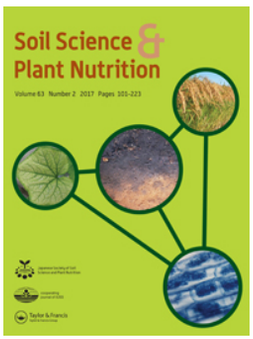

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ORIGINAL ARTICLE

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Comparative transcriptome analysis between *Solanum lycopersicum* L. and *Lotus japonicus* L. during arbuscular mycorrhizal development

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ABSTRACT

Arbuscular mycorrhizal (AM) fungi form symbiotic associations with diverse plant species. The AM fungi enhance mineral uptake from the soil, which benefits the growth of the host plants. Previous microarray and RNA-seq analyses have identified a large number of AM-responsive plant genes. However, little is known whether the gene expression profile of mycorrhiza is different among genetically distant plant species. The aim of this study was to assess the conservation and divergence of AM-responsive genes between two different hosts, *Solanum lycopersicum* L. and *Lotus japonicus* L., during AM development using RNA-seq data. In each host plant, gene expression was compared between AM roots and non-mycorrhizal (NM) roots. Potential orthologs of AM-responsible genes between *S. lycopersicum* and *L. japonicus* were identified with reciprocal BLAST searches. Only one quarter to one third of the AM-inducible genes in each plant species were co-upregulated in both species. The co-upregulated genes included those known to be essential for AM development and function. The co-upregulated genes exhibited a wide range of fold changes in the AM symbiosis, and the fold change value for individual co-upregulated genes was positively correlated between the two hosts. Most of the species-dependent upregulated genes exhibited low levels of induction. We also analyzed gene expression in AM fungi colonizing roots of *S. lycopersicum* and *L. japonicus*. Overall, the gene expression profiles of *Rhizophagus irregularis* were similar among the roots of the two different hosts, although hundreds of fungal genes were differentially expressed between the two hosts. In particular, genes related to the mitochondrial electron transport chain were highly expressed in AM fungi colonizing *L. japonicus* roots, indicating that adenosine triphosphate (ATP) production was enhanced in the *L. japonicus*–*R. irregularis* symbiosis. Overall, these results show that a certain proportion of AM-responsive genes is conserved across plant species. The species-dependent AM-responsive genes may be related to the physiological differences between AM and NM roots in each plant species.

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arbuscular mycorrhizal symbiosis; ortholog; *Rhizophagus irregularis*; RNA-seq; tomato

1. Introduction

Arbuscular mycorrhizal (AM) fungi form symbiotic associations with plants and provide the hosts with soil nutrients such as phosphorus, nitrogen, sulfur, zinc and copper, which promotes plant growth (Smith and Read 2008). In return, AM fungi receive carbon derived from the photoassimilates of their hosts. During AM development, AM fungi colonize plant roots and form highly branched structures known as arbuscules, where the nutrient exchange between the fungi and plants is thought to occur (Harrison *et al*. 2002; Parniske 2008; Gutjahr and Parniske 2013). More than 70% of terrestrial plant species establish this type of symbiotic association (Brundrett 2009). It has been suggested that some genetic systems of AM development are conserved among many land plants (Delaux *et al*. 2015).

The formation of the AM association begins with signal recognition between the plant and the AM fungus. Strigolactones secreted from plants are perceived by AM fungi, and these substances stimulate the hyphal branching and fungal metabolic activity (Akiyama *et al*. 2005; Besserer *et al*. 2006, 2008). The AM fungi release lipochitooligosaccharides and chitooligosaccharides that activate the common symbiosis signaling pathway (CSSP) in plant root cells (Maillet *et al*. 2011; Genre *et al*. 2013). The CSSP is required for the initial steps in the accommodation of both AM fungi and rhizobia in roots, and comprises several genes encoding receptor kinases (Endre *et al*. 2002; Stracke *et al*. 2002), cation channels (Ané *et al*. 2004; Imaizumi-Anraku *et al*. 2005; Charpentier *et al*. 2008, 2016), nucleoporins (Kanamori *et al*. 2006; Saito *et al*. 2007; Groth *et al*. 2010), calcium- and calmodulin-dependent kinases (Lévy *et al*. 2004; Mitra *et al*. 2004; Tirichine *et al*. 2006), and CYCLOPS/IPD3 (Messinese *et al*. 2007; Yano *et al*. 2008; Singh *et al*. 2014). These components of the CSSP were first identified in genetic studies on mutants of the model symbiotic legume plants *Lotus japonicus* L. and *Medicago truncatula* L. (Parniske 2008; Gutjahr and Parniske 2013; Oldroyd 2013). It is now known that genes in the CSSP are conserved among AM plants including angiosperms, gymnosperms, pteridophytes and bryophytes (Banba *et al*. 2008; Gutjahr *et al*. 2008; Wang *et al*. 2010; Delaux *et al*. 2015). Downstream of the CSSP, genes

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Supplemental data for this article can be accessed here.

involved in AM development and functioning are induced (Pimprikar *et al*. 2016). Transcriptomic microarray and RNA-seq analyses of legumes and non-legumes have revealed that many genes encoding transporters, peptidases, transcription factors, receptor kinases and germin-like proteins are highly upregulated during AM development in diverse plant species (Liu *et al*. 2003; Wulf *et al*. 2003; Manthey *et al*. 2004; Güimil *et al*. 2005; Hohnjec *et al*. 2005; Kistner *et al*. 2005; Gomez *et al*. 2009; Guether *et al*. 2009; Benedito *et al*. 2010; Hogekamp *et al*. 2011; Gaude *et al*. 2012; Hogekamp and Küster 2013; Handa *et al*. 2015). However, a comparative microarray analysis of tomato *Solanum lycopersicum* L. and the model legume *M. truncatula* showed that only a small number of orthologous genes were upregulated in AM roots – this may have been because non-overlapping probe sets were used in these analyses (Fiorilli *et al*. 2009). Currently, the conservation and divergence of AM-responsive genes among plant species are largely unknown.

Comprehensive gene expression profiles have been obtained by RNA-seq analysis using next-generation sequencing technology. Handa *et al*. (2015) performed *de novo* transcriptome assembly using RNA-seq data from the model legume *L. japonicus* and identified thousands of genes that were differentially expressed in AM roots compared with non-mycorrhizal (NM) roots (Handa *et al*. 2015). High-quality genome sequence data are now available for *S. lycopersicum* (Tomato Genome Consortium 2012), allowing us to comprehensively explore the gene expression profiles of tomato. In this study, we compared the changes in gene expression during AM development between *S. lycopersicum* and *L. japonicus* to investigate the conservation and divergence among plant AM-responsive genes. In addition, we analyzed the transcriptional profiles of the AM fungus *Rhizophagus irregularis* colonizing the roots of these two plant hosts.

2. Materials and methods

2.1. Biological materials

Seedlings of *S. lycopersicum* cv. Micro-Tom and *L. japonicus* MG-20 were grown in pots filled with autoclaved river sand, and inoculated with 500 spores plant^{-1} of *R. irregularis* DAOM 197198 (Mycorise, Premier Tech, Rivière-du-Loup, Canada). The inoculated and non-inoculated plants were supplied with half-strength Hoagland's solution containing a low concentration of phosphate (100 µM potassium dihydrogen phosphate) every other day. The plants were grown in a growth chamber (25°C, 16-h light/8-h dark photoperiod) for 4 weeks. Plant roots were frozen in liquid nitrogen and stored at −80°C until RNA extraction.

2.2. RNA-seq analysis

The RNA-seq procedures were as described previously (Handa *et al*. 2015). Briefly, total RNA was extracted from the frozen roots using RNAiso Plus (Takara Bio, Shiga, Japan) in combination with Fruit-mate (Takara Bio). Genomic DNA was removed from the RNA solutions by digestion with RNase-free DNase (Qiagen, Hilden, Germany) on an RNeasy column (Qiagen). The quality of the total RNA was checked using the Agilent 2100 Bioanalyzer system (Agilent Technologies, Santa Clara, CA, USA). The cDNA libraries were constructed using the TruSeq RNA Sample Prep Kit (Illumina, San Diego, CA, USA) according to the manufacturer's instructions. Paired-end sequencing (2 × 100 bp) was conducted with the Illumina HiSeq 2000 Sequencing System (Illumina). The data sets of the short reads have been deposited in the DNA Data Bank of Japan (DDBJ) Sequence Read Archive (accession number: DRA005187).

The sequence reads obtained from *S. lycopersicum* and *L. japonicus* roots were mapped using the Tophat program (Trapnell *et al*. 2009) to genome sequences obtained from the International Tomato Annotation Group (release ITAG2.4) (https://solgenomics.net/organism/Solanum_lycopersicum/genome) (Tomato Genome Consortium 2012) and the reference sequences of *L. japonicus* (Handa *et al*. 2015), respectively. The *L. japonicus* reference sequences consisted of the gene model of *L. japonicus* genome assembly build 2.5 (Lj2.5, http://www.kazusa.or.jp/lotus/release2/), the novel coding sequences in Lj2.5 sequenced regions predicted by the Cufflinks assembly (http://mycorrhiza.nibb.ac.jp), and *de novo* assembled transcriptome sequences encoding putative novel protein coding sequences of un-sequenced regions in Lj2.5 (http://mycorrhiza.nibb.ac.jp). To analyze the transcriptional profiles of the AM fungi, reads obtained from mycorrhizal roots of *S. lycopersicum* or *L. japonicus* were mapped to the *R. irregularis* genomic sequence obtained from the JGI MycoCosm database (http://genome.jgi.doe.gov/Gloin1/Gloin1.home.html) (Tisserant *et al*. 2013), excluding putative rRNA genes (protein ID: 67218, 67222, 73108, 102514, 235478, 247295, and 336739). Reads mapped against the reference sequences were counted with BEDTools (Quinlan and Hall 2010). The read counts were normalized using the iDEGES/edgeR method (Sun *et al*. 2013). Plant genes differentially expressed between AM and NM roots were detected with edgeR (Robinson *et al*. 2010), with a false discovery rate (FDR) cut-off of 0.01. The AM fungal genes that were differentially expressed in AM roots between *S. lycopersicum* and *L. japonicus* were also detected with edgeR (FDR < 0.01). Potential orthologs between *S. lycopersicum* and *L. japonicus* were identified with reciprocal BLAST searches using the *S. lycopersicum* coding sequence set (Tomato Genome Consortium 2012) and the *L. japonicus* putative coding sequence set (Handa *et al*. 2015). Each coding sequence set was searched against the other sequence set using TBLASTX (e-value < 1e-6). The two genes that were each other's best hit in the pairwise comparison were considered to be orthologs. A gene ontology (GO) functional enrichment analysis was conducted using Fisher's exact test with a weight algorithm in the TopGO R/Bioconductor package (Alexa and Rahnenführer 2016). The GO annotations of *R. irregularis* genes were obtained from the JGI MycoCosm database (Tisserant *et al*. 2013).

2.3. Mycorrhizal colonization

Roots were cleared with 10% (w/v) potassium hydroxide (KOH) and stained with 0.05% (w/v) trypan blue in lactic acid (Phillips and Hayman 1970). Hyphal, arbuscular and vesicular

colonization were determined as the percentage of root length colonized using the magnified intersection method (McGonigle *et al*. 1990). Data were analyzed with Student's t-test ($P < 0.05$) using JMP7 (SAS Institute, Cary, NC, USA).

3. Results

3.1. Differentially expressed genes in S. lycopersicum and L. japonicus during AM development

Roots of both *S. lycopersicum* and *L. japonicus* were well colonized with *R. irregularis* (Fig. 1A). There were no significant differences in any of the parameters of AM colonization between the two plants. The shoot dry weight of *L. japonicus* was significantly increased by AM fungal inoculation, whereas that of *S. lycopersicum* showed no positive response (Fig. 1B). To detect AM-responsive genes of *S. lycopersicum* and *L. japonicus*, RNA-seq analyses of AM and NM roots were performed. Approximately 55–88% of raw sequence reads were uniquely mapped against the reference sequence of *S. lycopersicum* or *L. japonicus* (Table 1). We identified 928 and 1697 differentially expressed genes (DEGs) in AM roots versus NM roots of *S. lycopersicum* and *L. japonicus*, respectively (Tables S1 and S2), which corresponded to approximately 3% of the putative protein-coding genes in the two plants (Table 2). The majority

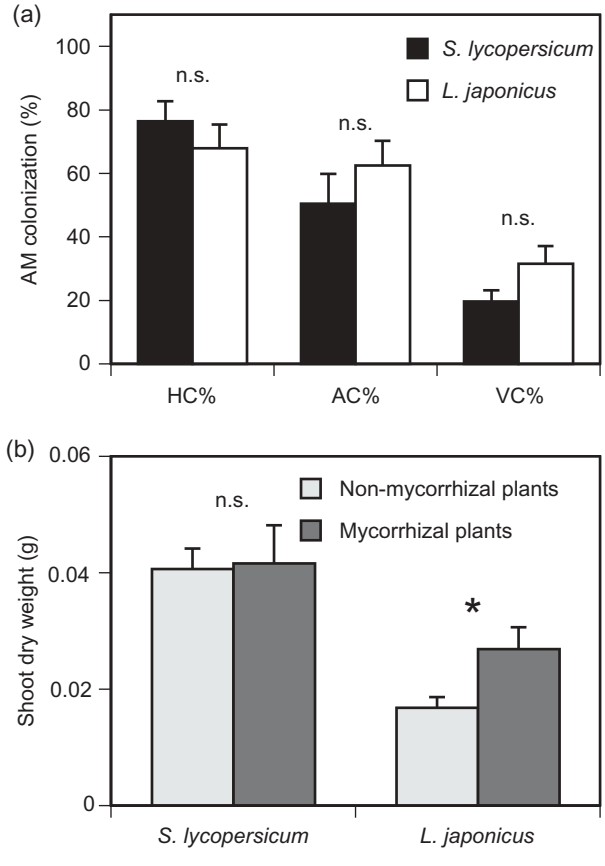

Figure 1. Arbuscular mycorrhizal colonization (a) and shoot dry weight (b) of *Solanum lycopersicum* L. and *Lotus japonicus* L. at 4 weeks after inoculation with *Rhizophagus irregularis*. HC%, hyphal colonization; AC%, arbuscular colonization; VC%, vesicular colonization. Error bars show standard errors ($n = 5$). *, Significant difference at $P < 0.05$ (Student's *t*-test); n.s., not significantly different at $P = 0.05$.

Table 1. Summary statistics for mapping Illumina RNA-seq reads of arbuscular mycorrhizal (AM) roots and non-mycorrhizal (NM) roots against reference sequences of *Solanum lycopersicum* L., *Lotus japonicus* L. and *Rhizophagus irregularis*.

	S. lycopersicum		*L. japonicus*	
	AM roots	NM roots	AM roots	NM roots
Total raw reads	25,554,717	24,200,612	8,923,628	10,029,123
S. lycopersicum/*L. japonicus*				
Reads mapped against *S. lycopersicum* reference sequence	21,306,768	21,337,381	-	-
Reads mapped against *L. japonicus* reference sequence	-	-	4,891,691	6,910,383
Mapped reads /total raw reads (%)	83	88	55	69
R. irregularis				
Reads mapped against *R. irregularis* reference sequence	1,076,008	-	772,325	-
Mapped reads /total raw reads (%)	4	-	9	-

Table 2. Differentially expressed genes (FDR < 0.01) in arbuscular mycorrhizal roots compared with non-mycorrhizal roots of *Solanum lycopersicum* L. and *Lotus japonicus* L.

	S. lycopersicum	*L. japonicus*
Putative protein-coding genes	34,725	57,103
Upregulation		
Upregulated genes	744	1,024
Upregulated genes with significant TBLASTX hit against database of the other species (A)	700	818
Co-upregulated genes in both *S. lycopersicum* and *L. japonicus* (B)	168	194
B/A (%)	24	24
Downregulation		
Downregulated genes	184	673
Downregulated genes with significant TBLASTX hit against database of the other species (C)	143	624
Co-downregulated genes in both *S. lycopersicum* and *L. japonicus* (D)	6	9
D/C (%)	4	1

of the DEGs were upregulated in AM roots. To identify AM-inducible genes shared between *S. lycopersicum* and *L. japonicus*, nucleotide sequences of the upregulated genes in one plant species were used in TBLASTX (e-value < 1e-6) searches against the reference sequence of the other plant species. Most of the AM-inducible genes in one host hit against a reference sequence in the other host (700/744 genes in *S. lycopersicum* and 818/1,024 genes in *L. japonicus*; Table 2). For both hosts, 24% of the AM-inducible genes were co-upregulated in AM roots of the other host, while the proportion of co-downregulated genes between *S. lycopersicum* and *L. japonicus* was 4% at the most (Table 2).

To compare the extent of gene induction or repression between *S. lycopersicum* and *L. japonicus* during AM development, we analyzed the expression profiles of orthologous genes that were predicted using a one-to-one reciprocal best BLAST hit (RBH) procedure. We identified 11,631 RBH pairs (TBLASTX, e-value < 1e-6). Ninety-two RBH pairs were co-upregulated in AM roots of both plants, accounting for 34 and 27% of RBH pairs that were upregulated in *S. lycopersicum* and *L. japonicus*, respectively (Fig. 2A). Only three RBH pairs were co-downregulated in *S. lycopersicum* and *L. japonicus* (Fig. 2A). We

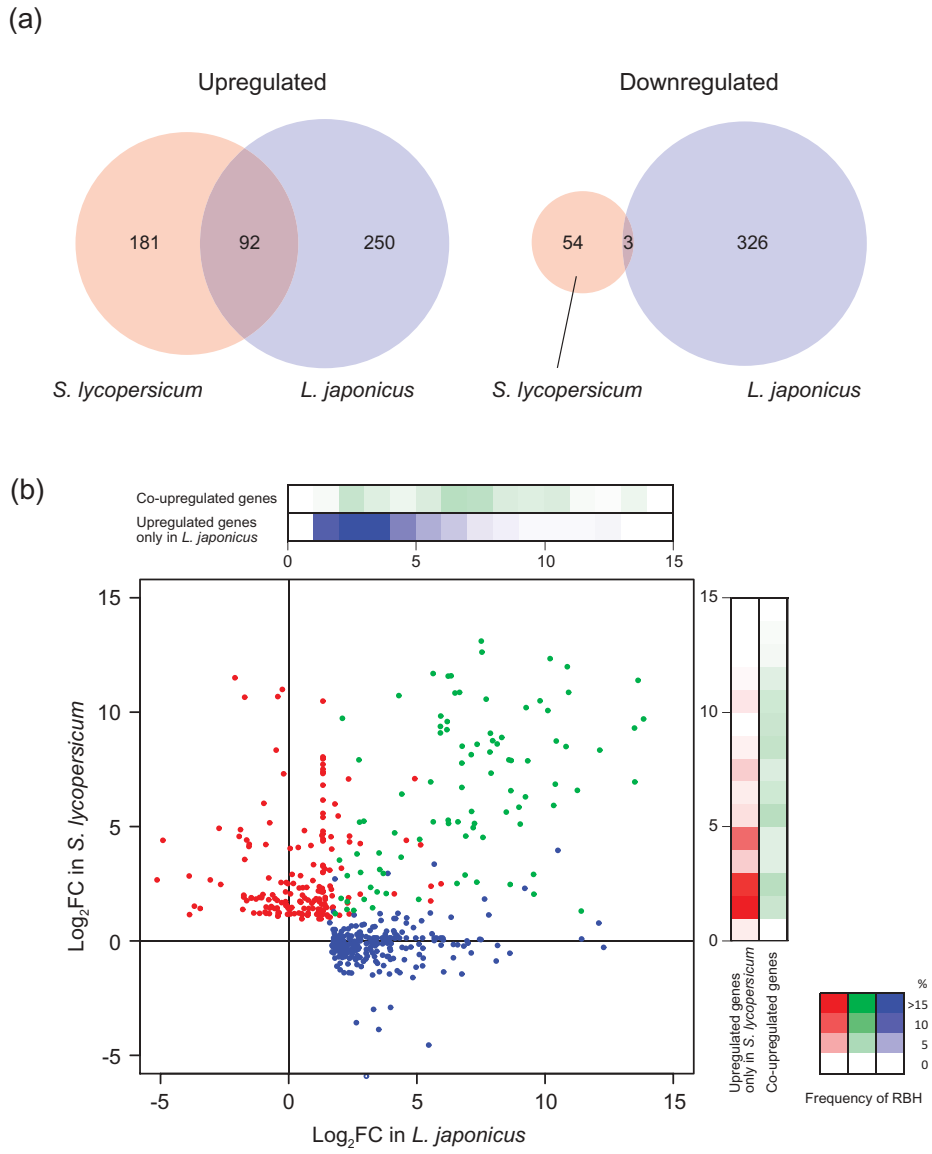

Figure 2. Comparative expression analysis of putative orthologs determined using reciprocal best hit (RBH) criterion between *Solanum lycopersicum* L. and *Lotus japonicus* L. Out of 11,631 RBH pairs identified, 330 and 671 genes were differentially expressed in arbuscular mycorrhizal (AM) roots of *S. lycopersicum* and *L. japonicus* compared with non-mycorrhizal (NM) roots, respectively (FDR < 0.01). (a) Venn diagram showing overlap of upregulated or downregulated orthologs between *S. lycopersicum* and *L. japonicus*. (b) Relationship of fold expression changes (FC) of RBH pairs in AM roots against NM roots between *S. lycopersicum* and *L. japonicus*. Figure shows RBH pairs significantly upregulated in AM roots of *S. lycopersicum* or *L. japonicus*. Green: co-upregulated RBH in *S. lycopersicum* and *L. japonicus*; red: upregulated RBHs only in *S. lycopersicum*; blue: upregulated RBHs only in *L. japonicus*. Heat maps adjacent to axes of scatter plot show relative frequency distribution of FC for upregulated RBH genes in *S. lycopersicum* or *L. japonicus*.

further analyzed the RBH pairs that were upregulated during AM development. The co-upregulated RBH pairs showed a wide range of fold changes in AM roots compared with NM roots, and the fold changes of individual genes were positively correlated between *S. lycopersicum* and *L. japonicus* (Spearman's rank correlation $\rho = 0.437$, $P < 0.0001$; Fig. 2B). The co-upregulated RBH pairs included well-known AM-inducible genes involved in AM or arbuscule development (Table 3): e.g., AM-specific phosphate transporter *PT4* (Javot *et al*. 2007; Yang *et al*. 2012), ABCG transporters *STR* and *STR2* (Zhang *et al*. 2010; Gutjahr *et al*. 2012; Kojima *et al*. 2014), acyl ACP-thioesterase *FatM* (Bravo *et al*. 2016), GRAS transcription factors *RAM1* (Gobbato *et al*. 2012; Rich *et al*. 2015; Xue *et al*. 2015; Pimprikar *et al*. 2016) and *RAD1* (Xue *et al*. 2015; Pimprikar *et al*. 2016), and exocyst complex component *Exo70I1* (Zhang *et al*. 2015). Hundreds of RBH pairs were induced in AM roots of either *S. lycopersicum* or *L. japonicus*, and most of them showed low-fold change values (Fig. 2B).

3.2. *Gene expression profiles of AM fungi colonizing* S. lycopersicum *and* L. japonicus *roots*

The gene expression analysis of *R. irregularis* colonizing roots of *S. lycopersicum* and *L. japonicus* was performed by mapping short reads against the *R. irregularis* genome. The proportion of *R. irregularis*-derived reads in AM roots was less than 10% (Table 1), consistent with the results of previous studies (Tisserant *et al*. 2013; Handa *et al*. 2015; Sugimura and Saito 2016). The expression levels of *R. irregularis* genes were significantly correlated between *S. lycopersicum* roots and *L.*

Table 3. Reciprocal best BLAST hit (RBH) pairs of genes co-upregulated between *Solanum lycopersicum* L. and *Lotus japonicus* L. during arbuscular mycorrhizal development.

	S. lycopersicum		*L. japonicus*		
RBH	Gene ID	Log_2FC[a]	Gene ID	Log_2FC[a]	Annotation in *L. japonicus*
RBH01	Solyc04g050940.1.1	13.1	CM0105.260.r2.a	7.5	Serine-threonine protein kinase
RBH02	Solyc11g007970.1.1	12.6	CM0909.780.r2.m	7.5	4-coumarate-CoA ligase
RBH03	Solyc02g090080.1.1	12.3	TCONS_00041135	10.2	Unknown protein
RBH04	Solyc05g053750.1.1	12.0	LjT47N10.60.r2.a	10.9	Triacylglycerol lipase 2
RBH05	Solyc07g054700.2.1	11.7	CM0432.310.r2.a	5.6	LysM domain containing protein
RBH06	Solyc02g083160.1.1	11.6	LjB12E19.100.r2.d	6.3	GDSL esterase/lipase
RBH07	Solyc12g096380.1.1	11.6	LjT04I16.80.r2.d	6.2	High affinity cationic amino acid transporter 1
RBH08	Solyc06g051860.1.1	11.4	CM2121.10.r2.a	13.6	AM-induced phosphate transporter *LjPT4*
RBH09	Solyc05g008570.1.1	10.9	CM0328.70.r2.d	10.9	Palmitoyl-acyl carrier protein thioesterase *LjFatM*
RBH10	Solyc03g117460.1.1	10.9	CM0573.170.r2.m	6.7	Major allergen Mal d 1
RBH11	Solyc07g065240.1.1	10.8	LjT48A12.120.r2.d	6.5	Leucine-rich repeat receptor-like protein kinase
RBH12	Solyc03g110950.1.1	10.7	CM1864.540.r2.m	4.3	GRAS family transcription factor *LjRAD1*
RBH13	Solyc09g098410.1.1	10.6	CM0177.350.r2.m	7.7	ABCG transporter *LjSTR2*
RBH14	Solyc08g062140.1.1	10.5	TCONS_00007973	9.8	Serine-threonine protein kinase
RBH15	Solyc12g007220.1.1	10.2	CM0617.810.r2.d	9.3	Peptide transporter
RBH16	Solyc07g054570.1.1	10.1	LjSGA_003462.2	10.1	Kelch-like protein
RBH17	Solyc07g006940.1.1	9.8	CM0244.1000.r2.m	5.9	Cysteine-rich receptor-like protein kinase
RBH18	Solyc06g007860.1.1	9.7	CM0127.320.r2.m	2.1	Unknown protein
RBH19	Solyc10g081520.1.1	9.7	CM0104.2930.r2.a	13.8	Blue copper protein
RBH20	Solyc08g077000.1.1	9.6	CM0021.530.r2.m	6.2	Palmate-like pentafoliata 1 transcription factor
RBH21	Solyc12g010490.1.1	9.4	CM0041.30.r2.a	5.9	AP2 domain-containing transcription factor
RBH22	Solyc11g068580.1.1	9.3	LjSGA_022237.1	13.5	Germin-like protein *LjGLP*
RBH23	Solyc03g097860.1.1	9.2	CM0042.2530.r2.d	6.2	Potassium transporter
RBH24	Solyc03g115620.1.1	9.1	CM0087.740.r2.m	5.9	Unknown protein
RBH25	Solyc03g119900.2.1	9.1	TCONS_00033391	7.9	Unknown protein
RBH26	Solyc02g085060.1.1	8.9	CM0249.1340.r2.m	8.3	Replication factor C subunit 5
RBH27	Solyc12g089230.1.1	8.7	CM0195.70.r2.d	7.9	Peptide transporter PTR1
RBH28	Solyc03g005950.1.1	8.7	CM0046.1690.r2.m	10.4	Peptide/nitrate transporter
RBH29	Solyc03g117170.1.1	8.6	LjSGA_026747.2	8.1	Unknown protein
RBH30	Solyc02g089150.2.1	8.6	CM0163.300.r2.d	7.3	PI-PLC X domain-containing protein
RBH31	Solyc02g069550.1.1	8.5	CM0797.40.r2.m	6.8	Prune homolog
RBH32	Solyc08g079780.1.1	8.5	CM0104.2940.r2.a	10.8	Blue copper protein
RBH33	Solyc12g056000.1.1	8.3	LjB03G07.10.r2.a	12.1	Cysteine proteinase
RBH34	Solyc09g072720.1.1	8.3	TCONS_00120316	7.8	Exocyst complex component
RBH35	Solyc04g077760.1.1	8.1	CM0096.900.r2.d	7.1	Exocyst complex component *LjExo70I1*
RBH36	Solyc01g105080.2.1	7.9	CM0314.250.r2.d	8.6	Leucine-rich repeat receptor-like protein kinase
RBH37	Solyc01g111790.1.1	7.9	CM1439.100.r2.d	2.7	Serine/threonine protein phosphatase 2A
RBH38	Solyc01g097430.2.1	7.9	CM0042.2570.r2.d	8.7	ABCG transporter *LjSTR*
RBH39	Solyc07g007080.1.1	7.9	comp286719_c0_seq1	9.3	Unknown protein
RBH40	Solyc08g007960.1.1	7.8	TCONS_00114768	6.8	Nuclear transcription factor Y subunit C-1 *LjCbf*
RBH41	Solyc11g008030.1.1	7.3	CM0909.730.r2.m	7.9	Monocopper oxidase-like protein SKU5
RBH42	Solyc01g094450.1.1	6.9	CM0284.730.r2.d	13.5	Ripening-related protein
RBH43	Solyc09g061240.1.1	6.9	CM0105.270.r2.a	5.5	Unknown protein
RBH44	Solyc01g010260.2.1	6.8	CM0318.690.r2.d	10.4	Cytochrome P450 93A1
RBH45	Solyc06g066390.1.1	6.7	CM0608.1100.r2.m	6.8	AP2 domain-containing transcription factor
RBH46	Solyc01g095250.1.1	6.6	CM0105.320.r2.a	11.3	Acidic chitinase
RBH47	Solyc04g080400.1.1	6.6	CM0071.1240.r2.a	8.7	Reticuline oxidase
RBH48	Solyc07g020870.1.1	6.4	CM0617.800.r2.d	4.4	U-box domain-containing protein
RBH49	Solyc02g092400.1.1	6.3	LjSGA_134299.1	9.2	Unknown protein
RBH50	Solyc02g094340.1.1	5.9	CM1852.30.r2.m	10.3	GRAS family transcription factor *LjRAM1*
RBH51	Solyc11g072830.1.1	5.9	CM0616.310.r2.d	9.0	Chitinase 2
RBH52	Solyc07g064120.1.1	5.7	CM1543.140.r2.m	7.1	ABCB transporter
RBH53	Solyc07g006610.2.1	5.6	comp12648_c0_seq1	8.5	PTI1-like tyrosine-protein kinase
RBH54	Solyc09g072780.1.1	5.3	CM0295.1000.r2.m	6.2	Peptide transporter
RBH55	Solyc09g091700.2.1	5.2	CM1323.380.r2.d	2.9	NADP-dependent alkenal double bond reductase
RBH56	Solyc01g081080.1.1	5.2	CM0017.1160.r2.a	6.6	Replication factor C subunit
RBH57	Solyc01g068380.2.1	5.2	CM0127.890.r2.m	5.6	Purple acid phosphatase
RBH58	Solyc00g170200.1.1	5.2	CM0153.40.r2.d	2.8	Alpha/beta-hydrolases superfamily protein
RBH59	Solyc09g008360.2.1	5.1	CM0012.1760.r2.d	6.2	Heparan-alpha-glucosaminide *N*-acetyltransferase
RBH60	Solyc03g112110.1.1	5.1	LjSGA_012459.1	7.2	Unknown protein
RBH61	Solyc05g009960.2.1	5.1	CM0124.80.r2.d	9.0	Unknown protein
RBH62	Solyc02g088310.1.1	4.9	comp41326_c0_seq1	7.2	Ethylene-responsive transcription factor
RBH63	Solyc03g080020.2.1	4.7	TCONS_00022353	4.1	Major facilitator superfamily transporter
RBH64	Solyc08g062200.1.1	4.6	CM0133.890.r2.m	6.9	Unknown protein
RBH65	Solyc08g067170.1.1	4.5	TCONS_00048122	7.6	U-box domain-containing protein
RBH66	Solyc09g011700.1.1	4.4	LjT44L17.30.r2.d	5.1	Copper transporter
RBH67	Solyc03g114080.1.1	3.8	LjT46F11.70.r2.a	3.5	Leucine-rich repeat receptor-like protein kinase
RBH68	Solyc05g009430.2.1	3.8	CM0903.40.r2.d	2.7	Nuclease S1
RBH69	Solyc09g065040.1.1	3.7	TCONS_00091213	4.4	Antifungal protein ginkbilobin-2
RBH70	Solyc02g086820.2.1	3.5	CM0081.350.r2.m	2.0	Carbonic anhydrase
RBH71	Solyc01g095720.2.1	3.1	CM0105.760.r2.a	3.5	Unknown protein

(*Continued*)

Table 3. (Continued).

	S. lycopersicum		*L. japonicus*		
RBH	Gene ID	Log_2FC[a]	Gene ID	Log_2FC[a]	Annotation in *L. japonicus*
RBH72	Solyc02g083700.2.1	3.0	comp13066_c0_seq1	2.8	Lysosomal alpha-mannosidase
RBH73	Solyc09g065750.2.1	2.9	CM0375.30.r2.m	3.7	Unknown protein
RBH74	Solyc09g009610.1.1	2.9	CM0010.40.r2.d	9.5	Purple acid phosphatase
RBH75	Solyc04g080480.1.1	2.9	LjSGA_124844.1	6.9	AMP-dependent synthetase and ligase
RBH76	Solyc08g082620.2.1	2.9	CM0004.1640.r2.a	2.3	Beta-ketoacyl-ACP synthase I
RBH77	Solyc02g084940.1.1	2.6	CM0249.1160.r2.d	7.3	Unknown protein
RBH78	Solyc01g111050.2.1	2.5	CM0113.40.r2.a	6.6	COBRA-like protein 1
RBH79	Solyc02g092060.1.1	2.5	LjSGA_065775.1	8.6	NAD(P)-binding Rossmann-fold superfamily protein
RBH80	Solyc09g015430.2.1	2.3	TCONS_00085591	3.2	Unknown protein
RBH81	Solyc08g062950.2.1	2.1	CM0133.560.r2.m	3.4	Cytochrome P450 711A1
RBH82	Solyc01g006380.2.1	2.1	CM0017.760.r2.a	3.8	U-box domain-containing protein
RBH83	Solyc03g031830.1.1	2.1	CM0367.670.r2.d	9.6	Unknown protein
RBH84	Solyc07g062810.2.1	1.9	LjSGA_054315.1	2.0	Unknown protein
RBH85	Solyc08g066650.2.1	1.8	LjB19M02.90.r2.m	2.9	Carotenoid cleavage dioxygenase 8 *LjCCD8*
RBH86	Solyc10g083180.1.1	1.8	comp63373_c1_seq1	5.1	Momilactone A synthase
RBH87	Solyc03g117470.2.1	1.7	comp40972_c0_seq1	2.3	Unknown protein
RBH88	Solyc01g059900.2.1	1.4	comp62669_c0_seq1	3.3	Dirigent protein
RBH89	Solyc01g081620.2.1	1.4	comp65143_c0_seq1	2.3	Unknown protein
RBH90	Solyc02g081050.2.1	1.3	CM0367.820.r2.m	2.5	LysM type receptor kinase
RBH91	Solyc06g069530.2.1	1.3	CM1882.210.r2.a	11.4	Acetyl-CoA carboxylase biotin carboxyl carrier protein
RBH92	Solyc04g081400.2.1	1.2	comp64780_c0_seq1	1.8	Hexokinase

[a] Fold change in normalized gene transcript level in arbuscular mycorrhizal roots compared with non-mycorrhizal roots.

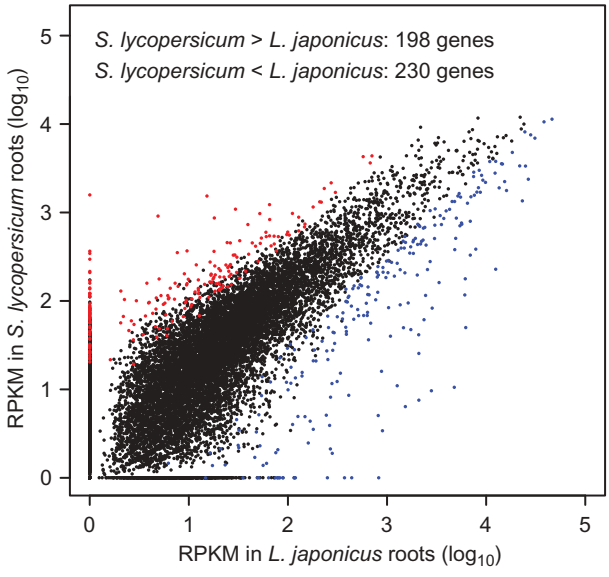

Figure 3. Global gene expression profile of *Rhizophagus irregularis* colonizing *Solanum lycopersicum* L. and *Lotus japonicus* L. roots. Reads per kilobase per million mapped reads (RPKM) of fungal genes are plotted. Red: *R. irregularis* genes with significantly higher expression in *S. lycopersicum* roots; blue: *R. irregularis* genes with significantly higher expression in *L. japonicus* roots; black: genes not differentially expressed between roots of *S. lycopersicum* and *L. japonicus*. Differentially expressed genes were detected with a false discovery rate cutoff of 0.01.

japonicus roots (Spearman's rank correlation $\rho = 0.833$, $P < 0.0001$; Fig. 3). However, a total of 428 *R. irregularis* genes (1.4% of the putative protein-coding genes) were differentially expressed between roots of *S. lycopersicum* and *L. japonicus* (Fig. 3 and Table S3). A GO enrichment analysis of genes highly expressed in *L. japonicus* roots revealed an overrepresentation of GO terms related to adenosine triphosphate (ATP) synthesis such as ATP synthesis coupled electron transport, cellular respiration, ATP synthesis coupled proton transport, NADH dehydrogenase (ubiquinone) activity, and proton-transporting ATP synthase complex (Table 4). Some *R. irregularis* genes encoding respiratory enzyme complexes involved in the mitochondrial electron transport chain were more highly expressed in *L. japonicus* roots than in *S. lycopersicum* roots (Fig. 4). Several GO terms related to sterol metabolism were enriched in *R. irregularis* genes showing higher transcript levels in *S. lycopersicum* roots than those in *L. japonicus* roots (Table 4).

4. Discussion

A large number of AM-responsive genes in legumes, tomato and *Oryza sativa* L. have been detected by comparing AM roots with NM roots using microarrays and RNA-seq data (Liu *et al*. 2003; Manthey *et al*. 2004; Güimil *et al*. 2005; Hohnjec *et al*. 2005; Fiorilli *et al*. 2009; Gomez *et al*. 2009; Benedito *et al*. 2010; Hogekamp *et al*. 2011; Handa *et al*. 2015). Our comparative transcriptome analysis between *S. lycopersicum* and *L. japonicus* revealed that only one fourth to one third of the AM-inducible genes (AM roots versus NM roots) of each plant species were co-upregulated, according to both reciprocal BLAST and unidirectional (non-reciprocal) BLAST searches. Furthermore, there were very few co-downregulated genes among the AM-repressive genes. The transcriptional responses of the co-upregulated RBH pairs, which included some essential genes for AM formation, were conserved between the two plants. That is, there was an enrichment of genes that play fundamental roles in AM development and function among the co-upregulated genes. We also detected some species-dependent upregulated genes, which generally showed low levels of induction. These genes are likely to be differentially expressed in relation to the physiological differences between AM and NM roots in each plant species, although some of them may function downstream of the CSSP. It is difficult to comprehensively survey the genes required for AM formation, maintenance and function by a simple comparison of gene expression profiles between AM and NM roots. Comparative

Table 4. Gene ontology (GO) enrichment analysis of *Rhizophagus irregularis* genes differentially expressed between roots of *Solanum lycopersicum* L. and *Lotus japonicus* L.

GO ID	GO term	Number of genes	*P* value
R. irregularis genes showing higher transcript levels in *L. japonicus* roots			
Biological process			
GO:0042773	Adenosine triphosphate (ATP) synthesis coupled electron transport	4	2.5E-05
GO:0045333	Cellular respiration	7	7.7E-05
GO:0015986	ATP synthesis coupled proton transport	3	7.6E-04
GO:0006334	Nucleosome assembly	3	1.7E-03
GO:0006366	Transcription from RNA polymerase II promoter	2	3.3E-03
GO:0006621	Protein retention in endoplasmic reticulum (ER) lumen	1	8.0E-03
GO:0015703	Chromate transport	1	8.0E-03
GO:0046087	Cytidine metabolic process	1	8.0E-03
Molecular function			
GO:0015078	Hydrogen ion transmembrane transporter activity	13	5.9E-14
GO:0008137	NADH dehydrogenase (ubiquinone) activity	5	1.8E-07
GO:0020037	Heme binding	9	4.8E-04
GO:0005506	Iron ion binding	8	1.1E-03
GO:0005507	Copper ion binding	3	3.6E-03
GO:0004126	Cytidine deaminase activity	1	8.2E-03
GO:0015109	Chromate transmembrane transporter activity	1	8.2E-03
GO:0046923	ER retention sequence binding	1	8.2E-03
GO:0008398	Sterol 14-demethylase activity	2	8.3E-03
Cellular component			
GO:0045259	Proton-transporting ATP synthase complex	4	8.4E-04
R. irregularis genes showing higher transcript levels in *S. lycopersicum* roots			
Biological process			
GO:0042364	Water-soluble vitamin biosynthetic process	3	4.1E-04
Molecular function			
GO:0033764	Steroid dehydrogenase activity, acting on the CH-OH group of donors, NAD or NADP as acceptor	5	3.9E-05
GO:0050051	Leukotriene-B4 20-monooxygenase activity	2	1.4E-03
GO:0000253	3-keto sterol reductase activity	3	3.9E-03
GO:0004495	Mevaldate reductase activity	3	3.9E-03
GO:0008875	Gluconate dehydrogenase activity	3	3.9E-03
GO:0018451	Epoxide dehydrogenase activity	3	3.9E-03
GO:0018452	5-exo-hydroxycamphor dehydrogenase activity	3	3.9E-03
GO:0018453	2-hydroxytetrahydrofuran dehydrogenase activity	3	3.9E-03
GO:0032442	Phenylcoumaran benzylic ether reductase activity	3	3.9E-03
GO:0043713	(R)-2-hydroxyisocaproate dehydrogenase activity	3	3.9E-03
GO:0048258	3-ketoglucose-reductase activity	3	3.9E-03
GO:0051990	(R)-2-hydroxyglutarate dehydrogenase activity	3	3.9E-03
GO:0000252	C-3 sterol dehydrogenase (C-4 sterol decarboxylase) activity	3	4.3E-03
GO:0033765	Steroid dehydrogenase activity, acting on the CH-CH group of donors	3	4.8E-03
GO:0004448	Isocitrate dehydrogenase activity	3	5.2E-03
GO:0004033	Aldo-keto reductase (NADP) activity	3	6.3E-03
GO:0046933	Proton-transporting ATP synthase activity, rotational mechanism	3	7.5E-03
GO:0046961	Proton-transporting ATPase activity, rotational mechanism	3	8.1E-03
Cellular component			
GO:0016469	Proton-transporting two-sector ATPase complex	3	9.6E-03

transcriptome analyses of diverse plant species or temporal transcriptome analyses using a synchronous fungal colonization system will be useful to explore AM-responsive genes in plants.

The intraradical hyphae, extraradical hyphae and spores of AM fungi are morphologically and physiologically differentiated (Bago *et al*. 2002). The differentiation of these fungal structures is supported by their differential gene expression (Tisserant *et al*. 2012, 2013; Tang *et al*. 2016). In intraradical hyphae, genes related to transmembrane transport, small secreted proteins, lipid metabolism, carbohydrate metabolism and DNA replication are more highly expressed (Tisserant *et al*. 2012, 2013; Tang *et al*. 2016). It has been demonstrated that several genes encoding sugar transporters, aquaporins, small secreted proteins and cell-cycle related proteins are involved in AM fungal colonization or function in the roots of the host (Helber *et al*. 2011; Ait Lahmidi *et al*. 2016; Fiorilli *et al*. 2016; Kikuchi *et al*. 2016; Sugimura and Saito 2016; Tsuzuki *et al*. 2016). Many diverse plant species can establish associations with AM fungi; that is, AM fungi have a very wide host range (Smith and Read 2008). Handa *et al*. (2015) showed that the gene transcript levels in AM fungi colonizing *L. japonicus* roots were correlated with those in AM fungi colonizing *M. truncatula* roots. In the present study, we detected similar expression patterns of *R. irregularis* genes between *S. lycopersicum* and *L. japonicus* roots, indicating that the gene expression profiles of *R. irregularis* are very similar even when the fungus colonizes host plants in different families. However, hundreds of *R. irregularis* genes were differentially expressed between the two hosts. The most remarkable difference was the higher expression of AM fungal genes related to the electron transport chain in *L. japonicus* roots than that in *S. lycopersicum* roots. In germinating spores of *Gigaspora margarita*, mitochondrial electron transport chain genes are induced in the presence of the endobacterium *Candidatus Glomeribacter gigasporarum* and by the application of strigolactones, which is accompanied by increased ATP production (Salvioli *et al*. 2016). It could be inferred, therefore, that the *L. japonicus*-

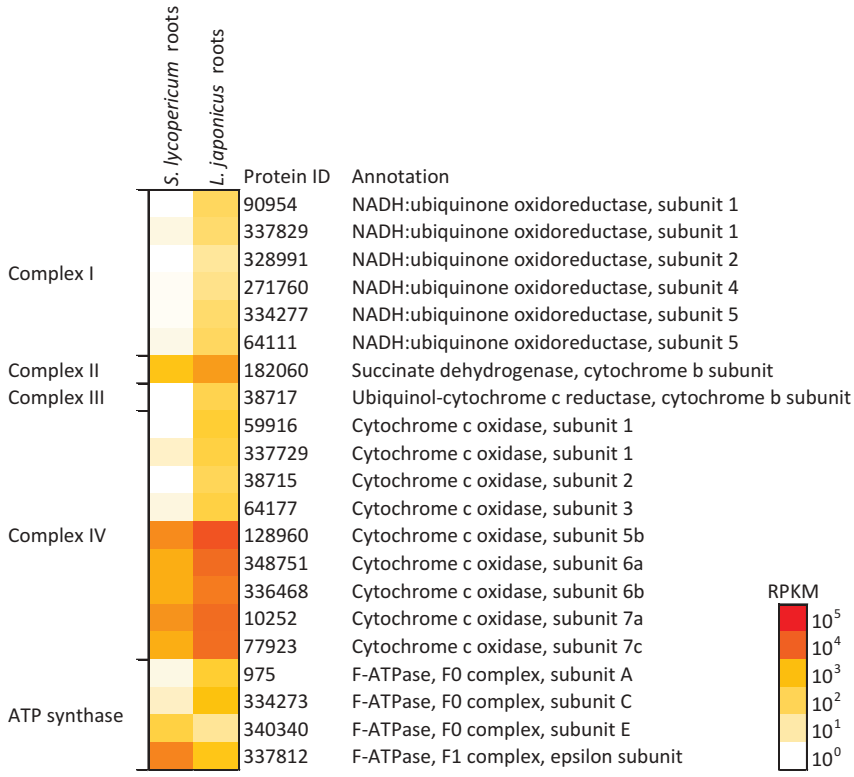

Figure 4. Transcript profiles of *Rhizophagus irregularis* genes related to electron transport chain. Differentially expressed genes (false discovery rate < 0.01) between AM fungi colonizing *Solanum lycopersicum* L. and *Lotus japonicus* L. roots are shown. Heat maps represent reads per kilobase per million mapped reads (RPKM) of *R. irregularis* genes.

induced expression of genes in the *R. irregularis* oxidative phosphorylation pathway enhances ATP production in the fungal hyphae. We observed positive growth responses to AM fungal colonization in *L. japonicus* but not in *S. lycopersicum*. It will be interesting to investigate the relationship between the mycorrhizal response of host plants and energy production in AM fungi in the future.

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