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Distinct gene expression and secondary metabolite profiles for suboptimal mycorrhizal colonization in wild-type and the jasmonic acid deficient *spr2* tomato mutant

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A previous study with *spr2* mutant tomato plants which are negatively affected in the synthesis of jasmonic acid (JA), suggested that JA regulates the arbuscular mycorrhizal fungi (AMF) colonization via the control of carbon (C) partitioning. Although this and other studies have suggested the important positive role played by JA in the regulation of AMF root colonization in tomato plants, it is currently unclear how different host plant genetic backgrounds affect gene expression and secondary metabolites variation during JA-dependent mycorrhization. In this study, wild type and *spr2* mutant tomato plants having “low”, “medium” and “high” mycorrhizal colonization with *Rhizophagus irregularis*, were analyzed independently using transcriptomic and untargeted metabolomic approaches. The results obtained revealed that the degree of mycorrhizal colonization efficiency could be associated with contrasting expression levels of certain key genes controlling gibberellin signaling, ethylene biosynthesis and signaling, and synthesis of apocarotenoids, phenylpropanoids and tomatine, in roots. Only a few wound responsive genes, including JA signaling and biosynthesis genes, such as *Prosystemin* and *JAZ2* were found to influence AMF colonization. Conversely, a systemic and JA-dependent induction/ repression of genes different from those altered in roots was detected in leaves of mycorrhizal plants. The most significant changes in metabolite abundance were detected in roots with reduced AMF colonization. Included among the latter were metabolites known to be associated with important aspects of AMF symbiosis, such as signaling, nutrient exchange and modulation of pathogen defense response. Alpha-tomatine levels appeared to be an important factor, whose abundance negatively correlated wit h AMF colonization levels in tomato, suggesting a regulatory role for JA in the synthesis of this metabolite during the AMF symbiosis.

Distinct gene expression and secondary metabolite profiles for suboptimal mycorrhizal colonization in wild-type and the jasmonic acid deficient *spr2* tomato mutant

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ABSTRACT

A previous study with *spr2* mutant tomato plants which are negatively affected in the synthesis of jasmonic acid (JA), suggested that JA regulates the arbuscular mycorrhizal fungi (AMF) colonization via the control of carbon (C) partitioning. Although this and other studies have suggested the important positive role played by JA in the regulation of AMF root colonization in tomato plants, it is currently unclear how different host plant genetic backgrounds affect gene expression and secondary metabolites variation during JA-dependent mycorrhization. In this study, wild type and *spr2* mutant tomato plants having "low", "medium" and "high" mycorrhizal colonization with *Rhizophagus irregularis*, were analyzed independently using transcriptomic and untargeted metabolomic approaches. The results obtained revealed that the degree of mycorrhizal colonization efficiency could be associated with contrasting expression levels of certain key genes controlling gibberellin signaling, ethylene biosynthesis and signaling, and synthesis of apocarotenoids, phenylpropanoids and tomatine, in roots. Only a few wound responsive genes, including JA signaling and biosynthesis genes, such as *Prosystemin* and *JAZ2* were found to influence AMF colonization. Conversely, a systemic and JA-dependent induction/ repression of genes different from those altered in roots was detected in leaves of mycorrhizal plants. The most significant changes in metabolite abundance were detected in roots with reduced AMF colonization. Included among the latter were metabolites known to be associated with important aspects of AMF symbiosis, such as signaling, nutrient exchange and modulation of pathogen defense response. Alpha-tomatine levels appeared to be an important factor, whose abundance negatively correlated with AMF colonization levels in tomato, suggesting a regulatory role for JA in the synthesis of this metabolite during the AMF symbiosis.

Keywords: arbuscular mycorrhizal colonization, jasmonic acid, ethylene, gibberellins, tomatine

Abbreviations: AMF, arbuscular mycorrhizal fungi; JA, jasmonic acid; MeJA, methyl jasmonate; *spr2, suppressor of prosystemin-mediated response2.*

INTRODUCTION

The roots of the majority of higher plants, and many other host plants including pteridophytes, a number of mosses, lycopods, and psilotales are associated symbiotically with biotrophic and aseptate filamentous fungi of the Glomeromycota phylum, the so-called arbuscular mycorrhizal fungi (AMF) (Redecker and Raab, 2006; Schüssler et al., 2001). An important feature of the AM symbiosis is the nutrient exchange between both partners, which occurs within individual cortical cells, where AMF form highly branched hyphae, called arbuscules, surrounded by a plant derived membrane, known as the periarbuscular membrane. This structure has a unique transport protein distribution designed to deliver mineral nutrients to the cortical cell in exchange for the 4-to-20% of the photosynthates allocated to the roots that are transferred to the fungal symbiont (Gutjahr and Parniske, 2013). The mineral exchange principally involves phosphorus (P), although zinc and copper are also thought to be mobilized. AMF substantially enhance P availability to plants considering that this element is mostly found as orthophosphate ions in the soil, which become very poorly mobile in the presence of Ca^{2+}, Fe^{3+} and Al^{3+} (Wang et al., 2017). AMF are also believed to represent a significant route of nitrogen (N) uptake by the plants (Délano-Frier and Tejeda-Sartorius, 2008). P uptake relies on phosphate transporters belonging to the phosphate transporter 1 (Pht1) family (Rausch et al., 2001).

In addition to improving plant nutrition and water uptake, root colonization by AMF confers fitness benefits to the host plants. Among these are enhanced root growth and significant changes in root architecture that increase their surface area in the soil and enhance water and nutrient acquisition by the plants. Other benefits include increased reproductive success and/ or tolerance to (a)biotic stresses. Several of the above effects are finely regulated by phytohormones, via a limitedly understood and highly context dependent interaction (Ruiz-Lozano et al., 2012; Gutjahr 2014; Selosse et al., 2014; Pozo et al., 2015). AMF colonization has been found to profoundly alter gene expression in roots of several plants species (Guimil et al., 2005; Fiorilli et al., 2009; Guether et al., 2009; Hogekamp and Kuester, 2013; Groten et al., 2015), whereas a number of large-scale gene expression studies have explored the systemic effects that AMF root colonization has in plant aerial tissues (Guimil et al., 2005; Cervantes-Gamez et al. 2016; Adolfsson et al., 2017; Wang et al., 2018). Changes in root and leaf metabolite levels in response to AMF colonization, have been also reported, particularly affecting amino acid (aa),

carbohydrate and organic acid contents (Fester et al. 2011; Schweiger et al. 2014; Rivero et al., 2015; Schweiger and Müller, 2015; Wang et al., 2018).

Jasmonic acid (JA), its methyl ester (MeJA) and its aa conjugates such as JA-isoleucine, form part of a family of bioactive molecules collectively referred as jasmonates. They are involved in multiple plant developmental and growth processes, activation of secondary metabolism and defense responses against insects and pathogens in various plant species (Wasternack and Hause, 2011; Erb et el., 2012; Ahmad et al., 2016; Larrieu and Vernou, 2016). JA is biosynthesized through the octadecanoid pathway, being the final product of a series of reactions initiated by the peroxidation of linolenic acid, an 18: 3 unsaturated fatty acid (Schaller et al., 2005). Recent models indicate that JA is the long-distance systemic wound signal in tomato. Wound-induced systemic signaling appears to be facilitated by a positive amplification loop in which systemin, a wound related bioactive peptide signal, is considered to play a crucial role (Schilmiller and Howe 2005; Sun et al., 2011). Most experimental evidence gathered so far strongly suggests that JA might also play an important regulatory role in the mycorrhizal symbiosis (Gutjahr, 2014; Pozo et al., 2015). Several mechanisms have been proposed to explain the mechanism(s) by means of which JA might exert a positive effect on the AMF symbiosis. Included are the regulation of flavonoid biosynthesis and defense gene expression, cytoskeleton rearrangements, sink strength reinforcement, lipid metabolism modifications and the regulation of C partitioning in the plant (Isayenkov et al., 2005; Stumpe et al., 2005; Tejeda-Sartorius et al., 2008). However, the role of JA in the mycorrhizal colonization process remains contentious due to the contradictory findings of several studies in which the application of JA (or derivatives such as MeJA) on mycorrhizal colonization, reported both positive and inhibitory effects, depending on various experimental factors (Gutjhar and Paszkowski, 2009). Additionally, it has been suggested that JA signaling might differentially affect AMF colonization and development by performing different roles in early and late stages of colonization (Foo et al., 2013).

Additional findings, derived from the use of the JA-biosynthesis impaired *spr2* tomato mutant as experimental model (Howe and Ryan, 1999) are reported in this work. They represent a further contribution to better understand JA's role in the AMF symbiosis in tomato. For this purpose, wild-type and *spr2* plants with different degrees of mycorrhizal colonization were analyzed to measure changes in gene expression and metabolic profiles. The results obtained suggest that

JA's effect on AMF colonization is dependent on several and diverse key factors. The proposed elements of this cross-talk gravitating around JA are examined and further discussed.

MATERIALS AND METHODS

Plant Growth and AMF Inoculation

Seeds of wild-type (WT) tomato (*Solanum lycopersicum* L. cv. Castlemart) and *spr2* mutant plants were surface-sterilized by soaking in a 70% ethanol solution for 60 s, with a 20% household bleach solution (5% w/ v sodium hypochlorite) for 5 min, and then rinsed three times with sterile water. All seeds were germinated in a sterile soil mixture constituted by equal parts of sand and loam which was autoclaved six times. One-week-old seedlings were removed and transplanted to 300-ml pots (one plant per pot, ten plants per genotype) containing the same sterilized soil mixture. At the time of transplanting, five plants per genotype were inoculated with 3 g of a soil-based (1: 1 sand-loam) inoculum of *Rhizophagus irregularis* (*Biofertilizante*, INIFAP, México) containing ca. 100 spores per g. Control plants (five per genotype) were supplied with 3 g of sterilized soil mixture only. The inoculated and control plants were watered 3 times per week and fertilized once a week with a Long Asthon solution in which the P content was reduced to 7 µM, until harvest. They were kept in a growth room with a 16 h/ 8 h light/ dark photoperiod at 27°C (light) and 23°C (dark) with an illumination of approximately 250 µmol m^{-2} s^{-1}. At 50 days post-inoculation (dpi), the root system was split lengthways. One half was stained to evaluate mycorrhizal colonization, whereas the remaining root and leaf tissues were frozen, ground in liquid nitrogen, stored at -80°C until required for analysis. Five independent experiments were performed employing the above procedure, but only three of them were used for further analysis. They were classified as experiments yielding "low", "medium" and "high" AMF colonization levels. Those described in this work were performed at the following dates: October 2015, November 2015 and April 2016.

Estimation of AMF Root Colonization

To determine total colonization, root fragments of control and mycorrhized plants (120 per genotype) were stained with trypan blue (Phillips and Hayman, 1970) and observed with a light microscope and the intensity of root colonization by AMF was determined (Trouvelot et al., 1986) using MYCOCALC software (www2.dijon.inra.fr/ mychintec/ Mycocalc-prg/ download.html). Three colonization parameters were analyzed: frequency of mycorrhization (F%), representing the percentage of root segments showing internal colonization; intensity of mycorrhization (M%), the average percentage of colonization of root segments, and arbuscule abundance (A%), the percentage of arbuscules in the whole root system.

Sample Preparation for Metabolomic Analyses

Frozen tomato roots of control and mycorrhizal plants, respectively, were lyophilized and finely ground in a Mixer Mill MM 400 (Retsch GmbH, Haan, Germany) for 12 s at 30 Hz. Subsequently, 25 mg of plant powder was extracted with 1 mL of an aqueous methanol-formic acid solution (75% *v/ v* and 0.15% *v/ v*, respectively). The mixture was sonicated for 15 min in a water bath at maximum frequency and centrifuged at 10,000 × *g* for 10 min at 4°C. The supernatants were filtered through a 0.22 µm syringe filter prior to analysis by mass spectrometry (see below). All samples were freshly prepared in triplicate.

Metabolic Fingerprinting of Tomato Root and Leaf Extracts by Mass Spectrometry

For non-targeted metabolite profiling, tomato root methanolic extracts were analyzed by direct liquid introduction electrospray ionization / mass spectrometry (DLI-ESI-MS) as described before (Montero-Vargas et al., 2013). Measurements were performed using a single quadrupole mass detector (SQD2, Waters, Milford, USA) in positive mode at a flow rate of 10 $\mu L \cdot min^{-1}$. Mass spectra were acquired in continuous mode in a range of 50-1300 *m/ z* during 1 min with a scan time of 10 s. The instrument settings were: capillary voltage 3.0 kV, cone voltage 35 V and extractor voltage 4 V. The source temperature and desolvation temperature were set to 80 and 150°C, respectively. The desolvation gas flow was set to 250 L h^{-1} and a cone gas flow was set to 50 L h^{-1}. Additionally, samples were analyzed to detect specific ions corresponding to α-tomatine, its biosynthetic precursors and catabolic products.

Raw Data Processing and Data Analysis

Raw mass spectrometry data were converted to .mzML format with msconvert (Chambers et al., 2012). The mass spectrum data were processed in R (http://www.rproject.org) using the package MALDIquant version 1.15 (Gibb and Strimmer, 2012) programed to execute the following tasks: .mzML data import, summary of all scans of each spectrum, smoothing by a Savitzky-Golay filter, peak alignment and peak selection detection. Raw data are available from Zenodo (https://zenodo.org/), DOI: 10.5281/zenodo.2550944. Finally, a comparison matrix (*m/ z* values for columns and file names for rows) with the intensity of peaks were exported in *csv* format for statistical analysis, applying binning with a bin width of 1 *m/ z* and intensity-based normalization. A total of 673 out of more than 1,000 ion signals were used after initial data cleaning steps.

Unsupervised techniques were employed to explore the effect of mycorrhizal on the metabolic fingerprints of the genotypes employed. These consisted of a hierarchical clustering (HCA) and a principal component Analysis (PCA), both implemented in ClustVis (Metsalu and Vilo, 2015). For multiple comparison of the means a Tukey's Honest Significant Difference (HSD) was applied with a confidence interval of 95 %. When the normality of the data was not achieved, the Kruskal-Wallis test was applied, considering *p* values below 0.05 as significant.

For putative metabolite identification, a homebuilt metabolite database for tomato was created based on previous reports (Moco et al., 2006; Itkin et al., 2011; Caprioli et al., 2015) and the SolCyc database (http://solcyc.solgenomics.net/). Subsequently, automatic matching of the *m/ z* list was performed employing the software SpiderMass (Winkler, 2015).

Extraction of Total RNA, cDNA Preparation and qPCR Analysis

Total RNA was extracted from 100-500 mg of frozen root and leaf tissues with the Trizol reagent (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's instructions, with modifications. These consisted of the addition of a salt solution (sodium citrate 0.8 M + 1.2 M NaCl) during precipitation in a 1: 1 *v/ v* ratio with isopropanol and further purification with LiCl (8 M) for 1 h at 4°C. All RNA samples were analyzed by formaldehyde agarose gel electrophoresis and visual inspection of the ribosomal RNA bands upon ethidium bromide staining. Total RNA samples (4 µg) were reverse-transcribed to generate the first-strand cDNA using an oligo dT20 primer and 200 units of SuperScript II reverse transcriptase (Invitrogen). The cDNA employed for the qRT-PCR assays was initially prepared from 4 µg total RNA. It

was then diluted 5-fold in sterile deionized-distilled (dd) water prior to qRT-PCR. Amplifications were performed using SYBR Green detection chemistry and run in triplicate in 96-well reaction plates with the CFX96 Touch Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA). Reactions were prepared in a total volume of 20 µl containing: 2 µl of template, 2 µl of each amplification primer (2 µM), 8 µl of iQ SYBR Green supermix (Bio-Rad) and 6 µl of sterile dd water. Quantitative real-time PCR was performed in triplicate for each sample using the primers listed in the Table S1. Primers were designed for each gene, based on cDNA sequences derived from the tomato genome (Sol Genomics Network; Mueller et al., 2005). Primer design was performed using DNA calculator software (Sigma-Aldrich; St. Louis, MO, USA). The following protocol was followed for all qRT-PCR runs: 15 min at 95°C to activate the JumpStart Taq Polymerase (Sigma-Aldrich), followed by 40 cycles of denaturation at 95°C for 15 s and annealing at 60°C for 1 min. Slow amplifications requiring an excess of 32 cycles were not considered for analysis. The specificity of the amplicons was verified by melting curve analysis after 40 cycles. Baseline and threshold cycles (Ct) were automatically determined using CFX Manager Software version 2.1. PCR efficiencies for all genes tested were greater than 95%. Relative expression was calculated using the comparative cycle threshold method (Livak and Schmittgen, 2001). Transcript abundance data were normalized against the average transcript abundance of two reference genes: *TIP41* and *SAND* (Expósito-Rodríguez et al., 2008). The fold change in expression of the target genes was calculated as described previously (Palmeros-Suárez et al., 2015). The values represent the mean ± SE of transcript abundance detected in three biological replicates per experiment, each of which involved triplicate technical repeats. The experiments analyzed were those having “low”, “medium” and “high” AMF colonization experiments. Similar to the metabolomic analysis, qPCR raw data are available from Zenodo (https://zenodo.org/), DOI: 10.5281/zenodo.2554756.

Statistical Analysis of Mycorrhizal Colonization Experiments

Data were analyzed by one-way ANOVA to determine whether or not the means of the different treatments tested were equal. A multiple comparison procedure with the Tukey’s test was performed to find significant differences between means. All tests were conducted using the

Minitab 15 statistical software package (Minitab Inc., USA). Differences at $p < 0.05$ were considered as statistically significant.

RESULTS

Variable degree of mycorrhizal colonization

Three independent experiments showing "low", "medium" and "high" colonization parameters were analyzed and compared. AMF colonization parameters in these three experiments are shown in Fig. 1. The M%, F% and A% parameters were consistently lower in mycorrhizal *spr2* roots, although they gradually ceased to be statistically significant from those in WT plants as the AMF colonization efficiency decreased.

Gene expression profiles

AMF colonization induced changes in the expression levels of a select set of the key genes analyzed in roots of WT and *spr2* mycorrhizal plants (Table 1). In leaves, transcriptional changes were more prominent (Table 2). The results obtained from roots include the expression data from samples taken from each individual experiment ("low", "medium" and "high" mycorrhization), whereas leaf gene expression data shown, included data obtained from the pooled leaves of "medium" and "high" mycorrhizal WT and *spr2* plants.

Gene expression profiles in roots

Mycorrhizal colonization marker genes

AMF colonization levels coincided with expression levels of the plant *LePT4* gene, a reliable indicator of mycorrhizal-colonization in roots (Javot et al., 2007; Wang et al., 2017). Conversely, both AMF *18S Ri* and *EF1-alpha* fungal gene markers tended to be higher in mycorrhizal roots of *spr2* plants with "low" and "medium" colonization levels.

Phytohormone-related genes

In roots, brassinosteroid (BR) gene expression appeared to be inversely proportional to AMF colonization. Thus, the highest expression values of BR-related genes were mostly detected in mycorrhizal WT and *spr2* roots having "low" colonization levels. Additionally, the *DWARF4* (*DWF4*) gene, coding for an enzyme involved in the catalysis of late hydroxylation steps in BR

277 synthesis (Bancos et al., 2002), was down-regulated in WT roots having "high" mycorrhizal
278 colonization. A notable exception was the *DET2* gene, which was induced in WT roots showing
279 "high" mycorrhizal colonization. *DET2* codes for a steroid 5a-reductase that is predominantly
280 active in the early steps of the BR biosynthetic pathway.
281
282 The expression patterns of the gibberellin (GA)-related genes *GA3ox* and *GAI*, coding for a 2-
283 oxoglutarate-dependent dioxygenase responsible for the downstream synthesis of bioactive GAs,
284 and for a DELLA member of transcriptional regulators that negatively regulate GA responses,
285 respectively, was also tested (Sun, 2008; Hedden and Thomas, 2012). Only the *GAI* gene
286 expression patterns showed an inverse correlation with AMF colonization levels in WT roots,
287 being repressed or induced in response to "high" or "low" mycorrhizal colonization,
288 respectively. Moreover, *GAI* remained, for the most part, up-regulated in mycorrhizal *spr2* roots.
289 The expression of the ABA marker genes was similar in all experiments and genotypes
290 examined, including *NCED1*, coding for one of the two tomato NCED enzymes that catalyze a
291 key rate-limiting step of ABA biosynthesis (Martín-Rodríguez et al., 2016).
292
293 *Apocarotenoid biosynthesis-related genes*
294 Generally, higher AMF colonization in WT roots correlated with increased *CCD7* expression
295 levels. This gene codes for one of the two dioxygenases required for strigolactone biosynthesis,
296 an essential component of the initial plant-AMF communication (López-Ráez et al., 2015). The
297 expression of the *CCD1b* gene was, in contrast, inversely related to mycorrhizal colonization in
298 WT roots, being induced in response to "low" mycorrhizal colonization but repressed under
299 "high" AMF colonization conditions. This pattern was partially reproduced in *spr2* roots, where
300 this gene was induced only when mycorrhizal colonization was "low". This gene codes for a
301 carotenoid dioxygenase enzyme involved not only in the biosynthesis of C13/ C14
302 apocarotenoids, but also in the generation of apocarotenoid cleavage products, such as β-ionone,
303 C_{13} α-ionol and C_{14} mycorradicin at late stages of the symbiosis (López-Ráez et al., 2015). The
304 expression levels of the *CGT1*, involved in the synthesis of apocarotenoid glycosyl esters in
305 fruits of other plants (Nagatoshi et al., 2012) and also in the final glucosylation steps of crocin, a
306 compound structurally similar to the core structure of the yellow pigment in mycorrhizal maize

roots (Fester et al., 2002), was only induced in mycorrhizal *spr2* roots with "low" to "medium" colonization levels.

9-LOX and 13-LOX pathway genes

The expression patterns of the *AOS1, LOXD* and *Jasmonate ZIM domain 2* (*JAZ2*) genes coincided to some extent with the contrasting AMF colonization levels observed in WT and *spr2* roots. The pattern of *AOS1* expression, neutral in all *spr2* roots and repressed in WT roots with "low" mycorrhizal colonization, partly coincided with reduced colonization levels. AMF colonization appeared to positively affect *LOXD* and *JAZ2* expression in WT roots only. However, mycorrhizal abundance appeared not to be a factor affecting *LOXD* expression, since the effect was observed in roots having "high" and "low" AMF colonization levels. In contrast, the expression of *JAZ2* appeared to be positively correlated with AMF colonization levels, considering its induced expression in roots having "medium" and "high" mycorrhization. The *JMT* gene, coding for an esterase catalyzing the release of active JA from MeJA, followed a similar expression pattern, characterized by its consistent induction in mycorrhizal WT roots, irrespective of colonization level. However, contrary to the above two genes, *JMT* was also highly expressed in *spr2* roots with "medium" colonization levels. Contrariwise, the observed induction of emblematic 9-LOX pathway genes appeared to be independent of the plant genotype, WT or *spr2*, and of the level of AMF colonization achieved.

Wound-response (WR) genes

Within the WR genes, the *Prosystemin* (*PS*) gene was steadily induced in mycorrhizal WT roots, although expression levels were highest in roots with "low" mycorrhizal colonization levels, whereas its expression remained neutral or repressed in *spr2* mycorrhizal roots. Conversely, the expression of the *NADPH oxidase* (*RBOH1*), an early WR gene proposed to be responsible for the generation of reactive oxygen species (Ryan, 2000), was inversely related to mycorrhizal abundance, apparently in a JA-independent way, given its repression in WT and *spr2* roots with "high" colonization levels. The effect of root AMF colonization on the expression of *LHA1*, one of the eight members that conform the plasma membrane H^+-ATPase gene family in tomato (Schaller and Oecking, 1999; Ryan, 2000; Liu et al., 2016), also seemed to be JA independent due to its induced expression in mycorrhizal *spr2* roots only. The expression of *PI II*, a late WR marker gene, was difficult to associate with a role in the AMF colonization process. The

observed repression of this gene in all *spr2* mycorrhizal roots analyzed, coincided with the confirmed JA-dependent regulation of this and other WR *PI* gene families. However, the *PI II* gene induction in WT mycorrhizal roots was erratic, showing opposite expression patterns in WT roots with "high" and "low" AMF mycorrhization levels. The role in tomato AMF colonization played by *AROGP3*, the JA–regulated tomato polygalacturonase non-catalytic subunit gene (Bergey et al., 1999) was also unclear, although a possible need for its down-regulation in mycorrhizal roots was supported by the repressed expression of this gene in WT roots with "high" and "low" AMF colonization levels. The observed lack of changes in the expression of polyphenol oxidase (*PPO*) cysteine proteinase 2 (*Cyp2*) and serine carboxypeptidase (*SCP*) WR genes, suggested that these genes were likely unaffected by AMF colonization.

Ethylene (ET) biosynthesis- and signaling-related genes

The expression of the *ACS6* and *ACO4* ET biosynthetic genes was inversely correlated with mycorrhizal colonization. They also showed a possible negative cross-talk with JA. The latter, considering their induction in WT roots with "low" mycorrhization colonization levels, which became widespread in mycorrhizal *spr2* roots. A similar behavior was shown by the *EIN 2* and *EIN 3* ET signaling genes. In contrast, the induction of *CTR4* in WT roots with "medium" and "high" AMF colonization levels suggested its role in promoting AMF colonization. Similar expression magnitudes of the *ERF1b* transcription factor (TF) gene in WT and *spr2* mycorrhizal roots advocated a JA-independent role for this TF during the mycorrhization colonization process.

Secondary metabolite biosynthesis genes

The analysis of key phenylpropanoid genes indicated that the expression of *PAL3* and *PAL4* was inversely correlated with AMF abundance in WT roots. This multi-gene family of genes, constituted by five members in tomato (Chang et al., 2008), codes for enzymes catalyzing the rate-limiting step of the phenylpropanoid biosynthetic pathway leading to trans-cinnamic acid, the precursor of diverse phenolic substances, including flavonoids. The expression pattern of *flavonol synthase* (*FLS*) in roots was relatively constant, suggesting it had no influence on the differential mycorrhizal colonization efficiencies observed between experiments and genotypes. This, despite the fact that *FLS* codes for a key enzyme for the synthesis of biologically active flavonols that presumably play a regulatory role in the mycorrhizal symbiosis (Steinkellner et al.

2007; Mandal et al. 2010,). Similar arguments could be used to explain the minimal changes in the gene expression pattern of *1-deoxy-D-xylulose 5-phosphate 2* (*DXS-2*). This gene codes for the critical regulatory enzyme of the plastidial methyl-erythritol-4-phosphate (or MEP) isoprenoid biosynthetic pathway leading to the generation, among many others, of C13-C14 apocarotenoid precursors. The small changes in the expression of 3-*hydroxy-3-methylglutaryl CoA reductase* (*HMGR1*), coding for the rate-limiting enzyme of the cytosolic isoprenoid biosynthetic pathway, also argued against a prominent role for this gene in the AMF colonization development in tomato. The expression pattern of the *benzyl alcohol acetyltransferase* (*BEAT*) gene, coding for an enzyme known to participate in synthesis of scent volatiles via benzenoid metabolic pathways (Bera et al., 2017), was inconsistent, showing augmented expression levels in WT roots -with "high" AMF colonization levels and also in *spr2* roots with "medium" and "low" mycorrhizal levels. Contrariwise, *farnesyl-diphosphate synthase 1* (*FPS1*), coding for a branch point enzyme of the isoprenoid pathway leading to both sesquiterpenes and sterols, and also considered to be a distal regulatory point of HMGR (Szkopińska, 2000), was repressed in WT roots having "high" mycorrhizal colonization levels. The expression of *cycloartenol synthase* (*CAS1*) and *glycoalkaloid metabolism 1* (*GAME1*) genes involved in the general pathway of steroid biosynthesis leading to the α-tomatine glycoalkaloid (Itkin et al., 2011; Moses et al., 2014, Jin et al., 2017) negatively correlated with mycorrhizal colonization, being induced in WT roots with "medium" and "low" mycorrhizal levels, and in most *spr2* roots examined.

Gene expression profile in leaves

The analysis of pooled leaves from "medium" and "high" mycorrhizal WT and *spr2* plants showed that AMF colonization induced a well-defined and largely JA-dependent systemic effect in leaves that was generally stronger than "local" expression in roots (Table 2). In WT plants, the high expression levels of the *FLS* and *CCD7* genes was noteworthy. Other genes, such as *GAME4, 6,* and *17*, involved in the tomatine biosynthetic pathway, as well as direct- and indirect-defense-related genes, such as *PPO*, *LOXD* and *JMT,* were induced in leaves of WT mycorrhizal plants. Conversely, foliar expression of *LePT4, GA3ox, AOS3, JMT, CCD7, GAME1, PPO* and *AroGP* was repressed mycorrhizal *spr2* plants. The above contrasted with the predominantly repressed expression of ET biosynthetic genes as well as the *ERF1b* TF and *BEAT* genes, in leaves of WT and *spr2* mycorrhizal plants. The induced expression of the *PI II* in

leaves of *spr2* mycorrhizal plants was contradictory to previously published data (Li et al., 2003). In contrast, the detection of similar expression levels of the BR-, ET-, ABA-, 9-LOX- and WR-related genes, in addition to *DET2*, *Cyp81*, *EIN 2*, *NCED1, HPL*, *DXS-2,* and *SCP* in leaves of WT and *spr2* plants, suggested that their systemic induction was JA-independent.

Metabolic fingerprinting of tomato roots

Direct liquid introduction electrospray mass spectrometric (DLI-ESI-MS) fingerprints were generated to examine the effect of mycorrhizal colonization on the global metabolic profile in roots of WT and *spr2* mutant plants. Data from control and mycorrhizal *WT* and *spr2* roots tended to separate into different regions of the plots in principal component analyses. Thus, AMF colonization influenced the metabolic profile of the roots characteristically for each genotype (Fig 2 and Fig. S1). Principal components (PCs) 1-3 of individual experiments explained the total variance to a similar degree (i.e., in plants with "low" [58.5%], "medium" [61.6%], and "high" [58.5%] mycorrhization levels).

Plants having "medium" colonization levels were those in which the effect of the mycorrhizal colonization on the plant metabolism of both genotypes was better observed, as indicated by the lack of overlapping on the 2D projection (Fig. 2). The results were robust enough to allow the prediction that any new observation from the same group would fall inside the ellipse with a 0.95 probability. Similar results were observed when data from roots having "medium" mycorrhizal colonization were plotted in a metabolic heat-map for the 100 most intense ions (Fig. 3). A clear discrimination between genotype and treatment was revealed by the formation of separate clusters after HCA. PCA and heat-map visualization of the metabolic data matrix derived from plants having "low" and "high" AMF colonization levels (Figs. S1 and S2) displayed less defined metabolic fingerprints for the separation between treatments and genotypes. This data set indicate that the main source of variance was the plant genotype. In plants with "low" mycorrhizal colonization levels, PC1 and PC2 clearly separated the control from mycorrhizal conditions, whereas in plants with "high" levels of AMF colonization this was observed only for components PC2 and PC3. These results suggested that differential changes in metabolism were induced in control and mycorrhizal WT and *spr2* root samples. Therefore, mass fingerprints were determined for each individual experiment. The results are illustrated in Table 3, where the 50 metabolite ions having the highest significantly altered abundance resulting from AMF

colonization in WT and/ or *spr2* roots are represented. The variability observed was in accordance with the inconsistent AMF colonization levels observed between experiments. Putative metabolites whose significant increase coincided with higher AMF colonization levels in roots were few. These were previously recognized to be associated with the mycorrhizal colonization process, such as hydroxypyruvate; L-valine/ L-aspartate-semialdehyde, cysteine, myricetin and O-feruloylquinate. Putative metabolites whose scarcity inversely correlated with "high" root mycorrhizal colonization included metabolites whose *m/ z* values corresponded to volatile organic compounds (VOCs), mostly aldehydes and alcohols, in addition to proline, succinic acid, nicotinic acid, N-acetylputrescine, salicylic acid (SA), $_p$normetanephrine, 5-hydroxyisourate, pyridoxal-5'-phosphate, phosphatated sugars, canthaxanthin, and 1-18:1-2-18:3-phosphatidylcholine.

In opposition, putative metabolites whose increased abundance was associated with decreased mycorrhization levels comprised methylglyoxal, histidine (His) and L-homocysteine, thiamin and 5-amino-6-(5'-phosphoribosylamino)-uracil, together with the secondary metabolites solavetivone, cyanidine, myricetin 3-O-(4'-O-acetyl-2"-O-galloyl)-alpha-L-rhamno pyranoside, tomatine and acetoxytomatine, as well as 1-18:1-2-18:3-phosphatidylcholine. In contrast, a significantly reduced accumulation of the VOCs 3-methyl-2-butenal, phenylacetaldehyde and 10-hydroxygeraniol in addition to histidinol, uracil, phosphorylethanolamine, orotate, 4-ureido-5-imidazole carboxylate, spermine, 1-deoxy-D-xylulose 5-phosphate, MeJA, sinapate, 1-sinapoyl-D-glucose, 2-cis,4-trans-xanthoxin/ xanthoxin, D-sorbitol-6-phosphate and 2',3'-cyclic nucleotides was detected in plants showing "low" to "medium" mycorrhization.

The targeted assay focusing on the analysis of the precursors of the tomatine biosynthetic pathway (Table 4) was coherent with the mycorrhizal colonization parameters (Fig. 1), and transcriptomic (Table 1) and untargeted metabolomics data (Table 3). Thus, α-tomatine, its biosynthetic precursors and catabolic products were more abundant in roots of WT plants having "medium" and "low" mycorrhizal colonization levels and were practically absent in "high" mycorrhyzal WT roots. These metabolites tended to be significantly reduced in mycorrhizal *spr2* roots, as well, thereby suggesting a regulatory role for JA in the synthesis of these metabolites during the AMF symbiosis. Another interesting set of data showed that the extent of GA anabolic and catabolic processes, leading to active or inactive GA chemical derivatives, was more intense

in WT roots having "high" mycorrhizal levels (Table 5). These findings were in agreement with transcriptional data shown in Table 1.

DISCUSSION

JA-biosynthesis-impaired *spr2* mutants are compromised in mycorrhizal colonization

Tomato *spr2* mutant plants consistently showed reduced AMF colonization, which was in agreement with prior reports (Tejeda-Sartorius et al., 2008; Song et al., 2015). Three independent experiments, representing "high", "medium" and "low" colonization levels were selected for further study, including a transcriptomic analysis of several genes (in roots and leaves), many of which have been shown to be presumably involved in the mycorrhizal colonization process. The selected genes are engaged in phytohormone biosynthesis and signaling, the 9- and 13-LOX pathways, and pivotal secondary metabolite pathways. Targeted and untargeted metabolomic analyses by mass spectrometry were also performed to evaluate the global variability in metabolism at a late AMF-colonization stage. A particular interest was given to the quantification of the α-tomatin steroidal glycoalkaloid biosynthetic pathway, considering that its prominent role in defense against fungal pathogens could be a factor responsible for the variable AMF colonization efficiencies observed (Moses et al., 2014; Piasecka et al., 2015).

The source(s) of variation between experiments remained undefined

Variable AMF colonization is frequently attributed to the known capacity of plants to alter the rate of mycorrhizal colonization in response to various factors (Graham and Eissenstat, 1994). However, it was difficult to identify those responsible for the variation observed in this study. Frequently cited determinants such as N/ P resource stoichiometry, the presence/ absence of other soil nutrients, soil water availability and fluctuating temperature and/ or light levels affecting photosynthesis rates were unlikely to have been influential factors considering that all experiments were conducted in controlled ambient growth chambers. The presence/ absence of non-mycorrhizal microbes, including fungal endophytes or other biotic factors known to modify AMF colonization levels were not examined and remain an unlikely source of variability. The task of finding the latter remains difficult, considering that previous meta-analyses were unable

to identify precisely the source of variability in plant AMF colonization (Hoeksema et al., 2010; Johnson, 2010; Carbonnel and Gutjahr, 2014; Turrini et al., 2016).

Root transcriptional data revealed genes possibly responsible for variation in AMF colonization levels between experiments and between WT and *spr2* genotypes

AMF colonization level parameters shown in Fig. 1 closely correlated with the expression patterns of the *LePT4* gene (Table 1), coding for a key phosphate transporter that is considered a reliable marker of a functional AMF mycorrhizal symbiosis (Xu et al., 2007). The high *LePT4* expression observed in WT roots with "high" AMF colonization was also found to correlate with their higher A% value (Fig. 1C), similarly to the tight correlation observed between *LePT4* expression levels and arbuscule abundance in Solanaceous plants and rice (Gutjahr et al., 2008; Wang et al., 2017). The high expression levels of the two AMF marker genes detected in JA deficient *spr2* mutants, although somehow unexpected, could, nevertheless, be interpreted as an aberrant proliferation of mycorrhizal fungi on the surface of the *spr2* roots due to their inability to penetrate the inner cortex for proper arbuscule formation (Hogekamp and Küster, 2013). This is supported by the presumed JA-dependent inhibition of fungal spread on the root surface and consequent promotion of arbuscule formation and function during the symbiotic stage of the AMF colonization process (Pozo et al., 2015).

The consistently lower AMF colonization levels observed in mycorrhizal *spr2* roots, supported the predominantly positive regulatory role assigned to JA in the mycorrhizal colonization process. According to the transcriptional data presented in Table 1, JA might act in coordination with GA to positively affect AMF colonization, via the repression of *GAI*. This result, although being in accordance with data presented in Table 5, disagreed with reported evidence indicating that DELLA repressors of GA signaling are required for an adequate AMF symbiosis (Yu et al., 2014; Carbonnel and Gutjahr, 2014). However, more recent studies evoke a more dynamic role for these phytohormones, in which GA levels in mycorrhizal roots fluctuate during the colonization process to maintain a suitable GA balance, necessary both for DELLA stability and proper AMF colonization. This concept was also in accordance with data in Table 5. Moreover, GAs are more likely to influence the latter stages of mycorrhizal colonization, when the plants under study were sampled, and when dynamic arbuscule formation is in process (Foo et al, 2013;

Martín-Rodríguez et al., 2015, 2016). This scenario agreed with the positive effect that GA signaling had on AMF colonization in *Lotus japonicus* (Takeda et al., 2015). BR gene expression patterns, were in general, opposite to AMF colonization abundance, and appeared not to be influenced by JA. This fits the contradictory and limited knowledge regarding BRs role in mycorrhizal symbiosis (Foo et al., 2013; Bitterlich et al., 2014). ABA-related gene expression patterns argued against their influence on mycorrhizal colonization, and also suggested an independence from JA regulation. Such outcome was inconsistent with previous information showing that ABA positively influenced mycorrhizal development (Herrera-Medina et al., 2007; Martín-Rodríguez et al., 2016), while the ET gene expression pattern concurred with ample experimental evidence indicating that ET inhibits AMF colonization (Foo et al., 2013, 2016: Pozo et al., 2015; Gomez Monteiro Fracetto et al., 2017). ET's negative influence on AMF colonization in tomato appeared to be repressed by JA, given that a majority of the ET genes examined were induced in mycorrhizal *spr2* roots. The above coincided with the shifting interaction known to exist between JA and ET for the coordination of plant defense, growth and development (Wasternack and Hause, 2013) and, indirectly, with their regulation of Nod factor signaling and nodulation in legumes (Hause and Schaarschmidt, 2009). The latter is believed to be coordinated by antagonistic or synergistic downstream associations of master symbiotic regulators such as JASMONATE ZIM DOMAIN (JAZ) and ETHYLENE INSENSITIVE 3 (EIN3) proteins, among others (Pozo et al., 2015). Interestingly, *EIN3* gene induction was consistently detected in WT roots having "low" AMF colonization levels and in all *spr2* mycorrhizal roots, whereas the opposite was true for the antagonistic *JAZ2* gene.

Only a few *13-LOX* oxylipin pathway and WR genes had an obvious correlation with mycorrhizal colonization patterns in WT and *spr2* roots. These included *PS*, consistently induced in the former but mostly repressed in the latter, *AROGP3*, whose expression was strongly repressed in mycorrhizal WT roots only, and *LHA1*, induced only in mycorrhizal *spr2* roots. Induced *PS* induction was in accordance with reports showing that mycorrhizal roots of *PS* overexpressing plants yielded significantly higher A% levels than WT roots (Tejeda-Sartorius et al., 2008) and that exogenous systemin promoted AMF symbiosis in the pre-symbiotic and early phases of the plant-AMF interaction (de la Noval-Pons, 2017a, 2017b). The repression of the *AROGP* was incompatible with arguments proposing that a partial degradation of complex

carbohydrates in the extracellular matrix is needed to allow fungal spread and the formation of the periarbuscular matrix (Liu et al., 2003). Likewise, the exclusive induction of the *LHA1* gene in *spr2* roots with reduced mycorrhizal colonization was contrary to data demonstrating that LHA1 was AMF-responsive in tomato, presumably to activate secondary transport systems at the symbiotic interfaces (Rosewarne et al. 2007; Liu et al., 2016). Additionally, the widespread induction of the 9-LOX pathway genes examined, irrespective of plant genotypes and mycorrhizal colonization levels, was discrepant with the presumed regulatory role assigned to the 9-LOX oxylipin pathway during the mycorrhization symbiosis (Morcillo et al., 2016).

The pattern of carotenoid cleavage dioxygenase gene expression was unexpected. Given the late sampling stage of the plants, a more pertinent scenario would have involved a shift from an early expression of *CCD7* genes required for strigolactone synthesis towards the induction of genes favoring the production of C13/ C14apocarotenoids (e.g., *CCD1a*, *CCD1b* and *CGT1*), to presumably avoid excessive AMF colonization (López-Raez et al., 2015). Further, the repression of *CCD1b* in WT roots with "high" mycorrhizal colonization levels contrasted with its induction in WT and *spr2* roots with "low" mycorrhization. These controversial results reinforce the consensus that further research is required to define how apocarotenoid flux is regulated in plants.

The neutral expression of *PAL5* in all root samples examined, together with the irregular expression pattern of *PAL3* in mycorrhizal WT roots having highly contrasting colonization levels, was contrary to consensual findings indicating its positive role in AMF colonization (Morandi, 1996). This role is believed to be conferred by their diversion of the C flow toward the synthesis of phenypropanoids and flavonoids that stimulate AMF root colonization (Mandal et al., 2010; Steinkellner et al., 2007). Curiously, the expression of *FLS* required for flavonol synthesis was only repressed in WT roots having "high" mycorrhizal colonization, contrary to the *BEAT* gene, whose expression was highest in these roots. The irregular *FLS* expression pattern in mycorrhizal roots of both genotypes was rather puzzling and implied that, contrary to previous reports (Scervino et al., 2005), *FLS*-dependent flavonol accumulation was not essential for AMF colonization in tomato under the experimental conditions employed in this study. Additionally, the presumably positive role played by the *BEAT* gene in the mycorrhizal process, perhaps via the synthesis of methy salicylate (Bera et al., 2017; see below) remains to be more

accurately determined. The expression patterns of key genes involved in the MEP and MVA isoprenoid biosynthetic pathways also suggested that they played no critical role in the mycorrhizal symbiosis in tomato, contrary to copious evidence showing the opposite (Walter et al., 2010; Floss et al. 2008a; Liu et al. 2003; Kuhn et al. 2010; however, see metabolic data discussion, below).

The analysis of the *CAS1* gene was included considering that tomatine biosynthesis is derived from the general steroid biosynthetic pathway. CAS1 catalyzes the cyclization of 2, 3-oxidosqualene, a precursor of cycloartenol, a key metabolic intermediary in sterol biosynthesis that is essential for plant cell viability. *CAS1-like* genes have also been found to be JA-inducible in a number of plants (Jin et al., 2017). Included as well was the analysis of the *GAME1* gene, coding for a galactosyltransferase involved in the synthesis of α-tomatine (Itkin et al., 2011). The expression of these genes was inversely correlated with AMF colonization, being predominantly induced in roots showing "low" AMF colonization levels (Table1). These results coincided with the repressed expression of *FPS1*, required for the synthesis of farnesyl diphosphate, an early biosynthetic precursor of sterols and triterpenoids (Abe et al., 1993), in WT roots with "high" AMF colonization levels. The above results were also in agreement with the higher accumulation of tomatine and tomatine biosynthetic precursors and catabolic products levels observed in roots having "medium" and "low" AMF colonization levels (Table 4). The combined data suggests that decreased anti-fungal α-tomatine and related compounds in WT roots with "high" AMF colonization levels may have contributed to a more efficient AMF colonization. Such argument concurs with the concept associating suppression of the defense responses against AMF with its successful establishment in the roots (García-Garrido and Ocampo, 2002; Montero-Vargas et al, 2018).

Leaf transcriptional data were indicative of AMF colonization having a systemic effect on above-ground plant tissues

Results shown in Table 2 indicate that AMF colonization in roots led to a widespread systemic induction of signaling- and defense-related genes in leaves and that this response was, to some extent, JA-dependent. This consideration is based on the strong expression levels of the *LOXD* and *JMT* genes in leaves of WT plants and on the observation that the majority of the genes examined were repressed in leaves of mycorrhizal *spr2* plants. On the other hand, the induction of BR-related genes, in addition to the general repression of ET biosynthetic and signaling genes,

was JA independent. Also relevant was the induction of several tomatine biosynthetic genes in leaves of mycorrhizal WT plants, which was opposite to the tendency observed in roots. In addition, a strong systemic induction of *CCD7* and *FLS* was detected. The only discordant finding was the up-regulation of *PI II* gene expression, a typical marker of the JA-regulated tomato wound response (Ryan, 2000) in leaves of mycorrhizal *spr2* plants. The systemic effect induced in leaves in response to AMF colonization in tomato roots agreed with the extensive gene expression changes reported in aerial plants parts of mycorrhizal tomato and other plant species (Liu et al. 2007; Fiorilli et al. 2009; Pieterse et al. 2014; Cervantes-Gámez et al., 2016). The high *CCD7* expression levels in WT mycorrhizal leaves could also represent part of a recently described positive cross-talk between ABA, JA and strigolactones in mycorrhizal tomato (Lanfranco et al., 2018). Moreover, the transient increase in JA levels in leaves of mycorrhizal *M. truncatula* plants was recently shown to activate flavonoid and terpenoid biosynthesis genes, presumably to improve the root´s ability to scavenge available iron (Adolfsson et al., 2017).

Differential metabolite accumulation in roots could have been a manifestation of the variability in AMF colonization levels observed between experiments

A supervised principal component analysis of signals derived from untargeted metabolomic analysis of the root extracts emphasized the variability observed between experiments (Figs. 2 and 3; Figs. S1 and S2). A selection of putative metabolites (Table 3), identified on the basis of their m/z ratio, were found to differentially accumulate in mycorrhizal roots. These metabolites are of interest as they may have influenced the mycorrhizal colonization process in either positive or negative ways, thereby partly explaining the variability reported in this study. The latter proposal somehow agreed with previous studies showing that the metabolomes of mycorrhizal and non-mycorrhizal tomato roots are significantly different (Rivero et al., 2015). The detection of three metabolite ions presumably associated with aa composition or synthesis (i. e., hydroxypyruvate [serine biosynthetic precursor; Igamberdiev and Kleczkowski, 2018]; and L-valine or L-aspartate-semialdehyde (Lys, Thr, Met, Ile biosynthetic precursor [Jander and Joshi, 2009]) was positively correlated with higher AMF colonization levels. This corresponded with the increased abundance of these amino acids (aas) detected in mycorrhizal tomato and sorghum roots (Schliemann et al., 2008; Rivero et al., 2015; Whiteside et al., 2012). Ser abundance was

important considering its multiple roles in biosynthesis of several biomolecules required for cell proliferation, including other aas, nitrogenous bases, phospholipids, and sphingolipids (Ros et al., 2014). The presence of myricetin was expected, considering that this and other flavonoids, such as quercetin and kaempferol, have a strong stimulatory activity on AMF (Akiyama and Hayashi, 2006). The abundance of O-feruloylquinate (Clifford et al., 2017) matched the increase in lignans observed in response to another beneficial root-fungus interaction (Baldi et al., 2010) and to the increased lignification of mycorrhizal roots cell walls (Ziedan et al., 2011). This modification is predicted to regulate AMF penetration and colonization during the establishment of the mycorrhizal symbiosis (Jung et al., 2012).

Compared to ectomycorrhizal fungi, where involvement of volatile organic compoundss (VOCs) in the establishment of the symbiosis is ample, the information regarding their effect on AMF colonization is scarce. In this study, the observed relationship between VOCs levels detected and AMF root colonization was not clear. Low levels of VOCs were generally observed, although some, such as aldehyde and alcohol volatiles coincided with "high" AMF colonization, whereas others, such as 3-methyl-2-butenal, phenylacetaldehyde and 10-hydroxygeraniol were detected in roots with "low" AMF colonization levels. An exception was the higher concentration of methylglyoxal detected in roots having "low" mycorrhization levels. The above results lent support to the suggestion that the AMF symbiosis can alter root´s VOCs emission profile, including terpenoids, as recently reported for sorghum and several other plants (Sun and Tang, 2013; Welling et al., 2016). The role of this and many other plant derived VOC signals in the soil, especially in associations between roots and microbial colonizers remains to be determined. VOCs have been proposed to permit a better adaptation of the plants to their respective soil environments, including hormonal regulation of plant growth and allopathic protection from herbivores and pathogens (Sun and Tang et al., 2013; Welling et al., 2016), although the above results suggest other possibilities. Reduced 10-hydroxygeraniol may indicate an inhibited plastidial MEP metabolic pathway and, perhaps, a scarcity of bioactive apocarotenoid precursors. The co-expression of MEP pathway and *geraniol 10-hydroxylase* genes observed in *Catharanthus roseus* was in conformity with this suggestion (Burlat et al., 2004). The significantly lowered levels of 1-deoxy-D-xylulose 5-phosphate, a key substrate of the MEP pathway was also in support of this scenario (Floss et al., 2008a). Another proposal is that the

682 inverse relationship between higher methylglyoxal contents and “low” AMF colonization was
683 representative of an imbalanced C budget caused by an inefficient utilization of assimilated CO_2
684 by roots with suboptimal colonization levels (Salomón et al., 2017).
685 Decreased PRO in WT roots with “high” mycorrhization could be rationalized by PRO’s
686 multifunctional roles, not only in protection against abiotic stresses, but also indirectly in plant
687 pathogen defense, via pyrroline-5-carboxylate, its catabolic intermediate (Qamar et al., 2015).
688 The accumulation of aas and related metabolites, different from those described above, coincided
689 with reduced AMF colonization efficiency. This was the case of His and L-homocysteine, a
690 methionine biosynthetic precursor, and cysteine. The depletion of histidinol, a His biosynthetic
691 precursor was in agreement with higher His levels. These results were inconsistent with an
692 increased uptake of these and other aas in mycorrhizal sorghum seedlings (Whiteside et al.,
693 2012). In contrast, succinic acid deficiency in WT roots with “high” mycorrhizal colonization
694 levels might have been an indirect indicator of mycorrhizal proliferation given its abundance in
695 secreted tomato C-rich exudates, secreted by the plant roots for AMF consumption (Kravchenko
696 et al., 2003). Such premise is congruent with AMF symbiosis causing a decrease in both formic
697 and succinic acid concentration in maize leaves (Sheng et al., 2011).
698 Reduced nicotinic acid content, together with other N-rich compounds such as guanine, 5-
699 hydroxyisourate (an allantoin precursor of the purine catabolic pathway regulated by N
700 availability) and pyridoxal-5'-phosphate, in roots with “high” AMF colonization, was contrary to
701 the AMF-dependent buffering of the N-rich metabolite depletion usually observed in tomato
702 plants grown under N-limiting conditions (Sánchez-Bel et al., 2018). The observed abundance of
703 other N-rich compounds such as cytidine, thiamin and 5-amino-6-(5'-phosphoribosylamino)-
704 uracil, a riboflavin synthesis precursor, in roots with “medium” and “low” colonization levels
705 was also in conflict with these reports. However, they agreed with the depletion of similar
706 compounds in these roots, such as orotate (possibly of fungal origin), 4-ureido-5-imidazole
707 carboxylate, an intermediate of the pathway converting 5-hydroxyisourate into *S*-allantoin,
708 dithiobiotin and uracil. It might be pertinent to suggest that reduced content of biosynthetic
709 precursors of allantoin, which plays an essential role in N assimilation, metabolism, transport,
710 and storage in plants, and also promotes root growth in tomato (Salvioli et al, 2012), might have
711 been an important factor that negatively impacted AMF colonization in tomato.

The negative correlation observed between N-acetylputrescine and AMF colonization abundance in WT roots with "high" mycorrhizal colonization levels was unexpected, considering that polyamines (PAs) are considered to have a positive effect on mycorrhizal colonization (Wu et al., 2010). PAs presumably enhance mycorrhization by increasing carbohydrate resource allocation to the roots and rates of glucose release from sucrose (Jiménez-Bremont et al., 2014). However, decreased spermine levels did coincide with lower AMF colonization parameters observed in this study. Decreased abundance of SA and normetanephrine were also in agreement with "high" AMF mycorrhization in WT roots. SA is known to negatively affect root colonization by AMF due its central regulatory role in plant pathogen defense responses (Blilou et al., 1999; Herrera-Medina et al., 2003), whereas catecholamines contribute to plant defense responses against pathogen infection (Kulma and Szopa, 2007). Reduced abundance of sinapate, 1-sinapoyl-D-glucose, xanthoxin, MeJA, D-sorbitol-6-phosphate and 2', 3'-cyclic nucleotides also coincided with low mycorrhizal colonization. Reduced sinapate and xanthoxin may have reflected lowered ABA levels given sinapic acid's negative effect on ABA content in *Arabidopsis thaliana* (Bi et al., 2017), and xanthoxin´s role in ABA biosynthesis (Taylor et al., 2005). Low ABA levels could have negatively affected AMF colonization in tomato via their proposed role in the regulation of strigolactone biosynthesis (López-Ráez et al., 2010). Low sinapate-1-glucose levels may have adversely affected AMF colonization by decreasing tomato's ability to adapt to the stressful conditions that are inherent to the establishment of this symbiosis (Fang et al., 2012). Among its many proposed regulatory mechanisms, reduced MeJA may have negatively affected AMF colonization by reducing its positive influence on N portioning towards the roots and lateral root proliferation, as demonstrated in *M. sativa* (Meuriot et al., 2004). The reduction of sorbitol-6-P content may have diminished AMF colonization by reducing C availability for the fungal symbiont, considering that polyols constitute important photosynthetic products in some plant species and can be efficiently synthesized and utilized as carbon sources by tomato (Schauer et al. 2005; Ohta et al., 2005). Lowered cyclic nucleotide contents might have adversely affected the function of cyclic nucleotide-gated channels that regulate nuclear Ca^{2+} oscillations that mediate responses to nitrogen-fixing rhizobial bacteria and AMF in *M. truncatula* (Charpentier et al., 2016). Other secondary metabolites whose abundance negatively correlated with AMF colonization were solavetivone; the cyanidine anthocyanin, 3-O-(4"-O-acetyl-2"-O-galloyl)-alpha-L-rhamno pyranoside, tomatine and acetoxytomatine. Solavetivone

and other sesquiterpene phytoalexins are known to have antifungal effects in solanaceous plants, including tomato (Yao et al., 2003). High cyanidin levels coincided with the detection of anthocyanin accumulation in mutant *A. thaliana* plants adversely affected in phosphate transporter activity (Bucher, 2004). Acylated flavonol glycosides similar to $_p$myricetin 3-O-(4'-O-acetyl-2''-O-galloyl)-α-L-rhamnopyranoside are known to have antifungal properties (Mahmoud et al., 2001), similar to tomatine and tomatidine, its aglycone derivative, which are steroidal alkaloids found in tomato plants and green fruits (Pusztahelyi et al., 2015). The coincidence in transcriptomic data (Tables 1 and 2) with targeted and untargeted metabolomic data (Table 3 and Table 4) associated with tomatine accumulation strongly suggest that this potent antifungal compound could be an important regulator of AMF symbiosis in WT tomato. The scarcity of the C40 canthaxanthin carotenoid in WT roots with "high" AMF colonization levels might have reflected a chemical shift toward the biosynthesis of AMF-colonization enhancing C_{13} and C_{14} bioactive apocarotenoid compounds (Floss et al., 2008b, 2009). Lower phosphate sugar levels could have indicated an active C flux from the plant roots to the AMF symbiont (Bago et al., 2000; Tian et al., 2018), whereas contrasting ratios of 1-18:1-2-18:3-phosphatidylcholine (PC) in roots with "high" AMF colonization/ low PC and "low" AMF colonization/ high PC might have evinced the utilization of phospholipids for the synthesis of lyso-phosphatidylcholine, recently identified as a lipophilic signal able to induce phosphate transporter genes in mycorrhizal potato roots and in tomato cell suspension cultures (Drissner et al., 2007). In this respect, low P-ethanolamine in tomato roots with "low" AMF colonization levels may have led to low phosphocholine synthesis in the root and, therefore, to deficient levels of this lipophilic signal (Martin and Tolbert, 1983).

CONCLUSIONS

Mycorrhizal colonization was not reproducible in this study for reasons that have yet to be determined. Despite the variability, JA-deficient plants had consistently lower mycorrhizal colonization parameters. Root transcriptomic and metabolomic data indicated that AMF colonization levels could be associated to the expression patterns of certain genes and metabolites. "Low" mycorrhization coincided strongly with the induction of ET-synthesis and

ET-signaling-related genes. Root mycorrhization systemically induced the expression of several genes in leaves, which proceeded in a partially JA-dependent manner. Prominent among the latter were the *FLS*, *CCD7*, a handful of wound response- and of tomatine-biosynthetic genes. PCA of metabolic fingerprints indicated that AMF mycorrhization modified metabolism in a genotype-dependent way. Most presumptive metabolites having significantly different abundance in mycorrhizal roots were predominantly associated with suboptimal AMF colonization levels. Many, including tomatine, may have inhibited mycorrhization due to their proven anti-microbial properties.

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Figure 1(on next page)

Degree of arbuscular mycorrhizal fungi (AMF) colonization, at 50 dpi, in roots of wild type (WT) and *spr2* tomato plants inoculated with *Rhizophagus irregularis*

The mean ± SE) of (**A**) colonization frequency (F%), (**B**) intensity of mycorrhizal colonization (**M%**) and (**C**) arbuscule abundance (A%) in the root system of wild type (WT) and the JA-deficient *spr2* mutant in three independent experiments having “low”, “medium” and “high” AMF colonization levels are shown. Different letters over the bars represent statistical difference at $P < 0.05$.

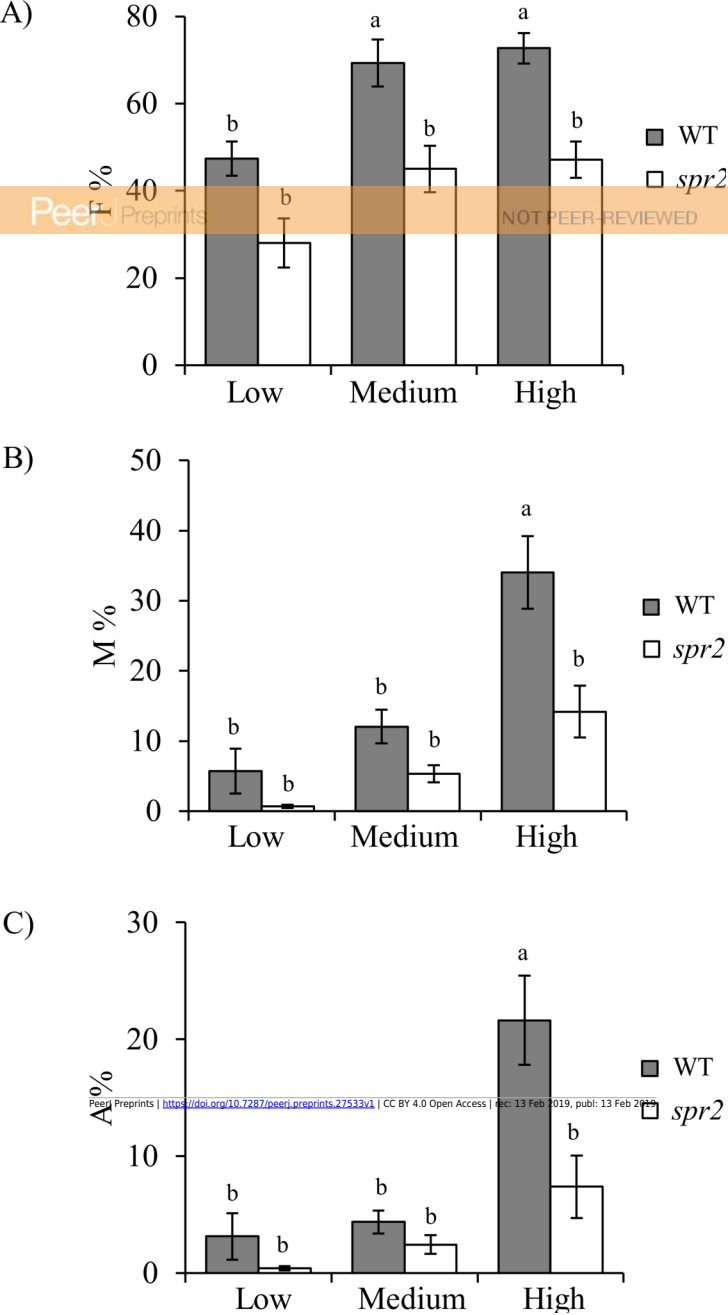

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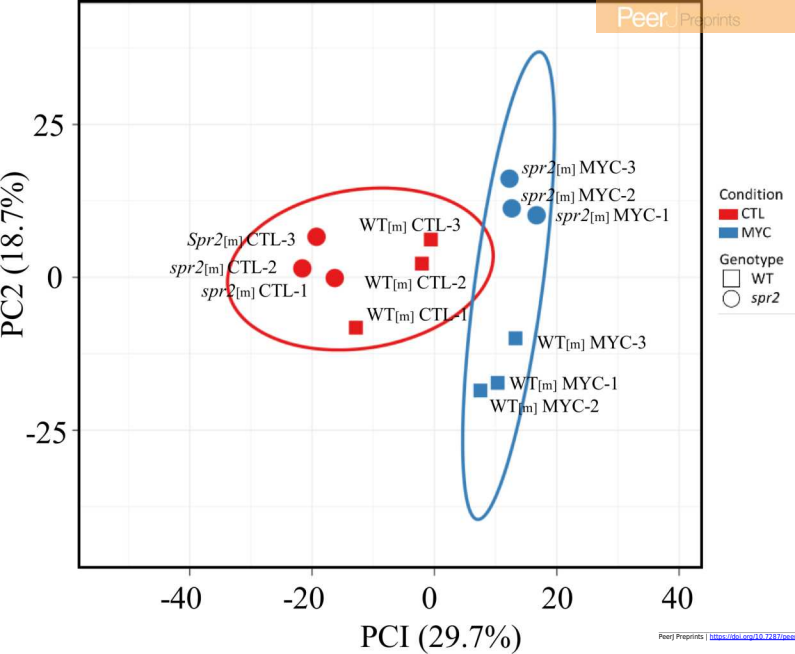
Figure 2(on next page)

Untargeted principal components analysis on metabolic fingerprinting of tomato roots under control (CTR) and mycorrhizal (MYC) conditions for the experiment yielding “medium” AMF colonization levels.

The intensity of 673 metabolites sampled from roots of wild type (WT) and mutant (*spr2*) tomato plants was used to construct a matrix to evaluate the global variance at the metabolic level in WT and mutant *spr2* genotypes in response to AMF colonization. The three principal components explain 61.6 % of the total variance. They indicate that the factor that most influenced variability was the plant genotype. Prediction ellipses are such that they predict with 95% probability that any new observation from the same group will fall inside the ellipse. n = 12 data points.



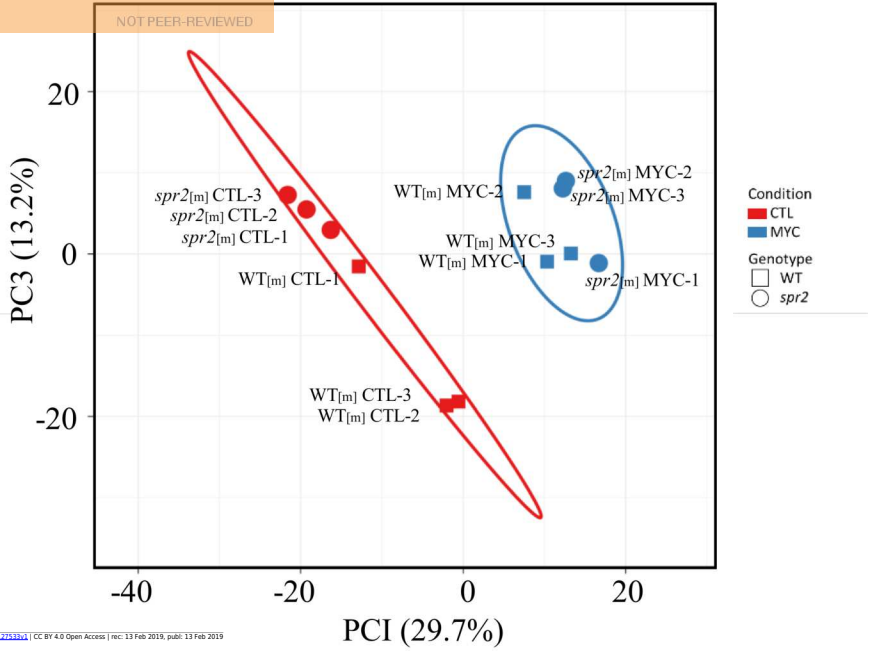


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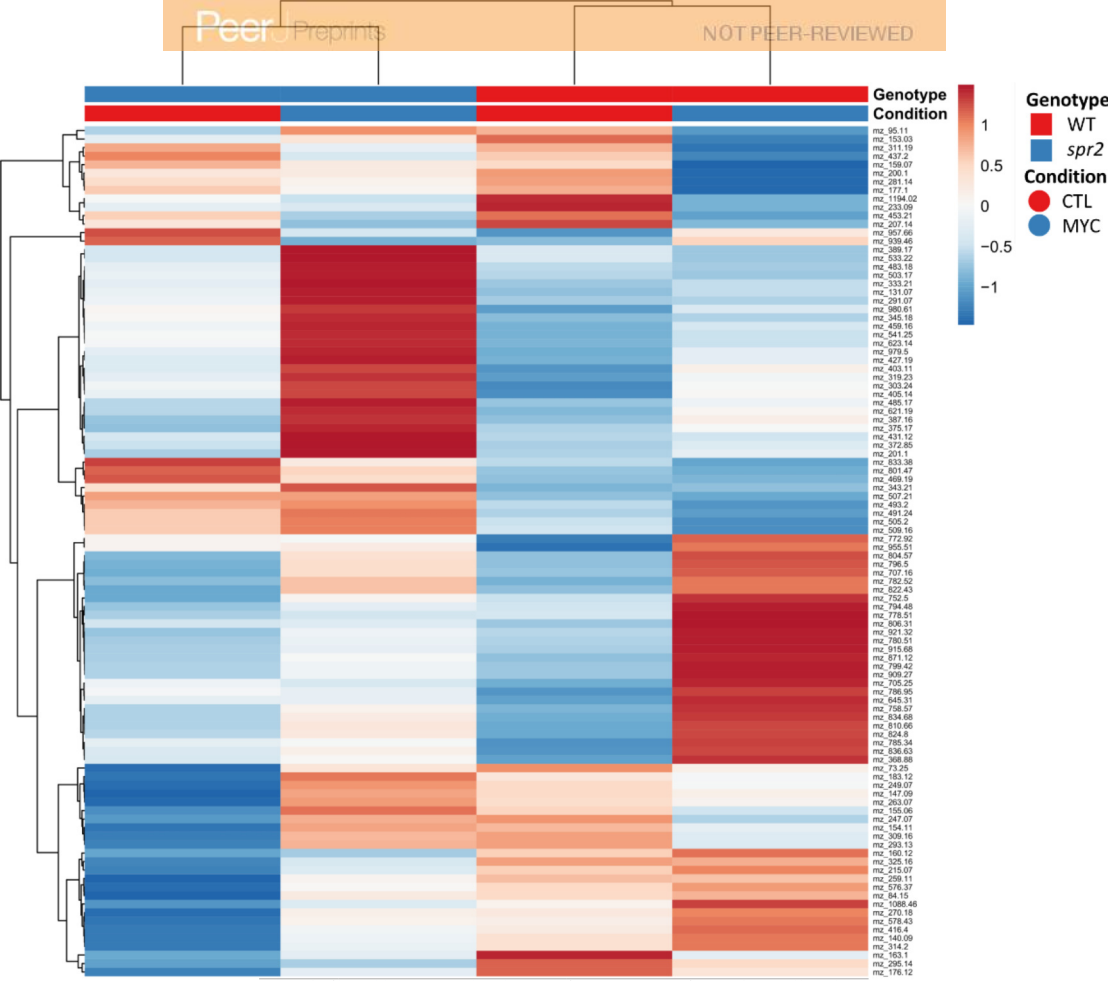
Figure 3(on next page)

Metabolic heat-map generated with the 100 most intense ions detected in tomato roots under control (CTR) and mycorrhizal (MYC) conditions for the experiment yielding “medium” AMF colonization levels.

Ions in aqueous methanol extracts sampled from roots of wild type (WT) and mutant (*spr2*) tomato plants under CTR and MYC conditions were detected by DLI-ESI-MS as described in Materials and Methods. The hierarchical clustering resulted in a correct assignment of plants into well-defined MYC and CTL clusters. From the different signals, the *m*/ *z* values were extracted for putative assignation.



spr2[m]-CTL *spr2*[m]-MYC WT[m]-CTL WT[m]-MYC

Table 1(on next page)

Gene expression levels in roots of mycorrhizal wild-type (WT) and jasmonic acid-deficient *spr2* mutant tomato plants.

These were determined 50 days after colonization of the roots with *Rhizophagus irregularis*. Shown are the results derived from three independent experiments yielding “low”, “medium” and “high” mycorrhizal colonization levels.

Genotype	WT			*spr2*		
	Colonization level					
	Low	Medium	High	Low	Medium	High
Gene						
			Mycorrhizal markers			
LePT4	**1252 ± 26**	**203 ± 36**	**2573 ± 407**	**78 ± 3.1**	**51 ± 4.0**	**188 ± 19**
18s Gi	**1938 ± 360**	**18808 ± 1221**	**14 ± 1.5**	**5749 ± 519**	**2346 ± 785**	**1677 ± 213**
EF1-α Gi	**118 ± 6.8**	**196 ± 37**	**23 ± 3.6**	**6662 ± 260**	**66 ± 26**	**51 ± 7.0**
			Brassinosteroids			
DET 2	0.7 ± 0.1	1.0 ± 0.0	**1.9 ± 0.2**	**2.4 ± 0.3**	1.0 ± 0.2	0.9 ± 0.1
DWF4	**1.6 ± 0.2**	1.3 ± 0.1	***0.3 ± 0.0***	1.3 ± 0.1	1.0 ± 0.1	1.1 ± 0.3
DWARF	**3.1 ± 0.6**	1.1 ± 0.2	0.8 ± 0.2	**2.9 ± 0.4**	**1.7 ± 0.1**	0.9 ± 0.1
Cyp81	1.3 ± 0.1	**2.7 ± 0.2**	0.8 ± 0.1	**1.6 ± 0.1**	1.2 ± 0.1	***0.2 ± 0.1***
CPD	**3.0 ± 0.4**	**1.8 ± 0.1**	1.1 ± 0.1	1.4 ± 0.2	1.1 ± 0.2	**1.8 ± 0.2**
			Gibberellins			
CPS	**3.8 ± 1.2**	**11.7 ± 1.2**	**8.4 ± 0.8**	**2.2 ± 0.3**	**23.3 ± 3.1**	**1.5 ± 0.3**
KS	1.3 ± 0.3	1.1 ± 0.0	0.6 ± 0.1	**2.1 ± 0.2**	1.4 ± 0.1	1.3 ± 0.3
GA3ox	**64.9 ± 7.5**	**32.0 ± 7.1**	**30.2 ± 3.2**	**2.3 ± 0.1**	**21.2 ± 1.9**	**35.1 ± 3.9**
GAI	**2.3 ± 0.3**	0.6 ± 0.1	***0.3 ± 0.0***	**1.9 ± 0.3**	***0.3 ± 0.1***	**1.7 ± 0.2**
			Abscisic acid			
TAS14	**3.0 ± 0.2**	1.2 ± 0.1	**1.6 ± 0.2**	**1.5 ± 0.2**	**1.8 ± 0.2**	**1.7 ± 0.2**
NCED1	0.6 ± 0.1	1.4 ± 0.1	**2.0 ± 0.2**	1.0 ± 0.1	**3.2 ± 0.4**	**1.5 ± 0.2**
			Apocarotenoids			
CCD7	**11.0 ± 1.2**	**13.1 ± 0.5**	**5.7 ± 1.0**	0.9 ± 0.1	**5.1 ± 0.8**	**2.1 ± 0.3**
CCD1a	1.4 ± 0.1	1.3 ± 0.2	0.7 ± 0.0	0.8 ± 0.2	**2.1 ± 0.4**	1.1 ± 0.2
CCD1b	**1.7 ± 0.1**	1.0 ± 0.2	***0.4 ± 0.1***	**1.5 ± 0.2**	1.4 ± 0.2	1.0 ± 0.2
CGT1	1.2 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	**1.6 ± 0.2**	**2.2 ± 0.5**	0.9 ± 0.1
			13-LOX			
LOX D	***0.2 ± 0.0***	**3.3 ± 0.2**	***0.5 ± 0.0***	0.6 ± 0.0	**1.7 ± 0.2**	0.7 ± 0.0
AOS 1	***0.5 ± 0.0***	**4.2 ± 0.6**	0.9 ± 0.1	0.8 ± 0.1	0.6 ± 0.2	1.0 ± 0.1
JAZ 2	***0.3 ±0.0***	**3.1 ± 0.6**	**1.7 ± 0.1**	1.0 ± 0.2	0.9 ± 0.3	***0.4 ± 0.0***
JMT	**3.4 ± 0.3**	**3.0 ± 0.5**	**3.1 ± 0.5**	0.9 ± 0.1	**9.8 ± 1.7**	0.8 ± 0.1
			9-LOX			
HPL	**1.7 ± 0.1**	**2.6 ± 0.4**	**2.9 ± 0.4**	**2.2 ± 0.3**	**3.0 ± 0.3**	**2.2 ± 0.2**
LoxA	**2.3 ± 0.1**	1.0 ± 0.3	**2.1 ± 0.1**	**2.7 ± 0.6**	**2.2 ± 0.5**	**1.5 ± 0.0**
DES	**10.8 ± 0.7**	1.3 ± 0.1	**3.1 ± 0.3**	**3.4 ± 0.5**	**1.8 ± 0.1**	**1.7 ± 0.2**

AOS 3	**1.6 ± 0.2**	**1.6 ± 0.4**	**6.5 ± 0.6**	**3.3 ± 0.7**	1.2 ± 0.3	**5.4 ± 0.5**
Wound response						
PS	**5.7 ± 0.7**	**2.1 ± 0.4**	**2.9 ± 0.3**	1.4 ± 0.2	***0.2 ± 0.1***	***0.4 ± 0.0***
RBOH1	0.8 ± 0.0	0.9 ± 0.1	***0.5 ± 0.0***	0.9 ± 0.0	1.0 ± 0.1	***0.5 ± 0.1***
LHA1	1.4 ± 0.1	0.8 ± 0.1	1.0 ± 0.0	0.9 ± 0.2	**2.6 ± 0.4**	**1.5 ± 0.1**
PLA2	1.0 ± 0.1	0.6 ± 0.0	0.9 ± 0.1	1.4 ± 0.2	**1.7 ± 0.4**	1.3 ± 0.3
PIN II	**9.0 ± 0.5**	0.7 ± 0.2	***0.3 ± 0.0***	***0.5 ± 0.1***	***0.3 ± 0.0***	***0.4 ± 0.0***
AroGP3	***0.2 ± 0.0***	0.7 ± 0.1	***0.2 ± 0.0***	1.1 ± 0.1	1.2 ± 0.1	1.0 ± 0.2
PPO	**4.2 ± 0.5**	0.9 ± 0.0	**2.0 ± 0.3**	**3.6 ± 0.5**	0.8 ± 0.1	**2.1 ± 0.3**
CYP 2	**1.7 ± 0.2**	0.7 ± 0.1	**1.7 ± 0.2**	0.9 ± 0.1	1.3 ± 0.1	**2.7 ± 0.6**
SCP	1.2 ± 0.2	0.6 ± 0.1	0.7 ± 0.0	1.3 ± 0.1	0.6 ± 0.1	**1.8 ± 0.2**
Ethylene Biosynthesis						
ACS2	**2.9 ± 0.3**	0.8 ± 0.2	***0.2 ± 0.0***	***0.4 ± 0.1***	***0.5 ± 0.1***	***0.5 ± 0.0***
ACS6	**4.1 ± 0.9**	0.7 ± 0.1	0.7 ± 0.0	**5.0 ± 0.6**	0.8 ± 0.1	**1.6 ± 0.2**
ACO4	**5.6 ± 0.4**	0.9 ± 0.2	0.9 ± 0.1	**1.6 ± 0.1**	**1.6 ± 0.1**	**2.1 ± 0.2**
Ethylene Signaling						
CTR4	1.4 ± 0.2	**1.6 ± 0.2**	**1.5 ± 0.1**	1.2 ± 0.1	1.2 ± 0.1	0.9 ± 0.1
EIN 2	**5.0 ± 0.8**	0.6 ± 0.1	0.8 ± 0.0	**3.1 ± 0.2**	0.6 ± 0.1	**3.3 ± 0.2**
EIN 3	**2.7 ± 0.3**	0.9 ± 0.2	0.9 ± 0.1	**2.0 ± 0.1**	**1.9 ± 0.1**	**3.9 ± 0.6**
ERF 1b	**3.7 ± 0.8**	**36.9 ± 2.9**	**12.1 ± 0.3**	**15.4 ± 2.3**	**23.3 ± 12.5**	**11.3 ± 0.9**
Phenyl propanoid/ phenolic compounds biosynthesis						
PAL3	**2.5 ± 0.2**	0.8 ± 0.0	***0.5 ± 0.0***	0.8 ± 0.1	0.8 ± 0.1	0.7 ± 0.1
PAL 4	**2.8 ± 0.3**	0.7 ± 0.1	***0.4 ± 0.1***	1.1 ± 0.2	***0.4 ± 0.0***	0.9 ± 0.1
PAL 5	1.3 ± 0.0	0.8 ± 0.1	1.4 ± 0.2	1.0 ± 0.1	1.1 ± 0.1	1.1 ± 0.0
FLS	**6.3 ± 0.3**	***0.4 ± 0.0***	**2.5 ± 0.2**	**2.0 ± 0.4**	**1.5 ± 0.1**	**2.3 ± 0.2**
Isoprenoid biosynthesis/ volatiles						
DXS-2	**2.0 ± 0.1**	**15.3 ± 4.4**	**5.7 ± 0.6**	**2.6 ± 0.2**	**22.0 ± 3.1**	1.4 ± 0.1
FPS1	0.6 ± 0.1	1.1 ± 0.1	***0.5 ± 0.0***	1.4 ± 0.1	0.8 ± 0.1	0.8 ± 0.1
HMGR 1	1.0 ± 0.1	**2.2 ± 0.3**	0.7 ± 0.0	1.3 ± 0.1	1.2 ± 0.0	1.0 ± 0.2
BEAT	1.2 ± 0.1	1.2 ± 0.1	**10.0 ± 0.5**	**2.3 ± 0.3**	**1.5 ± 0.4**	1.2 ± 0.1
Glycoalkaloid biosynthesis						
GAME 1	**1.4 ± 0.1**	**1.4 ± 0.4**	0.8 ± 0.1	**1.7 ± 0.1**	**4.4 ± 0.5**	1.4 ± 0.2
CAS 1	0.6 ± 0.1	**1.7 ± 0.3**	1.1 ± 0.1	1.0 ± 0.1	**2.1 ± 0.4**	**1.5 ± 0.4**

Table 2(on next page)

Gene expression levels in leaves of mycorrhizal wild-type (WT) and jasmonic acid-deficient *spr2* mutant tomato plants.

These were determined 50 days after colonization of the roots with *Rhizophagus irregularis*. Shown are the results derived from combined leaves sampled from from mycorrhizal plants having “medium” and “high” colonization levels.

Gene	WT	*spr2*
	Mycorrhizal marker	
LePT4	1.0 ± 0.2	***0.2 ± 0.0***
	Brassinosteroid	
DET2	**3.3 ± 0.7**	**3.0 ± 0.4**
Cyp81	**2.1 ± 0.6**	**5.4 ± 0.4**
	Gibberellins	
GA3ox	1.3 ± 0.2	***0.4 ± 0.1***
	13-LOX	
LOX D	**2.2 ± 0.2**	1.2 ± 0.1
JMT	**2.3 ± 0.1**	***0.5 ± 0.2***
	9-LOX	
HPL	**3.9 ± 0.3**	**2.2 ± 0.2**
AOS 3	1.1 ± 0.5	***0.5 ± 0.2***
	Abscisic acid	
NCED1	**2.0 ± 0.1**	**3.5 ± 0.2**
	Apocarotenoids	
CCD7	**17.9 ± 1.0**	***0.0 ± 0.0***
	Glycoalkaloids	
GAME 1	1.2 ± 0.2	***0.4 ± 0.1***
GAME 6	**2.4 ± 0.3**	1.0 ± 0.2
GAME 4	**1.9 ± 0.1**	**1.5 ± 0.1**
GAME 11	0.8 ± 0.1	1.4 ± 0.2
GAME 17	**2.0 ± 0.2**	1.4 ± 0.1
	Signaling	
PS	1.0 ± 0.3	0.9 ± 0.2
	Isoprenoid	
DXS-2	0.8 ± 0.1	**2.9 ± 0.3**
	Ethylene biosynthesis	
ACS2	***0.1 ± 0.0***	0.7 ± 0.1
ACO4	***0.5 ± 0.1***	***0.4 ± 0.1***
	Ethylene regulator	
EIN 2	**3.7 ± 0.6**	**1.8 ± 0.1**
ERF 1b	***0.1 ± 0.0***	***0.3 ± 0.0***
	Others	
PAL 5	0.7 ± 0.1	0.7 ± 0.2
AroGP3	0.6 ± 0.1	***0.5 ± 0.0***
PPO	**2.4 ± 0.2**	***0.1 ± 0.0***
PIN II	**5.4 ± 0.6**	**2.5 ± 0.3**
SCP	1.4 ± 0.1	**1.5 ± 0.2**
FLS	**102.1 ± 13.1**	**2.1 ± 0.1**
FPS1	0.8 ± 0.1	0.6 ± 0.1
BEAT	***0.5 ± 0.0***	***0.1 ± 0.0***

1

Table 3(on next page)

Identification of putative metabolites with significant changes in abundance in roots having contrasting AMF colonization efficiencies.

The accumulation pattern of these metabolites, with respect to untreated controls, was detected in mycorrhizal roots of wild-type (WT) and jasmonic-deficient *spr2* mutant tomato plants. Shown are the results of three independent experiments yielding “low”, “medium” and “high” colonization levels.

	Low		Medium		High			
m/z	WT	*spr2*	WT	*spr2*	WT	*spr2*	Effect on AMF	Putative metabolite[1, 2]
50.21							[+]	ND
57.08								ND
61.1								ND
66.13							[-]	ND
72.11							[-]	Methylglyoxal
72.26								ND
73.26								Aminopropanal; glyoxylic acid
82.05							[-]	ND
84.15							[-]	3-methyl-2-butenal
85.15								ND
86.15							[+]	ND
87.12								Pyruvate
97.13								ND
101.12							[+]	3-methyl-4-trans-hydroxy-2-butenal 4-methylpentanal Cis-3-hexenol Hexanal
104.21							[+]	Hydroxypyruvate
113.13							[-]	Uracil
115.06							[+]	Proline
118.12							[+]	L-valine L-aspartate-semialdehyde
119.13							[+]	Succinic acid
121.12							[-]	Phenylacetaldehyde Cysteine
123.05							[+]	Nicotinic acid
127.06								Thymine
129.00								ND
130.06								Itaconate
131.07							[+]	N-acetylputrescine
136.10							[-]	L-homocysteine
138.09							[+]	Salicylic acid
142.13							[-]	Histidinol Phosphorylethanolamine
151.07							[+]	Guanine
154.11							[-]	Histidine
156.12							[-]	Orotate
165.06							[-]	Ribonic acid
170.03								Dhap
171.13							[-]	10-hydroxygeraniol 4-ureido-5-imidazole carboxylate
173.12								ND
174.14								Aconitic acid
175.13								ND
179.01								Glucose aND isomers 3-(4-hydroxyphenyl)pyruvate
184.92							[+]	5-hydroxyisourate Normetanephrine
189.1								Cis-homoaconitate

								N6-acetyl-l-lysine
192.9								Citric acid
201.1							[-]	Spermine
211.11								ND
213.13								2-oxo-3-hydroxy-4-phosphobutanoate
215.07							[-]	1-deoxy-D-xylulose 5-phosphate
219.07							[-]	Solavetivone
225.21							[-]	(-)-Jasmonic acid methyl ester (2s)-flavanone Sinapate
229.18								5-phosphoribosylamine
231.04								ND
233.09								ND
241.1							[+]	Cystine
244.19							[-]	Cytidine Uridine
247.07							[+]	Marmesin Pyridoxal-5'-phosphate
249.07								Gamma-L-glutamyl-L-cysteine
251.14							[-]	2-cis,4-trans-xanthoxin Xanthoxin
261.05							[+]	D-fructose-6-phosphate D-galactose 6-phosphate D-mannose-6-phosphate D-myo-inositol (4)-monophosphate
263.07							[-]	D-sorbitol-6-phosphate
265.08								2,3-bisphospho-D-glycerate
266.17							[-]	Thiamin
267.29								Inosine
273.11								Phloretin Erythroidine
274.62								6-phosphogluconate
277.1							[-]	ND
283.08								Xanthosine
288.22							[-]	Cyanidin
291.07								ND
305.16							[-]	2',3'-cyclic CMP 2',3'-cyclic UMP
316.36								ND
317.19							[+]	Myricetin
319.23							[-]	ND
320.26								ND
330.24								3',5'-cyclic IMP
343.21								Sucrose and isomers
345.18								Ga24
353.12								Prostaglandin D2
354.15							[-]	5-amino-6-(5'-phosphoribosylamino)-uracil (Riboflavin precursor)
360.33								ND
362.13								ND
363.12							[+]	ND
367.22								ND
368.88							[+]	O-feruloylquinate
371.21								ND

376.73							[+]	ND
381.12								Trans,trans-farnesyl diphosphate
387.16							[-]	1-sinapoyl-D-glucose
389.17							[-]	ND
393.17								Ergosta-5,7,22,24(28)-tetraen-3-b-ol
399.19								ND
405.14								ND
416.4								Tomatidine
429.18							[+]	Idp
447.15								ND
453.21							[-]	ND
455.17								ND
457.17								ND
465.19								ND
493.2							[+]	ND
497.23							[-]	ND
499.15							[+]	ND
521.26							[+]	ND
544.93								Phytoene
557.99							[-]	ND
565.91							[+]	Canthaxanthin
583.3								ND
595.9							[-]	ND
657.19							[-]	Myricetin 3-O-(4"-O-acetyl-2"-O-galloyl)-alpha-L-rhamnopyranoside
659.2							[-]	ND
669.19							[-]	ND
691.18								ND
697.36								ND
701.28							[-]	ND
705.25							[-]	ND
707.16							[-]	ND
711.26								ND
713.23								ND
714.95								ND
718.99								ND
734.85								ND
739.35								ND
740.38								ND
754.97								ND
756.51								ND
782.52							[-]	1-18:1-2-18:3-phosphatidylcholine
796.5							[-]	ND
801.41								ND
802.55								ND
804.57							[+]	ND
808.69								ND
813.28								ND
815.44							[+]	ND
818.07							[+]	ND
820.49							[+]	ND
822.43							[-]	ND
830.57								ND
863.16							[-]	ND

929.50							[-]	ND
941.37								ND
953.43								ND
955.51								ND
979.50								ND
1029.38								Didehydrotomatine
1034.50							[-]	(3b,5a,22b,25S)-spirosolan-3-yl O-b-D-glucopyranosyl-(1->2)-O-[b-D-xylopyranosyl-(1->3)]-O-b-D-glucopyranosyl-(1->4)-b-D-Galactopyranoside (Tomatine)
1048.46								ND
1057.47								UGA
1092.50							[-]	Acetoxytomatine
1105.96								ND
1194.02								ND

[1]Increasingly darker shades of green indicate that these metabolites were significantly increased with respect to controls at $p = 0.05$, $p = 0.01$ and $p = 0.001$, respectively.

[2]Increasingly darker shades of red indicate that these metabolites were significantly reduced at $p = 0.05$, $p = 0.01$ and $p = 0.001$, respectively.

ND = Not determined.

Table 4(on next page)

α-Tomatine biosynthesis/ catabolism is significantly reduced in mycorrhizal WT roots having “high” AMF colonization efficiency.

Accumulation pattern of α-tomatine, its biosynthetic precursors and catabolic products, with respect to untreated controls, was detected in mycorrhizal roots of WT and *spr2* mutant tomato plants. Shown are the results of three independent experiments yielding “low”, “medium” and “high” colonization levels.

		Low		Medium		High	
Metabolite[1, 2]	***m/z* +/ - 0.3 mass**	**WT**	***spr2***	**WT**	***spr2***	**WT**	***spr2***
Tomatidenol	414.28						
Tomatidine	416.40						
Tomatidenol + galactose	576.37						
Tomatine + galactose	578.43						
Hydroxydehydrotomatidine trihexoside	916.56						
Tomatidine dihexoside dipentoside	1004.39						
Tomatidine dihexosi dependtose deoxyhexose	1018.35						
Didehydrotomatine	1030.51						
Dehydrotomatine	1032.48						
Tomatine	1034.50						
UGA[3]	1044.66						
UGA	1046.57						
Hydroxydehydrotomatine	1048.46						
Hydroxytomatine	1050.48						
UGA	1056.47						
Tomaditine tetrahexoside	1064.58						
Dihydroxytomatine	1066.47						
UGA	1068.48						
Acetoxydehydrotomatine + H-H20	1070.43						
Dehydrolycoperoside + H-2H2O-Hex	1072.45						
Acetoxydehydrotomatine	1090.50						
Acetoxytomatine	1092.50						
acetoxyhydrotomatine	1108.77						
UGA	1110.41						

[1]Increasingly darker shades of green indicate that these metabolites were significantly increased with respect to controls at $p = 0.05$, $p = 0.01$ and $p = 0.001$, respectively.

[2]Increasingly darker shades of red indicate that these metabolites were significantly reduced at $p = 0.05$ and $p = 0.01$, respectively.

[3]UGA = Unknown glycoalkaloid.

Table 5 (on next page)

Gibberellin (GA) biosynthesis/ catabolism is favored in wild-type (WT) roots having "high" AMF colonization levels.

Root of jasmonic-deficient *spr2* mutant plants tended to have the opposite effect. The colors indicate significant accumulation (green)/ depletion (red) of active GA, active GA precursors and catabolic products, with respect to untreated controls. Shown are the results of three independent experiments yielding "low", "medium" and "high" colonization levels.

		Low		Medium		High	
	m/z	**WT**	***spr2***	**WT**	***spr2***	**WT**	***spr2***
GA9 (GA4 precursor [p])-GA12 aldehyde	317.19+/-0.2						
GA51-catabolite (c)	329.16+/-0.2						
GA15	331.17+/-0.2						
GA12	333.21+/-0.2						
GA29-c, GA34-c	345.18+/-0.2						
GA24 [GA4p], GA44 [GA1p], GA37,GA110-c	347.17+/-0.2						
GA14 [GA4p], GA15 [GA4p], GA53 [GA1p], GA34-c,GA29-c	349.17+/-0.2						
GA25-c	360.33+/-0.2						
GA38 [GA1p]	362.13+/-0.2						
GA8-c, GA19 [GA1p], GA36 [GA4p], GA97-c	363.20+/-0.2						
GA28 [GA53-c], GA43 [GA12-c]	395.12+/-0.2						
GA46 [GA12-c]	493.20+/-0.2						

[1]Increasingly darker shades of green indicate that these metabolites were significantly increased with respect to controls at $p = 0.05$, $p = 0.01$ and $p = 0.001$, respectively.

[2]Increasingly darker shades of red indicate that these metabolites were significantly reduced at $p = 0.05$, $p = 0.01$ and $p = 0.001$, respectively.