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Review

Unraveling the network

Novel developments in the understanding of signaling and nutrient exchange mechanisms in the arbuscular mycorrhizal symbiosis

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The arbuscular mycorrhhiza (AM) symbiosis involves an intricate network of signaling and biochemical pathways designed to ensure that a beneficial relationship is established between the plant and fungal partners as a result of a mutual nutrient exchange. Emerging data has been recently published to explain why the relationship is not always fair, as observed in prevalent parasitic AM relationships in which the plant host receives no phosphorus (P) in exchange for carbon (C) delivered to the fungus. The theory behind this unorthodox view of the AM relationship, together with the description of other recent developments in nutrient mobilization as well as in key aspects of the bi-directional signaling that culminates in the symbiotic association, is the subject of this review.

The Arbuscular Mycorrhizal Symbiosis: An Ancient Friendship That Endured

The persistence today of a given type of plant-fungi associations in 90–95% of land plants-mycorrhizal and/or endophytic-reflects the crucial role that root colonization by filamentous fungi is assumed to have had on the successful terrestrial settlement of plants, most likely by permitting access to poorly mobile phosphate ions from soil, particularly before the evolution of roots.[1-4] Amongst the several types of mycorrhizal nonpathogenic and soil-based symbioses known (i.e., Arbuscular; Arbutoid; Ecto-; Ericoid; Monotropoid and Orchid), the arbuscular mycorrhizae are the most ubiquitous given that the roots of the majority (ca. 80%) of higher plants, and many other host plants including pteridophytes, a number of mosses, lycopods, and psilotales are associated symbiotically with biotrophic and aseptate filamentous fungi of the Glomeromycota phylum.[5-10]

Glomeromycotean fungi must transit across several developmental stages before the establishment of an arbuscular mycorrhiza (AM). Thus, in the so-called asymbiotic stage, AM fungal spores germinate and undergo limited hyphal growth, branching profusely in the presence of root exudates emanating from the host plant (see below) and eventually contacting the root surface by means of specialized appressoria. Penetration of the root epidermis follows, leading to symbiotic colonization of the root cortex tissue, a process characterized by the generation of intracellular arbuscules which are specialized hyphae sharing a resemblance with haustoria from plant pathogenic fungi, and/or hyphal coils. Arbuscules are a typical morphological characteristic of Arum AM, whereas hyphal coils predominate in Paris AM morphology. Lipid-rich vesicles accumulate also, in parallel with an extensive growth of a sporulative and highly branched extraradical mycelium (ERM) capable of exploring the soil for mineral nutrients and, in undisturbed habitats, colonizing the roots of other susceptible plants. The formation of asexual chlamydospores by the ERM represents the culmination of the fungal life cycle.[9,11,12]

In the AM symbiosis, which by definition involves a mutually beneficial, interaction between two organisms, the AM fungi (AMF) obtain their whole carbon supply from the plant host, an unavoidable condition for the completion of their life cycle. In return, their presence greatly enhances the plant's uptake of soil phosphorus, predominantly available as orthophosphate ions (Pi) that become very poorly mobile in the presence of Ca^{2+}, Fe^{3+} and Al^{3+}, and some other relatively immobile mineral nutrients (e.g., zinc, copper and, possibly, ammonium; see below).[13] This is possible thanks to extensive ERM networks that not only can reach several meters per cubic centimeter of soil, thereby providing a vast nutrient-absorbing interface between plant and soil, but are believed to be fundamental for the establishment, diversity, nutrition, and productivity of plant communities.[14,15] However, ERM networks are fragile and can be disrupted easily by tillage and other conventional agronomical practices,[16-18] and even by soil fungal-feeding invertebrates, such as the collembolan *Protaphorura armata*.[19]

Owing to the above, AM associations usually confer fitness benefits to the host plants, particularly when they are established under controlled conditions, which translate into enhanced growth, increased reproductive success and/or tolerance to (a)biotic stress.[20-23] However, AMF can adopt a mutualistic, or even parasitic life-style under certain environmental conditions. For instance, degeneration into parasitism is known to occur in well-fertilized soils in which the soluble phosphate (Pi) supply is plentiful.[24,25] On the other hand, the symbiosis may be interrupted or inhibited when the nutritional status of the plant is sufficient for optimal

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growth[26,27] or when the symbiosis-specific phosphate uptake mechanism of the plant is disrupted to such an extent that it prevents nutrient acquisition.[28,29]

Signaling in the AM Symbiosis: New Messages From the Underground

A plethora of experimental findings by diverse groups has dispelled some of the aura of mystery that still surrounds the mechanisms that lead to the establishment of the AM symbiosis. After many a summer, the long sought after plant signal, or bona-fide hyphal branching factor that triggers the plant-AMF interaction was found to be composed of constitutively released strigolactones (e.g., 5-deoxy-strigol).[30-32] However, subsequent studies in which a rapid up-regulation of several fungal genes by root exudates was observed,[33-35] contrary to strigolacone's relatively delayed effect on AMF gene expression[36] (see below) suggest that other yet unknown molecules (flavonoids?) may act together with strigolactones. Chemically, strigolactones are a group of sesquiterpene lactones derived from carotenoid biosynthesis previously identified as seed-germination stimulants for the parasitic weeds *Striga* and *Orobanche* which apparently occurs via the elicitation of ethylene biosynthesis.[37-40] In AMF, strigolactones were shown to be active at subpicomolar concentrations, promoting spore germination and inducing rapid changes in shape, density and motility of mitochondria. These alterations were hypothesized to be responsible for the switch from asymbiotic to presymbiotic growth characterized by increased metabolic activity and growth of AM fungi.[41] Recent data provided convincing evidence that the fungal mitochondria are indeed an early and important target of strigolactone action.[36] Thus, treatment of *Gigaspora rosea* with a strigolactone analogue (GR24) was found to rapidly increase, within minutes, NADH concentration, NADH dehydrogenase activity and the ATP content in fungal cells, specially at the hyphal tips, a situation that was indicative of high energy requirements.[42] The practically instantaneous activation of the oxidative metabolism did not require new gene expression, and was believed to proceed through post-translational regulation of some key enzymes. This resembled previously characterized rapid mitochondrial responses, in which the activation of matricial dehydrogenases (e.g., pyruvate dehydrogenase) and ATP synthase was found to require Ca^{2+}-mediated protein phosphorylation/dephosphorylation events.[43,44] It was only five days after the initial energy burst that an upregulation of gene expression was detected. In accordance with the growth stimulation observed, the induced genes are involved in mitochondrial metabolism (ATP synthase and cytochrome c oxidase V), hyphal growth and stimulation of the fungal mitotic activity (α-tubulin and sphingosine-1P lyase) or had been previously found to be responsive to root exudates (i.e., a CuZn superoxide dismutase).[45] Moreover, the observed upregulation of a putative 3-ketoacyl-CoA thiolase, which combined with the absent induction of pyruvate carboxylase, was strongly indicative of an increased lipid catabolism leading to an accumulation of acetylCoA, a consequent acceleration of the citric acid and glyoxylate cycles, and higher NAD(P)H and ATP synthesis required for an active anabolism. Interestingly, GR24 treatment was associated also with a higher number of nuclei, which preferentially accumulated in the apical area of growing hyphae, a location usually having the highest mitotic activity in filamentous fungi. This coincidence, coupled to the fungal apical growth model proposed by Steinberg,[46] which stresses the strong needs in energy and mRNA in this region, offered a possible explanation for the accumulation of both mitochondria and nuclei in *Gi. rosea*'s hyphal tips.

The discovery of strigolactones represents a significant forward step in the understanding of plant-AMF communication. Unfortunately, the mechanism(s) by means of which AMF are able to detect this and other plant signals is still a source of speculation. Experimental evidence showing that the flavonoid effect could be mimicked by estrogens and blocked by anti-estrogens led to an early proposal that flavonoids were detected by estrogen-like receptors in AMF.[47] This model was subsequently supported by convincing results showing that a specific binding site for flavonoid or structurally related compounds (estrogens and antiestrogens) exists in AM fungi.[32] The striking stimulation of fungal metabolic activity produced by strigolactones led to a further elaboration of the above model in which mitochondrial or cytoplasmic receptors were suggested to be the possible strigolactone's targets, similarly to the way thyroid hormones in mammalian cells stimulate mitochondrial biogenesis, lipid catabolism and respiration.[36,48,49] Other workers proposed the interaction of strigolactone with a hypothetical fungal receptor, followed by the quick inactivation of the lactone ligand through the removal of its D ring.[30]

Another interesting possibility, supported by the existence of a presumed linkage between the enhanced germination of parasitic plant seeds and fungal spores, suggests that AM fungi respond to strigolactones via an enhancement of endogenous ethylene synthesis which could then stimulate mitochondrial metabolism. However, the validity of this mechanism of action will depend on future findings capable of clarifying the rather controversial role played so far by ethylene in AM formation.[50-53]

Step-wise signaling events are also needed for subsequent events in AM formation, such as appressoria formation and symbiosis progression. These are presumed to be thigmotropic signals from the plant surface or secondary metabolites produced in planta after perception of the fungus. In addition, the fungus produces at least three as yet unidentified signals, or myc factors, whose mode of action remains similarly unclear: an early diffusible signal, a local positional signal that allows the plant to detect the location of appressoria, and a cell autonomous signal in colonized cells capable of inducing gene expression.[32,54-56]

The transition from the mutual exchange and perception of signals by both the fungal and green partners to the establishment of a functional symbiosis requires a signaling pathway known as the 'common sym pathway', which is thought to have evolved as a strategy designed to optimize P and N nutrition in legume plants by borrowing components of the ancestral AM recognition pathway in order to develop the rhizobial symbioses. Thus, the sym pathway is known to involve the perception of putative Myc or Nod factors by a receptor kinase (SYMRK; MtDM2 in *Medicago truncatula*),[57] followed by the presumed release of a plastidic factor via a putative plastid-localized ion channel (MtDM1), the involvement of a nucleoporin protein sharing homology with a component of the nuclear pore complex, and, ultimately, a down-stream induction of nuclear calcium signal spiking thought to be detected by a calcium- and calmodulin-dependent protein kinase (CCaMK; MtDM3), which leads to the induction of the complex genetic and metabolic re-programming required for the symbiosis.[31] An

intriguing possibility regarding the reason(s) why a minority of plants species is unable to establish an AM interaction could be related to differences in their related calcium/calmodulin-dependent protein kinases, as suggested for the nonmycotrophic *Arabidopsis thaliana* known to have CCaMKs that are different from functionally similar proteins.[58]

Novel findings have allowed an expansion of this basic scenario. Firstly, the important discovery that *SYMRK* genes are functional in a non-legume plant, *Casuarina glauca*, considered a pioneer plant due to its ability to grow in marginal soils, and most importantly, capable of establishing AM interactions and actinorhizal nodules with *Frankia* strains.[59] By RNA interference (RNAi)-mediated disruption of the function of *CgSymRK* by in transgenic *C. glauca* roots, these workers demonstrated that a reduction in *CgSymRK* expression severely affected nodulation and symbiotic nitrogen fixation and perturbed early steps of AM invasion, as well, in particular the fungal penetration of the root cortex. Secondly, evidence revealing an interaction between the catalytic domain of a putative mevalonate synthase (MtHMGR1) and the kinase domain of SYMRK of *M. truncatula*, showed it to be indispensable for nodule organogenesis. This meant a probable SYMRK-mediated modulation of the enzyme's ability to associate with uncharacterized vesicles and of its pivotal activity in the mevalonate pathway required for the synthesis of isoprenoid compounds. Such interactions would permit the regulation of the processes required for membrane formation and synthesis of defense-related steroidal compounds, respectively.[60] These results, combined with a previous report describing an oversensitivity to touch stress in legume *symrk* mutants, which was believed to be associated with defects in root hair formation in response to rhizobia[61] led to the elaboration of an improved signaling model, which proposes the combined action of two receptors: the SYMRK and a diffusible-signal-specific receptor-like-kinase. According to this model, the former receptor would be capable of detecting the mechanical stress produced externally by the formation of AMF appressoria and/or internally by root hair curling and formation of a closed invasion pocket elicited by the bacterial symbiont. This capacity would therefore, allow a precise modulation of the touch and defense responses related to either, or both, symbionts. In addition, the latter receptor would be poised to perceive the diffusible symbiont's signal(s). The perception of the physicochemical signals would lead to a downstream integration of their respective responses, permitting fungal and/or bacterial entry and subsequent intracellular progression and development into functional symbiotic structures. In the case of SYMRK, these steps could involve the polar recruitment of vesicles and vesicle-associated proteins and cargo.[62]

Additionally, strong evidence supporting the presumed involvement of calcium as an intracellular messenger in the arbuscular mycorrhizal (AM) symbiosis was reported in two recent reports. One employed soybean (*Glycine max*) cell cultures stably expressing the bioluminescent Ca^{2+} indicator aequorin as a tool to detect rapid and transient elevations in cytosolic free Ca^{2+} in response to small, heat-stable and lipophilic diffusible molecules produced by spores of *Gi. margarita*, and two *Glomus* species, independently of their germinating status.[63,64] Moreover, these workers confirmed the specificity of the diffusible-factor-induced-response by showing that it led also to an upregulated expression of their respective *DMI1*, *DMI2* and *DMI3* homologues, which contrasted with the lack of response shown by cell cultures of the non-host plant *A. thaliana*. In the other, which was somehow related with the above discovery of SYMRK in *C. glauca*, the use of reverse genetics and a cross-species complementation allowed the identification of a rice ortholog of *MtDMI3* which was found not only to be necessary for AM symbiosis in rice but able to complement a *M. truncatula dmi3* mutant as well.[65]

Nutrient Exchange: It is Only by Giving That We Receive

The symbiotic interaction concludes with the development of a symbiotic interface through which nutrients (N, P, other minerals and sugars), and possibly signaling molecules, are exchanged.[66] In most of the AM interactions this function is fulfilled by a specialized intracellular structure, the arbuscule, consisting of highly ramified hyphae with very fine terminal tips that greatly increase the surface-to-volume ratio of normal hyphae and, consequently, the efficiency of the bi-directional nutrient transfer.[67] Arbuscule formation requires extensive changes in the root's cortical cell organization, including vacuole fragmentation, centripetal nuclear migration, cytoskeleton rearrangement, and plastid modification.[68,69] In addition, arbuscules are surrounded by an extension of the plant cell membrane, the periarbuscular membrane (PAM), which is continuous with the plasmalemma. It must be emphasized, however, that the existing reports of Paris type mycorrhiza where arbuscules are completely absent, suggests that other plant–fungal interfaces might be involved also in the nutrient exchange.[70]

Carbon-phosphate transfer: Is it really a fair deal? Arbuscule-containing cells are known to respond to their new role as mediators of nutrient exchange by expressing, probably in response to a cell-autonomous fungal signal, the well characterized PAM-localized phosphate transporters needed for the uptake of the soil Pi delivered to the plant. Efficient Pi delivery to the roots represents the combined result of the exploration of large soil volumes by the ERM, an increased Pi absorbing surface area and the production of organic acids and phosphatases that facilitate the release of P from organic complexes.[71-74] Pi translocation is thought to be mediated by plant transporters embedded in the PAM together with mycorrhiza-inducible H^+-ATPases. The latter, found in tobacco and *M. truncatula*, are presumed to provide the H^+ ions needed for co-transportation of Pi across the membrane and create the acidic environment that characterizes the arbuscule's surroundings.[31,75,76] In support for their role in P transport, several AM symbiosis-induced Pi transporter genes have been isolated from roots of different plants, including several in the Solanaceae, rice, *M. truncatula*, barley, wheat, maize and *Lotus japonicus*.[25] Curiously, they all show a divergent evolution despite having similar putative physiological functions and mycorrhiza-regulated gene expression.[65] Also, root extracts of mycorrhizal plants in which the active principle was identified as the lysolipid lysophosphatidylcholine, were found to induce the mycorrhiza-specific phosphate transporter genes *StPT3* and *StPT4* in potato mycorrhizal roots.[57,77] Moreover, a pivotal role for the LePT4 phospahte transporter in mycorrhizal-mediated Pi uptake in tomato was implied on experimental evidence showing that the inherent P deficiency of the low affinity *lept4* P-transport mutant could not be fully compensated by other members of the family (i.e., *LePT3* and *LePT5*). Further analysis, including the characterization of the mycorrhiza-associated *lept4* mutant, and the expression pattern of *LePT3* and *LePT5*, suggested that different routes of mycorrhiza-mediated Pi uptake

might be operative in tomato plants,[78] although further research with multiple mutants was deemed to be needed for a full comprehension of the Pi transport mechanism in this plant.

Contrasting with the fairly well known mechanisms of P transport in the AM symbiosis is the limited knowledge available regarding the way in which the Pi-carbohydrate exchange is regulated. Practically all information in this respect has been obtained employing the in vitro AM split plate culture system developed by St-Arnaud and co-workers[79] given its relative simplicity and facilitated control of the experimental conditions. Thus, the use of monoxenic AM cultures of carrot (*Daucus carota*) roots fed with ^{14}C indicated that the amount of carbon assigned to the AMF in colonized roots increased in direct proportion to their P needs.[80] More recently, the utilization of an energy dispersive X-ray microanalysis to trace the uptake and transfer of ^{14}C-labelled carbohydrates and ^{33}P-phosphate in the symbiotic interaction between carrot roots and *G. fasciculatum* showed that increased carbohydrate availability stimulated Pi uptake by the ERM and its subsequent translocation to the mycorrhizal roots. It also altered the spatial distribution of P within the fungus, favoring its incorporation into phospholipids, nucleic acids and protein phosphates, in detriment of polyphosphate (polyP), and increasing cytoplasmic P levels in the intraradical mycelium (IRM) and in the root cortex.[81] Their results, in combination with previous findings, led to a model suggesting a coupled C and Pi flux between the fungus and the photobiont, initiated by an active absorption of Pi by the ERM, its subsequent transformation, transport and storage as polyP chains in the mycorrhizal root and its eventual translocation, as Pi, across the interfacial apoplast to the host plant. This last, critical, step would depend on an increased plant acid invertase activity in the interfacial apoplast, followed by hexose accumulation and subsequent absorption by the IRM, enhanced remobilization of polyP to supply the hexose phosphate intermediaries needed for trehalose and glycogen biosynthesis, increased intracellular Pi concentration in the hyphae, and finally, promoted Pi efflux through the fungal plasma membrane into the interfacial apoplast. On the other end of the symbiosis, the increased transfer of triacylglycerols and glycogen to the ERM would provide the energy required both for active P uptake processes from the soil and the synthesis of new C skeletons needed to extend the ERM in search of new P resources.[81]

A second, and in many aspects similar, C-P flux model in mycorrhizal roots was proposed not much later.[82] It was based on the assumption that: (i) the IRM, and not the arbuscules, are the principal organs of fungal hexose uptake;[83] (ii) the stimulation of sugar delivery by the plant host to IRM is highly localized in order to reach only those restricted patches within the root that are usually colonized by the AMF,[84] and (iii) the C-Pi exchange is strictly regulated by P-availability, a necessary check-point needed to reduce or eliminate the possibility of establishing a parasitic interaction in which no P is obtained as an exchange for hexoses. The above models were seriously questioned by computer-simulated model whose results supported the novel hypothesis that mycorrhizal nutrient transfers are driven only by the symbionts' internal needs, and that C and Pi transfers, instead of being simultaneously exchanged, are quantitatively unlinked.[85] It was devised also to correct the inability of the previous models to explain as the prevalence of parasitism[86] and the plant host's inability to expel AMF under lower soil P conditions, when the net cost of harboring the fungus becomes detrimental to the plant. Thus, in accordance to the premises of the model, based on economic mathematics of risk, rate of return, and return on investment, the plant will tend to invest in mycorrhizas and/or more roots under P-limiting conditions as a strategy to acquire more P, irrespective of the risk involved, whereas parasitism can be envisaged as the consequence of a failed investment, in which the C expense did not materialize in a reciprocal Pi supply to the plant. In the workers' opinion, this model is an adaptable tool can easily be utilized to study other phenomena, such as variation in organ lifespan, N exchange, litter exploitation by ectomycorrhizal fungus, or multiple plant-fungal interactions.

Carbon-nitrogen uptake: The added benefit of harboring a multifaceted soil scavenger. AM fungi are known to represent a significant route of nitrogen (N) uptake by the plants, as sustained by several reports describing their involvement in N transfer from one plant to another, the increased utilization of different N forms by plants and/or the direct acquisition of soil N and subsequent transfer to the host roots.[87-89] However, despite the presence of fungal enzymes and genes involved in primary nitrogen assimilation and catabolism (i.e., nitrate reductase, glutamine synthetase and glutamate dehydrogenase), little else was known about how AMF acquire nitrogen and transfer it to their host plants. This scenario was altered by the findings of Govindarajulu et al.[90] and Jin et al.,[91] who employed isotope labeling experiments with monoxenic AM cultures of carrot roots, as described above, to describe the relatively intricate mechanism by means of which inorganic N in the soil is absorbed by the AMF. The process begins with the favored uptake of ammonia (NH_4^+) at the ERM, which is believed to be the preferred N form taken up by AM fungi mostly because it avoids the extra energy expended in reducing NO_3^- to NH_4^+ prior its incorporation into amino acids.[92] It requires the activity of a nitrate reductase and the glutamine synthetase/glutamate synthase (GS/GOGAT) cycle. Subsequent translocation of N from the ERM to the IRM proceeds in the form of arginine (Arg), which is decomposed to C-free NH_4^+ by the sequential and synchronized activity of several enzymes before its final transfer to the plant, via ammonium transporters (see below). Apart from preventing an excessive accumulation of NH_4^+ in fungal hyphae when the external N is plentiful and uptake is rapid, the assimilation of N into Arg offers several added advantages, such as: (i) N translocation in a concentrated, non-toxic form, containing four nitrogens per molecule; (ii) P-N co-transportation due to its potential binding to polyP, and (iii) minimal loss of carbon by the fungus thanks to the use of the catabolic arm of the urea cycle that permits the transfer of N to the host plant in C-free inorganic form.

Consistent with this mechanism, the genes of primary nitrogen assimilation were found to be preferentially expressed in the ERM, whereas genes associated with Arg breakdown were more highly expressed in the IRM.[90] Besides, a high degree of synchronization between the spatially separated enzymatic reactions involved in the anabolic (e.g., GS and argininosuccinate synthetase) and catabolic (e.g., arginase and urease) arms of the urea cycle, indispensable for Arg to be a key component in nitrogen translocation in the AM mycelium, was demonstrated.[93] However, many questions regarding key regulatory, metabolic and transport aspects involved in N transfer remain unanswered, including the influence that changes in the C-to-N ratio in roots could have, similarly to the C-P relationship described above, on the activity of key enzymes of nitrogen metabolism and rates of N transfer.

Shortly before, the first report describing an AMF high affinity NH_4^+ transporter gene in *G. intraradices* was published.[94] This transporter showed a differential regulation by the N status of the AMF. The kinetics of NH_4^+ uptake from the surrounding soil media to ERM indicated also a maximum efficiency when the NH_4^+ ion was present at micromolar concentrations. In addition, a possible role in the retrieval of NH_3 leaked out of the ERM during amino acid catabolism and sensing of N-starvation conditions, was suggested. On the other hand, the nature and site of N transport across the fungus-plant interface and from the interfacial apoplast to the plant host remains unknown. Nevertheless, the participation of an Ato-like fungal NH_4^+ efflux system, similar to those identified in transcriptomic-wide studies in various ectomycorrhizal associations, could be involved in the release of ammonia at the fungal membrane, while plant Amt NH_4^+ transporters, non-specific channels such as aquaammoniaporins and voltage-dependent cation systems might mediate the final steps in ammonia transport across the interfacial apoplast to the plant cell cytoplasm.[95]

In addition to NH_4^+, the enhanced ability of the AMF symbiosis to exploit soil organic nitrogen sources of diverse complexity has been known ever since it was first reported by Hodge et al.[96] However, the molecular mechanism responsible for such an uptake are practically unknown, except for the recent discovery of an amino acid permease from the AMF *G. mosseae*, whose putative localization on the plasma membrane, localized expression in the ERM, favored binding of non-polar and hydrophobic amino acids and upregulation upon exposure to organic nitrogen in physiologically relevant concentrations revealed a potential role in the first steps of amino acid acquisition from the soil.[97,98]

Carbon uptake by the AMF: The cost of maintaining a friendly relationship. Arbuscular mycorrhizal fungi are obligate biotrophs, having developed a complete dependency on the host plant for photosynthetically-fixed C supply. The reasons remain obscure, although it is presumed that the long evolution of its symbiotic relationship with the host plant (more than 450 million years), led to the loss of the C acquisition capabilities needed for saprophytic growth.[99] The data registered by several C flux analyses calculate that 4–20% more photoassimilates than those normally directed to non-mycorrhizal plants are needed to cover the increased C demand represented by a stronger mycorrhizal root sink.[100-102] Isotopic labeling with nuclear magnetic resonance spectroscopy in AM roots[103] and radiorespirometry measurements on isolated intraradical hyphae[104] showed that hexose is the principal C form taken up by the AMF, although a restricted absorption of sucrose by the IRM has been suggested.[105] Hexose is rapidly converted to trehalose and glycogen, the main transient storage carbohydrates (CHOs).[103] Most of the time these CHOs are quickly metabolized in the IRM, ERM and germinating spores via the glycolytic and tricarboxylic acid cycles, as well as the pentose phosphate pathway, thereby explaining the substantially higher respiration rates usually detected in mycorrhizal roots.[100,103,105-107] Regarding trehalose, a transient accumulation of this disaccharide, coupled with the upregulated gene expression and/or enzyme activity of trehalose-6-P phosphatase (for trehalose synthesis) and neutral trehalase (for trehalose post-stress mobilization), respectively, was correlated also with the recovery process from heat and chemical stress treatments in AMF.[108]

In addition, a substantial amount of hexose C can be transformed to storage lipids in the IRM and spores, mostly in the form of triacylglycerols (45–95%), in which Cis 11,12 hexadecenoic acid is the predominant fungal fatty acid (FA).[109-111] Interestingly, the uniqueness of this FA, having an unusual saturation position and apparent specificity to certain AMF and bacteria, has been exploited as a specific biochemical marker to estimate the biomass of AM mycelia in soil,[112-114] and in addition to the levels palmitic (16:0) and oleic acid (18:1 n-9), to fathom the degree of arbuscular mycorrhizal colonization of roots, as well.[115] Lipids are also the major form of C storage in AMF (e.g., up to 70% of the dry weight of *G. caledonius* is composed of lipids)[116] and data strongly indicative of the exclusive expression of the FA synthase activity in the IRM of AMF suggests that FA metabolism may be a factor involved in their obligate biotrophism.[117]

Consequently, a drastic shift in the way carbohydrates are synthesized, metabolized and partitioned in the host plant during the AM symbiosis takes place. This is consistent with findings showing increased photosynthetic rates in mycorrhizal plants,[118,119] upregulated gene expression and/or activity of sucrose hydrolyzing enzymes (e.g., invertases and sucrose synthase) required for the release of hexose incorporated by the AMF,[118,120-124] sugars transporters[125,126] and H^+-ATPases.[75,76,127-129]

According to their sub-cellular location and their pH optima, plant invertases can be classified either as acidic cell wall-bound apoplastic invertases, acidic soluble vacuolar invertases, and alkaline soluble cytosolic invertases.[130] It is generally assumed that plant invertases and Suc synthases are crucial for the delivery of hexoses to the fungal partner, given the fact that no sucrolytic enzymes have been found yet in AMF. Extracellular invertases are considered to be particularly important for two reasons: (i) their activity leads to increased sink strengths, a characteristic of mycorrhizal roots, by promoting phloem unloading, and (ii) they may directly deliver utilizable hexoses to the apoplastic fungal structures. Support for their active role in AMF carbohydrate support comes from experimental evidence showing increased transcript accumulation and activity levels of apoplastic invertases in mycorrhizal roots, especially when carbohydrate demands were high.[118,124] The general regulation of AM formation by carbon availability and its close dependence on plant invertase(s) activity was further demonstrated by the observed suppression of mycorrhizal colonization using two ways to interrupt the flow of utilizable C to the mycorrhizal roots of transgenic tobacco plants. One was defective in phloem loading, and consequent translocation of sucrose to the sink tissue, due to the expression of a phloem-specific *Escherichia coli* inorganic pyrophosphatase, whereas the other had reduced invertase activity due to a root-specific overexpression of an Arabidopsis invertase inhibitor.[131] Conversely, no alteration in mycorrhization levels were observed by these workers when root hexose availability was increased by expressing yeast-derived invertase(s) in tobacco or in hairy roots of *M. truncatula*, thereby indicating that plants have a sufficient availability of carbohydrates under normal growth conditions and aspects other than the carbon supply may be the limiting factor(s) for the establishment of the AMF symbiosis, such as the Pi levels in the plant and/or plant defense responses that limit fungal growth to the root cortex. In a related series of experiments, the possible

deleterious effect on AMF colonization caused by the upregulated expression of sugar-induced defense responses generated in response to high invertase levels of activity was explored, using transgenic tobacco plants capable of foliar expression, at different levels, of an apoplastic chimeric invertase.[132] To the workers surprise, the levels of leaf invertase activity defined whether fungal colonization of the root was stimulated or repressed, and strongly suggested a regulatory function for apoplastic invertases at the whole plant level. Thus, weak and strong increases in source leaf invertase activity had an early stimulatory or late repressive effect on the AM symbiosis, respectively. The interpretation given to these results was that increased AM formation in transformed tobacco plants having low leaf invertase activity levels might have resulted both from a reduced defense status and increased abscisic acid (ABA) content in roots. This situation probably occurred as a result of a decreased hexose:sucrose ratio and/or a downregulated hexose sensing in the plant. This proposal is in agreement with a recent report showing that ABA contributes to increase the susceptibility of infection by AM fungi in tomato, and that it might play an important role also in the development of the complete arbuscule and its functionality.[52] In contrast, tobacco plants having a strongly increased leaf invertase activity, which were easily distinguishable due to the presence of several symptoms associated to excessive foliar hexose accumulation in the leaves, such as chlorosis and defective growth, led to reduced levels of AMF colonization due to the establishment of C limiting conditions in the roots caused by carbohydrate undersupply from the leaves.

A contemporary study extended the knowledge regarding the pivotal involvement of sucrolytic enzymes in the establishment of the AM symbiosis by showing a symbiosis-induced upregulation of the cell wall invertase *Lin6*, vacuolar invertase *TIV1* and sucrose synthase *TOMSSF* genes in mycorrhizal tomato plants, which appeared to be independent of improved phosphorus nutrition.[133] Moreover, transcriptional upregulation of sucrose-splitting enzymes during early colonization development correlated with the decreased levels of sucrose detected in these roots, whereas lower root glucose and fructose concentrations in mycorrhizal plants were indicative of their consumption by the root cells, the fungal symbiont, or both. In addition, promoter analysis of *Lin6*, *TIV1* and *TOMSSF* suggested a positive regulatory role for salicylic acid (SA) and ABA, which again coincided with the observed ABA-enhancing effects on AM colonization described above and with previous studies reporting the accumulation of SA[134] and ABA[135,136] in mycorrhizal roots. This study corroborated that the AM symbiosis upregulates sucrose metabolism genes, in accordance with an increased catabolism and utilization of sucrose in mycorrhizal tomato roots. It offered also, several possibilities for the future study of their regulation in the AM symbiosis, which could take advantage of their GUS fusions for detailed promoter analyses and/or the generation of plants with knocked-out or silenced versions of the *Lin6*, *TIV1* and *TOMSSF* genes.

Jasmonic acid (JA) is a phytohormone related to multiple developmental and growth processes, including photosynthesis gene modulation[137,138] and C partitioning.[139] The latter effect, studied in poplar tress, was attributed to a JA-mediated acceleration of photosynthate export from leaves, which was believed to occur due to a decreased leaf phloem loading time, a shift in carbon and nitrogen-based metabolites to stem and root storage, and/or the generation of long-distance signals from leaves to roots capable of affecting nutrient uptake and assimilation. Experimental evidence gathered so far indicates also that JA might play an important role in the mycorrhizal symbiosis.[51,115,140-142] Recently, the drastic reduction in mycorrhizal colonization observed in JA biosynthesis-defective tomato mutants coincided with a strong down-regulation of a number of genes involved in sucrose hydrolysis and transport and a significantly reduced cell wall invertase activity and AMF-specific fatty acid content in roots. These results suggested that JA might modulate the mycorrhization process through its influence on the regulation of C partitioning in the plant.[143]

Conclusion

Even though much progress has been made to elucidate the complex mechanism required for the establishment of an AM symbiosis, many more questions remain to be answered. A central concern is that the establishment of AM relationship seems to be a risky matter, more the result of a casual relationship than of an arranged marriage, as suggested by recent data uncovering the almost haphazard nature underlying the C-P interchange between both partners. Also needed is a clearer understanding of the mechanisms that permit and regulate C transfer from the photosymbiont to the fungus in addition to many aspects involved in the uptake and transport of inorganic and organic N from the soil to the plant. And last but not least, resolving the tantalizingly elusive nature of the Myc factors will represent a significant step in the efforts to understand the chemical language that endows the AMF with a safe-conduct to enter and inhabit the host plant undisturbed.

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