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Transcriptomic analysis reveals the possible roles of sugar metabolism and export for positive mycorrhizal growth responses in soybean

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To elucidate molecular mechanisms controlling differential growth responses to root colonization by arbuscular mycorrhizal (AM) fungi varying in colonization and cooperative behavior, a pot experiment was carried out using two soybean genotypes and three AM inocula. The results showed that inoculation by cooperative *Rhizophagus irregularis* (Ri) or less cooperative *Glomus aggregatum* with high AM colonization (Ga-H) significantly promoted plant growth compared with inoculation by *G. aggregatum* with low AM colonization (Ga-L). A comparative RNA sequencing analysis of the root transcriptomes showed that fatty acid synthesis pathway was significantly enriched in all three AM inoculation roots. However, sugar metabolism and transport were significantly enriched only in Ri and Ga-H inoculation, which was consistent with positive growth responses in these two inoculation treatments. Accordingly, the expression levels of the key genes related to sugar metabolism and transport were also upregulated in Ri and Ga-H roots compared with Ga-L roots. Of them, two sugars will eventually be exported transporters (*SWEET*) transporter genes, *GmSWEET6* (Glyma.04G198600) and *GmSWEET15* (Glyma.06G166800), and one invertase (Glyma.17G227900) gene were exclusively induced only in Ri and Ga-H roots. Promoter analyses in transgenic soybean roots further demonstrated that *GUS* driven by the *GmSWEET6* promoter was highly expressed in arbuscule-containing cortical cells. Additionally, Ri and Ga-H inoculation increased the contents of sucrose, glucose and fructose in both shoots and roots compared with those of Ga-L and non-mycorrhizal. These results imply that positive mycorrhizal growth responses in plants might mostly be due to the stimulation of photosynthate metabolism and transport by AM fungal inoculum with high colonization capabilities.

Abbreviations – 18:1, oleic acid; ACP, acyl carrier protein; Acyl-CoA, acyl-coenzyme A; AM, arbuscular mycorrhizal; DEGs, differentially expressed genes; EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycol-bis (*β*-aminoethyl ether)-N, N, N′, N′-tetraacetic acid; FatM, acyl-ACP thioesterase M; FPKM, fragments per kilobase of transcripts per million reads mapped (FPKM); GC–MS, gas chromatography-mass spectrometry; Ga-H, *Glomus aggregatum* with high AM colonization; GAI, GA INSENSITIVE; Ga-L, *Glomus aggregatum* with low AM colonization; GO, gene ontology; GRAS, GAI-RGA-SCR; HISAT, hierarchical indexing for spliced alignment of transcripts; Inv, invertase; KASII, ketoacyl-ACP synthase II; KEGG, Kyoto Encyclopedia of Genes and Genomes; MCAT, malonyl CoA-ACP transacylase; MGR, mycorrhizal growth response; MPR, mycorrhizal P response; NAC, NAM-ATAF1/2-CUC2; NM, non-mycorrhizal; Pi, phosphate; PIPES, piperazine-N,N′-bis(2-ethanesulfonic acid); PK, pyruvate kinase; RAM1, GRAS-domain transcription factor; RAM2, glycerol-3-phosphate acyltransferase; RGA, REPRESSOR of GAI; Ri, *Rhizophagus irregularis*; RSEM, RNA-Seq by Expectation Maximization; SCR, SCARECROW; STR, adenosine triphosphate-binding cassette transporter; SWEET, sugars will eventually be exported transporters; UDP, Uridine diphosphate.

Introduction

Symbiosis between plants and arbuscular mycorrhizal (AM) fungi is pervasive in nature. More than 80% of terrestrial plant species establish symbioses with AM fungi (Bonfante and Genre 2010). The most characterized advantage of AM symbiosis is the enhanced capacity of plants to acquire mineral nutrients from soil, especially phosphorus (P; Smith and Read 2008). However, different plant species or even varieties respond differently to colonization by the same individual AM fungal species or strain (Smith et al. 2009). For example, soybean and *Medicago* can respond positively to *Rhizophagus irregularis* colonization in low-P soils (Kiers et al. 2011, Wang et al. 2011), while growth of wheat and barley is depressed (Li et al. 2006, Grace et al. 2009). On the other hand, plant growth responses can vary depending on the AM fungal species (Klironomos 2003, Pringle and Bever 2008, Kiers et al. 2011). Soybean plants were previously found to show positive growth responses when inoculated with *R. irregularis*, and no or negative growth responses to colonization by *Glomus aggregatum* (Wang et al. 2016). These observations indicate that both hosts and AM fungi detect and respond to each other in symbiotic interactions. To date, the molecular mechanisms of differential growth responses remain poorly understood.

It has been hypothesized that the variety of plant growth responses to different AM fungi might result mainly from carbon (C) loss from plants to the fungal symbiont without concomitant return of nutrients to the host plant (Graham and Abbott 2000). Plants allocate about 20% of photosynthetically fixed C to AM fungi after formation of symbiotic association, which influences host C metabolism (Jakobsen and Rosendahl 1990). Variations in C and P supplies appear to be detected by both plants and AM fungi, and thereby C and P allocations are accordingly adjusted, which leads to promotion or inhibition of plant growth (Kiers et al. 2011). Sucrose or hexose is exported into periarbuscular spaces before acquisition by the mycobiont (Doidy et al. 2012a, Manck-Götzenberger and Requena 2016). Therefore, genes encoding sucrose synthases, invertases and transporters exhibit induced expression in mycorrhizal plants (Ravnskov et al. 2003, Schubert et al. 2004, Garcia-Rodriguez et al. 2007, Boldt et al. 2011, Doidy et al. 2012a, 2012b). Mycorrhiza-induced *SUGARS WILL EVENTUALLY BE EXPORTED TRANSPORTERS* (*SWEET*) genes that are also likely involved in sugar efflux into periarbuscular spaces (Manck-Götzenberger and Requena 2016). Recently, it has been found that, in addition to sugars, lipids are a major source of organic carbon delivered to AM fungi (Bravo et al. 2017, Jiang et al. 2017, Luginbuehl et al. 2017). As yet, uncertainty remains in regard to the molecular mechanisms of transport and partitioning of organic C at plant–fungal symbiotic interface in response to AM fungal colonization of host plants.

At the same time, regulation of phosphate (Pi) uptake pathway is thought to affect host plant growth responses (Harrison et al. 2002, Paszkowski et al. 2002, Wang et al. 2016). The upregulated expression of the AM-inducible *MtPT4* (phosphate transporter from *Medicago truncatula*) gene is correlated with the contribution of the mycorrhizal uptake pathway in positively responsive medicago inoculated with *R. irregularis* (Fellbaum et al. 2014). On the other hand, transcription levels of AM-inducible Pi transporter genes are not considered related to the growth depression of barley and wheat inoculated with *R. irregularis* or *Glomus geosporum* (Grace et al. 2009, Sisaphaithong et al. 2012). Overall, the expression of high-affinity Pi uptake transporters in plants depends on specific combinations of plant and AM fungal species.

Mycorrhizal fungal infection can induce genes that function in metabolism and transport of nutrients and organic C, cell wall remodeling and signal transduction. Therefore, the transcriptome of mycorrhizal plants must be considerably reprogrammed during colonization by AM fungi, which results in distinct physiological responses for specific combinations of AM fungus and host plant (Hohnjec et al. 2005, Hogekamp et al. 2011, Gutjahr et al. 2015). Transcriptomic studies allow for genome-wide identification of hundreds of AM-related genes (Hogekamp et al. 2011, Gutjahr et al. 2015, Afkhami and Stinchcombe 2016). However, expression profiling in mycorrhizal roots has mainly focused to date on interactions involving one or two cooperative AM fungi. There is limited information available on transcriptional responses of host plants colonized by cooperative or uncooperative AM fungal species. Even less is known about growth and transcriptomic responses of a host plant to variations in the extent of AM colonization by one fungal species.

Here, manipulative experiments and genome-wide expression analysis are combined in order to observe phenotypic and transcriptomic responses of mycorrhizal soybean. An emphasis is placed on comparing the metabolism and transport of organic C in mycorrhizal plants colonized not only by cooperative AM fungal species, but also by the less cooperative one with different extents of AM colonization. Importantly, we discovered promising candidate genes for regulating mycorrhizal growth responses (MGRs), which might be useful for future efforts to engineer soybean crops that are highly responsive to AM inoculation.

Materials and methods

Experimental materials

The two soybean (*Glycine max* L.) genotypes used were HN89 and HN112 that are characterized by their shallow or deep root architecture, and their high- or low-P acquisition efficiency, respectively (Wang et al. 2016). The AM fungal partners were *R. irregularis* (Schenck and Smith, DAOM 197198, Biosystematics Research Center), and *G. aggregatum* (Schenck and Smith, isolate 0165 collected from the Long-Term Mycorrhizal Research Site, University of Guelph, Guelph, Canada, Wang et al. 2016).

Experimental design and growth conditions

All plants were grown in low-P conditions (50 μM P supplied as KH_2PO_4) and four AM fungal inoculation treatments, including a *R. irregularis* inoculum (Ri) with about 45% AM colonization and 10 spores per gram soil, a *G. aggregatum* inoculum with about 70% AM colonization and 15 spores per gram soil (Ga-H), a *G. aggregatum* inoculum with about 25% AM colonization and 6 spores per gram soil (Ga-L), and a non-mycorrhizal (NM) control. The applied phosphate was available to both plants and fungi. Ga-H and Ga-L inocula were classified by their colonization capacity as expressed by both AM colonization and number of spores per gram soil. The inocula were produced on millet (*Setaria italica* L.), which was grown in autoclaved sand/soil (8:2) mixture for 4 months in a glasshouse. At the same time, the control NM inoculum was also prepared under the same conditions without mycorrhizal inoculation (Cruz et al. 2004, Zhang et al. 2015). Each inoculum was a mix of colonized root fragments and soils from pure pot culture containing spores and extraradical hyphae (the control without AM fungal propagules). There were four parallel plants within each treatment.

Soybean seeds were surface sterilized in chlorine gas for 8 h prior to sowing in pots filled with an autoclaved growth substrate (121°C for 40 min twice in a 24-h interval) of sand and soil (8:2, v:v) containing 20% dry inoculum. Available P of the sand and soil mixture was 7.83 mg kg^{-1}. Plants were grown in the greenhouse experiment under natural light in the intensity range of 500–1800 μmol m^{-2} s^{-1}. Diurnal air temperatures ranged consistently between 20 and 28°C, and relative humidity was between 50 and 75%. Plants were watered every week with modified half-strength Hoagland solution containing the following nutrients (in μM): KH_2PO_4 (50), KNO_3 (2.5×10^3), $Ca(NO_3)_2$ (2.5×10^3), $MgSO_4$ (1×10^3), K_2SO_4 (250), $C_{10}H_{12}FeN_2NaO_8$ (Fe-EDTA) (80), H_3BO_3 (20), $MnCl_2$ (4.50), $ZnSO_4$ (0.30), $CuSO_4$ (0.16) and $(NH_4)_6Mo_7O_{24}$ (0.16). Plants were harvested 30 days after inoculation.

Plant harvest

Shoots were harvested and dried to determine dry weight (DW). One shoot sample was flash-dried for 48 h, milled with a mechanical mixer and kept at −20°C until use (Gómez-González et al. 2010). Roots were harvested and weighed, and one root sample of known fresh weight (FW) was longitudinally taken and flash-frozen in liquid nitrogen for RNA extraction and sequencing. The rest of the roots were cut into small pieces of 1–2 cm lengths, and adequately mixed. Of them, about 0.1 g of the root pieces (about 70 of them) were taken to determine AM colonization and the percentage of mycorrhizal roots that were colonized with arbuscules, vesicles or intraradical hyphae by the magnified intersection method (McGonigle et al. 1990). Total time span for sampling was 2 h. After that, the remaining roots were weighed before and after drying, and the FW/DW ratio was used to calculate total root DW. Phosphorus concentrations of shoots and roots were measured using a San++ SKALAR continuous flow analyzer (Skalar, Delft, the Netherlands) after dry ashing. The P contents were expressed as milligram per dry plant. Mycorrhizal responses were calculated in terms of plant growth (MGR) and P content (mycorrhizal P response; MPR), respectively, using the following formula (Hetrick et al. 1992): $MGR/MPR = 100 \times (AM - NM)/NM$, in which AM and NM refer to the total DW or P content of individual mycorrhizal plants or the average of NM plants, respectively.

Analysis of soluble sugars

Soluble sugars were extracted from shoots and roots using the chloroform/methanol method as described by Lisec et al. (2006). A gas chromatography-mass spectrometry (GC–MS) system was employed for sugar measurements. The GC–MS system consisted of a 7890A gas chromatograph with a 7693 autosampler coupled to a MSD 5975C mass selective detector (Agilent Technologies, Santa Clara, CA). The contents of soluble sugars were expressed as milligram per gram DW.

RNA sequencing, de novo assembly and quantifying gene expression

The root cDNA libraries from three independent biological replicates of each AM treatment in HN89 were separately sequenced on a BGISEQ-500 platform (BGI, Shenzhen, China). Adapter sequences and low-quality reads

were removed from raw reads. High-quality reads were then mapped to the soybean reference genome sequence using HISAT (hierarchical indexing for spliced alignment of transcripts; Kim et al. 2015). The fragments per kilobase of transcripts per million reads mapped (FPKM) values were estimated further using RSEM (RNA-Seq by Expectation Maximization; Li and Dewey 2011), and the NOISeq method was used to screen differentially expressed genes (DEGs) among the different treatments. The basis of the fold change was the expression of the target genes in NM control roots. The absolute value of Log_2 fold change ≥1.0 with the probability ≥0.8 was used as the default threshold for assessing significance of differential gene expression. To assess whether any gene set with similar function plays important roles in AM roots, we conducted gene set functional enrichment analysis of DEGs. Both gene ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis were performed with TBtools (https://github.com/CJ-Chen/TBtools) by using Hyper-geometric test. GO terms or pathways with more than five genes in selection set and *P*-value lower than 0.05 were kept and visualized using ggplot2 (Wickham 2016).

Validation of DEG results by quantitative polymerase chain reaction

Total RNA was extracted from selected root subsamples using RNA-Solv reagent (Omega Bio-tek, Norcross, GA). RNA samples were used as templates for reverse transcription (RT) with the TransScript All-in-One First-Strand cDNA Synthesis SuperMix (TransGen Biotech, Beijing, China). Quantitative RT-polymerase chain reaction (qRT-PCR) was performed using the PowerUP SYBR Green Master Mix (Applied Biosystems, Foster City, CA) under a StepOne plus Real-Time PCR System (Applied Biosystems) to examine expression patterns of the selected genes. The expression of four Pi transporter genes (*GmPT4*, *GmPT8*, *GmPT9* and *GmPT10*) was analyzed in soybean roots using the specific primer pairs as shown in Wang et al. (2016). The expression of *GmSWEET6* and *GmSWEET15* was analyzed with specific primer sequences as follows: 5′-CGTTCGGGAGCGTAACATAG-3′ and 5′-TCGGACCAAAAGCGTAGAGT-3′ for *GmSWEET6*, and 5′- AATTGTCCTCTTAACCCACCT-3′ and 5′-CCGAATTACCACCCTAATAATGCT-3′ for *GmSWEET15*. The expression of a fungal 18S rRNA gene was analyzed to confirm mycorrhizal colonization (5′-TAACGAACGAGACCTTAACCT-3′,5′-CCTCACTAA GCCATTCAATCGG-3′). The soybean housekeeping gene *GmTefS1* encoding elongation factor EF-1a (Accession number: X56856) was used as an internal control to normalize the expression levels of the target genes. EF-1a is a highly conserved and ubiquitous protein translation factor (Durso and Cyr 1994). *TefS1* is widely used as a reference gene for gene expression normalization in soybean (Nunes et al. 2006, Guo et al. 2011, Li et al. 2015), the expression of which was not influenced by AM colonization (Zhang et al. 2015). The PCR protocols followed were 95°C for 10 min, followed by 40 cycles of 95°C for 15 s, and 60°C for 60 s. The qRT-PCR was conducted with four biological replicates. Expression data were normalized with the expression level of *EF-1a* by the $2-^{\Delta\Delta CT}$ method. The amplification efficiencies of the PCRs were between 90 and 110%.

GmSWEET6 promoter analysis

The genomic sequence 2176 bp upstream of the *GmSWEET6* translation start site was amplified by PCR from soybean leaf DNA. The utilized primers were 5′- CCACCTTGTTATACCTCATT-3′ and 5′-GGAATTTCTCTCTCTCTCTCT-3′. After ligation with the *GUS* reporter gene, the ligated *GmSWEET6* promoter-*GUS* fragment was cloned into the pTF102 vector (Li et al. 2014). The resulting construct was transformed into soybean by the cotyledonary-node transformation method (Li et al. 2014). T2 generation plants of homozygous independent transgenic lines were used for promoter analysis in *R. irregularis* inoculation treatments. At harvest, roots were collected for double staining of GUS and WGA-Alexafluor 488. Roots were incubated with a 5-bromo-4-chloro-3-indolyl glucuronide (X-gluc) solution overnight at 37°C. After staining, the roots were destained with 70% ethanol to improve contrast, and fixed with PEM buffer (50 mmol l^{-1} PIPES, 5 mmol l^{-1} EGTA and 5 mmol l^{-1} $MgSO_4$, pH 7.0) for 1 h. The GUS-stained roots were embedded in 7% low-melting-temperature agarose and sectioned into 30-µm slices using a Leica VT1200S vibratome (Leica, Wetzlar, Germany). Counterstaining of fungal structures was performed with Alexa Fluor 488 WGA conjugate (Invitrogen, Darmstadt, Germany). Images of double-stained roots were taken with a Zeiss LSM 780 laser scanning confocal microscope (Zeiss, München, Germany).

Statistical analyses

All data were analyzed in Microsoft Excel 2007. Significant differences (*P*-value ≤ 0.05) were determined by two-way ANOVA, which was followed by Duncan's multiple range mean separation test using SAS (SAS Institute Inc., Cary, NC).

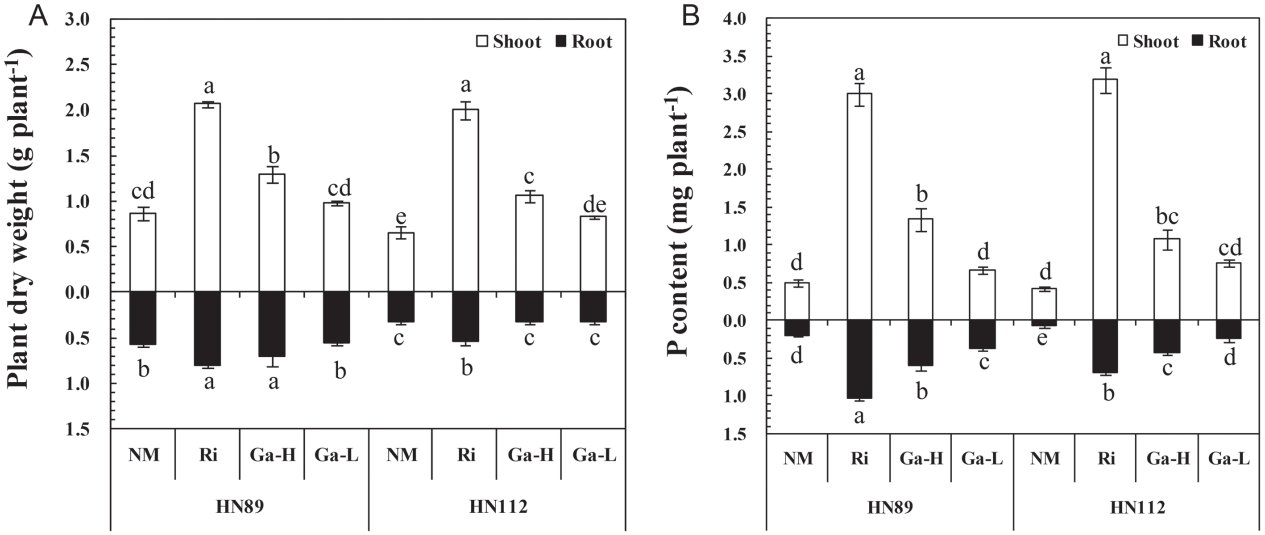

Fig. 1. Effects of AM treatments on plant DW (A) and P content (B) in the two soybean genotypes HN89 and HN112. Soybean plants were cultivated with different AM inocula for 30 days. Data are the mean of four biological replicates with standard error. Different letters indicate significant differences among AM treatments and genotypes (ANOVA followed by Duncan's multiple range test, $P \leq 0.05$).

Deposited data

The RNA-seq datasets generated in this study have been submitted to NCBI GEO database with the series record GSE107970.

Results

Effect of inoculum on growth and AM colonization of two soybean genotypes

In order to assess the responses of two soybean genotypes to different inoculation treatments, the plants were grown in sand/soil culture with different inocula. The results showed that both plant DW and P content responses to AM fungal inoculation treatments had similar pattern between the two soybean genotypes (Fig. 1). Inoculation with Ri dramatically increased plant DW and P content, while inoculation with Ga-L had no or minor effects on plant DW and P content in both soybean genotypes (Fig. 1 and Table S1, Supporting information). In contrast, both genotypes had intermediate response to inoculation with Ga-H. One observed difference between plants genotypes was that root DW and shoot P content in P-efficient genotype HN89 were higher at Ga-H than Ga-L inoculation treatments, while no statistically significant difference was observed between these two inoculation treatments in P-inefficient genotype HN112 (Fig. 1). Both soybean genotypes showed a similar mycorrhizal responsiveness in terms of plant DW (Fig. 2A) or P content (Fig. 2B), but HN112 had stronger MGR and MPR in Ri inoculation than HN89. Plants of both genotypes inoculated with Ri showed the highest MGR and MPR, followed by those treated with Ga-H inoculation but no significant difference in MGR of HN112 between Ga-H than Ga-L inoculation treatments was observed (Fig. 2A).

AM fungal colonization of soybean roots was high for Ri and Ga-H in both HN89 (89 and 86%, respectively) and HN112 plants (90 and 87%, respectively), and lower for Ga-L (73% in HN89 and 65% in HN112; Fig. 3A). We also checked the expression of a fungal 18S rRNA gene to determine mycorrhizal colonization. Confirming the lower degree of root colonization, the fungal 18S rRNA gene also showed lower expression level in Ga-L plants relative to Ri plants (Fig. 3B, Table S1). Consistent with overall AM colonization, the percentages of arbuscules and of intraradical hyphae in mycorrhizal roots of HN112 were lower in Ga-L plants relative to Ri plants (Fig. 3C, D). In addition, a higher number of vesicles were observed in the mycorrhizal roots of both soybean genotypes that were colonized with Ri compared with those colonized by Ga-H and Ga-L, but there was no significant difference between the soybean genotypes (Fig. 3E).

Overview of the gene expression profiles

RNA-seq was performed to investigate the molecular basis of the observed physiological differences in soybean plants in response to different inoculation treatments. RNA from three biological replicates of HN89 roots was sequenced. In total, 12 libraries were constructed and analyzed. After removing low-quality reads, more than 2.3×10^7 clean reads were obtained

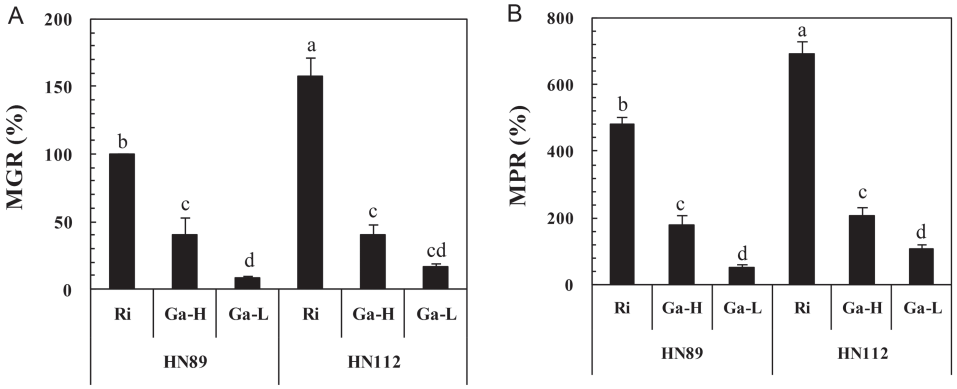

Fig. 2. Effects of AM treatments on MGR (A) and MPR (B) in the two soybean genotypes HN89 and HN112. Soybean plants were cultivated with different AM inocula for 30 days. Data are the mean of four biological replicates with standard error. Different letters indicate significant differences among AM treatments and genotypes (ANOVA followed by Duncan's multiple range test, $P \leq 0.05$).

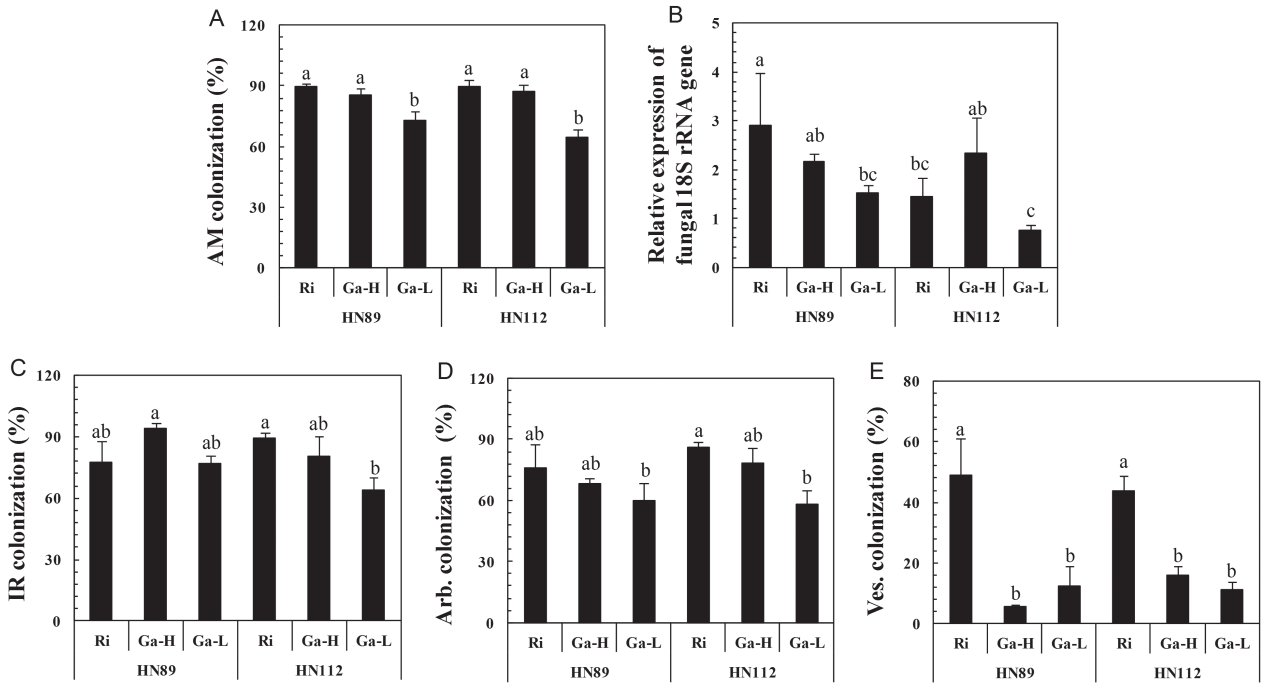

Fig. 3. Effects of AM treatments on AM colonization characteristics and the expression of fungal 18S rRNA gene in the two soybean genotypes HN89 and HN112. Soybean plants were cultivated with different AM inocula for 30 days. (A) AM colonization; (B) expression of fungal 18S rRNA gene; (C) intraradical hypha (IR) colonization; (D) arbuscule (Arb.) colonization; (E) vesicle (Ves.) colonization. Data are the mean of four biological replicates with standard error. Different letters indicate significant differences among AM treatments and genotypes (ANOVA followed by Duncan's multiple range test, $P \leq 0.05$).

for each sample, and the total length of clean reads reached 1.2×10^9 bp with quality exceeding Q20 for 98% of the bases (Table S2). Of the 56 044 genes, that are annotated in soybean database, 77, 78, 78 and 77% were detected as being expressed in roots inoculated with NM, Ri, Ga-H and Ga-L, respectively. The number of expressed genes was significantly higher in Ga-H roots than those in NM and Ga-L roots (Fig. S1).

To evaluate how different AM inoculation affects transcriptional responses in soybean root systems, the expression of transcripts was investigated in NM and AM roots. Using NM roots as a control, DEGs characterized by a $\log_2$ fold change with an absolute value of at least 1.0 and a probability of at least 0.8 were identified in Ri, Ga-H and Ga-L roots and then compared in order to highlight specific and shared expression patterns in each AM inoculation treatment. In total, 797 DEGs were simultaneously upregulated in all AM treatments, while 68 DEGs were downregulated. In contrast, 146 transcripts were upregulated, while 147 transcripts were downregulated specifically in Ri and Ga-H roots, but not in Ga-L roots, and 265 and 105 genes were specifically up- and downregulated in Ri roots (Fig. S2). The

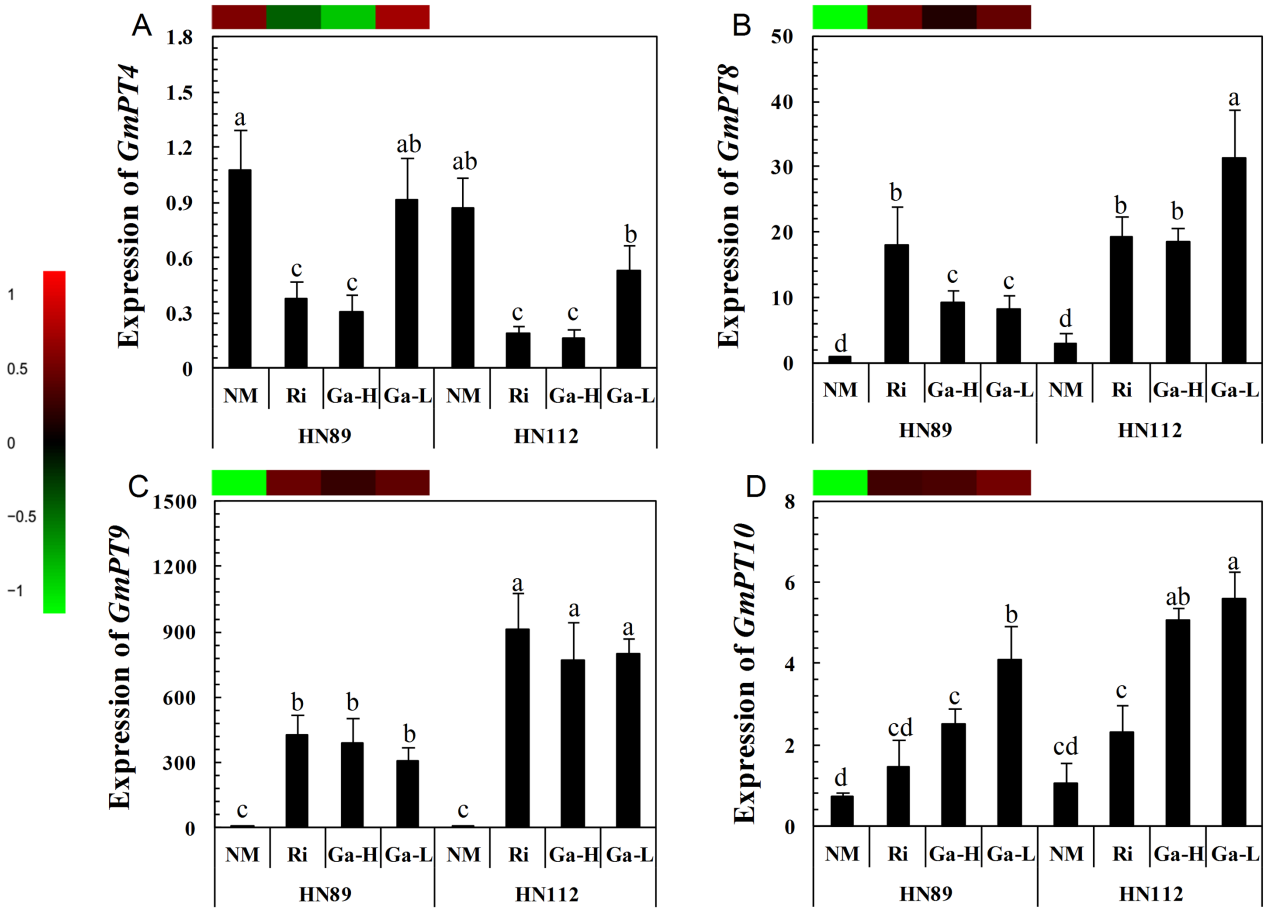

Fig. 4. Experimental validation of selected DEGs expression by qRT-PCR. qRT-PCR analysis was performed for four Pi transporter genes identified as DEGs in RNA-seq analysis of HN89 soybean roots subjected to different AM fungal inoculation treatments. (A) *GmPT4*; (B) *GmPT8*; (C) *GmPT9*; (D) *GmPT10*. The color of the bars above each AM treatment for HN89 represents the FPKM values observed in RNA-seq analysis, with the bar on the left side of the figure showing the relation between FPKM value and color. Red and green indicate higher and lower expression values, respectively. Values from RNA-seq analysis are the means of three biological replicates. Four biological replications were included in the qRT-PCR analysis of each gene in both soybean genotypes. Different letters indicate significant differences among AM treatments and genotypes (ANOVA followed by Duncan's multiple range test, $P \leq 0.05$).

growth differences among Ri, Ga-H and Ga-L treatments might be attributed to these differentially regulated transcripts.

Validation of the DEG results by qRT-PCR assays

qRT-PCR analysis was used to confirm the differential expression profiles observed in high-throughput sequencing for four genes (Fig. 4), including the low-P-induced Pi transporter gene *GmPT4*, and AM-induced Pi transporter genes, *GmPT8*, *GmPT9* and *GmPT10* (Wang et al. 2016). In both soybean genotypes, qRT-PCR analysis confirmed that *GmPT4* had higher expression in NM and Ga-L roots than those of Ri and Ga-H, while the expression of *GmPT8* and *GmPT9* was higher in AM roots than in NM roots (Fig. 4). The expression of *GmPT4* was higher in NM and Ga-L treatments compared with Ri and Ga-H treatments (Fig. 4A). *GmPT8* was most highly expressed in Ri roots (Fig. 4B), while *GmPT10* transcripts were most abundant in Ga-L roots (Fig. 4D). The strong correlation between the RNA-seq and qRT-PCR data of HN89 roots indicates that the transcriptomic profiling data were largely reliable (Fig. 4).

GO and pathway enrichment analyses of DEGs

GO enrichment analysis results of 797 upregulated genes in all AM roots, and 146 upregulated genes and 147 downregulated genes in Ri and Ga-H roots were further assigned to three categories: biological processes, cellular components and molecular functions (Figs S3

and S4). For biological processes, the transcripts upregulated in all AM roots were mainly related to proteolysis, defense response, response to biotic stimulus (Fig. S3). In contrast, the transcripts upregulated only in Ri and Ga-H roots were, within this category, largely associated with response to oxidative stress, oxidation–reduction process and transmembrane transport (Fig. S4A). Interestingly, the transcripts downregulated only in Ri and Ga-H roots were within the biological process category also mainly associated with response to oxidative stress (Fig. S4B). However, the degree of GO enrichment was greater for upregulated than downregulated DEGs in Ri and Ga-H roots (Fig. S4). With respect to cellular components, only two significantly enriched GO terms, membrane and ubiquitin ligase complex, were targeted by upregulated transcripts in all AM roots, whereas four significantly enriched GO terms were targeted by upregulated transcripts in Ri and Ga-H roots, but not in Ga-L roots, including integral component of membrane, intrinsic component of membrane, membrane and membrane part (Figs S3 and S4A). Only one significantly enriched GO term, membrane, was targeted by downregulated transcripts in Ri and Ga-H roots (Fig. S4B). This indicates that the upregulated transcripts related to membrane components were more active during Ri and Ga-H inoculation. Molecular functions were also active during AM inoculation. The differentially upregulated transcripts were enriched in peptidase activity, electron carrier activity and carboxypeptidase activity in all AM roots (Fig. S3). In contrast, the top three key GO terms of upregulated DEGs enriched only in Ri and Ga-H roots were antioxidant activity, peroxidase activity and oxidoreductase activity, while the dominant GO term of downregulated DEGs was 'hydrolase activity' enriched only in Ri and Ga-H roots (Fig. S4). Similarly, the degree of GO enrichment was greater for upregulated than downregulated DEGs (Fig. S4).

Although both Ri and Ga-H plants showed positive growth responses, MGR and MPR of Ri plants were significantly higher than Ga-H plants. Therefore, the differentially expressed transcripts between Ri and Ga-H roots were also analyzed using the GO enrichment analysis (Fig. S5). Totally, 127 upregulated and 181 downregulated DEGs in Ri roots relative to Ga-H roots were categorized into the two main categories of GO classification: biological processes and molecular functions. For biological process category, proteolysis and response to oxidative stress, and cellular homeostasis were the top abundant subcategories of upregulated and downregulated DEGs, respectively. Under the molecular function category, peptidase activity for upregulated DEGs, and heme and tetrapyrrole binding for downregulated DEGs were the top abundant subcategories (Fig. S5). The function of these DEGs might be associated with the differences in MGR and MPR between Ri and Ga-H plants.

The DEGs involved in pathways were further characterized in the KEGG database. A scatter plot was generated for the enriched results as shown in Fig. 5. In total, 40 KEGG-enriched pathways were observed for upregulated DEGs in all AM roots relative to NM roots (Fig. 5A). Among them, fatty acid biosynthesis was the most obvious enriched pathway. The enrichment factor for fatty acid biosynthesis reached 6.19, and the *P*-value was approximately zero. The enriched pathway included some key genes, which are involved in the biosynthesis and metabolism of lipids in AM plants recently reported by Jiang et al. (2017), such as genes encoding *FATTY ACYL-ACP THIOESTERASE* M (FatM), *KETOACYL-ACP SYNTHASE* II (KASII) and *MALONYL CoA-ACP TRANSACYLASE* (MCAT; Table S3). In contrast, only nine KEGG-enriched pathways were identified for the upregulated DEGs in both Ri and Ga-H roots, but not in Ga-L roots (Fig. 5B). Interestingly, at least five significantly enriched KEGG pathways were closely related to sugar metabolism, including pentose phosphate pathway, carbon fixation in photosynthetic organisms, fructose and mannose metabolism, glycolysis/gluconeogenesis and glycan synthesis and metabolism (Fig. 5B).

Specific transcriptional responses triggered by Ri and Ga-H inoculation

On the basis of the data obtained using the analyses of GO function and pathway enrichment, another in-depth analysis of the DEGs was conducted between Ri and Ga-H, and Ga-L roots. We focused on three groups of upregulated DEGs, which were related to transport activity, biosynthesis and metabolism of sugar and lipids, and transcription regulation, and might be responsible for the differences in growth observed among these three AM inoculation treatments (Figs 1 and 2).

In the transport activity group, the DEGs upregulated only in Ri and Ga-H roots included nitrate, sulfate, sugar, lipid and putative metabolite transporters (Fig. 6, Table S4). Expression of a Pi transporter gene, *GmPHO1*, was also highly induced in Ri and Ga-H roots. Remarkably, the two sugar efflux transporter genes, *GmSWEET6* (Glyma.04G198600) and *GmSWEET15* (Glyma.06G166800) named by Patil et al. (2015), were highly upregulated in Ri and Ga-H roots (Figs 6 and 7A,B, Table S4). The analysis of *GUS* gene expression in mycorrhizal transgenic roots also showed that the activity associated with the *GmSWEET6* promoter was strong in arbuscule-containing cortical cells (Fig. 7C,D).

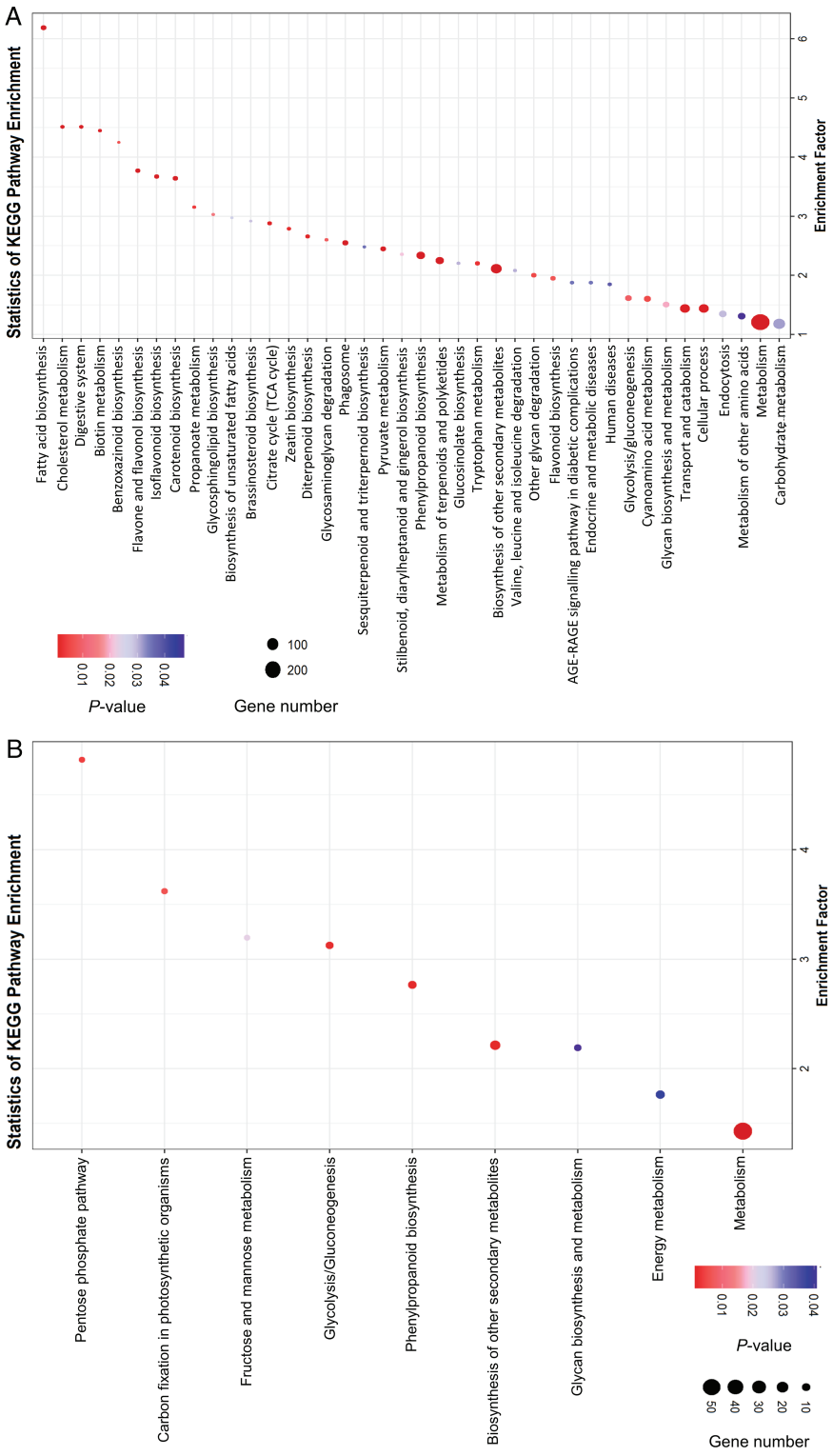

Fig. 5. KEGG enrichment analysis of genes upregulated in AM roots. (A) The pathway enrichment statistics of DEGs upregulated all three AM inoculation treatments. (B) The pathway enrichment statistics of DEGs upregulated only in Ri and Ga-H inoculation treatments. Vertical coordinates represent enriched pathways, and horizontal coordinates represent enrichment factors. The size of each point represents the number of DEGs in the pathway, and the color of the point represents the *P*-value. KEGG pathways with *P*-value < 0.05 were considered significantly enriched.

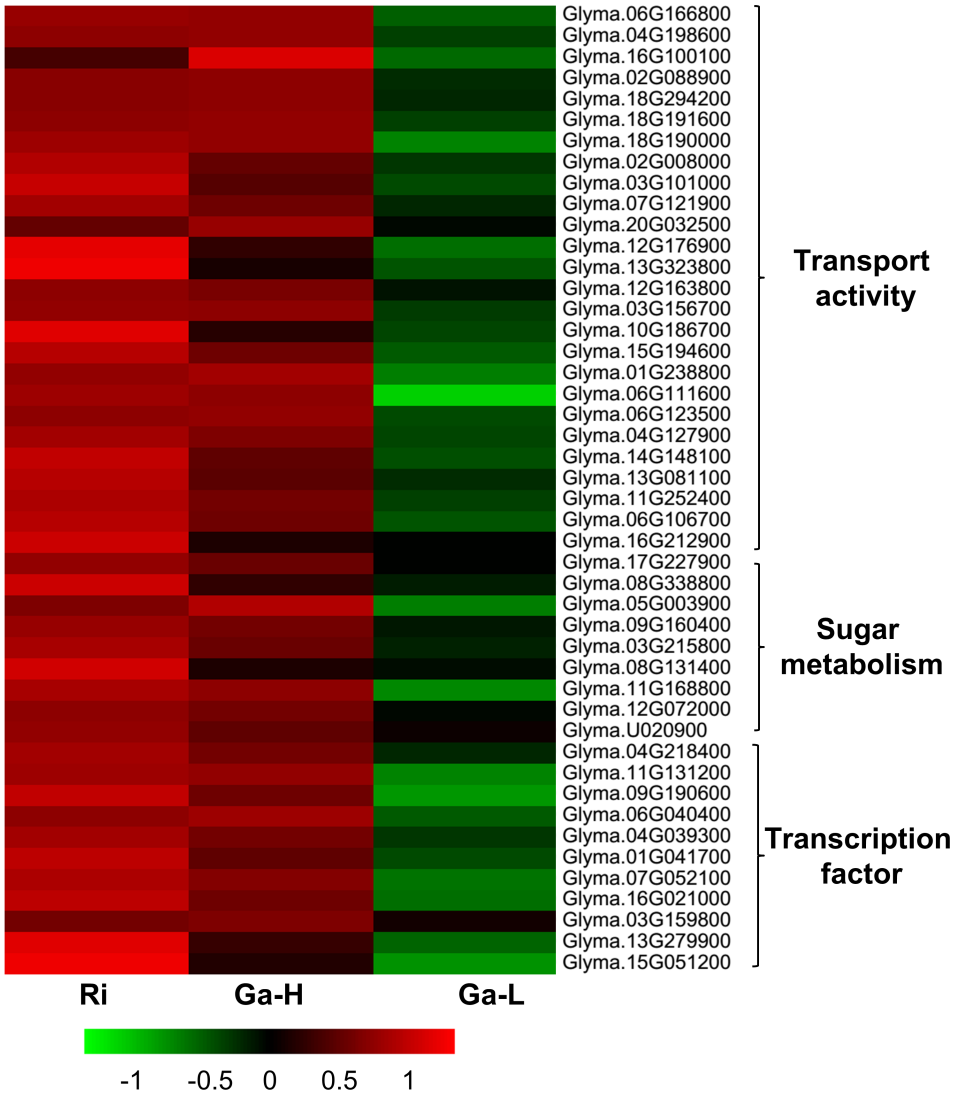

Fig. 6. Heat map of transporter, sugar metabolism and transcription factor genes upregulated in Ri and Ga-H treatments, but not in Ga-L. Red and green indicate higher and lower expression values, respectively. The absolute value of Log_2 fold change ≥1.0 with probability ≥0.8 was used as the threshold for determining significant differential gene expression.

Additional prominent transporter genes induced in Ri and Ga-H roots encode nodulin MtN21/EamA-like transporters, but very limited information is available on this particular transporter gene family. In contrast, the most overrepresented DEGs upregulated in all AM roots were ABCG transporters that are indicative of lipid export from host plants to AM fungi (Table S3; Bravo et al. 2017, Jiang et al. 2017), in addition to three previously reported AM-induced Pi transporter genes, *GmPT8*, *GmPT9* and *GmPT10* (Wang et al. 2016).

Nine genes involved in sugar metabolism were identified in the analysis of Ri and Ga-H specific expression (Fig. 6, Table S4). These included genes encoding invertase, UDP (Uridine diphosphate)-glycosyltransferase, raffinose synthase, alpha-galactosidase, pectinesterase, melibiase, acyl-coenzyme A (Acyl-CoA) N-acyltransferases and beta-N-acetylglucosaminidase. Interestingly, no genes involved in biosynthesis and metabolism of lipids were induced only in Ri and Ga-H inoculation treatments, which was consistent with the results of KEGG enrichment analysis. In contrast, the expression of genes encoding pyruvate kinase (PK) and glycerol-3-phosphate acyltransferase (*REQUIRED FOR ARBUSCULAR MYCORRHIZATION* 2, glycerol-3-phosphate acyltransferase [*RAM2*]) was upregulated in all AM roots (Table S3), which are involved in the biosynthesis and metabolism of lipids in AM plants as recently reported by Jiang et al. (2017).

In transcription regulation group, elevated transcript levels in Ri and Ga-H roots, but not in Ga-L roots, were identified for 11 transcription factors, with these belonging to WRKY family, phytochrome-interacting factor, heat shock transcription factor, abscisic acid responsive elements-binding factor, nucleic acid binding family,

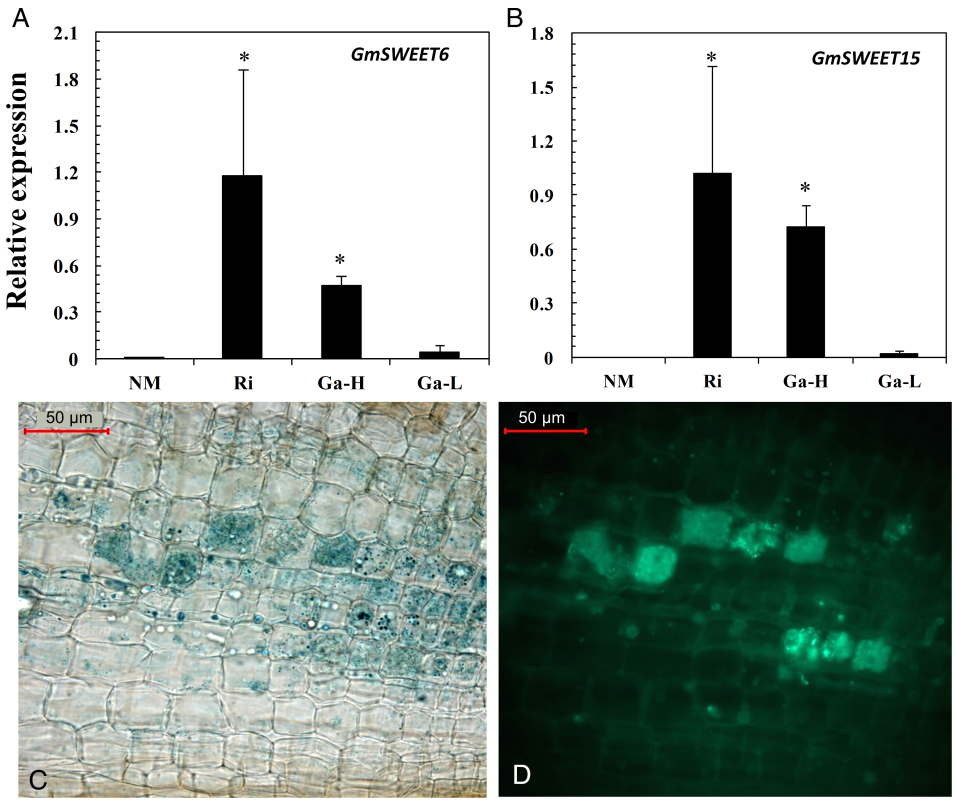

Fig. 7. The expression of *GmSWEET6* (A) and *GmSWEET15* (B), and localization of *GmSWEET6* expression in mycorrhizal soybean roots (C,D). (C) Light micrographs of mycorrhizal roots expressing the *GUS* gene under the control of the *GmSWEET6* promoter. (D) Corresponding fluorescence micrographs showing counterstaining of fungal structures with Alexa Fluor 488 WGA conjugate. Scale bars represent 50 μm.

ethylene responsive element binding factor and NAC (NAM, ATAF1/2, CUC2) family transcription factors (Fig. 6, Table S4). Interestingly, the gene expression of two GRAS-domain transcription factor (RAM1) subfamily members of GRAS (GAI-RGA-SCR) transcription factors was upregulated in all AM roots (Table S3), with a previous report indicating that the expression of these genes is required for the induction of AM-specific lipid biosynthesis (Luginbuehl et al. 2017).

In addition, we found 52 unknown genes upregulated in all AM roots (Table S5) and 14 unknown genes upregulated only in Ri and Ga-H roots (Table S6), which might be also partly responsible for the differences in growth observed among three AM inoculation treatments.

Effect of inoculum treatments on sugar content

Significantly higher fructose and glucose levels were found in roots than in shoots for all plants irrespective of inoculation status (Fig. 8). The plants colonized by Ri and Ga-H contained significantly higher amounts of glucose, fructose and sucrose than Ga-L or NM plants in both shoots and roots (Fig. 8).

Discussion

It is well recognized that specificity in AM symbiosis is very low in comparison to legume-rhizobium interactions. However, plant growth responses resulting from formation of AM associations vary widely among plant species and AM fungal species. Using ^{14}C and ^{33}P as tracers, it has been found that more sucrose is allocated to the cooperative species *R. irregularis* compared with the less cooperative species *G. aggregatum* (Kiers et al. 2011). However, the molecular mechanisms controlling the carbon allocation are poorly understood. In the present study, we present the first evidence that the beneficial effects of AM inoculation on soybean growth might be mostly due to the upregulated expression of specific genes involved in sugar metabolism and transport.

Previous studies have demonstrated that growth and P uptake of both P-efficient soybean genotype HN89 with shallow root architecture and P-inefficient soybean genotype HN112 with deep root architecture are promoted when their roots are colonized by *R. irregularis*, but not by *G. aggregatum* colonization (Wang et al. 2016). In the present study, we confirmed that Ri inoculation leads to the largest gains in growth and P uptake for the two soybean genotypes contrasting in P efficiency

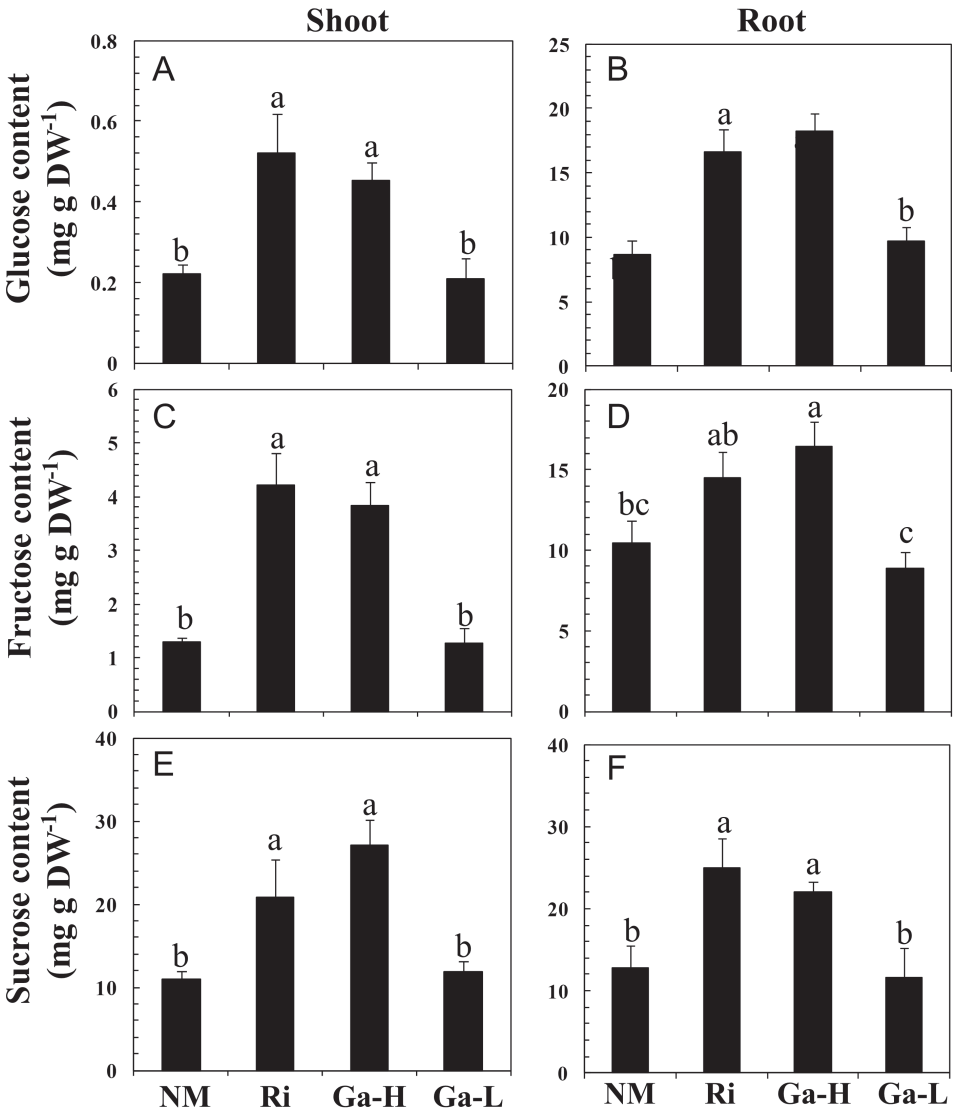

Fig. 8. Effects of AM treatments on sugar content in soybean genotype HN89. Soybean plants were cultivated with different AM inocula for 30 days. (A) Shoot glucose content; (B) root glucose content; (C) shoot fructose content; (D) root fructose content; (E) shoot sucrose content; (F) root sucrose content. Data are the mean of four biological replicates with standard error. Different letters indicate significant differences among AM treatments and genotypes (ANOVA followed by Duncan's multiple range test, $P \leq 0.05$).

(Fig. 1). Moreover, we demonstrated that Ga inoculation may not always produce little or no benefits to host plant growth. Inoculation by Ga-H obviously increased plant DW and P content compared with those colonized by Ga-L or no mycorrhizal colonization in both P-efficient and P-inefficient soybean genotypes (Fig. 1). Consistently, the highest MGR and MPR were observed in Ri, followed by Ga-H plants, and then Ga-L plants (Fig. 2). *G. aggregatum*, as a less cooperative fungus, was also found in *M. truncatula*, *Allium porrum* and carrot roots (Kiers et al. 2011). *R. irregularis* and *G. aggregatum* exhibited either high or low levels of cooperation based on plant growth responses, costs of carbon per unit P transferred, and resource hoarding strategies (Kiers et al. 2011). These results indicate that both plants and fungi may be able to detect variation in cooperative behavior of their partners, allowing them to adjust their own resource allocation accordingly.

It has been postulated that both the regulation of Pi uptake and the high carbon costs of AM symbiosis may explain different plant growth responses to these interactions (Smith et al. 2009, 2011, Kiers et al. 2011). The expression of the root-specific and low-P inducible Pi transporter gene *GmPT4* was suppressed in Ri and Ga-H roots compared with those in Ga-L and NM roots (Fig. 4A). This indicates that the direct pathway for P uptake is suppressed or less activated when soybean plants are colonized by responsive AM fungi, whereas plants exposed to unresponsive AM fungi remain capable of direct P uptake. The direct and via AM pathways for P uptake are not additive but complementary (Smith et al. 2009, 2011). However, 2 AM-inducible

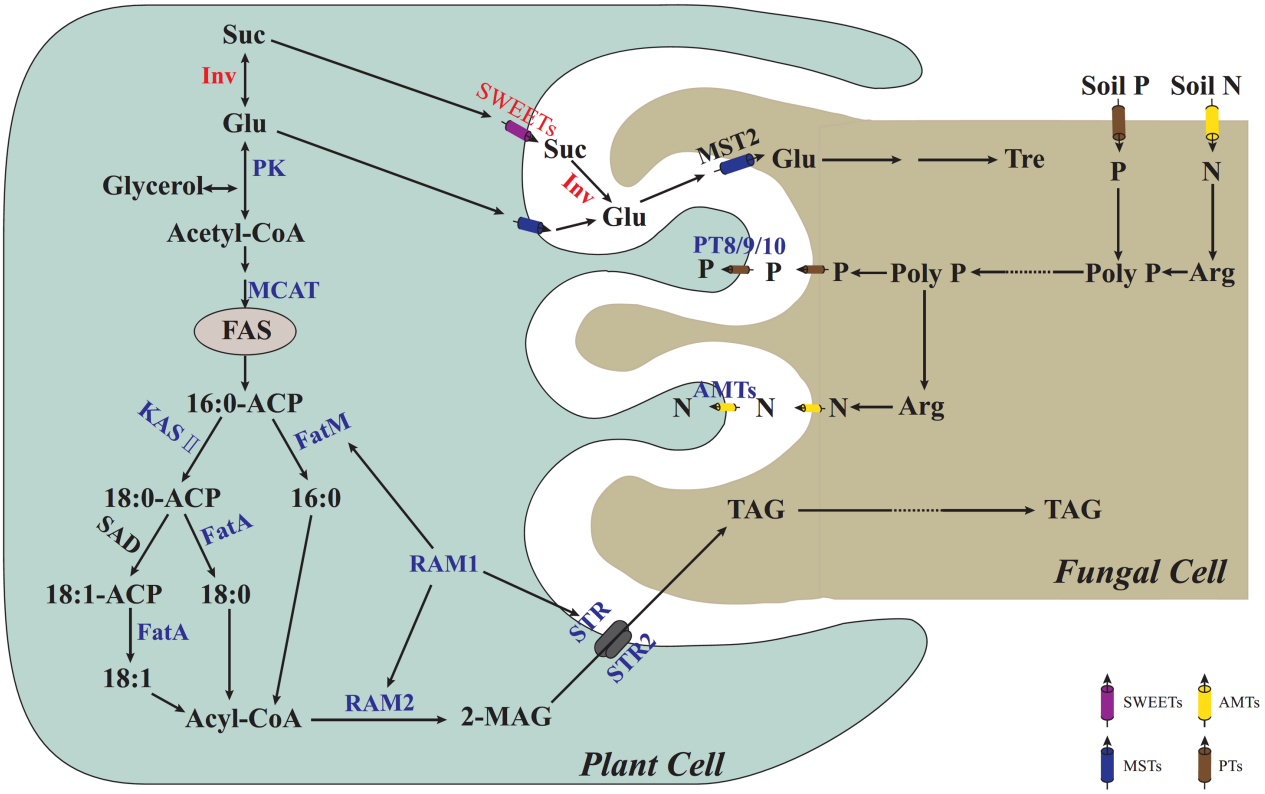

Fig. 9. Schematic model of the involvement of key gene products in metabolism and transport of lipids, sugar and nutrients between AM fungi and plant cells. The genes upregulated in all three AM inoculation treatments are shown in blue letters, while the genes only upregulated in Ri and Ga-H inoculation treatments are shown in red letters. 2-MAG, 2-monoacylglycerol; 16:0, palmitic acid; 16:0-ACP, palmitoyl-acyl carrier protein; 18:0, stearic acid; 18:1, oleic acid; 18:0-ACP, stearoyl-acyl carrier protein; 18:1-ACP, oleoyl-acyl carrier protein; Acetyl-CoA, acetyl-coenzyme A; AMTs, ammonium transporters; Arg, arginine; FAS, fatty acid synthase; FatA, acyl-ACP thioesterase A; Glu, glucose; Inv, invertase; MST2, a fungal monosaccharide transporter; Poly P: polyphosphate; PT, phosphate transporter; SAD, stearoyl-ACP desaturase; STR, adenosine triphosphate-binding cassette transporter; Suc, sucrose; TAG, triacylglycerol; Tre, trehalose.

Pi transporter genes, *GmPT8* and *GmPT9*, were greatly upregulated in all AM soybean roots, and *GmPT10* was upregulated in both Ga-H and Ga-L roots irrespective of MGRs (Fig. 4B-D). Additionally, soybean plants inoculated with Ga-L showed lower P contents than those inoculated with Ri even though both direct and mycorrhizal uptake pathways were opened (Figs 1, 2 and 4). These observations suggest that the expression level of AM-inducible Pi transporter genes does not always promote P uptake, and is not necessarily correlated with plant growth responsiveness, which is also reported in barley (Grace et al. 2009). Therefore, the expression levels of AM-inducible Pi transporter genes do not explain the differential growth responses observed for soybean plants in AM symbiotic interactions.

The establishment of AM symbiosis significantly altered transcription of genes involved in metabolism and transport of sugar and lipids in all mycorrhizal roots of HN89 plants. It is reported that FatM, RAM2, STR/STR2 appear to form an AM-specific operational unit for lipid biosynthesis and transport in AM symbiosis (Bravo et al. 2017, Jiang et al. 2017, Luginbuehl et al. 2017). In the present study, KEGG-enriched pathway analysis showed that fatty acid biosynthesis pathway was enriched in all AM roots of HN89 plants (Fig. 5A), and genes for FatM, RAM2, KASII, PK, MCAT, and STR/STR2 transporters all displayed enhanced expression (Table S3), indicating that lipids can play important roles in carbon nutrition of the fungal partner in AM symbiosis. In addition, two RAM1 subfamily members of GRAS transcription factors, which might target RAM2 to control lipid export towards AM fungi (Luginbuehl et al. 2017), also showed an upregulated gene expression in roots of HN89 at all AM treatments (Table S3). These results indicate that AM symbiosis triggers metabolism and transport of lipids in soybean roots. However, activation of these genes in all AM treatments demonstrates that regulation of these genes does not explain the differential growth responses observed among Ri, Ga-H and Ga-L plants.

Along these lines, sugar metabolism pathways were significantly enriched in both Ri and Ga-H roots, but not in Ga-L roots (Fig. 5B). Two sugar exporter genes, *GmSWEET6* (Glyma.04G198600) and *GmSWEET15* (Glyma.06G166800) named by SWEET gene family analysis in soybean (Patil et al. 2015), and one invertase gene (Glyma.17G227900) were exclusively induced only in Ri and Ga-H roots (Fig. 6, Table S4). At the plant–fungal interface, sucrose is hydrolyzed by sucrose synthases or invertases before efflux towards the mycobiont (Doidy et al. 2012a, Manck-Götzenberger and Requena 2016). The enhanced expression of the sugar exporter and invertase genes observed herein might regulate the sugar allocation to mycorrhizal fungi (Figs 6 and 7A,B). In Ri roots, *GUS* expression driven by the *GmSWEET6* promoter was quite evident in arbuscule-containing cortical cells (Fig. 7C,D). The differences in growth response among Ri, Ga-H and Ga-L plants might, therefore, primarily be the consequence of the differential expression of these genes. During AM symbiosis, more C is allocated to the cooperative species compared with the less cooperative species (Kiers et al. 2011). In return, the cooperative fungus preferentially allocates P to the host providing more carbohydrates. Therefore, a more cooperative species will obtain more plant C, and thereby induce positive growth and phosphate benefit for the host plant than a less cooperative species. Higher transcript accumulation of *GmSWEET6 and GmSWEET15* genes might help host plants to transfer more C to Ri and Ga-H. This still needs to be further clarified by functional analysis of these genes in future studies. Conversely, the DEGs encoding proteins predicted to be involved in lipid metabolism and transport, such as FatM, RAM2, KASII, PK and STR/STR2 transporters, were not induced by Ri and Ga-H inoculation relative to Ga-L inoculation. These observations indicate that differential growth responses to different AM inoculation treatments might not be attributed to regulation of lipid metabolism and transport. A schematic diagram showing metabolism and transport of sugar and lipids is shown in Fig. 9.

Root colonization by AM fungi adds a new carbon sink to the existing carbon sink in roots, and, therefore, increases the allocation of photosynthate to root systems (Johnson et al. 2002, Kaschuk et al. 2009). Simultaneously, carbon sink strength in mycorrhizal roots can stimulate an increase in the rate of photosynthesis (Kaschuk et al. 2009). In this experiment, soybean plants colonized by Ri and Ga-H contained more glucose, fructose and sucrose in both shoots and roots than either Ga-L or NM plants (Fig. 8). This indicates that the higher degree of AM colonization in Ri and Ga-H inoculation elevates the C sink strength of the colonized root systems, and thereby stimulates an increase in the rate of photosynthesis (Kaschuk et al. 2009), which further induces assimilation and transport of photosynthate in soybean plants. These might be the reason that photosynthate metabolism and transport were significantly enriched in Ri and Ga-H roots of HN89 plants, which might also partly explain the differential growth responses observed among Ri, Ga-H, and Ga-L plants.

It has been reported that the less cooperative *G. aggregatum* forms significantly fewer arbuscules, but significantly more vesicles per root length than the cooperative *R. irregularis* in *M. truncatula*. As a consequence, colonization by *G. aggregatum* results in significantly higher host C costs for more vesicle formation, and thereby inhibits plant growth (Kiers et al. 2011). However, this was not the case in our current study on soybean. The root colonization with arbuscules did not vary in the present study between Ri and Ga plants, while vesicle colonization was obviously higher in Ri roots than in Ga roots (Fig. 3D, E). Therefore, the high C cost of mycorrhizal colonization characteristics cannot be used to explain the differential growth responses among different inoculation treatments observed in soybean.

In conclusion, the present study revealed that Ri and Ga-H inoculation can both significantly promote plant growth and stimulate the assimilation and transport capacity of photosynthate in soybean. Moreover, AM symbiosis triggers considerable reprogramming in the transcriptome of soybean roots, which includes the induction of genes involved in the metabolism and transport of both sugars and lipids. Variations in MGRs of soybean plants might be mostly due to changes in sugar metabolism and transport affected by both the species of AM fungi and the extent of AM colonization.

Author contributions

X.W. conceived the experiments. The experiments were designed and performed by X.W., S.Z., A.C., C.C, C.L. and R.X. and X.W. drafted and edited the manuscript. All authors approved the final version.

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References

Afkhami ME, Stinchcombe JR (2016) Multiple mutualist effects on genomewide expression in the tripartite

association between *Medicago truncatula*, nitrogen-fixing bacteria and mycorrhizal fungi. Mol Ecol 25: 4946–4962
Boldt K, Pörs Y, Haupt B, Bitterlich M, Kühn C, Grimm B, Franken P (2011) Photochemical processes, carbon assimilation and RNA accumulation of sucrose transporter genes in tomato arbuscular mycorrhiza. J Plant Physiol 168: 1256–1263
Bonfante P, Genre A (2010) Mechanisms underlying beneficial plant–fungus interactions in mycorrhizal symbiosis. Nat Commun 1: 48
Bravo A, Brands M, Wewer V, Dörmann P, Harrison MJ (2017) Arbuscular mycorrhiza-specific enzymes FatM and RAM2 fine-tune lipid biosynthesis to promote development of arbuscular mycorrhiza. New Phytol 214: 1631–1645
Cruz C, Green JJ, Watson CA, Wilson F, Martins-Loução MA (2004) Functional aspects of root architecture and mycorrhizal inoculation with respect to nutrient uptake capacity. Mycorrhiza 14: 177–184
Doidy J, Grace E, Kuhn C, Simon-Plas F, Casieri L, Wipf D (2012a) Sugar transporters in plants and in their interactions with fungi. Trends Plant Sci 17: 413–422
Doidy J, van Tuinen D, Lamotte O, Corneillat M, Alcaraz G, Wipf D (2012b) The *Medicago truncatula* sucrose transporter family: characterization and implication of key members in carbon partitioning towards arbuscular mycorrhizal fungi. Mol Plant 5: 1346–1358
Durso NA, Cyr RJ (1994) A calmodulin-sensitive interaction between microtubules and a higher plant homolog of elongation factor-1α. Plant Cell 6: 893–905
Fellbaum CR, Mensah JA, Cloos AJ, Strahan GE, Pfeffer PE, Kiers ET, Bücking H (2014) Fungal nutrient allocation in common mycelia networks is regulated by the carbon source strength of individual host plants. New Phytol 203: 645–656
Garcia-Rodriguez S, Azcon-Aguilar C, Ferrol N (2007) Transcriptional regulation of host enzymes involved in the cleavage of sucrose during arbuscular mycorrhizal symbiosis. Physiol Plant 129: 737–746
Gómez-González S, Ruiz-Jiménez J, Priego-Capote F, Luque de Castro MD (2010) Qualitative and quantitative sugar profiling in olive fruits, leaves, and stems by gas chromatography–tandem mass spectrometry (GC-MS/MS) after ultrasound-assisted leaching. J Agr Food Chem 58: 12292–12299
Grace EJ, Cotsaftis O, Tester M, Smith FA, Smith SE (2009) Arbuscular mycorrhizal inhibition of growth in barley cannot be attributed to extent of colonization, fungal phosphorus uptake or effects on expression of plant phosphate transporter genes. New Phytol 181: 938–949
Graham JH, Abbott LK (2000) Wheat responses to aggressive and nonaggressive arbuscular mycorrhizal fungi. Plant and Soil 220: 207–218
Guo WB, Zhao J, Li XX, Qin L, Yan XL, Liao H (2011) A soybean β-expansin gene GmEXPB2 intrinsically involved in root system architecture responses to abiotic stresses. Plant J 66: 541–552
Gutjahr C, Sawers RJH, Marti G, Andrés-Hernández L, Yang SY, Casieri L, Angliker H, Oakeley EJ, Wolfender JL, Abreu-Goodger C, Paszkowski U (2015) Transcriptome diversity among rice root types during asymbiosis and interaction with arbuscular mycorrhizal fungi. Proc Natl Acad Sci USA 112: 6754–6759
Harrison MJ, Dewbre GR, Liu J (2002) A phosphate transporter from *Medicago truncatula* involved in the acquisition of phosphate released by arbuscular mycorrhizal fungi. Plant Cell 14: 2413–2429
Hetrick BAD, Wilson GWT, Cox TS (1992) Mycorrhizal dependence of modern wheat varieties, landraces, and ancestors. Can J Bot 70: 2032–2040
Hogekamp C, Arndt D, Pereira PA, Becker JD, Hohnjec N, Küster H (2011) Laser microdissection unravels cell-type-specific transcription in arbuscular mycorrhizal roots, including CAAT-box transcription factor gene expression correlating with fungal contact and spread. Plant Physiol 157: 2023–2043
Hohnjec N, Vieweg MF, Pühler A, Becker A, Küster H (2005) Overlaps in the transcriptional profiles of *Medicago truncatula* roots inoculated with two different Glomus fungi provide insights into the genetic program activated during arbuscular mycorrhiza. Plant Physiol 137: 1283–1301
Jakobsen I, Rosendahl L (1990) Carbon flow into soil and external hyphae from roots of mycorrhizal cucumber plants. New Phytol 115: 77–83
Jiang Y, Wang W, Xie Q, Liu N, Liu L, Wang D, Zhang X, Yang C, Chen X, Tang D, Wang E (2017) Plants transfer lipids to sustain colonization by mutualistic mycorrhizal and parasitic fungi. Science 356: 1172–1175
Johnson D, Leake JR, Ostle N, Ineson P, Read DJ (2002) In situ $^{13}CO_2$ pulse labeling of upland grassland demonstrates a rapid pathway of carbon flux from arbuscular mycorrhizal mycelia to the soil. New Phytol 153: 327–334
Kaschuk G, Kuyper TW, Leffelaar PA, Hungria M, Giller KE (2009) Are the rates of photosynthesis stimulated by the carbon sink strength of rhizobial and arbuscular mycorrhizal symbioses? Soil Biol Biochem 41: 1233–1244
Kiers ET, Duhamel M, Beesetty Y, Mensah JA, Franken O, Verbruggen E, Fellbaum CR, Kowalchuk GA, Hart MM, Bago A, Palmer TM, West SA, Vandenkoornhuyse P, Jansa J, Bücking H (2011) Reciprocal rewards stabilize cooperation in the mycorrhizal symbiosis. Science 333: 880–882
Kim D, Langmead B, Salzberg SL (2015) HISAT: a fast spliced aligner with low memory requirements. Nat Methods 12: 357–360

Klironomos JN (2003) Variation in plant response to native and exotic arbuscular mycorrhizal fungi. Ecology 84: 2292–2301
Li B, Dewey CN (2011) RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. BMC Bioinformatics 12: 323
Li HY, Smith SE, Holloway RE, Zhu YG, Smith FA (2006) Arbuscular mycorrhizal fungi contribute to phosphorus uptake by wheat grown in a phosphorus-fixing soil even in the absence of positive growth responses. New Phytol 172: 536–543
Li C, Zhang H, Wang X, Liao H (2014) A comparison study of *Agrobacterium*-mediated transformation methods for root-specific promoter analysis in soybean. Plant Cell Rep 33: 1921–1932
Li X, Zhao J, Tan Z, Zeng R, Liao H (2015) GmEXPB2, a cell wall *β*-expansin, affects soybean nodulation through modifying root architecture and promoting nodule formation and development. Plant Physiol 169: 2640–2653
Lisec J, Schauer N, Kopka J, Willmitzer L, Fernie AR (2006) Gas chromatography mass spectrometry-based metabolite profiling in plants. Nat Protoc 1: 387–396
Luginbuehl LH, Menard GN, Kurup S, Van EH, Radhakrishnan GV, Breakspear A, Oldroyd GED, Eastmond PJ (2017) Fatty acids in arbuscular mycorrhizal fungi are synthesized by the host plant. Science 356: 1175–1178
Manck-Götzenberger J, Requena N (2016) Arbuscular mycorrhiza symbiosis induces a major transcriptional reprogramming of the potato *SWEET* sugar transporter family. Front Plant Sci 7: 487
McGonigle TP, Miller MH, Evans DG, Fairchild GL, Swan JA (1990) A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. New Phytol 115: 495–501
Nunes AC, Vianna GR, Cuneo F, Amaya-Farfán J, de Capdeville G, Rech EL, Aragão FJ (2006) RNAi-mediated silencing of the myo-inositol-1-phosphate synthase gene (*GmMIPS1*) in transgenic soybean inhibited seed development and reduced phytate content. Planta 224: 125–132
Paszkowski U, Kroken S, Roux C, Briggs SP (2002) Rice phosphate transporters include an evolutionarily divergent gene specifically activated in arbuscular mycorrhizal symbiosis. Proc Natl Acad Sci USA 99: 13324–13329
Patil G, Valliyodan B, Deshmukh R, Prince S, Nicander B, Zhao M, Sonah H, Song L, Lin L, Chaudhary J, Liu Y, Joshi T, Xu D, Nguyen HT (2015) Soybean (*Glycine max*) SWEET gene family: insights through comparative genomics, transcriptome profiling and whole genome re-sequence analysis. BMC Genomics 16: 520
Pringle A, Bever JD (2008) Analogous effects of arbuscular mycorrhizal fungi in the laboratory and a North Carolina field. New Phytol 180: 162–175
Ravnskov S, Wu Y, Graham JH (2003) Arbuscular mycorrhizal fungi differentially affect expression of genes coding for sucrose synthases in maize roots. New Phytol 157: 539–545
Schubert A, Allara P, Morte A (2004) Cleavage of sucrose in roots of soybean (*Glycine max*) colonized by an arbuscular mycorrhizal fungus. New Phytol 161: 495–501
Sisaphaithong T, Kondo D, Matsunaga H, Kobae Y, Hata S (2012) Expression of plant genes for arbuscular mycorrhiza-inducible phosphate transporters and fungal vesicle formation in sorghum, barley and wheat roots. Biosci Biotech Bioc 76: 2364–2367
Smith SE, Read D (2008) Mycorrhizal Symbiosis, 3rd Edn. Academic Press, San Diego, CA
Smith FA, Grace EJ, Smith SE (2009) More than a carbon economy: nutrient trade and ecological sustainability in facultative arbuscular mycorrhizal symbioses. New Phytol 182: 347–358
Smith SE, Jakobsen I, Grønlund M, Smith FA (2011) Roles of arbuscular mycorrhizas in plant phosphorus nutrition: interactions between pathways of phosphorus uptake in arbuscular mycorrhizal roots have important implications for understanding and manipulating plant phosphorus acquisition. Plant Physiol 156: 1050–1057
Wang XR, Pan Q, Chen FX, Yan XL, Liao H (2011) Effects of co-inoculation with arbuscular mycorrhizal fungi and rhizobia on soybean growth as related to root architecture and availability of N and P. Mycorrhiza 27: 173–181
Wang XR, Zhao SP, Bücking H (2016) Arbuscular mycorrhizal growth responses are fungal specific but do not differ between soybean genotypes with different phosphorus efficiency. Ann Bot 118: 11–21
Wickham H (2016) Ggplot2: Elegant Graphics for Data Analysis. Springer, New York
Zhang S, Zhou J, Wang GH, Wang XR, Liao H (2015) The role of mycorrhizal symbiosis in aluminum and phosphorus interactions in relation to aluminum tolerance in soybean. Appl Microbiol Biot 99: 10225–10235

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Appendix S1. List of the genes specifically expressed in each mycorrhizal association.

Appendix S2. ANOVA analysis and RNA-seq data.

Table S1. F-values from ANOVA of the effects of inoculation treatment, genotype and their interaction on soybean growth, AM colonization characteristics and gene expression.

Table S2. Overview of RNA-seq data.

Table S3. List of the main annotated genes simultaneously upregulated in mycorrhizal roots inoculated by *Rhizophagus irregularis*, *Glomus aggregatum* with high AM colonization and *G. aggregatum* with low AM colonization.

Table S4. List of the main annotated genes upregulated in mycorrhizal roots inoculated by *Rhizophagus irregularis* and *Glomus aggregatum* with high AM colonization, but not by *G. aggregatum* with low AM colonization.

Table S5. List of the main unknown genes simultaneously upregulated in mycorrhizal roots inoculated by *Rhizophagus irregularis*, *Glomus aggregatum* with high AM colonization and *G. aggregatum* with low AM colonization.

Table S6. List of the main unknown genes upregulated in mycorrhizal roots inoculated by *Rhizophagus irregularis* and *Glomus aggregatum* with high AM colonization, but not by *G. aggregatum* with low AM colonization.

Fig. S1. Number of expressed genes in different inoculation treatments.

Fig. S2. The number of the genes upregulated or downregulated in soybean roots harvested from different inoculation treatments.

Fig. S3. GO enrichment analysis of genes upregulated in roots of all AM treatments.

Fig. S4. GO enrichment analysis of genes upregulated or downregulated only in Ri and Ga-H roots compared with NM roots.

Fig. S5. GO enrichment analysis of genes upregulated and downregulated in Ri roots relative to Ga-H roots.

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