrial plants (Smith and Read, 2008). Arbuscular mycorrhizal fungi (AMF) are obligate biotrophs that colonize the root cortex of the host plant inter- and intracellularly forming specialized and highly branched structures called arbuscules (Parniske, 2008). The fungus obtains carbohydrates from the host plant and, in return, they assist the plant in the acquisition of mineral nutrients (mainly phosphorus) and water. AM symbiosis is maintained throughout the life of the plant, affecting its physiology significantly (Parniske, 2008). Accordingly, the symbiosis not only influences plant nutrition, but also impacts the plant's ability to overcome biotic and abiotic stresses (Pozo and Azcón-Aguilar, 2007). While AMF are considered non-specific with respect to host range, there are differences in their growth patterns within the roots, functionality, and with regards to their effects on plant nutrition and resistance to stress (Cavagnaro *et al.*, 2001; Pozo *et al.*, 2002; Smith *et al.*, 2004).

AM establishment and functioning require a high degree of coordination between the two partners based on a finely regulated molecular dialogue that orchestrates complex symbiotic programmes (Paszkowski, 2006; Hause *et al.*, 2007; Requena *et al.*, 2007). Upon recognition of the fungal partner, the plant actively accommodates the fungus in the root tissue (Genre *et al.*, 2008) and controls its proliferation, which implies an important transcriptional reprogramming in the plant. Understanding the molecular basis of the AM symbiosis is an ongoing challenge, and, so far, some plant genes specifically associated with the establishment and development of the symbiosis have been identified in different plant species, mostly in legumes (Grunwald *et al.*, 2004; Hohnjec *et al.*, 2005; Liu *et al.*, 2007; Siciliano *et al.*, 2007; Guether *et al.*, 2009). Some of the changes in the host are related to modifications in the relative abundance of plant hormones, most of which are thought to play a role in the symbiosis (Hause *et al.*, 2007). Among plant hormones, ethylene (ET), salicylic acid (SA), abscisic acid (ABA), and jasmonic acid (JA) are known to be key elements in fine-tuning the plant defence response during interaction with other organisms (Pieterse *et al.*, 2009). In the case of the interaction with AMF, it is accepted that there is an inverse correlation between root colonization and the levels of ET and SA (Blilou *et al.*, 1999; Herrera-Medina *et al.*, 2003, 2007; Riedel *et al.*, 2008). Conversely, a positive correlation for ABA and mycorrhizal establishment has been evidenced (Herrera-Medina *et al.*, 2007). JA and its derivatives, known as jasmonates, have received special attention since they are believed to play a major role in the AM symbiosis. However, experimental data are highly controversial (Gutjahr and Paszkowski, 2009; Hause and Schaarschmidt, 2009). Increased JA levels in mycorrhizal roots compared with non-mycorrhizal controls have been described in *Medicago truncatula* (Hause *et al.*, 2002; Meixner *et al.*, 2005), while they remained unaltered in *Nicotiana attenuata* (Riedel *et al.*, 2008). In addition, studies using reverse genetics approaches with plant mutants affected in JA biosynthesis or signalling have shown positive and negative regulatory roles of the JA pathway in the symbiosis (Isayenkov *et al.*, 2005; Herrera-Medina *et al.*, 2008; Tejeda-Sartorius *et al.*, 2008). Besides the use of different plant and experimental systems, these controversies might be partly due to the overlapping yet distinct signalling activities of its precursor oxo-phytodienoic acid (OPDA) and jasmonate derivatives such as the isoleucine conjugate JA–Ile (Stintzi *et al.*, 2001; Taki *et al.*, 2005; Wang *et al.*, 2008). Moreover, jasmonates belong to a diverse class of lipid metabolites known as oxylipins that include other biologically active molecules (Wasternack, 2007; Mosblech *et al.*, 2009).

In the present study, the agriculturally and economical important crop tomato has been used as a model system to carry out integrative analysis of the transcriptional and metabolic changes that take place during AM symbiosis. The plant response to two related AMF, *Glomus mosseae* and *Glomus intraradices*, that showed in previous studies different colonization patterns and functionality, was compared in an attempt to provide insights into the common and differential host responses to AMF. The effects of AMF colonization on the content of ABA, SA, ET, and different JA-related compounds in the host plant were assessed, and correlated with the modifications in their transcriptional profiles. The results provide original insights into our understanding of the AM symbiosis and its impact on plant physiology, and they pave the way for further analyses of the regulatory network controlling this association.

## Materials and methods

### *Plant growth, AM inoculation, and chemical treatments*

The AMF *G. mosseae* (BEG 12) and *G. intraradices* (BEG 121) were maintained as a soil–sand-based inoculum. Tomato seeds (*Solanum lycopersicum* L. cv. MoneyMaker) were surface sterilized in 4% sodium hypochlorite containing 0.02% (v/v) Tween-20, rinsed thoroughly with sterile water and germinated for 3 d in a container with sterile vermiculite at 25 °C in darkness. Subsequently, individual seedlings were transferred to 0.25 l pots with a sterile sand:soil (4:1) mixture. Pots were inoculated by adding 10% (v:v) *G. mosseae* or *G. intraradices* inoculum. The same amount of soil:sand mix but free from AMF was added to control plants. All plants received an aliquot of a filtrate (<20 μm) of both AM inocula to homogenize the microbial populations. For each treatment, a total of nine plants were used. Plants were randomly distributed and grown in a greenhouse at 24/16 °C with a 16/8 h photoperiod and 70% humidity, and watered three times a week with Long Ashton nutrient solution (Hewitt, 1966) containing 25% of the standard phosphorus concentration. Plants were harvested after 9 weeks of growth, and the fresh weight of shoots and roots was determined. An aliquot of each individual root system was reserved for mycorrhizal quantification. For microarray and hormone analyses, three pools each consisting of roots from three independent plants were used.

For methyl jasmonate (MeJA) treatment tomato plants were grown hydroponically in 3.0 l plastic containers with Long Ashton nutrient solution containing 25% of the standard phosphorus concentration and with constant aeration. The nutrient solution was replaced once a week. Four-week-old plants were individually transferred to 50 ml plastic tubes filled with nutrient solution with or without 50 μM MeJA (Sigma-Aldrich) and maintained for 24 h. Then, the roots were rinsed with sterilized deionized water and stored at –80 °C until use.

### *Mycorrhizal colonization determination*

Roots were stained with trypan blue (Phillips and Hayman, 1970) and examined using a Nikon Eclipse 50i microscope and bright-field conditions. The percentage of total root colonization and frequency of intraradical fungal structures, arbuscules, and vesicles was determined by the gridline intersection method (Giovannetti and Mosse, 1980).

### *Phosphorus content*

The total phosphorus content of the leaves was measured at the CEBAS-CSIC (Spain). Shoots were briefly rinsed with deionized water and mature leaves were oven-dried at 60 °C for 72 h, weighed, and ground to a fine powder. Then, samples were extracted with deionized water. Tissue phosphorus concentrations were determined by inductively coupled plasma optical emission spectrometry (ICP-OES) (Iris Intrepid II, Thermo Electron Corporation) after acid digestion. Three biological replicates each consisting of a pool of leaves from three independent plants were measured for each treatment.

### Hormone quantification

OPDA, JA, JA–Ile, ABA, and SA were analysed by ultraperformance liquid chromatography coupled to mass spectrometry (UPLC-MS) as described by Flors *et al.* (2008). A 50 mg aliquot of dry tissue was used per sample. A mixture of internal standards containing 100 ng of [$^2H_6$]ABA, 100 ng of dihydrojasmonic acid, 100 ng of prostaglandin B1, and 100 ng of [$^2H_5$]SA was added to each sample prior to extraction. Individual calibration curves for each tested compound and internal standard were performed before the analysis. The tissue was immediately homogenized in 2.5 ml of ultra pure water and centrifuged at 5000 *g* for 40 min. Then, the supernatant was acidified and partitioned against diethyl-ether, dried, and resuspended in 1 ml of water/methanol (90:10, v/v). A 20 µl aliquot of this solution was injected into a Waters Acquity UPLC system (Waters). The UPLC was interfaced into a triple quadrupole tandem mass spectrometer (TQD, Waters). LC separation was performed using an Acquity UPLC BEH $C_{18}$ analytical column (Waters) at a flow rate of 300 µl $min^{-1}$. Quantifications were carried out with MassLynx 4.1 software (Waters) using the internal standards as a reference for extraction recovery and the standard curves as quantifiers.

Ethylene release was determined by gas chromatography–mass spectrometry (GC-MS). Excised roots were placed on wet filter paper in a 90 mm diameter Petri dish, sealed with sticky tape and a rubber stopper on the top, and incubated for 1 h at room temperature. A 1 ml aliquot of headspace gas per plate was sampled with a syringe, and ET was measured in a Hewlett Packard 5890 gas chromatograph fitted with a flame ionization detector (FID). Analyses were carried out at 65 °C with the injector and FID held at 120 °C and 105 °C, respectively. Five independent replicates per treatment were measured.

### RNA isolation

Total RNA was extracted using Tri-Reagent (Sigma-Aldrich) according to the manufacturer's instructions. The RNA was treated with RQ1 DNase (Promega), purified through a silica column using the NucleoSpin RNA Clean-up kit (Macherey-Nagel), and stored at –80 °C until use.

### Microarray hybridization and data analysis

The Affymetrix GeneChip Tomato Genome Array (Affymetrix) was used. A 5 µg aliquot of total RNA was used as starting material. cDNA synthesis, cRNA production, and fragmentation were carried out as described in the Expression Analysis Technical Manual (Affymetrix). The GeneChip Arrays were hybridized, stained, washed, and screened according to the manufacturer's protocol at the Unidad de Genómica of the Universidad Complutense de Madrid (http://www.ucm.es/info/gyp/genomica/) (Madrid, Spain). Three biological replicates, each consisting of pools of three independent plants, were used for microarray analysis of roots colonized or not by *G. mosseae* or *G. intraradices*. For the MeJA experiment, two biological replicates from MeJA- or mock-treated roots were used. Probe signal summarization, normalization, and background subtraction were performed using the multichip analysis RMA algorithm (Irizarry *et al.*, 2003) in the 'affy' package with default parameters. The statistical test for differentially expressed genes was performed using the software 'Cyber-T' (Baldi and Long, 2001), which allows a better variance estimation by calculating the moderated *t*-statistic using empirical Bayesian techniques. Genes were considered as differentially regulated if $P < 0.01$ and the ratio compared with the controls was $\geqslant 1.7$ or $\leqslant 0.6$. For the MeJA treatment, genes were considered as differentially regulated if $P < 0.01$ and the ratio compared with the controls was $\geqslant 2$ or $\leqslant 0.5$. Updated annotation of the differentially regulated genes was obtained by tblastx against the NCBI nr-database. An E-value $<10^{-15}$ was required to take into account the blast result.

Microarray data will be deposited in the Tomato Functional Genomics database (http://ted.bti.cornell.edu/cgi-bin/TFGD/array/home.cgi).

### Gene expression analysis by real-time quantitative RT-PCR (qPCR)

Real-time qPCR was performed using the iCycler iQ5 system (Bio-Rad) and gene-specific primers (Supplementary Table S1 available at *JXB* online). The first-strand cDNA was synthesized with 1 µg of purified total RNA using the iScript cDNA Synthesis kit (Bio-Rad) according to the manufacturer's instructions. Three independent biological replicates were analysed per treatment. Relative quantification of specific mRNA levels was performed using the comparative $2^{-\Delta(\Delta Ct)}$ method (Livak and Schmittgen, 2001). Expression values were normalized using the housekeeping gene *SlEF*, which encodes for the tomato elongation factor-1α.

### Statistical analysis

Data for hormone and phosphorus content and mycorrhization levels of tomato roots were subjected to one-way analysis of variance (ANOVA) using the software SPSS Statistics v. 14.1 for Windows. When appropriate, Fisher's LSD test was applied.

## Results

### *Root colonization by* G. mosseae *and* G. intraradices*, and physiological status of the plant*

In order to identify plant responses directly related to the AM symbiosis and not to potential nutritional effects, an experimental system that allowed a robust mycorrhizal colonization without significant changes in plant growth and nutrition was used. Nine weeks after inoculation the shoot and root mass and phosphorus (Pi) content of the tomato plants colonized by either *G. mosseae* or *G. intraradices* were not significantly different from those in the non-mycorrhizal controls (Supplementary Table S2 at *JXB* online). The colonization intensities of *G. mosseae* (23%) and *G. intraradices* (41%) differed significantly ($P < 0.01$). Moreover, both AMF showed different colonization patterns. They both developed intraradical hyphae, arbuscules, and vesicles, but with different frequencies. Although the relative abundance of arbuscules was similar in both interactions, the proportion of vesicles, fungal reservoir structures, was higher in *G. intraradices*- but scarce in *G. mosseae*-colonized roots (Fig. 1A, C, D). Besides the presence of fungal structures, the functionality of the symbiosis was also checked by molecular methods. The tomato gene *LePT4* encodes a phosphate transporter specific for the AM symbiosis which is expressed in arbusculated cells and considered a marker for a functional symbiosis (Balestrini *et al.*, 2007). A high *LePT4* expression was detected in all mycorrhizal roots, although the levels were almost 2-fold higher in *G. intraradices*- than in *G. mosseae*-colonized roots (Fig. 1B), in agreement with the differences observed in root colonization (absolute arbuscule abundance).

### *Impact of root colonization by* G. mosseae *or* G. intraradices *on defence-related hormones*

To investigate the responses of tomato plants to AMF and evaluate the impact of the interaction on hormone homeostasis, the levels of JA, ABA, SA, and ET in tomato

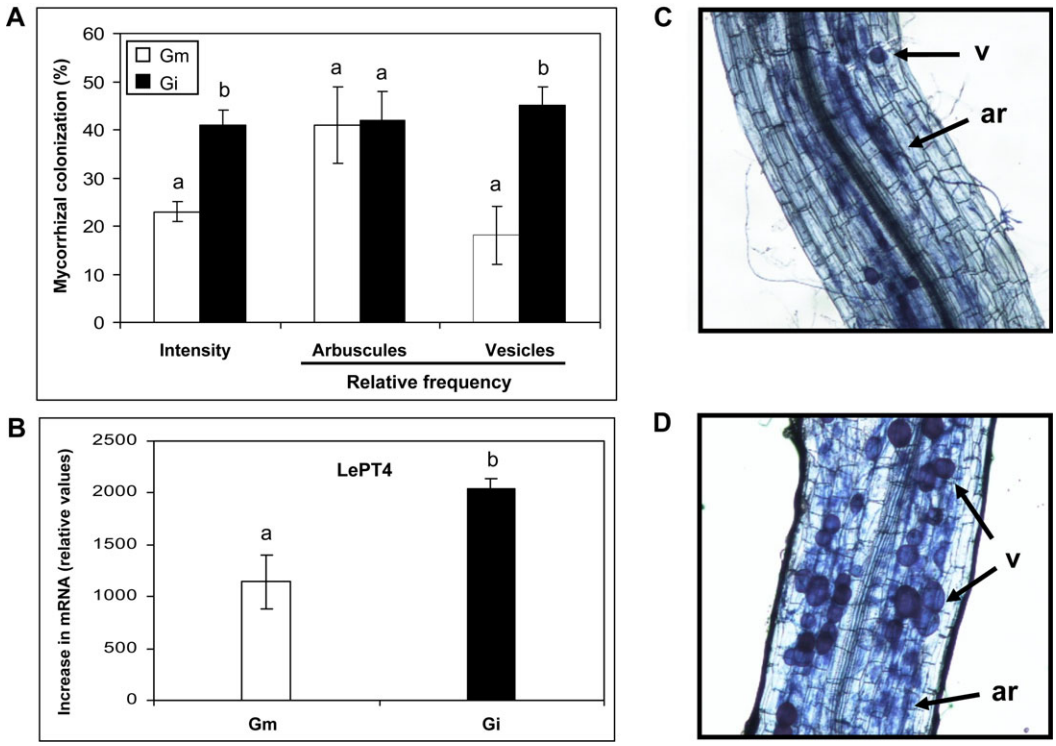


**Fig. 1.** Mycorrhizal colonization and expression analysis of the marker gene *LePT4* of tomato roots inoculated with *G. mosseae* or *G. intraradices*. (A) Intensity of mycorrhizal colonization by *G. mosseae* (Gm) or *G. intraradices* (Gi), and relative frequency of arbuscules and vesicles. (B) Gene expression analysis by real-time qPCR for the mycorrhizal marker gene *LePT4*. Data points represent the means of five (A) or three (B) replicates (±SE). Data not sharing a letter in common differ significantly ($P$ <0.01) according to Fisher's LSD test. The right-hand panels show photographs of root samples after trypan blue staining. (C) *Glomus mosseae* colonizes the root cortex to a lower extent, forming a large number of arbuscules but a limited number of vesicles. (D) *Glomus intraradices* extensively colonize the root cortex forming arbuscules and a large number of vesicles. Arrows indicate arbuscules (ar) and vesicles (v).

roots colonized by the two fungi were determined. Analysis by UPLC-coupled tandem mass spectrometry (LC-MS/MS) allowed the simultaneous quantification of free JA, the JA precursor OPDA, JA–Ile, ABA, and SA from each sample. The levels of OPDA were significantly ($P$ <0.05) higher in roots colonized by both AMF (Fig. 2A). In contrast, JA levels were not altered in mycorrhizal roots (Fig. 2B). Unlike free JA, the levels of its bioactive derivative JA–Ile were higher in *G. mosseae*-colonized roots compared with non-mycorrhizal controls, whereas no significant differences were observed upon *G. intraradices* colonization (Fig. 2C). In spite of this, differences between roots colonized by both fungi were not statistically significant. It was noteworthy that a clear increase on SA content was only detected in *G. mosseae*-colonized roots (Fig. 2D). Conversely, ABA content did not change upon mycorrhizal colonization by either of the AMF studied (Fig. 2E), but a clear reduction of ET levels in roots colonized by both AMF compared with non-mycorrhizal plants was observed by GC-MS (Fig. 2F).

### *Changes in plant gene expression during interaction with* G. mosseae *or* G. intraradices

To gain further insight into the plant changes related to the symbiosis, a global gene expression profiling of the roots from plants colonized by *G. mosseae* or *G. intraradices* as compared with non-mycorrhizal plants was performed. The Affymetrix Tomato Genome Genechip Array containing >9200 tomato genes was used. In agreement with the similarity in the Pi content of the plants, no differences were observed in the expression of Pi nutrition marker genes present in the array such as the Pi transporter *LePT1*, acid and purple phosphatases and kinases, and other marker genes for Pi starvation such as the iron deficiency-specific-4 (*IDS4*) and the tomato Pi starvation-induced (*TPSI1*). Thus, the transcriptional changes reported in the present study are not expected to be related to differences in Pi nutrition.

As a first approach, the aim was to determine whether the hormonal changes observed in mycorrhizal plants (Fig. 2) correlated with transcriptional regulation of their metabolic genes. For that, the expression of tomato genes present in the array involved in the metabolism of the hormones analysed was scrutinized (Supplementary Table S3 at *JXB* online). Consistent with the results from the hormonal analysis, only an induction of the genes encoding enzymes related to the metabolism and regulation of jasmonates, and more generally to oxylipins—LOXA, AOS1, AOS3, JAME, and the jasmonate ZIM domain 2 (JAZ2)—was observed in mycorrhizal plants. However, no changes for the genes

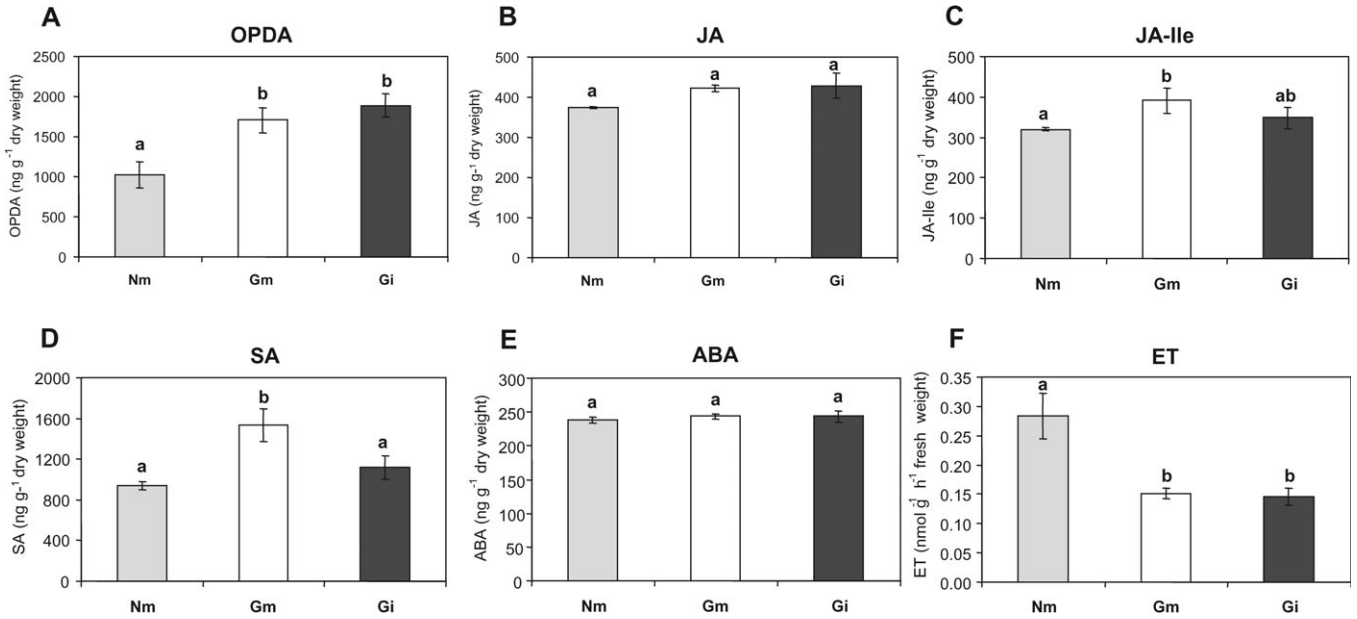


**Fig. 2.** Hormonal content in non-mycorrhizal (Nm) and *G. mosseae*- (Gm) and *G. intraradices*- (Gi) colonized roots. Levels of (A) oxo-phytodienoic acid (OPDA), (B) free jasmonic acid (JA), (C) jasmonic acid isoleucine (JA–Ile), (D) salicylic acid (SA), (E) abscisic acid (ABA), and (F) ethylene (ET). Data points represent the means of five replicates (±SE). Data not sharing a letter in common differ significantly ($P$ <0.05) according to Fisher's LSD test.

related to ABA, SA, and ET were observed, except for the gene encoding a 1-aminocyclopropane-1-carboxylic acid oxidase (ACO1) (Supplementary Table S3).

Comparing the global transcriptional profiles, 162 genes were differentially regulated ($P$ <0.01) in the roots upon *G. mosseae* colonization. Of these, 101 genes (62%) were up-regulated. In *G. intraradices*-colonized roots the expression of 173 genes differed significantly from non-mycorrhizal roots, of which 103 genes (60%) were up-regulated. When comparing the expression profiles in the two mycorrhizal systems, 59 genes were co-regulated during the interaction with both AMF (Table 1). The overlap was considerably higher for up-regulated genes. Among the co-induced genes, previously described markers of the AM symbiosis were found such as those encoding a chitinase, glutathione *S*-transferase, β-1,3-glucanase, patatin, β-D-xylosidase, a pathogenesis PR10-like protein, and DXS-2, a key enzyme of the mevalonate-independent pathway of carotenoid biosynthesis (Hohnjec *et al.*, 2005; Liu *et al.*, 2007; Siciliano *et al.*, 2007; Walter *et al.*, 2007; Floss *et al.*, 2008), suggesting a role in the AM interaction conserved across plant species. Besides these markers, the microarray analysis revealed the common induction in mycorrhizal roots of key genes in the biosynthesis of oxylipins. In plants there are two main branches of the oxylipin pathway, determined by two different types of lipoxygenases (LOXs), 9-LOX and 13-LOXs (Fig. 3). The 13-LOX pathway leads to the biosynthesis of JA and derivatives, and, as described above, some genes in this pathway were up-regulated during AM interaction (Fig. 3). In addition, the microarray analysis revealed the induction of genes encoding key enzymes of the 9-LOX branch, LOXA and AOS3. LOXA is involved in the production of lipid 9-hydroperoxides by adding molecular oxygen to either linolenic or linoleic acid at the C-9 position (Ferrie *et al.*, 1994). *AOS3* encodes a root-specific and jasmonate-regulated allene oxide synthase that catalyses the biosynthesis of γ- and α-ketols, and 10-OPDA, an isomer of the JA precursor 12-OPDA (Fig. 3) (Itoh *et al.*, 2002; Grechkin *et al.*, 2008). Furthermore, the gene encoding a divinyl ether synthase (DES), also related to the 9-LOX branch and involved in the formation of the divinyl ether fatty acids colnelenic and colnelic (Itoh and Howe, 2001), was also induced by the two AMF (Table 1 and Fig. 3).

Besides the common genes, there were specific sets of genes regulated only by either *G. mosseae* or *G. intraradices*. Among those significantly ($P$ <0.01) induced exclusively in *G. intraradices*-colonized roots, a group of genes related to the biosynthesis of carotenoids, namely *DXR*, *PSD*, *ZDS*, and *CtrL-b*, stood out (Supplementary Table S4 and Fig. S1 at *JXB* online). The production of the carotenoid cleavage products mycorradicin and cyclohexenone derivatives is associated with mycorrhization. Indeed, mycorradicin—known as the 'yellow pigment'—is responsible for the typical yellow coloration of some mycorrhizal roots and has been correlated with a functional symbiosis (Walter *et al.*, 2007). The first step in this biosynthetic pathway is catalysed by DXS-2, induced transcriptionally during interaction with both fungi but to a higher extent in *G. intraradices*- than in *G. mosseae*-colonized roots (Table 1). Although not significant under the present selection criteria, a moderate increase in the expression of *DXR*, *PSD*, *ZDS*, and *CtrL-b* was also observed in *G. mosseae*-colonized roots. Thus, the induction of the carotenoid pathway is not exclusive to *G. intraradices*-colonized roots; more probably it correlates with the root colonization level.

**Table 1.** Genes regulated in roots colonized by both *G. mosseae* and *G. intraradices* compared with non-mycorrhizal roots, and their changes in expression after MeJA treatment

| ID | Annotation | Ratio Gm | Ratio Gi | MeJA |
|---|---|---|---|---|
| BT014524 | Serine protease/subtilisin-like | 28.06 | 36.26 | 0.72 |
| X72729 | Ripening-related protein ERT1b | 25.96 | 38.42 | 1.25 |
| M69248 | Pathogenesis protein PR1b1 | 19.71 | 16.74 | 2.56[a] |
| AW622368 | Esterase/lipase/thioesterase | 17.59 | 42.64 | 0.11[a] |
| AI897365 | Putative proteinase inhibitor | 17.34 | 7.42 | 543.59[a] |
| AI487223 | Anthocyanin acyltransferase | 8.63 | 3.40 | 112.45[a] |
| AF454634 | Allene oxide synthase 3 (AOS3) | 8.49 | 3.42 | 236.13[a] |
| AB015675 | Copalyl diphosphate synthase | 7.69 | 10.41 | 1.25 |
| U09026 | Lipoxygenase A (LOXA) | 6.59 | 2.78 | 13.95[a] |
| AB010991 | 3b-hydroxylase (Le3OH-1) | 5.13 | 9.60 | 0.94 |
| BG631079 | β-1,3-Glucanase | 5.07 | 5.35 | 0.72 |
| BI933750 | 1-Deoxy-D-xylulose 5-phosphate synthase 2 (DXS-2) | 5.00 | 9.92 | 2.65[a] |
| AB041811 | β-D-Xylosidase (LEXYL1) | 4.72 | 4.49 | 1.00 |
| BG125734 | Calcium/lipid-binding protein | 4.49 | 15.13 | 0.91 |
| BT013355 | Pathogenesis protein PR-P2 | 4.45 | 6.57 | 9.79[a] |
| BG626023 | Electron carrier (ACD1-like) | 4.12 | 6.88 | 1.10 |
| BI423134 | Germin-like protein | 3.92 | 5.78 | 1.20 |
| BI423255 | Germin-like protein (GLP6) | 3.44 | 4.67 | 1.18 |
| X94946 | Proteinase inhibitor II (Cevi57) | 3.42 | 2.12 | 4.18[a] |
| BM412305 | EF-hand-containing protein | 3.24 | 4.56 | 0.81 |
| BF114155 | Glutathione *S*-transferase | 3.20 | 2.76 | 9.19[a] |
| AF090115 | Heat shock protein HSP17.4 | 3.20 | 7.48 | 1.58 |
| CK720570 | Patatin-like protein | 3.19 | 6.04 | 2.89[a] |
| AF515615 | Lysine-rich protein (TSB) | 3.18 | 3.67 | 0.47[a] |
| AI895164 | Fatty acid desaturase (FAD) | 3.05 | 3.12 | 0.46[a] |
| AJ785041 | Cytochrome P450 CYP81C6v2 | 2.89 | 2.65 | 360.27[a] |
| BT014484 | Glucosyltransferase | 2.83 | 3.91 | 6.26[a] |
| BG630947 | β-Galactosidase (TBG5) | 2.79 | 5.27 | 0.55 |
| BT014016 | Cysteine synthase (cs1) | 2.76 | 5.94 | 0.99 |
| BI923212 | Germin-like protein (GLP9) | 2.71 | 3.04 | 0.75 |
| CK716273 | Miraculin-like protein | 2.69 | 2.48 | 2.27[a] |
| AF049898 | Gibberellin 20-oxidase-1 (20ox-1) | 2.67 | 3.36 | 0.61 |
| AW220405 | Germin-like protein (ger2a) | 2.56 | 2.86 | 0.73 |
| AW626187 | Unkonwn | 2.48 | 2.83 | 0.74 |
| AW034398 | Subtilisin-like protease (sbt4a) | 2.47 | 3.19 | 1.70 |
| U30465 | Class II chitinase (Chi2;1) | 2.41 | 3.01 | 6.34[a] |
| CN384809 | 1-Aminocyclopropane-1-carboxylic acid oxidase (ACO) | 2.35 | 2.27 | 12.03[a] |
| AF317515 | Divinyl ether synthase (DES) | 2.29 | 1.96 | 3.55[a] |
| X79337 | Ribonuclease le | 2.24 | 1.88 | 2.44[a] |
| BG628191 | Jasmonate ZIM domain 2 (JAZ2) | 2.23 | 2.49 | 14.78[a] |
| L77963 | Metallothionein II-like protein (MTA) | 2.23 | 2.06 | 0.31[a] |
| AY455313 | Methylesterase/methyl jasmonate esterase (JAME) | 2.16 | 2.82 | 1.16 |
| AJ271093 | Allene oxide synthase 1 (AOS1) | 2.14 | 2.52 | 23.83[a] |
| AA824679 | Dihydrolipoamide *S*-acetyltransferase (LTA2) | 2.00 | 1.96 | 0.51 |
| Y15846 | Pathogenesis protein PR10-like | 1.93 | 2.72 | 0.52 |
| AI899627 | Unknown | 1.92 | 2.47 | 3.89[a] |
| BT013881 | Cytochrome P450 (CYP721A7v1) | 1.86 | 2.45 | 1.42 |
| BG625959 | Enoyl-[acyl-carrier-protein] reductase | 1.81 | 2.24 | 0.23[a] |
| AI780669 | Glutathione *S*-transferase | 0.49 | 0.50 | 7.29[a] |
| BM411685 | Zinc finger (B-box type) | 0.48 | 0.49 | 1.80 |
| AW649455 | GDSL-motif lipase/hydrolase | 0.48 | 0.54 | 0.33[a] |
| AF437878 | bHLH transcriptional regulator | 0.44 | 0.32 | 2.29[a] |
| AW218614 | UDP-glycosyltransferase | 0.43 | 0.36 | 4.85[a] |
| AW442015 | Cysteine-type endopeptidase | 0.40 | 0.60 | 0.67 |
| BI207994 | Cytochrome P450 CYP72A15 | 0.36 | 0.54 | 4.17[a] |
| M21775 | Metallocarboxypeptidase inhibitor | 0.36 | 0.35 | 0.82 |

**Table 1.** *Continued*

| ID | Annotation | Ratio Gm | Ratio Gi | MeJA |
|---|---|---|---|---|
| AI771889 | Cytochrome P450 CYP72A57 | 0.27 | 0.44 | 5.12[a] |
| BI205190 | UDP-glycosyltransferase | 0.25 | 0.39 | 21.66[a] |
| BM411019 | Unknown | 0.16 | 0.39 | 1.96 |

Genes significantly up- or down-regulated in roots colonized by *G. mosseae* (Gm) and *G. intraradices* (Gi) compared with non-mycorrhizal roots are sorted according to the fold change in expression in *G. mosseae*-colonized roots. MeJA shows the changes in expression levels of mycorrhiza-regulated genes upon treatment with 50 μM methyl jasmonate compared with mock-treated roots.
[a] Significant ($P$ <0.01) changes in MeJA-treated roots.

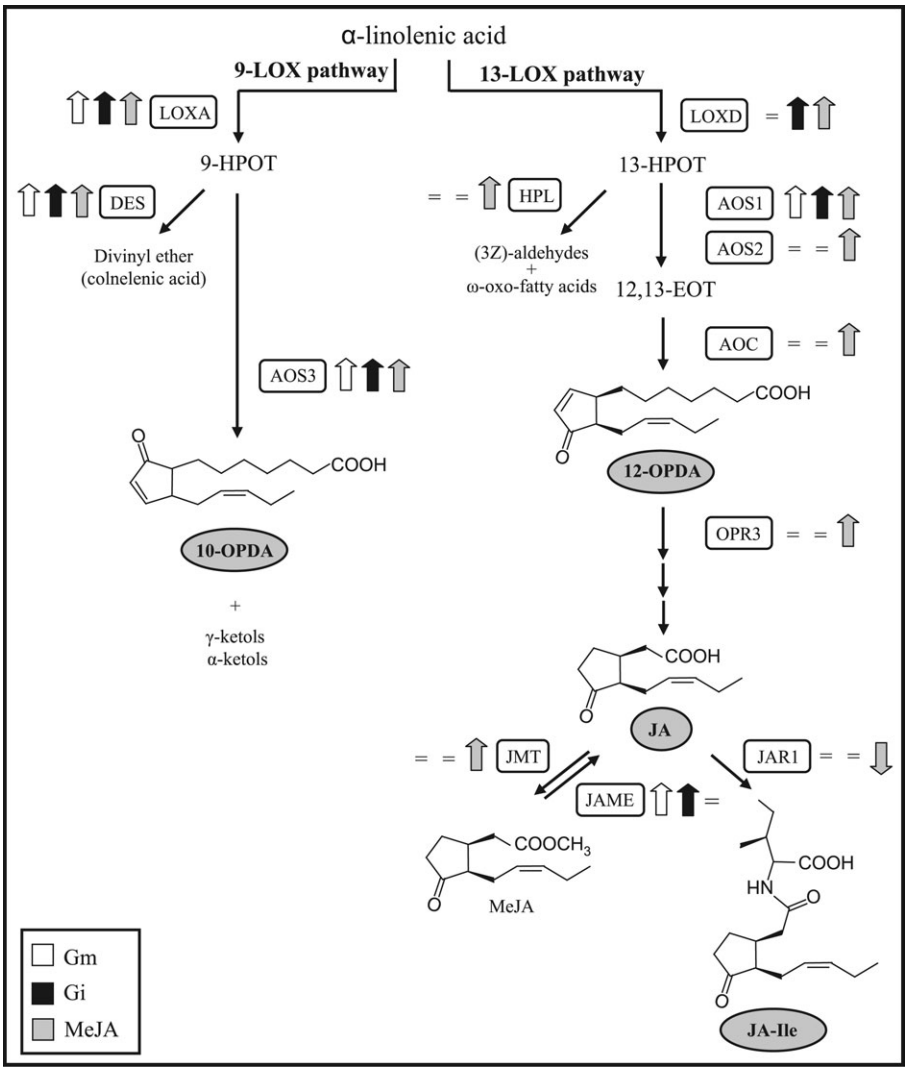


**Fig. 3.** Induction of the oxylipin biosynthethic pathway in arbuscular mycorrhizal symbiosis. Metabolic scheme of the oxylipin pathway including the 9-LOX and 13-LOX branches (modified after Wasternack, 2007). Shaded boxes show the metabolites analysed by LC-MS/MS in the present study. Thick arrows indicate the direction of the changes in expression levels compared with non-mycorrhizal control roots (up- or down-regulated) of the genes coding for the enzyme cited, as determined by the transcriptomic analysis; = indicates no changes in gene expression. LOX, lipoxygenase; AOS, allene oxide synthase; AOC, allene oxide cyclase; OPR3, oxo-phytodienoic acid reductase; JMT, jasmonic acid carboxyl methyltransferase; JAME, methyl jasmonate esterase; JAR1, jasmonate-amino synthetase; HPL, hydroperoxide lyase; and DES, divinyl ether synthase.

With regards to *G. mosseae*, a major part of the differentially regulated genes upon mycorrhization were related to defence and wounding responses associated with jasmonates (Supplementary Table S5 at *JXB* online), including the typical JA marker genes encoding proteinase inhibitors I and II (PinI and II), multicystatin (MC), polyphenol oxidase, and threonine deaminase (Wasternack *et al.*, 2006). Remarkably, despite the strong induction of

these genes in *G. mosseae*-mycorrhizal roots, they remained unaltered in *G. intraradices*-colonized roots.

To confirm the regulation of the different signalling pathways, the expression of the hormone biosynthetic genes and several response marker genes was analysed by real-time qPCR. In addition, potential markers of AM symbiosis, orthologues of genes previously described as AM induced in other plant species, such as *DXS-2*, *PR-10*, and the chitinase *Chi2;1*, were also checked (Table 2). There was a very good correlation between the expression data obtained by qPCR and microarray analysis, confirming the global transcript profiling analysis. The analysis highlights the induction of the key biosynthetic genes from the 9-LOX branch (*LOXA*, *AOS3*, and *DES*) in mycorrhizal roots, the induction of *LOXA* and *AOS3* being markedly higher in *G. mosseae*-colonized roots (Table 2), and the striking differential induction in those roots of the typical JA-regulated, wound-related genes such as PinII and MC.

According to the ABA content, the levels of *NCED*—encoding a 9-*cis*-epoxycarotenoid enzyme—involved in ABA biosynthesis, and the ABA-inducible gene *Le4* (Kahn *et al.*, 1993) remained unaltered in the mycorrhizal plants. The qPCR analysis showed unaltered expression of all the ET-related genes tested, not validating the up-regulation of *ACO1* observed in the microarray analysis. In relation to SA, the gene coding for PR1a, a common marker of SA-regulated responses (Uknes *et al.*, 1993), was only detected in *G. mosseae*-colonized roots (Table 2), supporting the increased SA levels detected previously by LC-MS/MS.

### *The mycorrhiza-related transcriptome is partially mimicked by MeJA treatment*

The induction of genes associated with jasmonates in mycorrhizal roots suggested a possible role for these hormones in the plant response to AMF. To assess whether this is the case, a microarray analysis was carried out to identify JA-regulated genes, and the transcriptional profile was compared with those obtained from mycorrhizal roots. Root treatment with MeJA resulted in 1398 differentially regulated genes ($P$ <0.01), most of them being up-regulated (~60%) (Supplementary Table S6 at *JXB* online). Even though two different systems were used for growing the plants, 66% of the genes co-regulated upon *G. mosseae* and *G. intraradices* colonization were also regulated by MeJA (Table 1). Among them, the jasmonate biosynthetic and regulatory genes derived from the 13-LOX branch and induced by mycorrhiza—*AOS1* and *JAZ2*—were also significantly ($P$ <0.01) induced by MeJA. Other JA metabolic genes such as *AOC*, *OPR3*, and the jasmonic acid carboxyl methyl transferase (*JMT*), not altered by mycorrhiza, were also up-regulated by MeJA, supporting the reported positive feedback in jasmonate biosynthesis (Fig. 3 and Supplementary Table S6). In addition, the genes involved in defence responses to wounding and herbivory, known to be regulated by JA in aerial parts of the plant (Wasternack *et al.*, 2006), were all up-regulated by MeJA in the roots. Remarkably, as stated before, most of these genes were induced in *G. mosseae*-mycorrhizal roots

**Table 2.** Expression analyses of marker genes for AM symbiosis and hormone pathways by quantitative real time RT-PCR (qPCR) in roots colonized by *G. mosseae* (Gm) or *G. intraradices* (Gi)

| ID | Annotation | Pathway/ marker | Microarray | | qPCR | |
|---|---|---|---|---|---|---|
| | | | Gm | Gi | Gm | Gi |
| BI933750 | 1-Deoxy-D-xylulose 5-phosphate synthase 2 (DXS-2) | AM | 5.00[a] | 9.92[a] | 8.59[a] | 20.08[a] |
| U30465 | Class II chitinase (Chi2;1) | AM | 2.41[a] | 3.01[a] | 2.70[a] | 2.23[a] |
| Y15846 | Pathogenesis protein PR10-like | AM | 1.93[a] | 2.72[a] | 4.82[a] | 5.81[a] |
| AY885651 | Phosphate transporter LePT4 | AM | – | – | 1141.69[a] | 2046.48[a] |
| U09026 | Lipoxygenase A (LOXA) | Oxylipins | 6.59[a] | 2.78[a] | 7.46[a] | 3.38[a] |
| AF454634 | Allene oxide synthase 3 (AOS3) | Oxylipins | 8.49[a] | 3.42[a] | 16.60[a] | 6.28[a] |
| AF317515 | Divinyl ether synthase (DES) | Oxylipins | 2.29[a] | 1.96[a] | 3.28[a] | 2.94[a] |
| U37840 | Lipoxygenase D (LOXD) | JA | 1.27 | 2.17[a] | 0.79 | 2.35[a] |
| AJ271093 | Allene oxide synthase 1 (AOS1) | JA | 2.14[a] | 2.52[a] | 2.51[a] | 3.32[a] |
| AF230371 | Allene oxide synthase 2 (AOS2) | JA | 0.97 | 0.89 | 0.98 | 0.84 |
| AF384374 | Allene oxide cyclase (AOC) | JA | 1.34 | 1.29 | 1.32 | 1.41 |
| AJ278332 | 12-Oxophytodienoate 3 reductase (OPR3) | JA | 0.94 | 1.05 | 1.09 | 1.15 |
| AF083253 | Multicystatin (MC) | JA | 9.27[a] | 1.64 | 8.35[a] | 0.76 |
| K03291 | Proteinase inhibitor II (PinII) | JA | 5.92[a] | 0.94 | 7.39[a] | 1.06 |
| CN384809 | 1-Aminocyclopropane-1-carboxylic acid oxidase (ACO1) | ET | 2.35[a] | 2.27[a] | 0.79 | 1.76 |
| X58885 | Ethylene-forming enzyme (EFE) | ET | 0.69 | 1.13 | 0.61 | 1.54 |
| AY394002 | CTR1-like protein kinase (CTR4) | ET | 1.11 | 1.13 | 0.71 | 0.83 |
| Z97215 | 9-*cis*-Epoxycarotenoid (NCED1) | ABA | 0.96 | 0.69 | 0.68 | 0.57 |
| X51904 | Le4 | ABA | – | – | 0.82 | 0.48 |
| M69247 | Pathogenesis-related protein PR1a | SA | ND | ND | $C_t$ 30.67 | ND |

Numbers indicate fold change in expression levels compared with non-mycorrhizal controls.
$C_t$, the threshold cycle; ND. non-detected;– not present in the tomato array.
[a] Significant ($P$ <0.01) changes.

(Supplementary Table S5), but not in those colonized by *G. intraradices*. The genes *LOXA*, *AOS3*, and *DES*, induced by both AMF and acting on the 9-LOX branch, were also induced by MeJA, illustrating a positive regulation of this branch by JA (Table 1 and Supplementary Table S6).

## Discussion

It is widely accepted that AM establishment induces transcriptional changes in the host plant (Grunwald *et al.*, 2004; Hohnjec *et al.*, 2005; Liu *et al.*, 2007; Fiorilli *et al.*, 2009), and the involvement of a number of plant hormones in mycorrhiza formation and functioning has been proposed (Hause *et al.*, 2007; Herrera-Medina *et al.*, 2007; Riedel *et al.*, 2008; Fiorilli *et al.*, 2009; Grunwald *et al.*, 2009). However, the precise mechanisms underlying plant–AMF interactions are still unknown. Here, the plant response to the colonization by the two AMF *G. mosseae* and *G. intraradices* was analysed using tomato as the model plant. A different level and structure of mycorrhizal colonization were observed when comparing both fungi, *G. intraradices* being the most effective colonizer. This higher colonization level intensity was accompanied by a larger induction of host genes coding for symbiosis-related elements such as the mycorrhiza-specific phosphate transporter LePT4 and those involved in the biosynthesis of mycorrhiza-related carotenoids (Walter *et al.*, 2007). Differences in the colonization by *G. intraradices* and *G. mosseae* isolates have been previously shown in several plant species (Pozo *et al.*, 2002; Feddermann *et al.*, 2008), supporting that at least part of the morphological features in the AM colonization, and probably of the hormonal and transcriptional changes in the host, are related to the AMF genotype.

Simultaneous quantification of several hormones by LC-MS/MS and GC-MS revealed significant changes in the levels of defence-related hormones in mycorrhizal tomato roots, depending on the AMF involved. While ABA and free JA levels remained unaltered in mycorrhizal plants compared with controls, ET and OPDA levels were significantly down- and up- regulated, respectively, during the interaction with both fungi. In contrast, the levels of the JA derivative JA–Ile and SA were elevated exclusively in roots colonized by *G. mosseae*, the AMF with a lower colonization level. The significant reduction in ET production in roots colonized by both AMF is in agreement with studies in other mycorrhizal plants and during plant interaction with other beneficial endophytic fungi (Barazani *et al.*, 2007; Riedel *et al.*, 2008). This reduction, together with data from pharmacological and genetic approaches to analyse the role of ET in the symbiosis, indicates that a precise regulation of ET levels is required for AM establishment (Azcón-Aguilar *et al.*, 1981; Zsogon *et al.*, 2008).

As mentioned above, OPDA levels were elevated in roots colonized by both AMF. Surprisingly, the higher content of OPDA was not accompanied by increased levels of free JA. Free JA increased up to several fold in roots of barley, cucumber, *M. truncatula*, and soybean upon mycorrhization (Hause *et al.*, 2002; Vierheilig and Piche, 2002; Meixner *et al.*, 2005; Stumpe *et al.*, 2005). Accordingly, a conserved role for JA in the establishment and functionality of the AM symbiosis was proposed. However, analysis of the JA content in *N. attenuata* showed unaltered levels in mycorrhizal roots (Riedel *et al.*, 2008). It is also noteworthy that no changes in OPDA content were found in mycorrhizal barley or *Medicago* roots (Hause *et al.*, 2002; Stumpe *et al.*, 2005). Therefore, it is possible that OPDA and other oxylipins, but not free JA, are the main players in orchestrating the plant response to mycorrhizal fungi in the Solanaceae. In this regard, organ- and plant species-specific patterns of accumulation of different JA-related compounds have been described, tomato being among the plants with higher levels of OPDA relative to the JA concentration before and after wounding (Miersch *et al.*, 2008) and in response to pathogen attack (Vicedo *et al.*, 2009).

At the transcriptional level, some of the genes related to the biosynthesis and metabolism of jasmonates showed a moderate increase in their expression in mycorrhizal roots. LOXD and AOS1 are involved in the early steps of the pathway, so their higher expression levels might support the increase in the JA precursor 12-OPDA. Induction of the JA biosynthetic genes *AOS* and *AOC* was previously shown in arbusculated cells in barley and *M. truncatula* roots (Hause *et al.*, 2002). However, changes in the expression of *AOC* and later biosynthetic genes were not detected in the tomato array. The picture is more complex because, besides JA, other oxylipins play important roles in biological processes such as plant defence and development (Wasternack, 2007; Mosblech *et al.*, 2009). Interestingly, the microarray analysis revealed a strong induction of the genes coding for LOXA and AOS3, key enzymes in the 9-LOX branch of the oxylipin pathway which give rise to the formation of ketols and 10-OPDA. 10-OPDA is a structural isomer of the JA precursor 12-OPDA (Itoh *et al.*, 2002; Grechkin *et al.*, 2008). Because of the transcriptional activation of the 9- and 13-LOX branches in mycorrhizal roots and the inability of LC-MS/MS to discriminate between the two isomers, it is likely that the increased OPDA levels observed correspond to a mixture of both isomers. In addition to its function as a JA precursor, 12-OPDA *per se* plays a role in plant defence signalling, regulating the expression of a specific subset of genes (Stintzi *et al.*, 2001; Taki *et al.*, 2005). Despite the lack of information about 10-OPDA *in vivo*, a similar role for this OPDA isomer in plant defence has been postulated (Itoh *et al.*, 2002). Further supporting the activation of the 9-LOX branch, up-regulation of *DES* in mycorrhizal tomato roots was also found. The encoded enzyme catalyses the biosynthesis of colnelenic and colneleic acids, for which a role in defence against plant pathogens has been proposed in tobacco and potato (Mosblech *et al.*, 2009). It is noteworthy that the 9-LOX branch is largely root specific. Indeed, *AOS3* is exclusively expressed in the roots (Itoh *et al.*, 2002), and *LOXA* and *DES* show only very low basal

expression levels in shoots (Ferrie *et al.*, 1994; Itoh and Howe, 2001). Recently, the relevance of the 9-LOX pathway in plant interactions with nematodes and pathogens has been demonstrated (Vellosillo *et al.*, 2007; Gao *et al.*, 2008). Thus, it is plausible that the activation of the 9-LOX pathway is part of the strategy of the plant to control AMF development within the roots. Supporting this hypothesis, increased mycorrhization levels were found in the tomato mutant *jai1* (Herrera-Medina *et al.*, 2008; JAL-R *et al.*, unpublished data), in which *AOS3* expression is undetectable (Itoh *et al.*, 2002). Moreover, although *LOXA* and *AOS3* were significantly induced in roots colonized by both AMF, a higher up-regulation was found in the interaction with the lower colonization rate, tomato–*G. mosseae*. As a side effect, higher levels of 9-LOX-derived products may be responsible for the enhanced resistance to root pathogens in mycorrhizal plants. We previously compared *G. mosseae* and *G. intraradices* in terms of their ability to protect tomato plants against *Phytophthora parasitica* var. *nicotianae*, and only *G. mosseae* efficiently induced resistance (Pozo *et al.*, 2002). More recently, resistance to this pathogen has been demonstrated to depend on the 9-LOX branch in tobacco roots (Fammartino *et al.*, 2007). Accordingly, the stronger activation of this branch of the oxylipin pathway in *G. mosseae*-colonized roots may contribute to the enhanced resistance of these roots against *P. parasitica*.

In addition to the higher up-regulation of 9-LOX biosynthetic genes, *G. mosseae* differentially triggered other metabolic changes related to defence in tomato roots. Only roots colonized by this AMF showed a moderate but significant increase in the levels of JA–Ile, one of the most active forms of JA with specific biological roles (Staswick and Tiryaki, 2004; Fonseca *et al.*, 2009). JA–Ile is formed by the action of JAR1, which catalyses the conjugation of JA to isoleucine synthesized from threonine by a threonine deaminase (TD). Remarkably, a gene encoding a TD was up-regulated >6-fold in roots colonized by *G. mosseae*, but not by *G. intraradices*, which is in agreement with the differences in JA–Ile content between roots colonized by the two AMF. In *N. atenuatta* the defensive role of this enzyme is linked to its mediation of JA–Ile signalling, leading to the accumulation of direct defences such as protease inhibitors (Kang *et al.*, 2006). In agreement with the elevated levels of JA–Ile only in *G. mosseae*-colonized roots, all wound-inducible marker genes (coding for proteinase inhibitors I and II, polyphenol oxydase, arginase 2, multicystatin, etc.) were up-regulated exclusively in those roots. It is noteworthy that only JA–Ile, and not JA or OPDA, is able to promote interaction of the SCFcoi1 ubiquitin ligase complex and JAZ proteins in *Arabidopsis*. This interaction liberates MYC2, a positive regulator of JA (reviewed in Memelink, 2009) which is essential in rhizobacteria-induced systemic resistance (Pozo *et al.*, 2008). Thus, it is tempting to speculate that elevated JA–Ile levels and related transcripts in *G. mosseae*-colonized plants may be related to its ability to induce mycorrhiza-induced resistance.

As for JA–Ile, SA content and expression of its marker gene *PR1a* were significantly elevated only in *G. mosseae*-colonized roots. SA is a key phytohormone in the regulation of plant defence responses, especially in interactions with biotrophic pathogens. In agreement with the biotrophic character of AMF, a negative regulatory role of SA in the AM symbiosis has been proposed (Gutjahr and Paszkowski, 2009). Indeed, an inverse correlation between SA levels and AM colonization was found in pea and tobacco (Blilou *et al.*, 1999; Herrera-Medina *et al.*, 2003). Accumulation of SA in *G. mosseae*-colonized barley roots has also been described (Khaosaad *et al.*, 2007). Therefore, the enhanced SA levels could modulate the plant control of AMF proliferation within the roots. Additionally, because of the role of SA in regulating systemic defence responses and induced resistance (Vlot *et al.*, 2009), the increase in SA levels might be key in the induction of resistance by mycorrhiza.

Taken together, the results show common and divergent responses of the plant to mycorrhizal colonization by different AMF at the hormonal and transcriptional levels. Remarkably, the differences in the response are very significant: only ~35% of the genes regulated during the interaction with *G. mosseae* and *G. intraradices* overlap. A similar overlap (~30%) was found in the response of *M. truncatula* to the same fungi (Hohnjec *et al.*, 2005). The differential responses found in tomato plants interacting with *G. mosseae*, namely stronger induction of the oxylipin 9-LOX branch, increased SA and JA–Ile contents, and the associated induction of *Pr1a* and jasmonate-related defence genes points to a more exhaustive control of the fungal partner by the plant that may explain the reduced colonization level of *G. mosseae* when compared with *G. intraradices*. Moreover, it might be that these changes in the host also contribute to the bioprotection ability of this AMF.

In conclusion, it is shown here that the maintenance of AM symbiosis implies changes in the content of several phytohormones, which correlate with changes in the expression of genes involved in their biosynthesis and the responses they regulate. A crucial role for oxylipins in this mutualistic symbiosis has been proposed for the first time, and indications of their regulation by jasmonates suggested. Additionally, a different plant response to the colonization by particular AMF is demonstrated, which may underlie the differential impact of individual AMF on plant physiology and, particularly, on its ability to cope with biotic stresses. Further research is required to elucidate the role of the 9-LOX-derived oxylipins, as well as the metabolic and transcriptional changes needed for the long-term maintenance of the AM symbiosis and its benefits to the host.

## Supplementary data

Supplementary data are available at *JXB* online.

**Fig. S1.** Scheme of the biosynthesis of the apocarotenoids cyclohexenone and mycorradicin in mycorrhizal roots (adapted from Walter *et al.*, 2007). Thick arrows indicate significant up-regulation of the corresponding genes as determined by the transcriptomic analysis of mycorrhizal roots (*G. mosseae* or *G. intraradices* colonized) compared

with non-mycorrhizal controls; = indicates no changes in gene expression. DXS-2, 1-deoxy-D-xylulose 5-phosphate synthase 2; DXR, 1-deoxy-D-xylulose reductase; PSY, phytoene synthase; PDS, phytoene desaturase; ZDS, zeta-carotene desaturase; CrtL-b, lycopene cyclase b; and CCD1, carotenoid cleavage dioxygenase 1.

**Table S1.** Primer sequences used in the real-time qPCR analysis.

**Table S2.** Mycorrhization, root and shoot fresh weights, and phosphorus content in tomato plants colonized by *G. mosseae* (Gm) or *G. intraradices* (Gi). Nm indicates control non-mycorrhizal plants.

**Table S3.** Expression level of genes involved in hormone metabolism in roots colonized by *G. mosseae* (Gm) or *G. intraradices* (Gi).

**Table S4.** Genes specifically induced or repressed in tomato roots colonized by *G. intraradices* (Gi). Fold change in their expression level compared with non-mycorrhizal plants or upon methyl jasmonate (MeJA) treatment.

**Table S5.** Genes specifically induced or repressed in tomato roots colonized by *G. mosseae* (Gm). Fold change in their expression level compared with non-mycorrhizal plants or upon methyl jasmonate (MeJA) treatment.

**Table S6.** Genes showing significant differential expression in tomato roots upon treatment with 50 μM MeJA.

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