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Review

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Partner communication and role of nutrients in the arbuscular mycorrhizal symbiosis

Author for correspondence:
Luisa Lanfranco
Tel: +39 0116705969
Email: luisa.lanfranco@unito.it

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Luisa Lanfranco[1], Valentina Fiorilli[1] and Caroline Gutjahr[2]

[1]Department of Life Sciences and Systems Biology, University of Torino, Viale P.A. Mattioli 25, 10125 Torino, Italy; [2]Plant Genetics, School of Life Sciences Weihenstephan, Technical University of Munich (TUM), Emil Ramann Str. 4, D-85354 Freising, Germany

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Summary

The evolutionary and ecological success of the arbuscular mycorrhizal (AM) symbiosis relies on an efficient and multifactorial communication system for partner recognition, and on a fine-tuned and reciprocal metabolic regulation of each symbiont to reach an optimal functional integration. Besides strigolactones, *N*-acetylglucosamine-derivatives released by the plant were recently suggested to trigger fungal reprogramming at the pre-contact stage. Remarkably, *N*-acetylglucosamine-based diffusible molecules also are symbiotic signals produced by AM fungi (AMF) and clues on the mechanisms of their perception by the plant are emerging. AMF genomes and transcriptomes contain a battery of putative effector genes that may have conserved and AMF- or host plant-specific functions. Nutrient exchange is the key feature of AM symbiosis. A mechanism of phosphate transport inside fungal hyphae has been suggested, and first insights into the regulatory mechanisms of root colonization in accordance with nutrient transfer and status were obtained. The recent discovery of the dependency of AMF on fatty acid transfer from the host has offered a convincing explanation for their obligate biotrophism. Novel studies highlighted the importance of plant and fungal genotypes for the outcome of the symbiosis. These findings open new perspectives for fundamental research and application of AMF in agriculture.

I. Introduction

Soil is a complex matrix with diverse geochemical properties that is inhabited by wide range of prokaryotic and eukaryotic organisms (Nielsen *et al.*, 2015). The soil volume in direct contact with the plant root is defined as the rhizosphere and represents a particularly biologically rich environment, in which microbial communities profit from metabolites released by roots (Sasse *et al.*, 2017). Some of the soil inhabitants, such as arbuscular mycorrhizal fungi (AMF) establish a very intimate association with plant roots, leading to the formation of a mutualist interaction called the arbuscular mycorrhizal (AM) symbiosis (Martin *et al.*, 2017).

AMF show peculiar features: besides their obligate biotrophism, they are characterized by coenocytic hyphae and multinucleated spores (Kamel *et al.*, 2016; Lanfranco *et al.*, 2016). No sexual reproduction has been described so far, although evidence for the

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potential of mating-related processes has been obtained (Corradi & Brachmann, 2017). They have a rather long history of taxonomic revisions, which reflects the general difficulty in resolving the earliest branches in the fungal genealogy. Ribosomal DNA-based phylogenies placed them in the Glomeromycota phylum which is considered a sister group to Dikarya (Schüssler *et al.*, 2001). An extensive phylogenomic study, based on kingdom-wide sampling of fungal species and genome-scale sampling of loci, placed AMF in the subphylum Glomeromycotina with a close relationship with Mortierellomycotina (Spatafora *et al.*, 2016).

AM is one of the most ancient and widespread symbioses in nature (Lanfranco *et al.*, 2016). The main advantage of the AM symbiosis is the exchange of nutrients: the plant provides up to 20% of the photosynthetically fixed organic carbon to the AMF (Roth & Paszkowski, 2017), whereas the AMF transfers mineral nutrients to the plant thanks to its efficiency in exploring and acquiring these resources from the soil (Smith *et al.*, 2011). In addition, plants colonized by AMF often show higher tolerance to biotic and abiotic stresses compared to nonmycorrhizal plants and this is not a mere consequence of a better nutritional status (Jung *et al.*, 2012; Augé *et al.*, 2015). At the ecosystem level, AM improves soil quality (Rillig *et al.*, 2015) and increases plant biodiversity (van der Heijden *et al.*, 1998).

Root colonization by AMF occurs in successive steps. Before physical contact between plant and fungus, diffusible molecules mediate reciprocal recognition. When fungal hyphae touch the root epidermis, they form adhesion structures called hyphopodia. Subsequently, AMF enter the root and grow into the root cortex taking an intercellular and/or intracellular route. In the cortex, hyphae penetrate single cells, where they develop highly branched hyphal structures, the arbuscules (Gutjahr & Parniske, 2013; Lanfranco *et al.*, 2016). Arbuscules are surrounded by a plant-derived periarbuscular membrane (PAM), which, together with the arbuscule membrane, forms an extensive interface for nutrient exchange (Fig. 1).

Excellent recent reviews describe the latest advances in plant regulatory and cell biological mechanisms required for accommodation of AMF inside roots (Luginbuehl & Oldroyd, 2017; MacLean *et al.*, 2017; Pimprikar & Gutjahr, 2018). Herein we discuss new findings in understanding the molecules and mechanisms that control partner recognition, the importance of nutrients in the formation and maintenance of arbuscular mycorrhizas, and the role of plant–fungal genotype combinations for the outcome of the symbiosis.

II. Interkingdom communication enabling symbiosis

The rhizosphere is a preferential niche for large microbial communities. Unequivocal and efficient communication systems are therefore required to enable specific interactions such as the AM symbiosis.

1. Plant exudates activate the fungus

AMF and plants rely on reciprocal recognition before physical contact (Nadal & Paszkowski, 2013; Bonfante & Genre, 2015). Plant roots, particularly under inorganic phosphate (Pi) limiting conditions, release strigolactones (SLs), carotenoid-derived molecules with hormone functions in plants (Waters *et al.*, 2017). These stimulate branching and elongation of AMF hyphae (Akiyama *et al.*, 2005; Besserer *et al.*, 2006; Fig. 2), thus promoting the chances of encountering the host. Furthermore, a general activation of the fungal mitochondrial metabolism (visible as organelle division, ATP production and gene expression) has been associated with SL exposure (Besserer *et al.*, 2008; Lanfranco *et al.*, 2018). Notably, SL treatment also led to an increase in the release of chitin oligomers by AMF (Genre *et al.*, 2013), which act as signalling molecules on the plant (Sun *et al.*, 2015a). SLs also contribute to the induction of fungal genes (Tsuzuki *et al.*, 2016; Kamel *et al.*, 2017). One of them, encoding a putative secreted protein 1 (SIS1), is important for symbiosis as host-induced gene silencing (HIGS) led to stunted arbuscules and reduced root length colonization (Tsuzuki *et al.*, 2016). The fungal receptor for SL is currently unknown and its identification is a matter of active

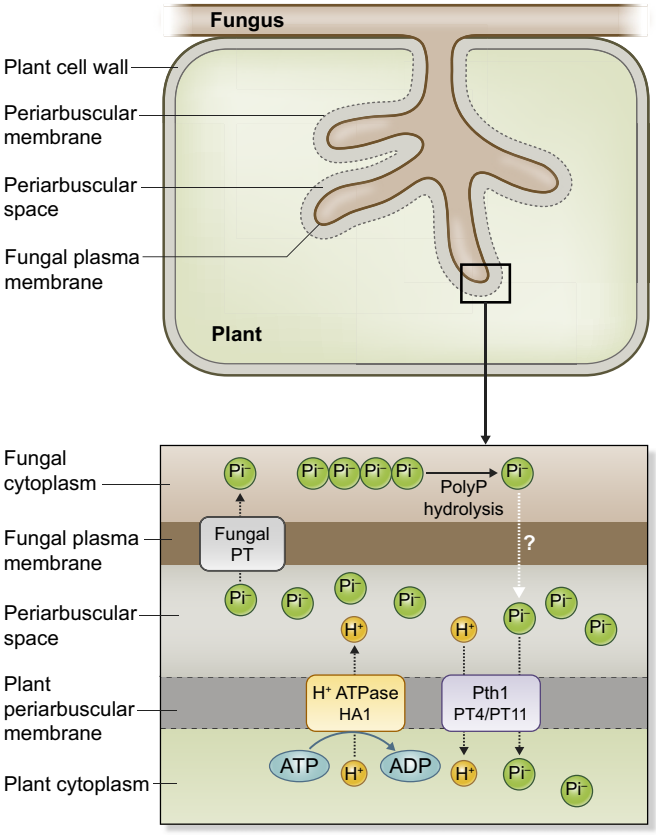

Fig. 1 A simplified scheme of an arbuscule-containing cell showing the periarbuscular membrane (PAM), the periarbuscular space (PAS) and details of the phosphate ion (Pi) transfer. Pi derived from polyP hydrolysis in the fungal cytoplasm, are delivered to the periarbuscular space, by a still unknown mechanism. Pi is then imported into plant cells by arbuscular mycorrhizal (AM)-inducible, PAM-localized plant phosphate transporters (PT), such as Medicago PT4 and rice PT11 (Javot *et al.*, 2007b; Yang *et al.*, 2012). This transport is suggested to be driven by an H^+ energy gradient produced by an H^+-ATPase (Krajinski *et al.*, 2014; Wang *et al.*, 2014). The expression of fungal *PT* genes in the intraradical mycelium suggests a possible role in Pi reabsorption from the PAS (Benedetto *et al.*, 2005; Balestrini *et al.*, 2007; Fiorilli *et al.*, 2013; Xie *et al.*, 2016).

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investigation. Nevertheless, the importance of SLs for efficient symbiosis formation is clear, as plants defective in the biosynthesis or the exudation of SLs display a lower colonization level, whereas arbuscule morphology is normal (summarized in Waters *et al.*, 2017; Lanfranco *et al.*, 2018).

Although SLs are plant-derived, they do not appear to play an important role at the host side because rice mutants defective in the alpha-beta hydrolase SLs receptor D14, are not perturbed in AM colonization (Yoshida *et al.*, 2012; Gutjahr *et al.*, 2015). During SLs perception, D14 interacts with the F-box protein MAX2/D3/RMS4 in a receptor complex (Hamiaux *et al.*, 2012). MAX2/D3/RMS4 is also involved in the perception of karrikins together with the alpha-beta fold hydrolase KAI2/D14LIKE (Nelson *et al.*, 2010; Waters *et al.*, 2012). Karrikins are butenolide molecules found in smoke extracts that promote seed germination of many plant species (Flematti *et al.*, 2004). Interestingly, rice *d3* and pea *rms4* mutants displayed aborted colonization attempts and reduced arbuscules formation, respectively (Yoshida *et al.*, 2012; Foo *et al.*, 2013; Gutjahr *et al.*, 2015), and a rice mutant defective in the karrikin receptor D14-LIKE/KAI2 is characterized by an absence of hyphopodia (Gutjahr *et al.*, 2015). In addition, the rice *d14l/kai2* mutant lacks the transcriptional response to fungal germinating spore exudates, indicating that the karrikin receptor complex may be involved in perception of the fungus. However, it is not yet clear whether a karrikin-like compound of fungal or plant origin acts as ligand of the D14L receptor in plant–AMF recognition (Gutjahr *et al.*, 2015; Waters *et al.*, 2017).

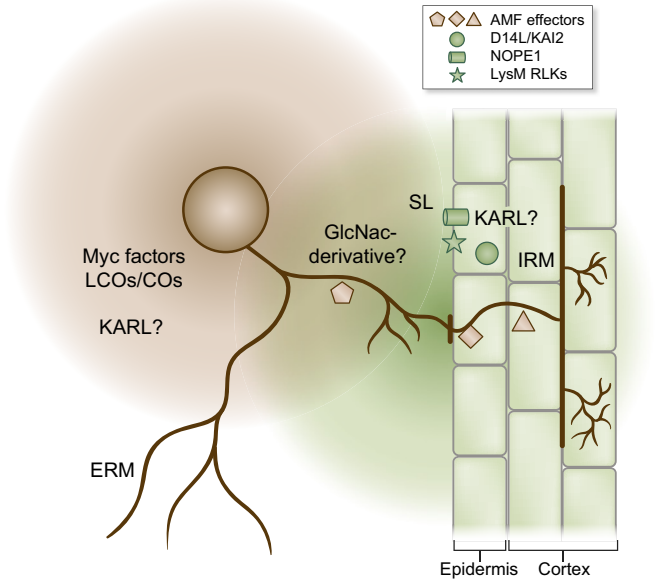

Fig. 2 Molecules involved in the communication between arbuscular mycorrhizal fungi (AMF) and host plants. Plant roots release strigolactones (SL) which stimulate AMF metabolism and hyphal branching to promote colonization (Akiyama *et al.*, 2005; Besserer *et al.*, 2006, 2008). A rice mutant deficient for the *D14L* gene is characterized by an absence of hyphopodia (Gutjahr *et al.*, 2015). The D14L/KAI2 protein localizes to the nucleus and cytoplasm. It is yet unclear whether the karrikin-like (KARL) ligand of D14L/KAI2, relevant for AM symbiosis is of plant or fungal origin. The recent finding that a plasmamembrane-resident plant *N*-acetylglucosamine (GlcNAc) transporter (NOPE1) is required for early signalling in AM suggests the existence of GlcNAc-based diffusible plant molecules, which may trigger presymbiotic fungal reprogramming (Nadal *et al.*, 2017). Also AMF use GlcNAc-based molecules, which include lipo-chito-oligosaccharides (LCOs; Maillet *et al.*, 2011) and short chitin tetra- and pentamers (COs; Genre *et al.*, 2013); these are perceived by plant LysM-RLKs (Zipfel & Oldroyd, 2017) and activate plant symbiotic responses. AMF effector candidates, thought to interfere with host cellular processes to favour colonization at early and/or late stages of the AM symbiosis, have been predicted from fungal genomes and transcriptomes (Sędzielewska Toro & Brachmann, 2016; Kamel *et al.*, 2017). SLs stimulate the production of chitin oligomers (Genre *et al.*, 2013) and secreted proteins (Tsuzuki *et al.*, 2016; Kamel *et al.*, 2017) by AMF. Note that the tissue-specific expression of D14L/KAI2 and NOPE1 is currently unknown. IRM, intraradical mycelium; ERM, extraradical mycelium.

The recent discovery that an *N*-acetylglucosamine (GlcNAc) transporter of rice and maize, called NOPE1, is required for early signalling in the AM symbiosis, points to the existence of additional and GlcNAc-based diffusible plant molecules, which may trigger presymbiotic fungal reprogramming (Nadal *et al.*, 2017; Fig. 2). *nope1* mutants display very low levels of root colonization and root exudates from the mutant differ from wild-type (WT) exudates in their ability to induce transcriptome changes associated with the GO-term 'signalling' in the AMF *Rhizophagus irregularis* (Nadal *et al.*, 2017). Although the exact molecular function of NOPE1 and its substrate are so far unknown, the strong mycorrhizal phenotype of the *nope1* mutant indicates a crucial role in plant–fungal communication. Identification of the NOPE1 substrate will add an exciting new aspect to plant biology in general, as GlcNac-based signalling molecules are currently only known from bacteria and fungi but not – to our knowledge – from plants.

2. Fungal chitin-based molecules elicit symbiotic plant responses

AMF use GlcNAc-based molecules as pre-contact signals to activate symbiotic responses in the host plant such as calcium spiking, lateral root formation, starch accumulation and gene expression (Gutjahr *et al.*, 2009; Mukherjee & Ané, 2011; Czaja *et al.*, 2012; Genre *et al.*, 2013; Camps *et al.*, 2015; Sun *et al.*, 2015a). These so called 'Myc Factors' include lipo-chito-oligosaccharides (Myc-LCOs, Maillet *et al.*, 2011) and short chitin tetra- and pentamers (Myc-COs; Genre *et al.*, 2013) (Fig. 2). Although the Myc-LCOs show strong similarity to Nod Factors, which are released by nitrogen-fixing rhizobia (Gough & Cullimore, 2011), the metabolic pathways leading to their synthesis in AMF are not yet known.

Both Myc-COs and Myc-LCOs are able to elicit repetitive nuclear calcium (Ca^{2+}) oscillations, known as Ca^{2+}-spiking, which is considered a hallmark of symbiotic signalling (Oldroyd, 2013; Sun *et al.*, 2015a) in legumes. Interestingly, in rice only Myc-COs and not Myc-LCOs were able to elicit Ca^{2+} oscillations in root epidermal cells (Sun *et al.*, 2015a), indicating differences in the ability to perceive chitin-based symbiotic signalling molecules among nodulation-competent legumes and the monocot rice. So far, the biological significance of producing both Myc-COs and Myc-LCOs remains obscure. It is possible that a diversity of signalling molecules contributes to the ability of AMF to interact with a wide range of AM host plants or to the robustness of the system.

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However, GlcNAc-containing molecules can be produced by many microorganisms, including plant pathogens, and it is puzzling how plants can distinguish AMF from the others. One possibility is that this is facilitated by fine-tuned Myc Factors ligand-receptor specificities (Zipfel & Oldroyd, 2017). Small molecules with a GlcNAc backbone are perceived by LysM-domain containing receptor-like kinases (LysM RLKs) and receptor like proteins (LyM RLPs), with different ligand specificities (Gust *et al.*, 2012). The repertoire of LysM-receptors differs significantly among plant species (Zhang *et al.*, 2009), which may have favoured the co-evolution or maintenance of several different Myc Factors. Due to the functional redundancy of AMF-responsive LysM-receptor kinases in the genome of AMF-host plants, and the multitude of different Myc Factors, definitive receptors for Myc-COs or Myc-LCOs have not yet emerged (Buendia *et al.*, 2016; Zipfel & Oldroyd, 2017). Good candidates are SlLYK10 from tomato and NFP from *Parasponia*, as virus-induced and RNAi-mediated gene silencing of the corresponding genes, respectively, partially perturbed AM formation (Op den Camp *et al.*, 2011; Buendia *et al.*, 2016). However, there is currently no evidence that both LysM-RLKs bind Myc-COs or Myc-LCOs and it cannot be excluded that VIGS and RNAi affected the expression of additional redundant LysM-RLKs. The rice LysM RLK OsCERK1, which has a dual role in both interactions with pathogenic fungi and AMF (Miyata *et al.*, 2014), was shown to play a central role in the perception of Myc-COs because an *oscerk1* mutant does not respond to these molecules with Ca^{2+}-spiking (Carotenuto *et al.*, 2017). In addition, it fails to induce lateral roots in response to AMF (Chiu *et al.*, 2018). However, root colonization of *oscerk1* is only delayed and not entirely abolished (Miyata *et al.*, 2014; Zhang *et al.*, 2015; Chiu *et al.*, 2018), pointing towards redundant recognition mechanisms. By contrast, OsCEBiP, a LysM RLP, which acts as co-receptor of OsCERK1 in the perception of long-chain chitin oligomers from pathogenic fungi (Kaku *et al.*, 2006), is not required for the AM symbiosis and is not essential for Myc-CO-induced Ca^{2+} spiking (Carotenuto *et al.*, 2017). Therefore, an unknown LysM-containing protein likely associates with OsCERK1 to mediate specificity for the interaction with AMF.

An additional level of complexity may be added by the possibility that AMF may produce different amounts and/or a different repertoire of Myc Factors at different life-stages. Additionally, the composition of the Myc Factor cocktail may differ among AMF species. Thus, our understanding of how plants distinguish beneficial microbes and limit the invasion by detrimental ones will rely, at least in part, on the characterization of the blend of GlcNAc-containing molecules produced by AMF and their specific receptors and downstream signalling components.

Also volatile signals may participate in the belowground communication with the plant. Fungal volatile organic compounds (VOCs) can reprogram root growth and architecture and influence the defence system of the host plants (Werner *et al.*, 2016). Using an elegant split Petri-dish system, Sun *et al.* (2015b) found that volatiles, released by germinating spores of the AMF *Gigaspora margarita*, stimulated lateral root formation in *Lotus*, as well as in the AM nonhost *Arabidopsis*, indicating that these volatiles trigger a response, which is conserved in both host and nonhost species. The SLs biosynthesis gene *LjCCD7*, was upregulated following exposure to these VOCs, suggesting that SLs may act as mediators of such a response (Sun *et al.*, 2015b).

3. An emerging role for fungal effectors in AM symbiosis

In addition to GlcNAc-containing molecules, other AMF-produced factors contribute to interkingdom communication. A growing interest, coming from studies on pathogenic interactions, is given to effectors: they serve to dampen defence responses and/or to interfere with host cellular processes to favour colonization of the host (Lo Presti *et al.*, 2015).

AMF effector candidates have been predicted from fungal genomes and transcriptomes (Sędzielewska Toro & Brachmann, 2016; Kamel *et al.*, 2017). The number of identified genes depends on the criteria used to define effectors. A first criterion is the presence of a signal peptide that guides proteins towards secretion. In addition, the presence of cysteines, internal repeats, PFAM domains and nuclear localization signals also has been considered (Sędzielewska Toro & Brachmann, 2016; Kamel *et al.*, 2017). A large majority (95%) of *R. irregularis* secreted proteins (SPs) is conserved in the related species *R. clarus*, whereas only 194 of 872 (22%) of *R. irregularis* SPs show similarity with those from *Gigaspora rosea*, a distantly related AMF (Sędzielewska Toro & Brachmann, 2016; Kamel *et al.*, 2017). The AMF secretome therefore seems to be characterized by the prevalence of lineage-specific proteins, which is in agreement with data obtained from comparative analyses in other fungal groups including parasitic, mutualistic or saprotrophic fungi (Schirawski *et al.*, 2010; Heard *et al.*, 2015; Pellegrin *et al.*, 2015). Secretome variations have been ascribed to several factors such as phylogenetic history, life style as well as host specificity. Indeed, a comparison of the transcriptomes from *R. irregularis* and *G. rosea,* when colonizing three different host plants (the dicotyledon *M. truncatula*, the monocotyledon *Brachypodium distachyon* and the liverwort *Lunularia cruciata*), revealed that the expression of putative SPs can differ depending on the host plant. Among 87 SP genes expressed in the intraradical mycelium of *R. irregularis* only 33 were expressed in all three plant species (Kamel *et al.*, 2017), suggesting that these 33 fulfill core functions, whereas the others may act host-specifically (Fig. 3). Remarkably, a larger proportion (74%) of host-specific SPs was found in *G. rosea* with respect to *R. irregularis* (44%) and this may reflect differences in the their host range. Host-specifically expressed effector candidates also have been observed for the endophyte *Piriformospora indica*, when colonizing roots of barley or Arabidopsis (Lahrmann *et al.*, 2013). A tight host-specificity is more common in plant–pathogen interactions and host shifts can produce the most devastating disease outbreaks (Woolhouse *et al.*, 2005). In plant–pathogen interactions effectors can play a significant role in host specificity (Hung *et al.*, 2014). Regarding SPs, a recent study compared the complete genome sequence of six isolates of *Magnaporthe* species obtained from three different host plants. An inventory of SPs showed that many new SPs have evolved in different isolates and, interestingly, some of these SPs are only

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present in groups of isolates from the same host plant suggesting that the evolution of SPs is under host-directed selection (Zhong *et al.,* 2016).

However, Kamel *et al.* (2017) also identified a small set of SPs, shared by *R. irregularis and G. rosea,* with similar expression patterns in the different host plants. These genes, which have been referred to as the AM symbiotic core secretome, encode proteases or protein with unknown function. It has been hypothesized that proteases may play a role in the inactivation of plant defence proteins (Jashni *et al.*, 2015), the cleavage of fungal/plant signalling proteins and even the generation of peptides and amino acids with nutritional or signalling functions. The characterization of these common and lineage-specific SPs will highlight their mode of action inside the plant and reveal similarities and specificities between AMF and fungal pathogens.

The seminal work by Kloppholz *et al.* (2011) provided currently the only functional characterization of a putative AMF effector. The protein, named secreted protein 7 (SP7), from *R. irregularis* increased the degree of root colonization by AMF, when the corresponding gene was ectopically expressed in *M. truncatula* hairy roots (Kloppholz *et al.*, 2011). Using *Magnaporthe* as a heterologous system, the authors provided evidence of SP7 translocation into the plant cell nucleus, where it was suggested to counteract the plant immune response by interacting with the pathogenesis-related-transcription factor ethylene response factor ERF19 (Kloppholz *et al.*, 2011). However, the *SP7* gene is not only expressed in intraradical fungal structures; *SP7* transcripts also strongly accumulate in extraradical fungal mycelia (Kamel *et al.*, 2017), suggesting that SP7 may play a role in addition to suppressing plant immunity inside the root. SP7 contains several sequence repeats, which are separated by computationally predicted KEX2 protease cleavage motifs. This could mean that SP7 can be cleaved into small peptides, which may act on the fungus or the plant (Kamel *et al.*, 2017).

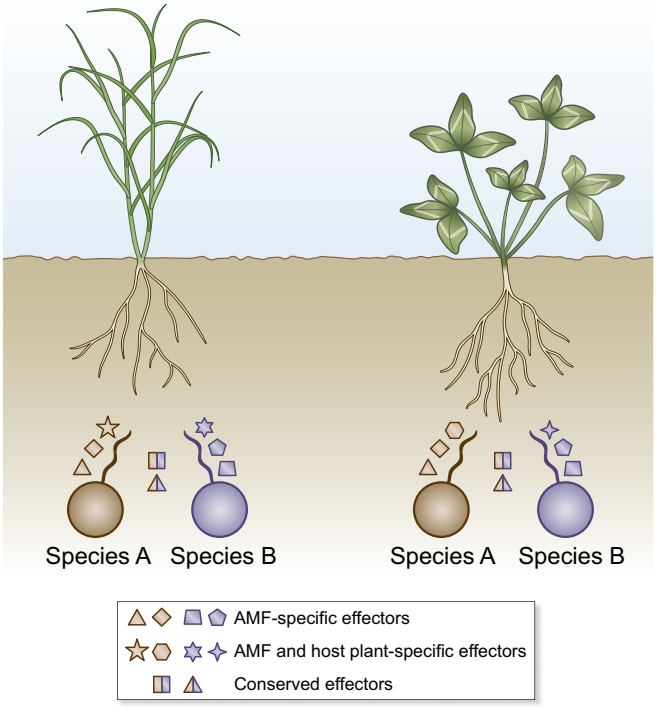

Fig. 3 Scheme of the variety of symbiotic effectors produced by arbuscular mycorrhizal fungi (AMF) during the interaction with host plants (based on data from Kamel *et al.*, 2017). For a single AMF species some effectors are expressed in association with all plant species, whereas others are expressed in a host plant-specific manner. Some effectors are conserved among AMF and may play core symbiotic functions.

Tsuzuki *et al.* (2016) recently described a gene encoding the putative secreted protein SIS1 from *R. irregularis,* which was among the genes upregulated in both SL-treated germinating spores and symbiotic extraradical mycelium; therefore, it has been proposed as a marker gene for fungal SLs response (Tsuzuki *et al.*, 2016). In the absence of genetic transformation protocols for AMF, SIS1 silencing was obtained by HIGS. This led to reduced colonization and stunted arbuscules.

Another *R. irregularis* gene has been identified with a putative role in the accommodation of fungal structures in the root (Fiorilli *et al.*, 2016). The gene was called *RiPEIP1* (Preferentially Expressed *In Planta*) because it is strongly induced in the intraradical phase, including arbuscules, as demonstrated by laser microdissection. *RiPEIP1* expression in *Oidiodendron maius*, an ericoid endomycorrhizal fungus, for which transformation protocols are available, led to enhanced colonization capacity compared to the *O. maius* WT strain (Fiorilli *et al.*, 2016). Because it encodes a four-transmembrane domain protein, *RiPEIP1* does not fit to the canonical definition of effectors; further studies are needed to define the mechanism of action of RiPEP1 and its specific role in the process of AM colonization.

In addition to proteins, small RNAs of the pathogenic fungus *Botrytis cinerea* were shown to target mRNAs of defence genes in the host plant, thus acting as effectors (Wang *et al.*, 2017). It is possible that such cross-kingdom RNAi also is exploited by AMF. The interference with RNA metabolism of the host plant also can be envisaged for the so-called RALPH (RNase-Like Proteins associated with Haustoria) the secreted avirulence effectors described in the obligate biotroph pathogenic fungus *Blumeris graminis* (Spanu, 2017).

III. Nutritional and regulatory roles for key metabolites in the AM symbiosis

After the AM symbiosis has been established, both symbionts benefit from nutrient supply by the other partner. Accumulating evidence indicates that the exchanged nutrients not only function as nourishment, but also act as signals that can drastically influence AM development. Thus, AM development is strongly linked to symbiotic function.

1. AMF receive lipids as well as carbohydrates from the host

Based on stable isotope labelling experiments, it has long been established that AMF receive carbohydrates and specifically glucose from the plant (Pfeffer *et al.*, 1999; Trépanier *et al.*, 2005). How the sugars are transported from the plant to the fungus is still unclear. A number of genes encoding sugar transporters with activities

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towards monosaccharides (MSTs) and sucrose (SUTs), as well as members of the SWEET family, are upregulated in mycorrhizal roots (Harrison, 1996; Doidy *et al.*, 2012; Manck-Götzenberger & Requena, 2016), but genetic evidence for their function is still missing. So far, only the function of the PAM-located sucrose transporter SUT2 from tomato has been investigated by reverse genetics (Bitterlich *et al.*, 2014). Roots of *sut2* antisense plants are significantly more colonized than WT roots. Together, this suggests that SUT2 may be involved in competition with the fungus for sucrose, for example by pumping the metabolite from the periarbuscular space (PAS; Fig. 1) back into the plant cell (Bitterlich *et al.*, 2014). A high affinity monosaccharide transporter MST2 from the AMF *R. irregularis* has been characterized. *RiMST2* is expressed in arbuscules and intercellular hyphae and is possibly responsible for sugar uptake from the plant apoplast surrounding the fungus. Silencing of *RiMST2* by HIGS led to reduced root colonization and impaired arbuscule branching (Helber *et al.*, 2011), indicating an important role of *RiMST2* function for fungal intraradical development. Interestingly, expression of *RiMST2* was triggered also in the extraradical mycelium, when it was supplied with xylose. Furthermore, the extraradical mycelium was able to take up ^{14}C-labelled glucose and xylose from the medium (Bücking *et al.*, 2008; Helber *et al.*, 2011) and this uptake was inhibited by the protonophore carbonyl cyanide m-chlorophenyl hydrazone, demonstrating that it occurred by active transport and not simple diffusion across the membrane (Helber *et al.*, 2011). The finding that AMF can actively take up pentoses and hexoses from the medium challenges the notion that obligate biotrophy of AMF is based upon strict dependence on plant-derived sugars.

Genome and transcriptome sequencing of the first AMF species shed more light on the biology and the evolution of AMF (Tisserant *et al.*, 2013; Lin *et al.*, 2014; Kamel *et al.*, 2016; Ropars *et al.*, 2016; Tang *et al.*, 2016). Surprisingly, it was found that genes encoding the cytosolic fatty acids (FA) synthase subunits, which are responsible for the bulk FA production in fungi, are absent from AMF genomes (Wewer *et al.*, 2014; Tang *et al.*, 2016). At approximately the same time it was discovered that legume mutants with stunted arbuscules and with reduced colonization were defective in three AM-induced lipid biosynthesis genes: *DISORGANIZED ARBUSCULES* (*DIS*), *FatM* and *REDUCED ARBUSCULAR MYCORRHIZA 2* (Wang *et al.*, 2012; Bravo *et al.*, 2016, 2017; Jiang *et al.*, 2017; Keymer *et al.*, 2017; Luginbuehl *et al.*, 2017). *DIS* encodes a β-keto-acyl-ACP synthase I (KASI), which is specific to genomes of AM-competent gymnosperms and dicots and catalyses FA chain elongation from C4 to C16 (Keymer *et al.*, 2017). *FatM* encodes a thioesterase, which terminates FA chain elongation by hydrolysis of the acyl-ACP, and FatM shows a preference for C16-ACP (Bravo *et al.*, 2017; Brands *et al.*, 2018). *RAM2* encodes an sn-2 glycerol-3-phosphate acyltransferase 6, which transfers a fatty acyl residue to the sn-2-position of a glycerol, thereby creating β-mono-acyl-glycerol (β-MAG, Luginbuehl *et al.*, 2017). Both *FatM* and *RAM2* have been found only in genomes of AM-competent land plants (Delaux *et al.*, 2015; Bravo *et al.*, 2016). Consistent with the phenotype, the promoters of all three genes *DIS*, *FatM* and *RAM2* are specifically active in arbuscule-containing cells (Gobbato *et al.*, 2013; Bravo *et al.*, 2017; Jiang *et al.*, 2017; Keymer *et al.*, 2017).

Comprehensive lipid profiling in *L. japonicus* and *M. truncatula* supported the hypothesis that DIS, FatM and RAM2 act in an AM-specific lipid-biosynthesis pathway because *ram2* mutants accumulate unusual phospholipids enriched in palmityl moieties, which are the predicted products of the concerted action of DIS and FatM (Bravo *et al.*, 2017; Keymer *et al.*, 2017; Brands *et al.*, 2018).

AMF store lipids mainly as tri-palmityl-triacylglyerol (16:0 – TAG) and desaturate the 16:0 fatty acyl chain at a specific ω5 position, permitting distinction of fungal from plant lipids by using 16:1ω5 FAs as an AMF-specific signature (Olsson *et al.*, 2005). The lipid profile of *dis*, *fatm* and *ram2* mutants contained hardly any 16:1ω5 FAs (Bravo *et al.*, 2017; Keymer *et al.*, 2017; Brands *et al.*, 2018), and the fungus *R. irregularis* did not form lipid-containing vesicles in *dis* and *ram2* mutant roots, and formed only very small vesicles in roots of *L. japonicus fatm* knock-down mutants (Keymer *et al.*, 2017; Brands *et al.*, 2018). This suggests that in the roots of these mutants the fungus is deprived of lipids. Lipid transfer from host plants to AMF was shown by two independent experimental approaches (Keymer & Gutjahr, 2018): Luginbuehl *et al.* (2017) and Jiang *et al.* (2017) used a synthetic approach and transformed *Medicago* hairy roots with the *Umbellularia californica* fatty acyl-ACP thioesterase gene (*UcFatB*) that produces the 12:0 FA, lauric acid, which occurs neither in *Medicago* nor in *R. irregularis*. Transgenic *Medicago* roots carrying *UcFatB* synthesized lauric acid and it also was detected in the spores of colonizing *R. irregularis* (Jiang *et al.*, 2017; Luginbuehl *et al.*, 2017), unequivocally demonstrating that lauric acid containing lipids were transferred from the host to AMF. Keymer *et al.* (2017) measured lipid transfer in non-transgenic plants by isotopolog profiling of 16:0 and 16:1 FAs. To this end, *Lotus* plants and carrot root organ culture were fed with ^{13}C labelled glucose. The isotopolog profile of 16:0 FAs in *Lotus* and carrot roots differed significantly. However, in each case the root profile was precisely mirrored by the 16:0 FAs in the fungal extraradical mycelium, as well as by the fungus-specific 16:1 FAs (Keymer *et al.*, 2017). This demonstrated that the fungal FA isotopolog profile was determined by the plant and, therefore, the FAs were transferred from the plant to the fungus. In the *dis*, *fatm* and *ram2* mutants, lipid transfer was impaired as well as in *str* mutants, which are deficient in an ABC-half transporter gene (Jiang *et al.*, 2017; Keymer *et al.*, 2017; Brands *et al.*, 2018). STR together with its complex partner STR2 (Zhang *et al.*, 2010) is considered a good candidate transporter for lipid transfer across the PAM (Gutjahr *et al.*, 2012; Bravo *et al.*, 2017; Keymer & Gutjahr, 2018).

Taken together, these recent findings indicate that AMF are entirely dependent on lipid supply by the plant for their growth, development and reproduction. The dependence on lipids may be the prime reason for their obligate biotrophy. This may explain why AMF store a large amount of lipids in their spores. They are probably used as resources for membrane construction during

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spore germination and during the first phase of root colonization, until the first developing arbuscules can obtain lipids from the host. These findings also change our view on the energy balance of the symbiosis, in which the burden of organic carbon compound biosynthesis is more significantly shifted towards the plant than was assumed previously.

2. Mechanisms of phosphate transfer from AMF to plant hosts

Phosphorus (P) is a major macronutrient limiting for plant growth. It occurs in soils predominantly as dihydrogen phosphate ion ($H_2PO_4^-$, Pi; Nussaume *et al.*, 2011). Due to the low mobility of this ion a phosphate depletion zone forms rapidly around the root. To overcome Pi starvation stress and increase access to Pi, plants have evolved several strategies. Under low Pi availability, plants activate a Pi starvation response system that regulates root and shoot architecture and physiology (Poirier & Bucher, 2002). In addition, plants can exploit the AM symbiosis to optimize Pi acquisition. Almost the entire Pi taken up by plants is contributed by AMF independent of the magnitude of the plant growth response (Smith *et al.*, 2004). Thanks to the extraradical hyphal network in the soil AMF greatly increase the absorbing surface area (up to 100-fold that of root hairs) extending well beyond the Pi depletion zone (Javot *et al.*, 2007b). AMF also were suggested to be able to mineralize soil organic P (Feng *et al.*, 2003; Shibata & Yano, 2003); and some initial evidence was provided by Sato *et al.* (2015) demonstrating that extraradical hyphae of the AMF *R. clarus* release an acid phosphatase of *c.* 187 kDa, which may be involved in mobilizing organic P. However, is not yet clear how far AMF are dependent on soil bacterial for phosphate mineralization. Colonization by AMF also induces the expression and secretion of acid phosphatases on the plant side (Ezawa *et al.*, 2005), indicating that the symbiosis may also increase the plant's ability to solubilize organic P from the soil.

Fungal Pi:H^+ symporter (PT) homologues of the yeast high-affinity transporter PHO84 (Bun-Ya *et al.*, 1991), are thought to be responsible for Pi uptake from the soil (Harrison & van Buuren, 1995; Maldonado-Mendoza *et al.*, 2001; Benedetto *et al.*, 2005; Xie *et al.*, 2016). Consistent with this, the fungal PT genes are expressed in the extraradical mycelium (ERM). However, their additional expression in the intraradical mycelium (IRM) suggests a second role in Pi reabsorption from the PAS (Benedetto *et al.*, 2005; Balestrini *et al.*, 2007; Fiorilli *et al.*, 2013; Xie *et al.*, 2016; Fig. 1).

Once absorbed by the ERM, Pi is quickly converted inside vacuoles into polyphosphate (polyP) chains, linear polymers comprising up to hundreds of Pi molecules (Solaiman *et al.*, 1999; Ezawa *et al.*, 2003). It has been hypothesized that AMF synthesize polyP through the VTC complex (Tani *et al.*, 2009; Ezawa & Saito, 2018), as described in yeast (Hothorn *et al.*, 2009). PolyP is then translocated to the IRM *via* cytoplasmic streaming and/or along a motile tubular vacuolar network (Olsson *et al.*, 2002; Uetake *et al.*, 2002; Hijikata *et al.*, 2010). Interesting new insights into the mechanism of long-distance polyP translocation in AM associations were obtained from the characterization of the *R. clarus* aquaporin 3 (RcAQP3), an aquaglyceroporin responsible for water transport across the plasma membrane (Kikuchi *et al.*, 2016). *RcAQP3* is strongly expressed in intraradical mycelia and downregulation of *RcAQP3 via* VIGS through the host plant, as well as the suppression of host plant transpiration, slowed polyP translocation. Thus, Kikuchi *et al.* (2016) proposed a model in which transpiration provides a primary driving force for polyP translocation by creating water flow through the fungal RcAQP3 and the AM-inducible plant aquaporins.

PolyP breakdown in the IRM possibly involves acid and alkaline phosphatases (Ezawa *et al.*, 2001; Aono *et al.*, 2004; Kojima & Saito, 2004) and produces a large amount of negative charges. A compensatory mechanism maintains a neutral charge inside the cell by accompanying the massive accumulation of polyP in fungal mycelia with near-synchronous and near-equivalent uptake of Na^+, K^+, Ca^{2+} and Mg^{2+} (Kikuchi *et al.*, 2014).

Pi is delivered to the periarbuscular space by a still unknown mechanism. It is then imported into plant cortical cells by AM-inducible, PAM-localized plant PTs, such as Medicago PT4 and rice PT11 (Javot *et al.*, 2007b; Yang *et al.*, 2012; Fig. 1). This transport is suggested to be driven by an H^+ energy gradient produced by a H^+-ATPase. Similar to PT4/PT11 this H^+-ATPase has been found to be important for arbuscule maintenance and AM-mediated phosphate uptake (Krajinski *et al.*, 2014; Wang *et al.*, 2014; Fig. 1). AM-inducible PT genes have been identified in different host plants (Rausch *et al.*, 2001; Harrison *et al.*, 2002; Paszkowski *et al.*, 2002; Nagy *et al.*, 2005; Balestrini *et al.*, 2007; Javot *et al.*, 2007a; Xu *et al.*, 2007; Loth-Pereda *et al.*, 2011; Hong *et al.*, 2012; Yang *et al.*, 2012; Willmann *et al.*, 2013; Xie *et al.*, 2013; Walder *et al.*, 2015; Volpe *et al.*, 2016; Sawers *et al.*, 2017). They are homologues of the yeast PHO84 and belong to the Phosphate transporter 1 (Pht1) class (Poirier & Bucher, 2002) of the plant H^+/Pi symporters. In a phylogenetic tree of PHT1 proteins, they cluster in a separate clade that does not contain Pht1 transporters from AM nonhost plants (Hong *et al.*, 2012; Yang *et al.*, 2012), indicating that an AM-specific *PT* genes duplication was maintained for symbiotic Pi transport in the plant kingdom. Interestingly, in addition to AMF, the root endophyte *Colletotrichum tofieldiae* was shown to transfer Pi to the AM nonhost plant Arabidopsis and to promote plant growth only under P-deficient conditions (Hiruma *et al.*, 2016). Although the Arabidopsis genome does not contain AM-specific *PT* gene duplications, several Arabidopsis genes of the *Pht1* family were induced during colonization. It will be interesting to investigate whether they, in a similar way to AM-specific PTs, localize to plant membranes close to fungal hyphae for direct Pi uptake from the fungus. Promoters of AM-specific PT genes have been mostly reported to be specifically expressed in arbuscule-containing cells. However, the *PT4* promoters of *M. truncatula* and *L. japonicus* are also expressed in root tips when grown in Pi starvation conditions (Volpe *et al.*, 2016). Interestingly, *mtpt4* mutants and *Lotus* hairy roots silencing *PT4* by RNAi do not fully respond to low Pi conditions with changes in lateral root formation (Volpe *et al.*, 2016). This suggests that PT4 is involved in root architecture responses to low Pi, in addition to symbiotic Pi uptake.

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3. Phosphate status influences AM development

When a fungal *PT* or plant *PT* genes essential for symbiosis are mutated or silenced arbuscule development is affected (Javot *et al.*, 2007a; Yang *et al.*, 2012; Volpe *et al.*, 2016; Xie *et al.*, 2016) by accelerated arbuscule turnover (Javot *et al.*, 2007a). This indicates that arbuscule lifetime is related to successful Pi delivery, a possible mechanism to avoid fungal parasitism (Gutjahr & Parniske, 2017). Interestingly, the accelerated arbuscule turnover in the *Medicago pt4* mutant can be suppressed when the plant is grown in nitrogen starvation conditions (Javot *et al.*, 2011; Breuillin-Sessoms *et al.*, 2015). This indicates that under these conditions, symbiotic nitrogen delivery becomes an advantage even if Pi is not delivered, according to Liebig's law of the minimum (Gutjahr & Parniske, 2017). However, a double mutant of *MtPT4* and the PAM-localized ammonium transporter *MtAMT2.3* (Breuillin-Sessoms *et al.*, 2015) retained a phenotype of premature arbuscule degeneration under N starvation conditions, pointing towards a particular importance of ammonium as compared to nitrate, at least in Medicago. Together, this indicates that fungus-delivered nutrients can act as cell-autonomous signals in the regulation of arbuscule maintenance. The molecular mechanism for this is currently unknown, but it has been suggested that PAM-localized PTs could act as transceptors similar to PHO84 in yeast (Popova *et al.*, 2010; Yang *et al.*, 2012; Breuillin-Sessoms *et al.*, 2015; Volpe *et al.*, 2016). This was based on the observation that in rice the *OsPT13* gene, which is specifically expressed in arbuscule-containing cells, is not required for AM-mediated Pi uptake, in contrast to the major player *OsPT11* (Yang *et al.*, 2012). However, mutation of *OsPT13* still leads to accelerated arbuscule turnover, indicating that *OsPT13* may be important for Pi sensing. The same may apply to ammonium transporters, as only *AMT2.3* was essential for arbuscule branching in the *pt4* mutant background, whereas the other AM-induced *AMT2.2*, *AMT2.4* and *AMT2.5* genes were not required or were redundant, although AMT2.4 showed a higher affinity for ammonium than AMT2.3 in yeast complementation assays (Breuillin-Sessoms *et al.*, 2015). This could indicate that the receptor activity of AMT2.3 is more important than its transport activity. Remarkably, the recently described PT gene from the AMF *Gigaspora margarita*, which is expressed in both ERM and IRM, was shown to act as a transceptor (Xie *et al.*, 2016). Thus, coupling of Pi uptake and sensing seems to be also important for the fungus.

An innovative RNAi-based suppressor screen for *pt4* and focusing on transcription factors led to the identification of MYB1, the first transcriptional regulator of arbuscule degeneration (Floss *et al.*, 2017). MYB1 is involved in the regulation of a range of hydrolase genes possibly involved in dismantling the arbuscule inside the cortex cell. The *myb1* mutant does not show an increased arbuscule lifetime in comparison to wild-type plants, (Volpe *et al.*, 2013; Floss *et al.*, 2017). Ectopic expression of MYB1 is associated with a decreased root length colonization, stretches of hyphae without arbuscules and a high incidence of degenerated arbuscules (Floss *et al.*, 2017). Together, these results indicate genetic redundancy at the level of MYB1 when Pi is delivered normally. MYB1 interacts with the GRAS proteins NODULATION SIGNALLING PATHWAY1 (NSP1) and with the suppressor of gibberellin signalling DELLA in binary interaction studies (Floss *et al.*, 2017), pointing towards a link between the regulation of arbuscule degeneration and plant hormone signalling.

In addition to its cell-autonomous influence on arbuscule maintenance, Pi also regulates AM formation in a systemic manner. It has long been known that AM colonization is repressed when plants are grown under high Pi supply (Mosse, 1973; Branscheid *et al.*, 2010; Balzergue *et al.*, 2011; Kobae *et al.*, 2016). In addition, in split-root experiments, in which only one side of the split root system was fertilized with high Pi concentrations, AM formation was suppressed on both sides (Branscheid *et al.*, 2010; Breuillin *et al.*, 2010; Balzergue *et al.*, 2011). Members of the miR399 family, which are systemic Pi-starvation signals, have been proposed as signalling molecules in the regulation of AM by Pi, as they are induced by AM fungal colonization (Branscheid *et al.*, 2010). miR399 overexpression did not restore AM fungal colonization at high Pi concentration (Branscheid *et al.*, 2010), suggesting that other mechanisms are involved. Perturbed early communication between plant and fungus also is a possible cause of reduced AM colonization. However, Ca^{2+} spiking in epidermal cells is still generated in response to AMF hyphopodia at high Pi conditions, indicating that the host plant maintains the ability to perceive and respond to the fungal partner (Balzergue *et al.*, 2013). On the plant side, SLs biosynthesis is reduced under high Pi. The exogenous application of GR24, a synthetic SLs analogue, failed to increase AM colonization levels at high Pi (Breuillin *et al.*, 2010; Balzergue *et al.*, 2011), suggesting that other factors or phytohormones such as auxin or gibberellin may be involved in suppressing AM at high Pi (Floss *et al.*, 2013; Carbonnel & Gutjahr, 2014; Pozo *et al.*, 2015).

Interesting further clues are emerging from metagenomics studies: the plant immune system (Lebeis *et al.*, 2015) and soil nutrient composition (Hacquard *et al.*, 2015; Castrillo *et al.*, 2017) were shown to play a key role in the coordination of root colonization by specific microbial taxa. Castrillo *et al.* (2017) demonstrated that the genetic network controlling the Pi stress response influences the composition of the microbial community of *Arabidopsis* roots. An *Arabidopsis* double mutant defective in *PHR1* and *PHL1*, encoding two redundant master transcriptional regulators of Pi starvation responses, showed an upregulation of plant defence genes leading to an atypical composition of a synthetic bacterial community at low as well as high Pi conditions. These results are in line with the observation that *Arabidopsis* roots induce defence genes when colonized at high Pi conditions by the fungal endophyte *C. tofieldiae* (Hacquard *et al.*, 2016), which promotes plant growth under low-Pi conditions by translocating Pi to the host (Hiruma *et al.*, 2016), reminiscent of what occurs in AM symbiosis. A similar activation of defence-related genes was observed in field-grown maize when the plants were grown at high soil Pi concentrations. This was accompanied by alterations in the root-inhabiting fungal community and with reduced root-length colonization by AMF (Yu *et al.*, 2018). It appears that lowering plant defences at low Pi serves to increase the chances of recruiting beneficial soil microbes to overcome the nutritional stress.

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Conversely, it is tempting to speculate that in Pi-sufficient plants, similar defence mechanisms may participate in limiting AM formation.

An RNAseq analysis of *R. irregularis* colonizing *Lotus* roots represents the first investigation of fungal responses to high Pi (Sugimura & Saito, 2017). Fungal cell-cycle regulatory genes, cyclin-dependent kinase CDK1 and several DNA replication- and mitosis-related genes were repressed under high Pi conditions in the IRM (Sugimura & Saito, 2017). The same genes are not regulated by a high Pi treatment in the ERM (Kikuchi *et al.*, 2014), suggesting that the transcriptional change in cell-cycle related genes may be mediated by the Pi-sufficient plant and not triggered by Pi itself. High Pi treatment also led to downregulation of 29 putative secreted proteins, including the SLs-induced putative secreted protein (SlS1) (Sugimura & Saito, 2017), pointing to an effect of the reduced SLs production of the plant.

IV. The plant–fungus genotype combination determines the outcome of the symbiosis

1. Plant growth responses cannot be predicted by AMF phylogeny

Despite a rather modest morphological variation, AMF show a high level of genetic variability. The characterization of ribosomal sequences revealed an unusually high sequence divergence, especially in the Internal Transcribed Spacer region (Thiéry *et al.*, 2016). Thus, the small rDNA subunit (SSU) is nowadays commonly used as a more reliable marker to define species in the Glomeromycotina (Öpik & Davison, 2016). However, SSU rDNA may suffer from a limited resolution and many exceptions to the correlation between SSU alone and morphological species were reported. Indeed, the concept of species for AMF is currently a matter of debate and resolution of this issue will possibly require multilocus data (Bruns *et al.*, 2018).

AMF also display a high functional diversity: the efficiency to stimulate plant growth of AMF genera and isolates belonging to the same species is highly variable. Also, depending on the host plant, the effect can vary in magnitude and can have either positive or negative growth consequences (Hart & Reader, 2002; Munkvold *et al.*, 2004; Feddermann *et al.*, 2008; Antunes *et al.*, 2011; Hong *et al.*, 2012; Fig. 4). The high functional variation, measured as the growth effect on the host plant, contrasts with the low intraspecific morphological variation shown by isolates of the same species.

In a large comparative study looking for relationships between fungal traits/phylogenetic position and plant growth responses, 56 AMF isolates belonging to six different families and 17 genera were inoculated on three different host plants (Koch *et al.*, 2017). Even if most isolates originated from geographically distant areas, traits such as extraradical hyphal volume or total spore weight were relatively constant within AMF families. Surprisingly, AMF phylogeny and species identity could not predict the plant growth response. Moreover, with the exception of total spore volume, none of the considered fungal traits (total fungal volume, extra- and intraradical fungal volumes) was positively correlated with plant performance (Koch *et al.*, 2017). This suggests that molecular features such as the repertoire of fungal signalling molecules, effectors or the abundance and efficiency of nutrient transport proteins may play a more important role for plant performance than AMF growth and morphology. Deciphering the origin of this intraspecific functional diversity is challenging and will require genomics and functional genomics studies at intra- and interspecific levels such as that of Chen *et al.* (2018). The effects on plant performance are likely under the control of a number of loci showing intraspecific polymorphisms. As suggested by host-specific expression patterns of candidate effector genes (Kamel *et al.*, 2017) the host plant may also play a role in the regulation of such loci. In addition, plant growth promotion may not be the only trait that should be considered: other benefits such as tolerance to abiotic or biotic stresses could provide a different picture. This knowledge will be fundamental to predict the impact of inoculation with specific AMF on plant performance.

The recent discovery of homokaryotic as well as dikaryotic strains of *R. irregularis,* and the identification of putative mating-type (MAT) loci (Ropars *et al.*, 2016; Corradi & Brachmann, 2017) highlighted the potential of AMF for sexual reproduction. The characterization of MAT loci will be instrumental to understand whether they are involved in dikaryon formation and, eventually, in karyogamy and meiosis. These new findings and expected advances in the understanding of AMF genetics and life cycle may even pave the way to genetic strain improvement for applied purposes.

2. Plant responsiveness to AMF is subject to genetic diversity

Not only the AMF, but also the plant genotype strongly affects the outcome of the symbiosis (Smith *et al.*, 2004; Fig. 5). The performance response of plants to AMF has been defined as responsiveness as opposed to dependence, which refers to genetically determined, poor nutrient use efficiency that can be compensated by AMF (Paszkowski & Boller, 2002; Janos, 2007;

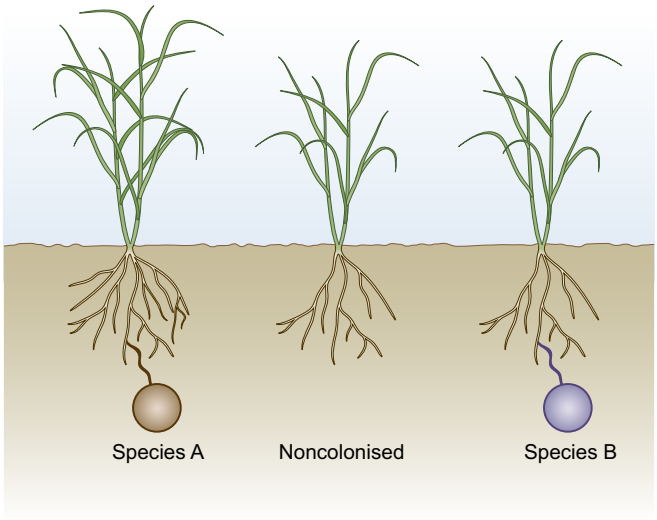

Fig. 4 The magnitude of plant growth promotion depends on the arbuscular mycorrhizal fungal (AMF) genotype.

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Sawers *et al.*, 2008). Responsiveness can differ among cultivars of the same species and, in addition, it is affected by soil nutrient content (Sawers *et al.*, 2010; Chu *et al.*, 2013), indicating a complex genotype–environment interaction. Sawers *et al.* (2017) identified a first symbiotic parameter, which may determine AM-responsiveness in maize. They investigated AM-responsiveness in 30 American maize lines, including the founder lines of a nested association mapping population (McMullen *et al.*, 2009), when colonized with the fungus *Funneliformis mosseae* in glasshouses. Interestingly, the capacity of the maize lines to profit from the symbiosis in terms of shoot dry weight and shoot Pi content correlated with the amount of associated extraradical hyphae (Sawers *et al.*, 2017; Fig. 5). This suggested an influence of plant genetics on fungal growth performance and, conversely, an impact of fungal morphology on plant performance when comparisons are based on only one fungal isolate. The plant molecular mechanisms determining fungal performance are entirely unknown and may be related to the amount of carbohydrates and lipids released to the fungus. Indeed, the expression pattern of monosaccharide transporter genes from the AMF *R. irregularis* in intraradical vs extraradical hyphae depended on the host plant (Ait Lahmidi *et al.*, 2016). This may be symptomatic of differences in monosaccharide supply or plant signals, which influence carbohydrate uptake strategies of the fungus.

Moreover, an ionomics screen for 19 mineral ions in shoots and roots using the same cohort of 30 maize lines, allowed the identification of clusters of ions, which changed in response to AMF and to maize genotype in a coordinated manner (Ramírez-Flores *et al.*, 2017). It will be interesting to understand how the coordinated uptake of or protection from certain ions occurs and whether these correlations also can be found in a field setting. Plant genetic variation also determines the root colonization level of a given fungus. However, according to our current knowledge, the amount of colonization is not a major determinant of plant performance benefit (Koch *et al.*, 2017; Sawers *et al.*, 2017). In a major study, 94 bread wheat genotypes were analysed for root length colonization by a mixed inoculum of three AMF species and six QTLs associated with colonization level were identified (Lehnert *et al.*, 2017). Interestingly, these QTLs contained genes related to defence and cell wall metabolism, which may be involved in limiting root colonization.

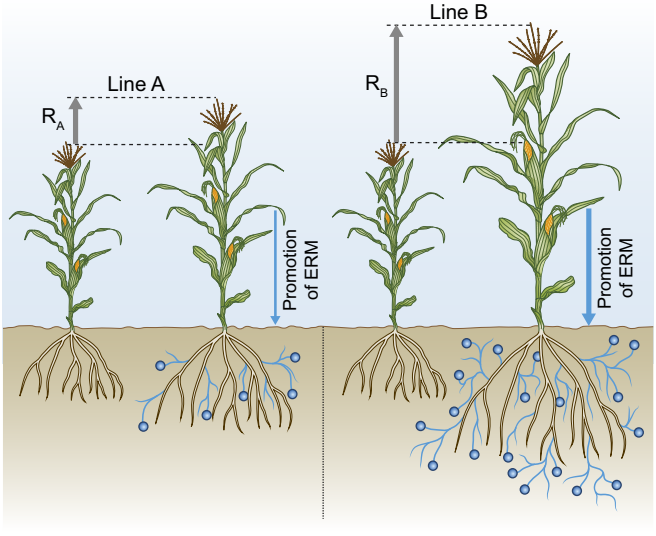

Fig. 5 Distinct plant genotypes of the same species show differences in responsiveness (R) to arbuscular mycorrhizal fungi (AMF). In maize, responsiveness was correlated with the ability of the line to promote the growth of the extraradical mycelium (ERM) of *Funnelliformis mossae* (Sawers *et al.*, 2017).

Some plant genotypes respond to AMF with growth depression. The mechanism behind the depression is not yet clear and depends partially on soil conditions (Sawers *et al.*, 2010). In other studies on wheat and barley growth depression was partially uncoupled from Pi uptake as well as from fungal growth (Li *et al.*, 2008; Grace *et al.*, 2009). It has been suggested that domestication may have decreased the ability of plants to respond positively to AMF (Lehmann *et al.*, 2012). This was investigated in a comparison of 27 crops with their wild progenitors (Martín-Robles *et al.*, 2018). Both wild and domesticated species responded to AMF at low Pi conditions. However, the response was not strictly correlated to Pi in the green leaves, indicating either a variety of Pi partitioning strategies in the different species or a range of mechanisms contributing to the growth response. A subset of 14 pairs of wild and domesticated species was also tested at high Pi conditions. Interestingly, the growth response of wild progenitors to AMF was similar at low and high Pi, whereas it was strongly reduced at high Pi in the domesticated counterparts. In addition, suppression of root colonization at high Pi was more pronounced in the domesticated plants (Martín-Robles *et al.*, 2018). Together, this indicates that – at least in the tested species – domestication selected for AM independence at high Pi concentrations, which possibly increased yield in the absence of the fungus-associated carbon drain. However, as AMF provide other services to plants such as increased resistance to abiotic stress and certain pathogens, it remains to be investigated whether other stresses would enhance AM-responsiveness of domesticated plants under high Pi fertilization.

V. Perspectives

It is now commonly accepted that soil biodiversity promotes multiple ecosystem functions and that the tailored management of soil communities, including AMF, has the potential to enhance agricultural sustainability (Bender *et al.*, 2016). Understanding the biology of AMF and the AM symbiosis is therefore crucial for their full exploitation. A significant enlargement of our current knowledge in several fields of AM research can be envisaged in the near future.

Comparative genomics and transcriptomics from a larger number of AMF species will expand our knowledge of their genome organization, genetic and regulatory complexity. The complexity of AMF genetics is increased by the presence of endobacteria, which live inside many AMF (Bonfante & Desirò, 2017) and may influence fungal fitness. For example, the endobacterium Candidatus *Glomeribacter gigasporarum* was shown to increase sporulation, ATP production, reactive oxygen detoxification and responsiveness to the plant signal strigolactones of the

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fungal host, *G. margarita* (Salvioli *et al.*, 2016). In addition, viruses can thrive inside AMF, yet our knowledge on the AMF virome is limited to few AMF species (Ikeda *et al.*, 2012; Kitahara *et al.*, 2014; Turina *et al.*, 2018). In particular, Ikeda *et al.* (2012) demonstrated that a virus-free fungal strain produced more spores and promoted plant growth more efficiently than the virus-containing strain. The full complement of the microbiota living inside AMF certainly deserves further investigation to define their influence on the metabolism of the fungal host and the potential impact on plant performance.

The characterization of putative AMF effectors and the identification of factors involved in the perception of plant signals, nutrient uptake, transport and metabolism also will be an active field of research and should involve AMF species-comparisons to foster an understanding of AMF functional diversity. Current limitations in the direct genetic manipulation of AMF can be circumvented using heterologous systems such as *Nicotiana benthamiana* leaf and legume hairy root assays or transgenic expression in transformable biotrophic fungi such as *O. maius* (Fiorilli *et al.*, 2016) or pathogenic oomycetes such as *Phytophtora palmivora* (Rey & Schornack, 2013). HIGS or VIGS and the emerging tool Spray-Induced Gene Silencing (SIGS; Wang & Jin, 2017) can be exploited for silencing fungal genes; however, the efficiency and reliability of these methods still need to be improved.

We expect to see progress in the description and characterization of plant receptors for AMF signalling molecules as well as in the identification of substrates of receptors and transporters such as D14L/KAI2 and NOPE1 (Gutjahr *et al.*, 2015; Nadal *et al.*, 2017). Physiological and molecular investigation is needed to resolve mechanisms and regulation of nutrient transfer between the symbionts and, in particular, the flux of carbohydrates and lipids towards the fungus (Rich *et al.*, 2017). It is becoming increasingly clear that despite their large host range, the efficiency of AMF in promoting plant performance differs strongly among fungal species and isolates, and the ability of the plant to respond to the symbiosis depends on the plant genotype. The molecular basis of AM-responsiveness is entirely unclear but it may depend on a diversity of traits such as nutrient partitioning, hormone homeostasis or (in)compatibilities of AMF effector–plant target pairs. The identification of the genetic polymorphisms underlying differences in symbiotic performance of plants and AMF will be key to smart breeding for profitable application of the AM symbiosis in sustainable agricultural systems with reduced chemical fertilizer and pesticide input.

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ORCID

Luisa Lanfranco http://orcid.org/0000-0002-3961-2552
Valentina Fiorilli http://orcid.org/0000-0001-9805-1559
Caroline Gutjahr http://orcid.org/0000-0001-6163-745X

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