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Signals and host cell remodeling in arbuscular mycorrhizal symbiosis

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Abstract

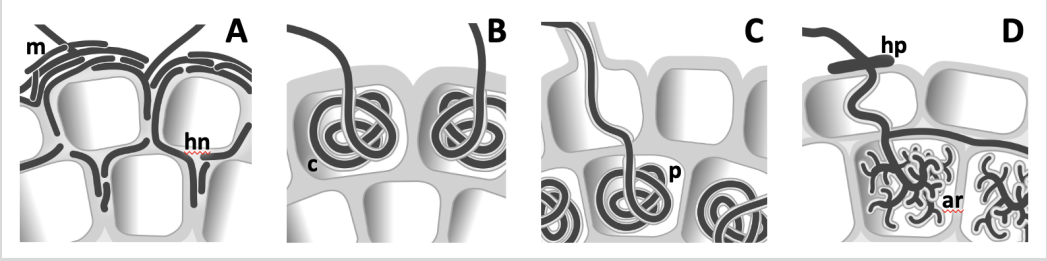
Mycorrhizas are mutualistic interactions that the majority of land plants establish with a heterogeneous group of soil fungi; their distribution and diversity have supported the success of plants on the planet. Among all different types of mycorrhizas, arbuscular mycorrhiza (AM) is the most ancient and the most common in host plants of all major crops. The functional core of AM is a finely branched fungal structure called the arbuscule. Arbuscules are hosted inside living root cells, within a specialized cell compartment that is generated through a precise sequence of molecular and cellular events. Over the last 10 years, the application of novel technologies, such as genome sequencing, high-throughput transcriptomics and live cell imaging have generated substantial advances in our knowledge of such events. Here we present a synopsis of the recent literature on the interactions between AM fungi and their hosts, with an evolutionary-developmental focus on the intimate contact that develops between plant cells and fungal hyphae, in terms of molecular signaling, nutrient exchange and cell organization.

CONTENTS

Our planet hosts around 390,000 plant species. Within their hypogeous and epigeous organs, plants store more than 450 Gigatons (Gt) of carbon, roughly corresponding to 80% of the planet biomass (Bar-On et al 2018). Interestingly, the belowground biomass is mainly composed of plant roots (130 Gt C) and soil-dwelling microbes. Among them, fungi (with their 12 Gt C) are the second most conspicuous soil microbes, only outnumbered by bacteria (70 Gt. C). Such figures provide solid experimental support to the acknowledged claim that mycorrhizas -

symbiotic associations between around 340,000 plant species and 50,000 species of soil fungi - represent one of the most powerful and pervasive ecological processes in terrestrial environments. In these mutually beneficial interactions, fungi receive photosynthesis-derived carbon from the plant, while supporting the plant diet with mineral nutrients, mostly phosphorus and nitrogen. Present in both natural and human-controlled agricultural niches, mycorrhizas offer several ecosystem services. Mycorrhizal fungi are in fact well known drivers of carbon sequestration and soil particle aggregation. They also have a major impact on the composition of microbial and plant communities, as originally demonstrated in a seminal study by Van der Heijden and colleagues (1998) where the below-ground diversity of arbuscular mycorrhizal fungi emerged as a major factor in the maintenance of plant biodiversity. Consensus is growing on the role of biodiversity in ecosystem functioning, and - in this respect - mycorrhizal symbioses are considered to give a major contribution to the mechanisms that support the whole biosphere and its functioning (Eisenhauer et al 2022). The link between mycorrhizas and planet biodiversity is quite well defined: 90% of extant vascular and non-vascular plants can form mycorrhizas and, among them, angiosperms account for the largest and most diverse group of mycorrhizal species (85–90%). They include annual herbs and perennial trees as well as the majority of staple crops such as rice, wheat, maize, potato, sweet potato, tomato and cassava. The number of fungal mycorrhizal species is more difficult to ascertain and is currently estimated to account for around 10% of known fungal species (Hyde, 2022), belonging to the three major phyla of Ascomycota, Basidiomycota and Mucoromycota.

BOX 1 - Main types of mycorrhizal symbioses



The figure illustrates four major types of mycorrhizal interactions. In ectomycorrhizas (A), the epidermal cells of lateral roots are targeted by extraradical hyphae that proliferate on the surface of the whole root tip, engulfing it in a pseudoparenchymatous tissue known as the mantle (m). Inner mantle hyphae

further penetrate between epidermal cells, proliferating between epidermal and outer cortical cells to generate the so-called Hartig net (hn), a dense mesh of hyphae hosted in an expanded apoplast.

In ericoid mycorrhizas (B), extraradical hyphae directly penetrate epidermal cell walls without the development of apparent adhesion structures. Convoluted hyphal coils (c) are then produced inside each epidermal cell, enclosed within the symbiotic interface compartment and the perifungal membrane (8).

In orchids (C), mycorrhizal symbiosis involves both germinating seeds (protocorms) and seedling roots, but the best described colonization process is in protocorms. Here, epidermal hair cells are the main penetration site for external hyphae, which then reach the cortical parenchyma to form large intracellular hyphal coils, or pelotons (p), within a membrane-delimited symbiotic interface.

Lastly, in arbuscular mycorrhizas (D), presymbiotic hyphae contact the epidermal cells of young lateral roots forming an adhesion structure called the hyphopodium (hp). The hyphopodium, often flattened and branched, adheres strongly to the epidermal cell walls. A penetrating hypha then develops in this area, crosses the epidermis and outer cortical cell layers with both loose intracellular coils and branched hyphae, and finally reaches inner cortical cells, where tree-like arbuscules are formed (a). Also in this case, all intracellular hyphae and arbuscules are accommodated in the symbiotic interface. This interface, outlined by the periarbuscular membrane, is believed to be the main site of nutrient exchange, thanks to its extensive surface.

These diverse mycorrhizal types involve different groups of fungi: Basidiomycota and Ascomycota are the preferential partners in ectomycorrhizas and ericoid mycorrhizas, while Basidiomycota taxa associate with orchids and Glomeromycotina develop arbuscular mycorrhizas (Genre et al 2020).

The resulting plant-fungal diversity, supported by over 400 million years of co-evolution, has resulted in four main mycorrhizal types, defined according to their major morphological traits (BOX 1): ectomycorrhizas (ECM), ericoid mycorrhizas (ERM), orchid mycorrhizas (ORM) and arbuscular mycorrhizas (AM). Their specific cell-to-cell interactions were initially described by ultrastructural observations between 1970s and 1990s, and these pioneering studies provided the foundation for subsequent investigations on signalling and gene regulation in these

symbioses (Bonfante 2018). This corpus of studies revealed that several cellular and molecular traits are shared among the four types of mycorrhizas, even if they originated independently at different times during plant evolution (Genre et al 2020) and have an uneven phylogenetic distribution (Tedersoo et al 2020): AMs are the dominant type (involving 72% of plant species), followed by ORM (10%), ECM (2%) and ERM (1.4%). Nevertheless, this distribution between plant species does not mirror the real ecological success of each mycorrhizal type. In fact, ECMs dominate the vast boreal forests, despite the low number of gymnosperm and angiosperm species involved. By contrast, AMs - with their extremely broad host ranges - are present across most land ecosystems, from arid sub-arctic environments to sub-tropical rainforests. A reason for the ecological success of AMs can be found in their evolutionary history. AMs are in fact acknowledged as the most ancient form of symbiosis, with the earliest fossil evidence of such plant–fungus associations dating back to 400 million years ago. In fact, the study of the uniquely preserved ecosystem of the Rhynie chert revealed that extinct plants such as *Aglaophyton majus* hosted intracellular structures that are very similar to modern arbuscules (Remy et al, 1994).

Besides wild plants, AMs also develop in most crop plants, which has focused the interest of scientists, producers and politicians on this symbiosis. Understanding the mechanisms that regulate plant-fungal interplay has in fact emerged as a social responsibility for solving the dilemma of saving space for wild nature preservation (Wilson, 2016) or growing plants to feed a growing human population.

In the framework of this complex ecological and ethical scenario, we propose a synopsis of the recent literature on the interactions between AM fungi and their hosts, with a particular focus on the intimate contact that develops between plant cells and fungal hyphae, in terms of molecular signaling, nutrient exchange and cell organization. Novel technologies, such as genome sequencing, high-throughput transcriptomics and proteomics as well as live sub-cellular imaging are providing the tools to decipher the genetic, molecular and cellular bases of such plant-fungal interactions.

II. An insight in the biology of arbuscular mycorrhizal fungi

Arbuscular Mycorrhizal (AM) fungi are the most relevant representatives of Glomeromycotina, a subphylum of the Mucoromycota, which also embraces

Geosiphon pyriformis, a rare fungus hosting cyanobacteria (Schüßler, 2012). Even if their ranking as Glomeromycota or Glomeromycotina is still controversial (Tedersoo et al., 2018; Spatafora et al., 2016), the assignment of AM fungi to Mucoromycota satisfies morphological, genomic and functional parameters. As in other Mucoromycota, they are aseptate and multinucleated fungi which often host endobacteria in their cytoplasm (Bonfante and Venice 2020), but also possess unique features: they are described as asexual, obligate symbionts, which depend on the reduced carbon obtained by the host plants, while they provide plants with minerals. Glomeromycotina which contain different orders interact with a huge number of plants: liverworts, ferns, gymnosperms (from *Ginkgo biloba* to *Cupressus*) and angiosperms (from trees such as poplar to herbs and shrubs). It is also relevant to remind that AM fungi are probably not alone to colonize plants producing intracellular branched hyphae: Mucoromycotina 'fine root endophytes', previously known as *Glomus tenue*, are a group of soil fungi that form endosymbioses with different land plants (Sinanaj et al 2021), even if significant questions on their distribution and functions are still open.

Genomics has improved some of our views on AM fungal biology: AMF genomes are among the largest in the fungal kingdom, ranging from 125 Mbp for the A1 strain of *Rhizophagus irregularis* (Tisserant et al 2013) to the unexpected sizes of 570 Mbp of *Gigaspora rosea* (Morin et al., 2019) and 770 Mbp of *G. margarita* (Venice et al., 2020). By contrast, the gene number is comparable among the AM sequenced species (around 25000-26000 genes), even if in the largest genomes, the size was inflated by a huge amount of transposable elements, which occupy around 70% of their genomes. However, *Paraglomus occultum* which belongs to the the early diverging order of Paraglomales has a very reduced genome, i.e. 39.6 Mb, fewer genes, and lacks the genes involved in the production of fatty acids and sugars (see below), suggesting that the MRCA of Glomeromycotina was already an obligate plant biotroph (Malar et al., 2022). As for some plant pathogens, AM genomes encode for a large number of effectors, that might guarantee the communication between AM fungi and their hosts, and for a limited presence of plant cell wall degrading enzymes. This trait may be involved in eluding host defense and maintaining the viability of host cells. AM fungi are fully dependent on plant-derived lipids, since their genomes lack a cytosolic Fatty Acid Synthase complex that is common to fungi (FASI), but possess a mitochondrial FASII for the

synthesis of lipoic acid (reviewed in Bonfante and Venice, 2020). This feature, together with the apparent lack of the pathway for thiamine biosynthesis and the limited uptake of sugars from soil (Tisserant et al., 2013), contributes to the signature of AMF as obligate biotrophs. However, *R. irregularis* could be grown and reproduced axenically on a medium added with fatty acids, among which myristate (Kameoka et al., 2019, Sugiura et al., 2019). Some AM fungal genomes have revealed the presence of pathways leading to the synthesis of secondary metabolites, as polyketides (Venice et al 2020b). The multinucleate status of AM fungi on one hand complicates transformation procedures and on the other raises many debates on their level of genetic variability. Genome sequences and an in-depth analysis of MAT-loci, which are related to fungal sexuality events, revealed that while most of the isolates are homokaryotic i.e., containing nuclei of one genetically similar nucleotype, a few are dikaryotic, i.e. containing nuclei of two genetically divergent nucleotypes (Ropars et al 2016; Kokkoris et al 2021). Furthermore, the availability of multiple sequenced strains from the same species, revealed an impressive amount of intra-specific variability (the *R. irregularis* strains only share around 8000 conserved genes), such that the concept of "pangenomes" has been suggested to describe AM genomes (Mathieu et al., 2018). In conclusion, current views indicate a long evolutionary story of AM fungi mirroring their capacity to adapt to their plant hosts and changing environments, and to interact with endobacteria. However, due to the multinucleate status of AM fungi, their genes cannot be easily tested for their functionality, with the rare exception of the fungal genes involved in Pi uptake (Xie et al., 2022). As a consequence, our current physiological knowledge is mostly based on the host plant side, while many of the striking biological features of AM fungi (i.e. obligate biotrophy, multinucleated condition, unknown sexual status) make them remain a rather intractable experimental material.

III. The development of Arbuscular Mycorrhiza

The establishment of AM symbiosis (Fig. 1) has been elucidated in detail and is largely conserved, with minor variations depending on the plant and fungal partners involved (Choi et al., 2018). Fungal spore germination is activated in response to optimal moisture and temperature conditions and generates a loose mycelium that

grows for a few days at the expense of spore-stored lipids (Genre et al., 2020). The resulting hyphae explore the surrounding soil in search of a host root. Following reciprocal plant-fungus recognition through an exchange of diffusible molecules, fungal hyphae contacting the root epidermis develop swollen and often branched adhesion structures called hyphopodia. In response to both chemical and physical stimuli produced by the hyphopodium, the contacted epidermal cell develops an intracellular accommodation structure, called the prepenetration apparatus (PPA) (Genre et al., 2005), a columnar cytoplasmic aggregation that concentrates the whole exocytic pathway and anticipates the route of the penetrating hypha across the epidermal cell. PPA-associated exocytosis drives the biogenesis of the symbiotic interface, a novel cell compartment surrounded by an extension of the host plasma membrane, that contains all intracellular hyphae (Bonfante 2001). Once the epidermal layer is crossed, hyphae can branch and grow both inter- and intracellularly to reach the inner tissues. The PPA mechanism is replicated in each colonized cell and reaches the highest complexity and extension in the deepest cortical cells where hyphae branch repeatedly to generate the core structure of AM symbiosis: arbuscules (Luginbuehl and Oldroyd, 2017). Arbuscules are highly branched intracellular hyphae that can occupy up to 90% of the host cell volume. Arbuscules are ephemeral structures that collapse and degenerate within a few days (Gutjahr and Parniske, 2013; Kobae and Hata, 2010), with the host cell regaining its previous organization.

While root colonization proceeds, the extraradical mycelium also develops extensively in the surrounding soil, fed by plant-derived sugars and lipids. Besides scavenging water and mineral nutrients to be delivered to the host plant, the extraradical mycelium is also responsible for the production of a new generation of spores.

IV. Signaling as a prerequisite for symbiosis

AM fungi perceive the vicinity of a host via root-exuded molecules that induce spore germination and hyphal branching (Buée et al, 2000; Nagahashi and Douds, 2004). The most studied plant symbiotic signals are carotenoid-derived phytohormones called strigolactones (SL), which have a primary role in plant development (Al-Babili and Bouwmeester, 2015; Ruyter-Spira et al., 2013). AM

fungi sense SLs in root exudates (Figure 2) at concentrations as low as 10 nM. Fungal responses to GR24, a synthetic molecule commonly used to study SL actions, include stimulation of mitochondrial activity, a rapid increase in ATP and NADH and nuclear proliferation (Akiyama et al, 2005; Besserer et al., 2006; Besserer et al 2008; Akiyama et al., 2010, Salvioli et al 2016). GR24 exposure also causes a sharp increase in Ca^{2+} concentration in the fungal cytoplasm (Moscatiello et al., 2014). Although fungal SL receptors remain unknown, such observations suggest that SLs are perceived via a Ca^{2+} mediated signaling pathway and trigger a cellular and molecular prelude to root colonization (Besserere et al., 2006, 2008; Bonfante and Genre, 2015).

AM fungi also release signal molecules (Figure 2) that trigger plant symbiotic responses (Bonfante and Requena, 2011), including transcriptional regulation, nucleus associated Ca^{2+} signals, starch accumulation in roots and lateral root formation (Lanfranco et al., 2016). Repeated oscillations in nuclear Ca^{2+} concentration (spiking) have been observed in the root epidermal cells contacted by AM fungal hyphopodia, but also when the same cells were treated with exudates from germinated AM fungal spores (Chabaud et al., 2011). Similarly, the expression of the early symbiotic gene ENOD11 in *M. truncatula* is upregulated upon both fungal contact (Chabaud et al., 2002) and the perception of fungal exudates (Kosuta et al., 2003).

Different N-acetylglucosamine oligosaccharides have been characterized in AM fungal exudates as bio-active molecules responsible for such plant responses. They include tetra- and penta-chito-oligosaccharides (CO4 and CO5) (Genre et al., 2013) as well as lipo-chito-oligosaccharides (LCOs), which are very similar to nodulation (Nod) factors released by nitrogen-fixing rhizobia (Maillet et al., 2011). When applied as purified molecules, such Myc-factors mimic the perception of fungal exudates in the host roots, including nuclear Ca^{2+} spiking and the regulation of symbiosis-related genes. Interestingly, the release of CO4 and CO5 in *R. irregularis* exudate is boosted upon GR24 treatment (Genre et al., 2013), suggesting the existence of a positive loop between plant and fungal signal perception and production of these oligosaccharides (Lanfranco et al., 2016; Volpe et al., submitted).

Recent investigations are shedding light on the role of alternative receptor complexes, with central roles played by CERK1 and MYR1 LysM receptor-like

kinases (Miyata et al., 2014; He et al., 2019) in the plant perception of different fungal-derived molecules, such as short- and long-chain COs (Zhang et al., 2021), leading to the activation of symbiotic or defense-related plant responses (Chiu and Paszkowsky, 2021).

The study of plant signaling mechanisms involved in the perception of AM fungal signals has been developed in legumes such as *Medicago truncatula*, largely following the research on rhizobial Nod factor signaling. Such comparative investigations have revealed the existence of a so-called 'common symbiotic signalling pathway' (CSSP), which includes several genes that are essential for both symbioses (Oldroyd, 2013; Gobbato, 2015). Evidence that the same genes are also involved in diverse symbiotic, pathogenic and parasitic plant interactions is accumulating (Genre and Russo, 2016).

The CSSP (Figure 3) starts on the plant cell membrane, with a malectin-like domain (MLD) leucine-rich repeat (LRR) receptor-like kinase (known as SYMRK, in *Lotus japonicus*). SYMRK is believed to be able to form alternative complexes with either Nod factor or Myc factor receptors. In its cytoplasmic domain, SYMRK interacts with a MAP kinase kinase (Chen et al., 2012), and HMGR1, a 3-hydroxy-3-methylglutaryl-CoA reductase involved in mevalonate synthesis. Indeed, mevalonate has been demonstrated to trigger downstream symbiotic responses such as nuclear Ca^{2+} spiking and ENOD11 expression (Venkateshwaran et al., 2015).

The remaining known CSSP proteins are either localised to the nuclear envelope or the nucleoplasm: three nucleoporins - NUP85, NUP133, and NENA (Kanamori et al., 2006; Saito et al., 2007; Groth et al., 2010) - are likely involved in nuclear targeting of downstream CSSP actors, such as the nuclear envelope localized ion channel CASTOR/POLLUX (Charpentier et al., 2008), the Ca^{2+} channel CNGC15 (Charpentier et al., 2016) and the SERCA-type ATPase MCA8 (Capoen et al, 2011). The nuclear envelope lumen is a major Ca^{2+} store, and indeed Ca^{2+}-mediated signaling is a central hub in the CSSP, with the induction of characteristic oscillations (spiking) in nuclear Ca^{2+} concentration (Genre and Russo., 2016). Ca^{2+}-spiking is interpreted by the nucleoplasm-localized Ca^{2+}-and-calmodulin-dependent protein kinase CCaMK (Mitra et al., 2004). Its activation regulates gene expression through its interacting partner CYCLOPS (Miwa et al., 2006) and the transcription factors NSP1, NSP2, NIN and RAM1 (Oldroyd 2013; Genre and Russo, 2016).

However, this largely characterized pathway is probably not exclusive (Bonfante and Requena, 2011) and emerging evidence suggests the presence of additional, CSSP-independent signalling. On this line, Gutjahr et al. (2015) identified loss of responsiveness to AM fungi in a rice mutant, which was also mirrored by the absence of physical contact and of characteristic transcriptional responses to AM fungal diffusible signals. The responsible gene, DWARF 14 LIKE (D14L), encodes an alpha/beta-fold hydrolase involved in the perception of karrikins and unrelated to the recognition of known Myc-factors, demonstrating the existence of parallel fungal recognition strategies in AM host plants.

Despite the remaining unclear aspects, over twenty years of research have conclusively demonstrated that a common genetic basis underpins AM colonization in 72% of land plants, through the perception of fungal signals and the activation of conserved accommodations strategies.

V. The symbiotic interface

The perifungal or periarbuscular membrane surrounding intracellular hyphae and arbuscules, respectively (Harrison, 2012) outlines the so-called symbiotic interface, a novel cell compartment containing a thin layer of unstructured cell wall materials (Bonfante, 2001; Gutjahr and Parniske, 2013; Balestrini and Bonfante, 2014) where the fungus is hosted and where most of the signal and nutrient exchanges are believed to occur (Luginbuehl and Oldroyd, 2017; Choi et al., 2018).

A number of plant genes is required for arbuscule accommodation and symbiotic interface development and functioning. Besides cell reorganization-related proteins, such as Vesicle-Associated Membrane Proteins (Ivanov et al., 2012), EXO70I (Zhang et al., 2015), proteases (Takeda et al., 2009; Rech et al., 2013), Vapyrin (Pumplin et al., 2010), arbuscule-associated genes include lipid biosynthetic enzymes such as FATM and RAM2 (Bravo et al., 2017) and a specific set of membrane-associated proteins that reside on the periarbuscular membrane throughout arbuscule functioning and are directly involved in nutrient exchange. These include a proton ATPase (Krajinsky et al., 2014; Wang et al., 2014), ATP-binding cassette (ABC) transporters, Stunted Arbuscule (STR) and STR2 (Zhang et al., 2010), phosphate transporters PT4 (Javot et al., 2007;) and PHT1 (Yang et al., 2012), ammonium transporters AMT2 (Guether et al., 2009). Interestingly, trafficking of some of these proteins, such as phosphate transporters to the

periarbuscular membrane requires their genes to be expressed during arbuscule branching (Pumplin et al 2012), leading to the hypothesis that PAM construction is achieved by synchronizing the massive reorientation of exocytosis with the transcription of periarbuscular membrane-resident proteins (Gutjahr and Parniske 2013). In line with this, a few plant transcription factors required for AM symbiosis and arbuscule development have been identified, such as CYCLOPS (Horváth et al., 2011), the gibberellin repressor protein DELLAs (Takeda et al., 2015), Reduced Arbuscular Mycorrhiza1 (RAM1; Gobbato et al., 2012), Required for Arbuscule Development1 (RAD1; Xue et al., 2015), and DELLA-Interacting Protein1 (DIP1; Yu et al., 2014). Additional evidence suggests a model where DELLA proteins regulate arbuscule development through modulation of RAM1 and RAD1 that in turn regulate genes required to support arbuscule branching (Park et al., 2015).

Arbuscule formation and functioning is also expected to be under fungal control, however, our understanding of such processes is limited. Fungal effectors are thought to promote compatibility or to suppress plant defense responses by interfering with metabolism or signaling pathways, in analogy with other plant-microbe interactions (Lo Presti et al., 2015). Indeed, Secreted Protein 7 (Kloppholz et al., 2011) is secreted into the host cell and localizes to the plant nucleus, where it counteracts the plant immune response by interacting with the pathogenesis-related transcription factor Ethylene Response Factor 19. In this frame, AM fungal genomes code for a large number of putative secreted proteins (Sędzielewska-Toro and Delaux, 2016), and a few of them have been given a possible role in the accommodation of fungal structures (Tsutzuki et al., 2016; Fiorilli et al., 2016). Even if the mechanisms of action for these putative secreted proteins have not been elucidated yet, the functional characterization of the periarbuscular interface is currently the focus of AM research, and important advances are expected in the coming years.

VI. Evolutionary and developmental perspectives on fungal accommodation by plant tissues

It was recently shown (Russo et al., 2019 a,b; Carotenuto et al., 2019 a,b) that prepenetration responses culminating in interface construction include cell cycle reactivation in cortical cells, with the onset of both anticlinal cell divisions and

recursive endoreduplication events in advance of arbuscule accommodation (Russo and Genre, 2021).

On the one hand, the combined use of gene expression and confocal microscopy has highlighted the appearance of ectopic cell divisions in the inner cortex a few hours after hyphopodium formation (Figure 4), when colonizing hyphae are normally confined to the root epidermis (Russo et al., 2019a,b). Sparse couples of 'split cells' (half the length of the surrounding parenchymal cells) were consistently observed in the inner root cortex of diverse AM host plants (Russo et al., 2019a). The occurrence of cell division in the fully differentiated tissue was confirmed by the direct observation of dividing cells as early as 48 hours post hyphopodium formation in *Daucus carota* expressing a GFP fusion with TPLATE (Russo et al., 2019a), an adaptin-related protein that accumulates on the cell plate membrane and plasmalemma at the cortical division zone (Van Damme et al., 2006).

On the other hand, flow cytometry, confocal imaging and gene expression studies revealed the diffuse occurrence of endoreduplication (DNA duplication in the absence of cell division) in the root cortex (Carotenuto et al., 2019b), allowing the precise localization of inner cortical cells with different levels of increased ploidy in the colonized areas of the root (Carotenuto et al., 2019a).

Altogether, these observations outlined a diffuse context of cell cycle reactivation in association with AM fungal accommodation (Russo and Genre, 2021). Firstly, cell divisions in the inner cortex have been observed when intraradical hyphae were limited to epidermal and outer cortical layers but not in later stages; by contrast, recursive endoreduplication cycles appear to be active for a longer period of time, with arbusculated and neighbouring cells reaching levels of 128 and even 256C ploidy, corresponding to up to 6 cycles of endoreduplication. In more detail, endoreduplication appeared to surge at the front of fungal expansion and reach the highest peaks in the central area of infection units, suggesting the existence of a proportion between ploidy and the abundance (or age) of intraradical fungal structures. Importantly, Carotenuto et al (2019b) also observed that the couples of 'split' cortical cells derived from cell division often displayed different ploidy levels, with higher ploidy in cells that were closer to the fungus or hosting an older arbuscule. This confirms that cell division takes place before endoreduplication, or at least that endoreduplication can proceed after cell division.

Secondly, the observation that both cell division and ploidy increase at a distance from arbuscules or colonizing hyphae suggests the existence of a yet unidentified signaling process reactivate the cell cycle before fungal arrival.

In addition, the concentration of both ectopic cell divisions and endocycle events to the inner cortex envisages a remarkable correlation with the accommodation of arbuscules, which normally develop in the same cell layer. Cell proliferation, with its limited occurrence, appears to have a secondary role, if any, in the generation of additional space for arbuscule accommodation. By contrast, the sparse cell divisions observed in AM colonized areas might relate to the developmental fate of cortical cells. In the roots of most plants, in fact, cortical cell differentiation is determined by an endocycle that doubles their DNA content from 2C to 4C (Cebolla et al, 1999; Edgar et al., 2014) with a consequent size increase (Robinson et al., 2018). In line with that*, in situ* studies of cell ploidy in uncolonized roots of *M. truncatula* (Carotenuto et al., 2019a) revealed that most cortical cells had 4C nuclei, while a few of them displayed 8C and 16C ploidy levels. Even if experimentally challenging, it would be very interesting to investigate if there is a relationship between initial cell ploidy and the occurrence of ectopic cell division in early AM interaction.

Besides tissue differentiation, endoreduplication is also common in plant interactions with diverse microbes: replicating DNA produces multiple copies of each gene, intensifying cell responsiveness to microbial signals. Examples are numerous from pathogens and parasites (de Almeida-Engler and Gheysen, 2013; Chandran et al., 2016; Wildermuth et al., 2017) to symbionts (Suzaki et al., 2014; Lace and Ott, 2018). Furthermore, endoreduplication-related cell enlargement is typically associated with the accommodation of several microbes, and specifically with arbuscules in AM (Balestrini and Bonfante, 2014; Heck et al., 2016).

The requirement of a specific rearrangement in the host cell organization for arbuscule accommodation is apparent from a simple observation of the structural and functional complexity of the periarbuscular interface (Luginbuhel and Oldroyd 2017; Choi et al., 2018; Roth et al., 2019; Ivanov et al., 2019), compared to the tunnel-like interface hosting linear hyphae in outer root tissues. In fact, while epidermal and outer cortical PPAs are structured as roughly linear cytoplasmic bridges across the vacuole, the PPAs that generate periarbuscular interfaces are much more complex and extensive, appearing as large accumulations of cytoplasm

that extend from the hyphal penetration site and occupy most of the host cell central volume (Genre et al., 2008). Such a massive, centripetally oriented exocytic event has striking ultrastructural and molecular similarities with the assembly of the cell plate on the cell equatorial plane at the end of mitosis, and indeed an evolutionary correlation between symbiotic interface biogenesis and cell plate deposition has been envisaged in both AM (Russo et al., 2019b) and N-fixing nodulation (Downie, 2014). In support of this hypothesis, *in vivo* imaging of GFP-TPLATE fusions revealed a strong accumulation of TPLATE at sites of PPA assembly and at sites of cell-to-cell hyphal passage, where the perifungal membrane fuses with the plasmalemma in striking analogy with cell plate fusion with the cell membrane at the end of mitosis (Russo et al., 2019b).

If the recruitment of cell division processes to assemble the extensive symbiotic interface surrounding each arbuscule now appears more convincing, developmental restraints could contribute to explain why sparse cell division and diffuse endoreduplication are limited to the cortex. Dong et al. (2021) have recently highlighted that a SHR-SCR module (known to regulate cortex/endodermis initial cell division in the root meristem) maintains its activity and is required for cell cycle reactivation in legume inner cortex during nodule organogenesis (Suzaki et al., 2014). Even if analogous studies in rice (which does not form root nodules, but hosts AM fungi) did not confirm SHR-SCR expression in cortical cells, it is reasonable to speculate that analogous mechanisms involving meristematic transcription factors maintain a disposition to reactivate the cell cycle in the inner cortical cells. This peculiarity is likely related to the evolution of root branching (Xiao et al., 2019), but appears to have later been co-opted in several plant interactions, from N-fixing symbioses (Dong et al., 2021) to nematode parasitism (de Almeida-Engler and Gheysen, 2013), where both cell division and endoreduplication are required for microbe accommodation. While such processes involve the formation of new organs (i.e. lateral roots, N-fixing nodules or nematode-hosting cysts), their occurrence in AM, where organogenesis is absent, appears puzzling; even more so, if we consider that AM symbiosis appeared in land plants before the evolution of true roots (Strullu-Derrien et al., 2014; 2018).

By discussing the developmental and evolutionary context where cell cycle processes interweave with AM symbiosis, a scenario emerges (Fig. 1) in which the perception of AM fungal colonization in outer root tissues triggers a so-far

unknown intraradical signaling process activating cell cycle-related processes ahead of the penetrating intraradical hyphae. Inner cortical cells, due to their peculiar developmental status deploy two downstream responses: a few of them (possibly depending on their ploidy) complete mitosis, splitting into two smaller cells, as cell elongation is very limited in a mature tissue (Russo et al., 2019a, b); the remaining majority of inner cortical cells enter the endocycle, duplicating their DNA content up to several times, continuously stimulated by the approaching fungal symbiont - in fact endoreduplication also extends to those cells that had divided earlier (Carotenuto et al., 2019a, b). Such a model implies that host cells largely anticipate and direct fungal colonization, in line with previous suggestions that the plant holds substantial control over symbiosis development (Gutjahr and Parniske, 2013).

VII. Conclusions

We currently have no information on how the earliest land plants acquired the ability to host a symbiotic fungus inside their cells. One can speculate that initial surface interactions provided an advantageous exchange of nutrients, pressing towards more intimate contacts, such as the penetration of fungal hyphae between the plant cells and eventually inside their lumen. In this context, creating *de novo* a fully functional symbiotic interface - as in modern plants - appears unrealistic. By contrast, stimulating cell divisions in differentiated organs could have been a more amenable strategy to generate both crack openings in the surface tissues (an entry route that is conserved in many extant plant-microbe interactions; Ibáñez et al., 2017) and irregular intercellular spaces in the inner ones, producing a protected niche for the fungus. The subsequent re-routing of cell plate formation towards the creation of a more efficient symbiotic interface appears achievable, especially in the light of the current findings, and the observation of split-cells in some of the earliest fossils of AM hosts indicates that this is indeed an ancient response associated with fungal accommodation (Strullu-Derrien et al., 2018).

Based on the information originated by genome sequencing, it will be interesting to focus future investigation on AM fungi (such as Paraglomerales) and plants (such as bryophytes) belonging to more ancient taxa, in order to characterize their root colonization process: are hyphopodia crucial for root penetration or could crack-

entry play a role in such interactions as it does in other plant-fungus and plant-bacterium interactions? Is the PPA model a common hallmark of fungal accommodation? Is arbuscule morphology conserved, alongside the expression of known markers of their functionality? In conclusion, a combination of cellular and molecular biology applied to plant-fungal couples different from the model organisms that have focused research so far, may offer new ideas on how AM fungi became the favorite guests for 72% of land plant species.

Figures

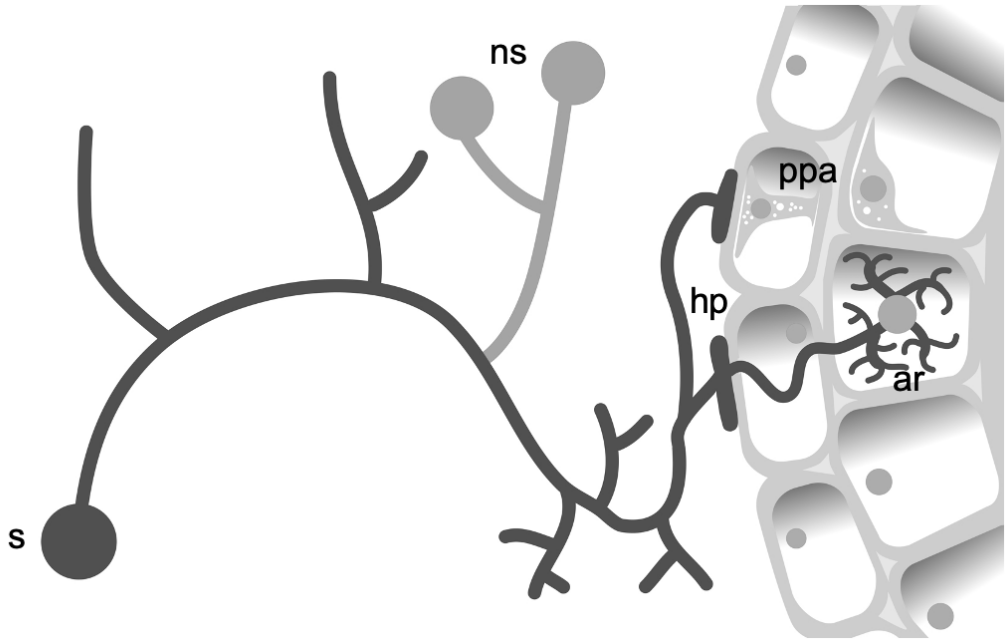


Figure 1. Arbuscular mycorrhiza development starts with the germination of resting spores (s) in the rhizosphere. This generates a loose explorative mycelium, whose hyphae eventually reach the host root surface, where they develop adhering hyphopodia (hp). Hyphopodium perception triggers the assembly of a prepenetration apparatus (ppa) in the underlying epidermal and cortical cells, which builds the symbiotic interface where intracellular hyphae and arbuscules (ar) are hosted. Symbiosis functionality allows the extraradical mycelium to generate new spores (ns).

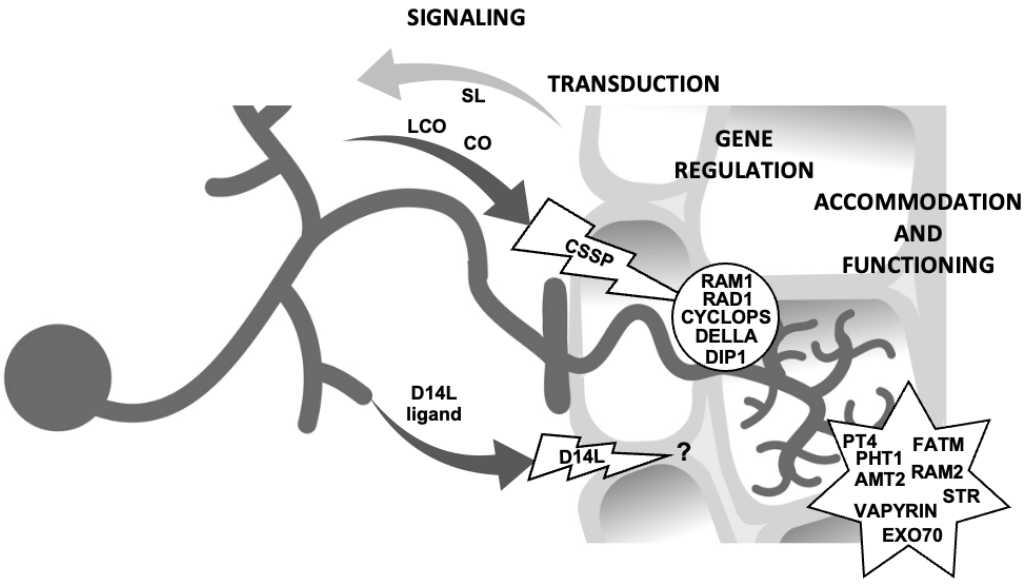


Figure 2. Root colonization by AM fungi involves a sequence of molecular mechanisms. Firstly, an exchange of signals ensures reciprocal recognition and triggers symbiotic responses: root secreted strigolactones (SL) activate fungal metabolism and branching; Myc factors (LCO and CO) trigger the common symbiotic signaling pathway (CSSP), in parallel with CSSP-independent signal transduction mediated by D14L. Downstream of the CSSP, several transcription factors (RAM1, RAD1, CYCLOPS, DELLA, DIP1) control the expression of a number of genes that are required for fungal accommodation and arbuscule functioning, such as transporters (PT4, PHT1, AMT2, STR), lipid biosynthetic enzymes (FATM, RAM2) and structural proteins related to the symbiotic interface assembly (VAPYRIN, EXO70).

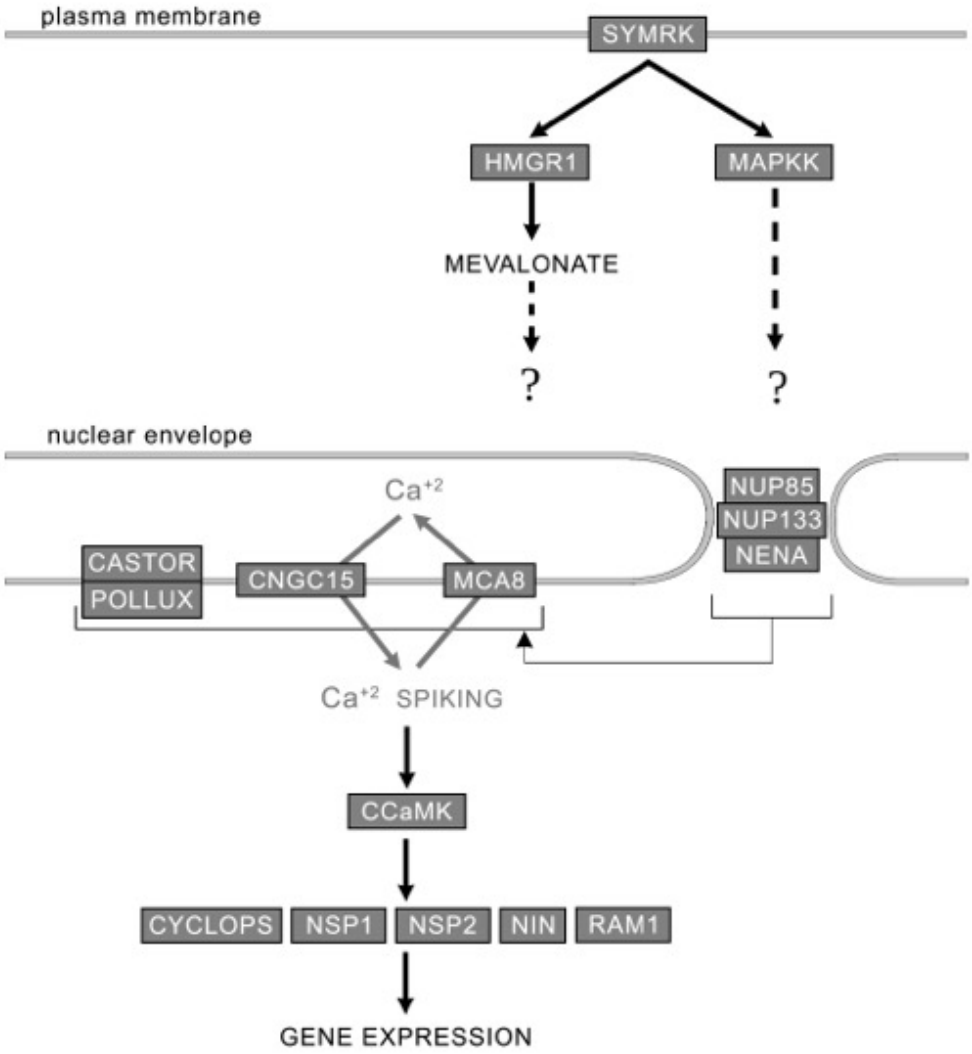


Figure 3. Scheme of the 'common symbiotic signalling pathway' (CSSP), mediating the perception of AM fungal signals. The plasma membrane bound leucine-rich repeat (LRR) receptor-like kinase SYMRK is a central element on receptor complexes for Nod factors or Myc factors. In its cytoplasmic domain, SYMRK interacts with a MAP kinase kinase, and HMGR1, an enzyme involved in mevalonate biosynthesis. Downstream this membrane-associated module act three nucleoporins - NUP85, NUP133, and NENA - likely involved in nuclear targeting of the ion channel CASTOR/POLLUX, the Ca^{2+} channel CNGC15 and the SERCA-type ATPase MCA8. In turn, these generate Ca^{2+}-mediated signals (spiking) in the nucleoplasm. Ca^{2+}-spiking is interpreted by the nuclear Ca^{2+}-and-calmodulin-dependent protein kinase CCaMK, its interacting partner CYCLOPS and the transcription factors NSP1, NSP2, NIN and RAM1, leading to gene regulation.

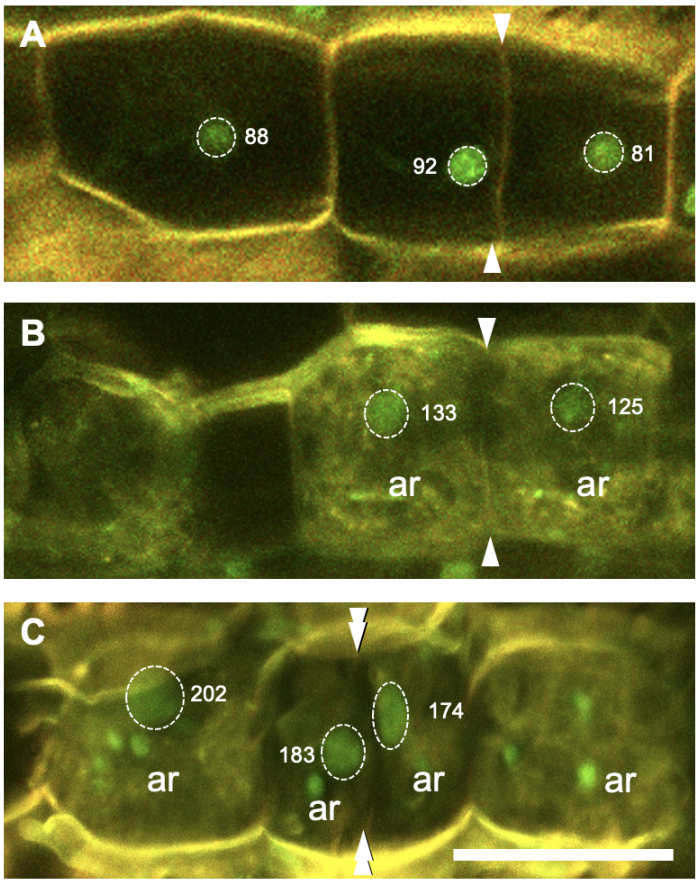


Figure 4. Confocal images of DAPI (green, nuclei) and and SR2200 (gold, cell walls) stained sections of *M. truncatula* (cv 'Jemalong' A17) roots colonized by the AM fungus *G. margarita* (Strain BEG34). The images are representative of the induction of cell cycle-related processes for arbuscule accommodation. **A.** Cell division (arrowheads) generates 'split cells' in the inner cortex during early root colonization. **B.** Arbuscule (ar) accommodation in split cells is preceded by an increase in cell ploidy, represented by the numbers indicating nuclear volume in μm^3 (estimated by image analysis according to Carotenuto et al., 2019b). **C.** Recursive events of both cell division and endoreduplication may lead to higher ploidy and couples of shorter 'split cells' (double arrowheads). All four cells in this image host an arbuscule. Bar = 50µm.

References

Akiyama K, Matsuzaki K, Hayashi H (2005) Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. Nature 435:824-827

Akiyama K, Ogasawara S, Hayashi H (2010) Structural requirement of strigolactones for hyphal branching in AM fungi. Plant Cell Physiol 51:1104-1117

Al-Babili S, Bouwmeester HJ (2015) Strigolactones, a novel carotenoid-derived plant hormone. Ann Rev Plant Biol 66:161-18

Balestrini R, Bonfante P. 2014. Cell wall remodeling in mycorrhizal symbiosis: a way towards biotrophism. Frontiers in Plant Science 5: 237

Bar-On YM, Phillips R, Milo R (2018) The biomass distribution on Earth. Proc Natl Acad Sci USA 115:6506–6511

Besserer A, Becard G, Roux C et al (2008) GR24, a synthetic analogue of strigolactones, stimulates mitosis and growth of the arbuscular mycorrhizal fungus Gigaspora rosea by boosting its energetic metabolism. Plant Physiol 148:402-413

Besserer A, Puech-Pages V, Kiefer P et al (2006). Strigolactones stimulate arbuscular mycorrhizal fungi by activating mitochondria. PLoS Biol 4:1239-1247

Bonfante P (2018) The future has roots in the past: the ideas and scientists that shaped mycorrhizal research. New Phytol 220:982-995

Bonfante P, Genre A (2015) Arbuscular mycorrhizal dialogues: do you speak 'plantish' or 'fungish'? Trends Plant Sci 20:150-154

Bonfante P, Requena N (2011) Dating in the dark: how roots respond to fungal signals to establish arbuscular mycorrhizal symbiosis. Curr Opin Plant Biol 14:451-457

Bonfante P, Venice F (2020) Mucoromycota: going to the roots of plant interacting fungi. Fungal Biology Reviews 34:100-113

Bonfante, P (2001). At the Interface Between Mycorrhizal Fungi and Plants: the Structural Organization of Cell Wall, Plasma Membrane and Cytoskeleton. In:

Hock, B. (ed) Fungal Associations. The Mycota, vol 9. Springer, Berlin, Heidelberg, pp. 45-61

Bravo A, Brands M, Wewer V et al (2017) Arbuscular mycorrhiza-specific enzymes FatM and RAM2 fine-tune lipid biosynthesis to promote development of arbuscular mycorrhiza. New Phytol. 214:1631-1645

Buée M, Rossignol M, Jauneau A et al (2000) The pre-symbiotic growth of arbuscular mycorrhizal fungi is induced by a branching factor partially purified from plant root exudates. Mol Plant-Microbe Interact 13:693-698

Capoen W, Sun J, Wysham D, Otegui MS et al (2011) Nuclear membranes control symbiotic calcium signalling of legumes. Proc Natl Acad Sci USA 108:14348-14353

Carotenuto G, Sciascia I, Oddi L et al (2019b) Size matters: three methods for estimating nuclear size in mycorrhizal roots of *Medicago truncatula* by image analysis. BMC Plant Biology (2019) 19:180

Carotenuto G, Volpe V, Russo G et al (2019a) Local endoreduplication as a feature of intracellular fungal accommodation in arbuscular mycorrhizas. New Phytologist 223: 430-446

Cebolla A, Vinardell JM, Kiss E et al (1999) The mitotic inhibitor ccs52 is required for endoreduplication and ploidy-dependent cell enlargement in plants. EMBO J. 18:4476–4484

Chabaud M, Genre A, Sieberer BJ et al (2011) Arbuscular mycorrhizal hyphopodia and germinated spore exudates trigger Ca^{2+} spiking in the legume and non legume root epidermis. New Phytol 189:347-55

Chabaud M, Venard C, Defaux-Petras A et al (2002) Targeted inoculation of *Medicago truncatula* in vitro root cultures reveals MtENOD11 expression during early stages of infection by arbuscular mycorrhizal fungi. New Phytol 156:265-273

Chandran D, Wildermuth MC (2016) Modulation of host endocycle during plant-biotroph interactions. Enzymes 40:65–103

Charpentier M, Bredemeier R, Wanner G, Takeda N, Schleiff E, Parniske M (2008) Lotus japonicus CASTOR and POLLUX are ion channels essential for perinuclear calcium spiking in legume root endosymbiosis. Plant Cell. 20:3467-79.

Charpentier M, Sun J, Vaz Martins T, Radhakrishnan GV, Findlay K, Soumpourou E, Thouin J, Véry AA, Sanders D, Morris RJ, Oldroyd GE (2016) Nuclear-localized cyclic nucleotide-gated channels mediate symbiotic calcium oscillations. Science 352:1102-1105.

Chen T, Zhu H, Ke D et al (2012) A MAP kinase kinase interacts with SymRK and regulates nodule organogenesis in *Lotus japonicus*. Plant Cell 24:823-38

Chiu CH, Paszkowsky U (2021) How membrane receptors tread the fine balance between symbiosis and immunity signaling. Proc Nat Acad Sci USA 118:e2106567118

Choi J, Summers W, Paszkowski U (2018) Mechanisms underlying establishment of arbuscular mycorrhizal symbioses. Annual Review of Phytopathology 56:135-160.

Czaja LF, Hogekamp C, Lamm P et al (2012) Transcriptional responses toward diffusible signals from symbiotic microbes reveal MtNFP- and MtDMI3-dependent reprogramming of host gene expression by arbuscular mycorrhizal fungal lipochitooligosaccharides. Plant Physiol 159:1671-1685

de Almeida Engler J, Gheysen G (2013) Nematode-induced endoreduplication in plant host cells: why and how? Mol. Plant. Microbe. Interact. 26:17–24

Dong W, Zhu Y, Chang H et al. (2021) An SHR- SCR module specifies legume cortical cell fate to enable nodulation. Nature. 589:586–590

Downie JA (2014) Legume nodulation. Curr. Biol. 24:184–190

Edgar B, Zielke N, Gutierrez C (2014) Endocycles: a recurrent evolutionary innovation for post-mitotic cell growth. Nat. Rev. Mol. Cell. Biol. 15:197–210

Eisenhauer N, Bonfante P, Buscot F et al (2022) Biotic Interactions as Mediators of Context-Dependent Biodiversity-Ecosystem Functioning Relationships. Research Ideas and Outcomes 8: e85873

Fiorilli V, Belmondo S, Khouja HR et al. (2016) RiPEIP1, a gene from the arbuscular mycorrhizal fungus Rhizophagus irregularis, is preferentially expressed in planta and may be involved in root colonization. Mycorrhiza, DOI 10.1007/s00572-016-0697-0

Genre A, Chabaud M, Balzergue C et al (2013) Short-chain chitin oligomers from arbuscular mycorrhizal fungi trigger nuclear Ca^{2+} spiking in *Medicago truncatula* roots and their production is enhanced by strigolactone. New Phytol 198:190-202

Genre A, Chabaud M, Faccio A et al (2008) Prepenetration apparatus assembly precedes and predicts the colonization patterns of arbuscular mycorrhizal fungi within the root cortex of both *Medicago truncatula* and *Daucus carota*. Plant Cell. 20:1407–1420

Genre A, Chabaud M, Timmers T et al (2005) Arbuscular mycorrhizal fungi elicit a novel intracellular apparatus in *Medicago truncatula* root epidermal cells before infection. Plant Cell 17:3489-3499

Genre A, Lanfranco L, Perotto S et al (2020) Unique and common traits in mycorrhizal symbioses. Nature Reviews Microbiology 18:649–660

Genre A, Russo G. 2016. Does a common pathway transduce symbiotic signals in plant-microbe interactions. Front Plant Sci 7:96

Gobbato E (2015) Recent developments in arbuscular mycorrhizal signaling. Curr Opin Plant Biol 26:1-7

Groth M, Takeda N, Perry J et al (2010) NENA, a *Lotus japonicus* homologue of Sec13, is required for rhizodermal infection by arbuscular mycorrhizal fungi but dispensable for cortical endosymbiotic development. Plant Cell 22:2509-2526

Guether M, Neuhauser B, Balestrini R et al (2009) A Mycorrhizal-Specific Ammonium Transporter from Lotus japonicus Acquires Nitrogen Released by Arbuscular Mycorrhizal Fungi. Plant Physiol. 150:73-83

Gutjahr C, Gobbato E, Choi J et al (2015). Rice perception of symbiotic arbuscular mycorrhizal fungi requires the karrikin receptor complex. Science 350:1521-1524

Gutjahr C, Parniske M (2013) Cell and developmental biology of arbuscular mycorrhiza symbiosis. Ann Rev Cell Develop Biol 29:593-617

Harrison MJ. 2012. Cellular programs for arbuscular mycorrhizal symbiosis. Curr Opin Plant Biol 15:691-8

He J, Zhang C, Dai H et al (2019) A LysM Receptor Heteromer Mediates Perception of Arbuscular Mycorrhizal Symbiotic Signal in Rice. Molecular Plant 12:1561-1576

Heck C, Kuhn H, Heidt S et al (2016) Symbiotic fungi control plant root cortex development through the novel GRAS transcription factor MIG1. Curr. Biol. 26:2770–2778

Heckmann AB, Lombardo F, Miwa H et al (2006) *Lotus japonicus* nodulation requires two GRAS domain regulators, one of which is functionally conserved in a non-legume. Plant Physiol 142:1739-1750

Horváth B, Yeun LH, Domonkos A et al (2011) *Medicago truncatula* IPD3 is a member of the common symbiotic signaling pathway required for rhizobial and mycorrhizal symbioses. *Mol Plant Microbe Interact* 24:1345-1358

Hyde KD (2022) The numbers of fungi. *Fungal Diversity* 114:1

Ibáñez F, Wall L, Fabra A (2017) Starting points in plant-bacteria nitrogen- fixing symbioses: intercellular invasion of the roots. J. Exp. Bot. 68:1905–1918

Ivanov S, Austin J, Berg RH et al (2019) Extensive membrane systems at the host-arbuscular mycorrhizal fungus interface. Nat. Plants. 5:194–203

Ivanov S, Fedorova EE, Limpens E et al (2012) Rhizobium-legume symbiosis shares an exocytotic pathway required for arbuscule formation. Proc Natl Acad Sci USA 109:8316-8321

Javot H, Penmetsa RV, Terzaghi N et al (2007) A Medicago truncatula phosphate transporter indispensable for the arbuscular mycorrhizal symbiosis. Proc Natl Acad Sci USA 104:1720-1725

Kameoka H, Tsutsui I, Saito K (2019) Stimulation of asymbiotic sporulation in arbuscular mycorrhizal fungi by fatty acids. Nat. Microbiol. 4:1654-1660

Kanamori N, Madsen LH, Radutoiu S et al (2006) A nucleoporin is required for induction of Ca^{2+} spiking in legume nodule development and essential for rhizobial and fungal symbiosis. Proc Natl Acad Sci USA 103:359-364.

Kloppholz S, Kuhn H, Requena N. 2011. A secreted fungal effector of *Glomus intraradices* promotes symbiotic biotrophy. *Curr Biol* 21:1204-1209

Kobae Y, Hata S (2010) Dynamics of periarbuscular membranes visualized with a fluorescent phosphate transporter in arbuscular mycorrhizal roots of rice. Plant Cell Physiol 51:341-53

Kokkoris V, Chagnon PL, Yildirir G et al (2021) Host identity influences nuclear dynamics in arbuscular mycorrhizal fungi. Current Biology 31:1531-1538

Kosuta S, Chabaud M, Lougnon G et al (2003) A diffusible factor from arbuscular mycorrhizal fungi induces symbiosis-specific *MtENOD11* expression in roots of *Medicago truncatula.* Plant Physiol 131:952-962

Krajinski F, Courty PE, Sieh D et al (2014) The H+-ATPase HA1 of Medicago truncatula is essential for phosphate transport and plant growth during arbuscular mycorrhizal symbiosis. Plant Cell 26:1808-1817

Lace B, Ott T (2018) Commonalities and differences in controlling multipartite intracellular infections of legume roots by symbiotic microbes. Plant Cell Physiol. 59:666–677

Lanfranco L Bonfante P, Genre A (2016) The mutualistic interaction between plants and arbuscular mycorrhizal fungi. Microbiology Spectrum. 4:1-20

Lo Presti L, Lanver D, Schweizer G, Shigeyuki T, Liang L, Tollot M, Zuccaro A, Kahmann R. 2015. Fungal effectors and plant susceptibility. Ann Rev Phytopathol 66:513-545

Luginbuehl LH, Oldroyd GE (2017) Understanding the arbuscule at the heart of endomycorrhizal symbioses in plants. *Curr. Biol.* **27**:R952–R963

Maillet F, Poinsot V, André O, Puech-Pagès V, Haouy A, Gueunier M, Dénarié J. 2011. Fungal lipochitooligosaccharide symbiotic signals in arbuscular mycorrhiza. Nature 469:58-63

Malar C, Wang M, Stajich Y et al (2022) Early branching arbuscular mycorrhizal fungus Paraglomus occultum carries a small and repeat-poor genome compared to relatives in the Glomeromycotina. Microbial Genomics 8:000810

Mathieu S, Cusant L, Roux C et al (2018) Arbuscular mycorrhizal fungi: intraspecific diversity and pangenomes. New Phytologist 220:1129–1134.

Mitra RM, Gleason CA, Edwards A et al (2004) A Ca^{2+}/calmodulin-dependent protein kinase required for symbiotic nodule development: gene identification by transcript-based cloning. Proc Natl Acad Sci USA 101:4701-4705.

Miwa H. Sun J, Oldroyd GE, Downie JA (2006) Analysis of calcium spiking using a cameleon calcium sensor reveals that nodulation gene expression is regulated by calcium spike number and the developmental status of the cell. Plant J 48:883-894

Miyata K, Kozaki T, Kouzai Y et al. (2014) The bifunctional plant receptor, Os CERK1, regulates both chitin-triggered immunity and arbuscular mycorrhizal symbiosis in rice. Plant and Cell Physiology 55: 1864–1872

Morin ES, Miyauchi H, San Clemente ECH et al (2019) Comparative genomics of *Rhizophagus irregularis, R. cerebriforme, R. diaphanus* and *Gigaspora rosea* highlights specific genetic features in Glomeromycotina. New Phytol 222:1584-1598

Moscatiello R, Sello S, Novero M et al (2014) The intracellular delivery of TAT-aequorin reveals calcium-mediated sensing of environmental and symbiotic signals by the arbuscular mycorrhizal fungus *Gigaspora margarita*. New Phytol 203:1012-1020

Nagahashi G, Douds DD (2004) Isolated root caps, border cells, and mucilage from host roots stimulate hyphal branching of the arbuscular mycorrhizal fungus, Gigaspora gigantea. Mycol Res 108:1079-1088

Oldroyd GED (2013) Speak, friend, and enter: signalling systems that promote beneficial symbiotic associations in plants. Nature Rev Microbiol 11:252-263

Park H-J, Floss DS, Levesque-Tremblay V et al (2015) Hyphal branching during arbuscule development requires *Reduced Arbuscular Mycorrhiza1*. Plant Physiol 69:2774-2788

Pumplin N, Mondo SJ, Topp S, Starker CG, Gantt JS, Harrison MJ. 2010. *Medicago truncatula* Vapyrin is a novel protein required for arbuscular mycorrhizal symbiosis. Plant J 61:482-494

Pumplin N, Zhang X, Noar RD et al (2012) Polar localization of a symbiosis-specific phosphate transporter is mediated by a transient reorientation of secretion. *Proc Natl Acad Sci USA* 109:665-672

Rech SS, Heidt S, Requena N (2013) A tandem Kunitz protease inhibitor (KPI106)-serine carboxypeptidase (SCP1) controls mycorrhiza establishment and arbuscule development in Medicago truncatula. Plant J 75:711-25

Remy W, Taylor TN, Hass H et al (1994) Four hundred-million-year-old vesicular arbuscular mycorrhizae. Proc Natl Acad Sci U S A. 91(25):11841-11843

Robinson DO, Coate JE, Singh A et al. (2018) Ploidy and size at multiple scales in the arabidopsis sepal. *Plant Cell*. 30:2308–2329

Ropars J, Toro KS, Noel J et al (2016) Evidence for the sexual origin of heterokaryosis in arbuscular mycorrhizal fungi. Nat Microbiol. 1:16033

Roth R, Hillmer S, Funaya et al (2019) Arbuscular cell invasion coincides with extracellular vesicles and membrane tubules. Nat. Plants. 5:204–211

Russo G, Carotenuto G, Fiorilli V (2019b) Ectopic activation of cortical cell division during the accommodation of arbuscular mycorrhizal fungi. New Phytologist 221: 1036-1048

Russo G, Carotenuto G, Fiorilli V et al (2019a) TPLATE Recruitment Reveals Endocytic Dynamics at Sites of Symbiotic Interface Assembly in Arbuscular Mycorrhizal Interactions. Frontiers in Plant Science 10: 1628

Russo G, Genre A (2021) Divide and Be Conquered - Cell Cycle Reactivation in Arbuscular Mycorrhizal Symbiosis. Frontiers in Plant Science 12:753265

Ruyter-Spira C, Al-Babili S, van der Krol S et al (2013) The biology of strigolactones. Trends Plant Sci 18:72-83

Saito K, Yoshikawa M, Yano K et al (2007) NUCLEOPORIN85 is required for calcium spiking, fungal and bacterial symbioses, and seed production in *Lotus japonicus*. Plant Cell 19:610-624.

Salvioli A, Ghignone S, Novero M et al (2016) Symbiosis with an endobacterium increases the fitness of a mycorrhizal fungus, raising its bioenergetic potential. ISME J 10:130–144

Schüßler A (2012) The *Geosiphon–Nostoc* Endosymbiosis and Its Role as a Model for Arbuscular Mycorrhiza Research. In: Hock B (ed) Fungal Associations. The Mycota, vol 9. Springer, Berlin, Heidelberg, pp. 77-91

Sędzielewska-Toro KA, Delaux P-M. 2016. Mycorrhizal symbioses: today and tomorrow. New Phytol 209:917-920

Sinanaj B, Hoysted GA, Pressel S et al. (2021) Critical research challenges facing Mucoromycotina 'fine root endophytes'. New Phytol, 232:1528-1534

Spatafora JW, Chang Y, Benny GL et al (2016) A phylum-level phylogenetic classification of zygomycete fungi based on genome-scale data. Mycologia 108:1028-1046.

Strullu-Derrien C, Kenrick P, Pressel S et al (2014) Fungal associations in Horneophyton ligneri from the Rhynie Chert (c. 407 million year old) closely resemble those in extant lower land plants: novel insights into ancestral plant-fungus symbioses. New Phytol. 203:964–979

Strullu-Derrien C, Selosse MA, Kenrick P et al (2018) The origin and evolution of mycorrhizal symbioses: from palaeomycology to phylogenomics. New Phytol. 220:1012–1030

Sugiura Y, Akiyama R, Tanaka S et al (2020) Myristate can be used as a carbon and energy source for the asymbiotic growth of arbuscular mycorrhizal fungi. Proc Natl Acad Sci U S A. 117:25779-25788

Suzaki T, Ito M, Yoro E et al (2014) Endoreduplication-mediated initiation of symbiotic organ development in *Lotus japonicus*. Development. 141:2441–2445

Takeda N, Handa Y, Tsuzuki S et al (2015) Gibberellins interfere with symbiosis signaling and gene expression and alter colonization by arbuscular mycorrhizal fungi in *Lotus japonicus*. Plant Physiol 167:545-557

Takeda S, Asamizu E, Tabata S et al (2009) Apoplastic plant subtilase support arbuscular mycorrhiza development in Lotus japonicus. Plant J 58:766-777.

Tedersoo L, Sánchez-Ramírez S, Kõljalg U et al (2018) High-level classification of the *Fungi* and a tool for evolutionary ecological analyses. Fungal Divers 90:135-159

Tedersoo L, Bahram M, Zob M (2020) How mycorrhizal associations drive plan population and community. Science 367:eaba1223

Tisserant E, Malbreil M, Kuo A et al (2013) Genome of an arbuscular mycorrhizal fungus provides insight into the oldest plant symbiosis. Proc. Natl. Acad. Sci. U.S.A., 110:20117-20122

Tsuzuki S, Handa Y, Takeda N, Kawaguchi M. 2016. Strigolactone-induced putative secreted protein 1 is required for the establishment of symbiosis by the arbuscular mycorrhizal fungus Rhizophagus irregularis. Mol Plant-Microbe Interact 29:277-286

Van Damme D, Coutuer S, De Rycke R et al (2006) Somatic cytokinesis and pollen maturation in Arabidopsis depend on TPLATE, which has domains similar to coat proteins. Plant Cell 18:3502–3518

van der Heijden M, Klironomos J, Ursic M et al (1998)Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. Nature 396:69–72

Venice F, Desirò A, Silva G et al (2020b) The Mosaic Architecture of NRPS-PKS in the Arbuscular Mycorrhizal Fungus *Gigaspora margarita* Shows a Domain With Bacterial Signature. Frontiers in Microbiology 11:581313

Venice F, Ghignone A, Salvioli A et al (2020) At the nexus of three kingdoms: the genome of the mycorrhizal fungus *Gigaspora margarita* provides insights into plant, endobacterial and fungal interactions. Environ Microbiol 22:122–141

Venkateshwaran M, Jayaraman D, Chabaud M et al (2015). A role for the mevalonate pathway in early plant symbiotic signaling. Proc Natl Acad Sci USA 112:9781-9786

Wang E, Yu N, Bano SA et al (2014) A H^+-ATPase that energizes nutrient uptake during mycorrhizal symbioses in rice and Medicago truncatula. Plant Cell 26:1818-183

Wildermuth MC, Steinwand MA, McRae AG et al (2017) Adapted biotroph manipulation of plant cell ploidy. Annu. Rev. Phytopathol. 55:537–564

Wilson EO (2016) We must keep every scrap of nature in and around our cities. Nature holds the key to our aesthetic, intellectual, cognitive and even spiritual satisfaction. Landscapes 18: 26-35.

Xie X, Lai W, Che X., Wang S et al (2022) A SPX domain-containing phosphate transporter from *Rhizophagus irregularis* handles phosphate homeostasis at symbiotic interface of arbuscular mycorrhizas. New Phytol, 234:650-671

Xue L, Cui H, Buer B, Vijayakumar V, Delaux PM, Junkermann S, Bucher M. 2015. Network of GRAS transcription factors involved in the control of arbuscule development in *Lotus japonicus*. Plant Physiol 167:854-871

Yang SY, Grønlund M, Jakobsen I et al (2012) Non-redundant regulation of rice arbuscular mycorrhizal symbiosis by two members of the PHOSPHATE TRANSPORTER1 gene family. Plant Cell 24:4236-4251

Yu N, Luo D, Zhang X et al (2014) A DELLA protein complex controls the arbuscular mycorrhizal symbiosis in plants. Cell Res 24:130-133

Zhang C, He J, Dai H et al (2021) Discriminating symbiosis and immunity signals by receptor competition in rice. Proc Nat Acad Sci USA 118: e2023738118

Zhang Q, Blaylock LA, Harrison MJ. 2010. Two Medicago truncatula half-ABC transporters are essential for arbuscule development in arbuscular mycorrhizal symbiosis. Plant Cell 22:1483-97.

Zhang X, Pumplin N, Ivanov S, Harrison MJ (2015) EXO70I is required for development of a sub-domain of the periarbuscular membrane during arbuscular mycorrhizal symbiosis. Curr Biol 25:2189-2195