Plants transfer lipids to sustain colonization by mutualistic mycorrhizal and parasitic fungi

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Arbuscular mycorrhizal (AM) fungi facilitate plant uptake of mineral nutrients and draw organic nutrients from the plant. Organic nutrients are thought to be supplied primarily in the form of sugars. Here we show that the AM fungus *Rhizopogon irregularis* is a fatty acid auxotroph and that fatty acids synthesized in the host plants are transferred to the fungus to sustain mycorrhizal colonization. The transfer is dependent on RAM2 (REQUIRED FOR ARBUSCULAR MYCORRHIZATION 2) and the ATP binding cassette transporter–mediated plant lipid export pathway. We further show that plant fatty acids can be transferred to the pathogenic fungus *Golovinomyces cichoracearum* and are required for colonization by pathogens. We suggest that the mutualistic mycorrhizal and pathogenic fungi similarly recruit the fatty acid biosynthesis program to facilitate host invasion.

A bout 80 to 90% of plant species are colonized by arbuscular mycorrhizal (AM) fungi, which facilitate the uptake of mineral nutrients such as phosphate and nitrogen from the soil (1–3). Evidence suggests that the fungus receives carbon from the plant in the form of sugars (hexoses) in return (4–7); however, plants could transfer alternative carbon sources. The AM fungal genome of *Rhizopogon irregularis* lacks genes encoding type I multidomain fatty acid synthases (FASs), which synthesize palmitic acid (C16:0) in fungi (8–10). Although the mitochondrial type II FAS genes are present in *R. irregularis*, the genes support production of octanoic acid (C8:0) rather than palmitic acid (10–12).

To investigate whether AM fungi can synthesize fatty acids de novo, we performed isotope labeling experiments. Isotope ratios can be used as tracers to understand complex substrate metabolism (fig. SIA) (13). We used the 1. *R. irregularis–carrot* (*Daucus carota*) root monoxenic culture system in divided petri plates, which prevents diffusion of nonvolatile solutes between compartments (fig. S1, A to C) (4, 5, 7). When isotopically labeled [1,3-13C]glycerol was added to the fungal extraradical mycelium (ERM) (fig. S1B), we found that 10.52 ± 2.02% and 3.47 ± 0.53% of glycerol moieties in the ERM and IRM/R, respectively, were labeled at the fragment containing C2 and C3 atoms (Fig. 1A), indicating that glycerol absorbed by the ERM can be transferred between it and the IRM/R. Also, 6.54 ± 0.73% and 1.01 ± 0.21% of glucose moieties in the ERM and IRM/R, respectively, were labeled at the fragment containing C2 and C3 atoms (Fig. 1A), indicating that fatty acids synthesized in the host plants are transferred to the fungus to sustain mycorrhizal colonization. The transfer is dependent on RAM2 (REQUIRED FOR ARBUSCULAR MYCORRHIZATION 2) and the ATP binding cassette transporter–mediated plant lipid export pathway. We further show that plant fatty acids can be transferred to the pathogenic fungus *Golovinomyces cichoracearum* and are required for colonization by pathogens. We suggest that the mutualistic mycorrhizal and pathogenic fungi similarly recruit the fatty acid biosynthesis program to facilitate host invasion.

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(1, 4, 5) Overexpression of *MtPK*, *MtKAS II*, *MtKAR*, and *MtFatM* increased AM colonization, whereas knockdown of these genes reduced AM colonization (Fig. 1, C and D, and fig. S2, C and D). Furthermore, a *MtFatM* mutant obtained from a *Tnt1*-insertion line was impaired in fungal colonization (fig. S3, A and B), consistent with previous observations (16). *MtFatM* is expressed in arbuscle-containing cells, revealed by a β-glucuronidase (GUS) reporter driven by the *MtFatM* promoter (fig. S5, C and D). Thus, *M. truncatula* fatty acid biosynthesis genes support AM symbiosis.

To determine whether fatty acids synthesized in the host plant can be transferred to the AM fungus, we genetically engineered lauric acid (C12:0 fatty acid) synthesis in *M. truncatula* roots. *Umbellularia californica* lauroyl-ACP thioesterase (*UcFatB*) terminates acyl-chain elongation in fatty acid biosynthesis early by releasing the medium-chain fatty acid, lauric acid, from ACP (17). We found that overexpression of *UcFatB* increased the amount of lauric acid to 2.5 mol % in root fatty acids, representing a >25-fold increase relative to the empty vector–transformed control (Fig. 1E and fig. S4). *Cis*-palmitic acid (C16:0) is normally absent from plant roots and has been used as an AM fungal marker (6). Plants overexpressing *UcFatB* displayed a >12-fold increase compared with the empty vector–transformed control in fungal lauric acids or triglyceride (TAG) (C14:2, C16:1–C16:1–C12:0) containing C16:1o5 fatty acid in addition to C12:0 in the ERM or IRM/R (Fig. 1F and fig. S4). This result supports that fatty acids are transferred from the host root to the AM fungus.

*RAM2* synthesizes 2-monoyglycerols for accumulation of extracellular lipid polyesters such as cutin on the surface of shoot organs (18–20). Mutant *ram2* plants are deficient in AM fungal colonization when grown as a monoculture (individual plant) (20) or cultivated together with another *ram2* plant (Fig. 2A). However, this mutant can be colonized with high expression of *PHOSPHATE TRANSPORTER 4* by *R. irregularis* when cultivated together with a wild-type nurse plant to increase inoculum strength, although *ram2* roots contain fewer fully developed arbuscules than wild-type roots in nurse plants (Fig. 2, A and B, and fig. S5). The fact that *R. irregularis* colonization of *ram2* roots occurs in the presence of a nurse plant (Fig. 2, A and B) allowed us to test whether *RAM2* is required for the accumulation of plant-derived fatty acids in AM fungi. When we expressed *UcFatB* in *ram2* roots (*ram2–UcFatB*), the fungal TAG (C14:2) and lauric acid did not accumulate in the IRM/R and ERM associated with these plants in the presence of a wild-type nurse plant (Fig. 2, C and D, and fig. S6). These data suggest that 2-monoyglycerols synthesized by *RAM2* are required for the transport of fatty acid species from the root to the AM fungus.

The heterodimeric adenosine triphosphate (ATP)–binding cassette (ABC) transporters STR (stunted arbuscule) and STR2 are specifically localized in the peri-arbuscular membrane and are required for AM symbiosis (21, 22). STR and STR2 are half-transporter proteins of the ABCG subfamily, of which ABCG11 and -12 are required.
for the accumulation of wax and cutin (23). Co-overexpressing STR and STR2 in Medicago roots and Arabidopsis leaves led to higher accumulation of extracellular lipid polyesters, such as cutin monomers (Fig. 3A and figs. S7 and S8). The effect was limited to specific monomers—notably, 16:0-c, o-dicarboxylic acid (DCA) and 18:2-DCA (Fig. 3B). Consistent with the low level of STR and STR2 expression under nonmycorrhizal conditions (21), we found no differences in the composition of cutin monomers in leaves or roots of wild-type and str plants without mycorrhizal infection (fig. S9). The R. irregularis colonization of str roots occurs with more fully developed arbuscules in the presence of a nurse plant than when str is grown as a monoculture (fig. S10), reminiscent of the ram2 phenotype. When we expressed UcFatB in str roots (str-UcFatB), the fungal TAG (C44:2) and lauric acid content of WT-EV, WT-UcFatB, ram2-EV, and ram2-UcFatB plants. ram2 plants grew in the presence of a WT nurse plant (fig. S6A). WT plants grew as a monoculture. ram2 plants are deficient in AM fungal colonization when cultivated together with another ram2 plant (A). The ERM was collected from R. irregularis—carrot root culture system. (C) Lauric acid content of UcFatB-overexpressing (UcFatB) and empty vector (EV) roots without mycorrhizal fungus infection, expressed as mole percent of total fatty acids. (F) Lauric acid content in the ERM in the UcFatB-overexpressing and EV plant inoculation system (fig. S4A). The ERM was collected from R. irregularis colonization sand at 42 dpi. These experiments were repeated three times with similar results. Values are the mean ± SE of measurements from three independent plates, 8 to 12 plants of each genotype, or the ERM from six pots. *P < 0.05; **P < 0.01; ns, not significant (Student’s t test).
acid did not accumulate in the IRM/R and ERM associated with these plants in the presence of a wild-type nurse plant (Fig. 3, C and D, and fig. S11). Altogether, we conclude that the heterodimeric ABC transporters STR and STR2 are responsible for delivery of lipids to the AM fungus. Powderly mildew (PM) and Ustilago maydis fungi are parasitic pathogens that infect a large number of plant species. We noticed that these pathogenic fungi induce expression of fatty acid biosynthesis genes in Arabidopsis and maize, respectively (fig. S12A and table S2) (24, 25). The induction of KAR and KAS I by PM was further verified by a GUS reporter driven by the AtKAR and AtKAS I promoters in Arabidopsis (fig. S12B). Columbia-0 (Col-0) plants are susceptible to the PM pathogen Golovinomyces cichoracearum UCSC1, supporting the production of a large number of spores (26, 27) that can be used for lipid analysis. We expressed UcFatB in Col-0 and observed a 3- to 4.5-fold increase relative to the empty vector–transformed control in lauric acid of G. cichoracearum fatty acids associated with UcFatB overexpression in Arabidopsis leaves (Fig. 4, A and B, and fig. S13). Furthermore, we found that kas1, kar1, and fatb-1 mutant plants were smaller and showed enhanced disease resistance compared with wild-type plants: Fungal colonies on mutant leaves had fewer spores and conidiophores (Fig. 4, C and D, and fig. S14). Clones containing KAS I, KAR, and FatB genes complemented the kas1, kar1, and fatb-1 mutations, respectively (fig. S15). Altogether, our data indicate that G. cichoracearum can take up fatty acids from the plant and that reduced plant fatty acid biosynthesis impairs pathogenic fungal infection.

Fig. 3. STR and STR2 mediate lipid export from the plant to the AM fungus. (A and B) Cutin analysis of total load (A) and monomer load (B) in EV and STR-STR2 co-overexpression (STR-STR2-OE) roots. Each value is the mean ± SE of measurements performed on 15 to 20 plants. FW, fresh weight. (C) Lauric acid content of WT-EV, WT-UcFatB, str-EV, and str-UcFatB hairy roots without R. irregularis infection. (D) Lauric acid content in ERM associated with WT-EV, WT-UcFatB, str-EV, and str-UcFatB plants. DCA, a,w-dicarboxylic acid; OHFA, hydroxylated fatty acid. str plants grew in the presence of a WT nurse plant (fig. S11A). WT plants grew as a monoculture. The ERM was collected from R. irregularis–colonized sand at 42 dpi. These experiments were repeated three times with similar results. Values are the mean ± SE of measurements performed on 8 to 12 plants. *P < 0.05; **P < 0.01 (Student’s t test).

Fig. 4. Plant hosts supply fatty acids to the pathogenic fungus. (A) Lauric acid content in 21-day-old WT, EV, and UcFatB-overexpression plant rosette leaves (three independent transgenic lines: 16, 20, and 22). (B) Lauric acid content of G. cichoracearum collected from WT, EV, and UcFatB-overexpression rosette leaves at 8 dpi. (C) Four-week-old wild-type (Col-0), kas1, kar1, fatb-1, and fatb-2 plants were inoculated with G. cichoracearum. Trypan blue staining of the leaves was used to visualize fungal structures and plant cell death at 8 dpi. Extensive plant cell death was observed in the kas1 mutant. Scale bar, 100 μm. (D) Quantitative analysis of conidiophore formation on 4-week-old wild-type and mutant plants at 5 dpi. Values are the mean ± SD of measurements performed on 30 independent colonies in one experiment. The experiment was repeated twice with similar results. *P < 0.05; **P < 0.01 (Student’s t test).
We suggest that sources of carbon for mutualistic AM fungi include fatty acids exported from the host plant, as well as sugars (fig. S16). Consistent with the nutrient role of fatty acids in fungi, genes encoding enzymes involved in fatty acid degradation (fig. S17) and fatty acid elongation (fig. S18) are found in the *R. irregularis* genome (28). 2-monoacylglycerols, likely exported by ABCG transporters, accumulate as extracellular lipid polyesters at the plant surface in Arabidopsis leaves (18, 19, 29). In mycorrhizal symbiosis, the 2-monoacylglycerols synthesized by RAM2 are likely exported by the periarbuscular membrane-localized heterodimeric ABC transporters STR and STR2 into the interface space and then taken up by AM fungi (fig. S16) (16, 21, 22). Induction of RAM2 expression by the Myc factor signal recognition pathway requires the GRAS-domain transcription factor RAM1 (29). The expression of fatty acid biosynthesis genes appears to be regulated by transcription factors that regulate arbuscule development (fig. S19) (30). Fatty acid biosynthesis in plants is usurped by parasitic pathogenic fungi to secure fatty acids and promote infection.

REFERENCES AND NOTES


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SUPPLEMENTARY MATERIALS

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Materials and Methods

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References (SI–55)

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Food for fungi
A wide variety of plants form symbiotic relationships in their roots with arbuscular mycorrhizal fungi. The fungi channel inorganic and micronutrients from soil to the plant, and the plant supplies the fungi with organic nutrients. Jiang et al. and Luginbuehl et al. found that as part of this exchange, the plant supplies lipids to its symbiotic fungi, thus providing the fungi with a robust source of carbon for their metabolic needs.

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