New Phytologist

Forum

Viewpoint

At the core of the endomycorrhizal symbioses: intracellular fungal structures in orchid and arbuscular mycorrhiza

Summary

Arbuscular (AM) and orchid (OrM) mycorrhiza are the most widespread mycorrhizal symbioses among flowering plants, formed by distinct fungal and plant species. They are both endosymbioses because the fungal hyphae can enter inside the plant cell to develop intracellular fungal structures that are surrounded by the plant membrane. The symbiotic plant–fungus interface is considered to be the major site of nutrient transfer to the host plant. We summarize recent data on nutrient transfer in OrM and compare the development and function of the arbuscules formed in AM and the pelotons formed in OrM in order to outline differences and conserved traits. We further describe the unexpected similarities in the form and function of the intracellular mycorrhizal fungal structures observed in orchids and in the roots of mycoheterotrophic plants forming AM. We speculate that these similarities may be the result of convergent evolution of mycorrhizal types in mycoheterotrophic plants and highlight knowledge gaps and new research directions to explore this scenario.

Introduction

Mycorrhizal symbioses are an ancient and beneficial association between plant roots and fungi. They have been found in the earliest fossils of land plants and have since accompanied plant evolution and adaptation to diverse terrestrial environments (Smith & Read, 2008). Arbuscular mycorrhiza (AM) is the most ancient and widespread mycorrhizal type, formed by >80% of plants and involving *c.* 240 fungal species mostly grouped in the subphylum Glomeromycotina (Spatafora *et al.*, 2016). During evolution, certain plant lineages have switched to different fungal symbionts and the AM symbiosis has been replaced by at least three main other types of mycorrhizal associations (Brundrett & Tedersoo, 2018; Genre *et al.*, 2020). Among flowering plants, the Orchidaceae and the Ericaceae have switched to mycorrhizal fungi in the Basidiomycetes or Ascomycetes to form orchid (OrM) and ericoid (ErM) mycorrhiza, respectively. Like AM, both OrM and ErM are endomycorrhizal symbioses because the fungus forms intracellular structures in the host plant (van der Heijden *et al.*, 2015). Basidiomycetes or Ascomycetes have also replaced AM fungi in some gymnosperm and angiosperm lineages, where they form ectomycorrhiza (Brundrett & Tedersoo, 2018; Genre *et al.*, 2020). How these mycorrhizal types are related in form and function is a key evolutionary question, and in this viewpoint, we will discuss the hypothesis that endomycorrhizal symbioses may share important traits, and that convergent evolution may have occurred in the form and function of the intracellular fungal structures at the core of these endosymbioses.

The paradigm of mycorrhiza functioning is that the plant provides the fungal partner(s) with photosynthesis-derived organic carbon in exchange for essential nutrients (Smith & Read, 2008). In this respect, OrM is considered a peculiar symbiosis because all orchids require, at least during the early developmental stages, an external supply of organic carbon that is provided by the fungal partner (Rasmussen *et al.*, 2015). Germination of orchid seed does not yield a normal seedling but a postembryonic structure called protocorm (Yeung, 2022) that usually cannot fix its own carbon as it lacks chlorophyll (Fig. 1). Thus, orchid seed germination and protocorm development in nature are highly dependent on the mycorrhizal fungi, a physiological condition named 'initial mycoheterotrophy' (Hynson *et al.*, 2013). The protocorm will eventually form a seedling through the development of the first leaflets, which are photosynthetic in most orchid species. However, the protocorm of *c.* 235 orchid species develops into adult plants that remain achlorophyllous and therefore dependent on the mycorrhizal fungal partner for carbon throughout their life cycle (full mycoheterotrophy; Merckx *et al.*, 2013). In addition, many chlorophyllous orchid species, especially those living in shaded forest habitats, complement their inefficient photosynthesis with carbon derived from the fungal partner (partial mycoheterotrophy; Gebauer & Meyer, 2003; Selosse & Roy, 2009; Stöckel *et al.*, 2011).

Partial or full mycoheterotrophs form OrM with ectomycorrhizal or wood-decomposing fungi (Hynson & Bruns, 2010; Suetsugu *et al.*, 2020), and the origin of carbon in the orchid leaf tissues can be traced to the fungal partner because the ^{13}C signature of these fungi is clearly distinct from photosynthesis-derived carbon (Gebauer & Meyer, 2003). This ^{13}C signature was detected not only in neighboring fungal sporocarps, but also in fungal pelotons extracted from OrM root tissues (Gomes *et al.*, 2023; Zahn *et al.*, 2023). Nutrient exchange in photosynthetic orchids from meadow habitats is expected to conform to the mycorrhiza paradigm, with photosynthesis-derived carbon exchanged for mineral nutrients. However, experimental proof of net carbon delivery to the OrM fungal partner in photosynthetic orchids is scanty (Cameron *et al.*, 2008) and partial mycoheterotrophy has been demonstrated in some meadow orchid species (Gebauer *et al.*, 2016; Schiebold *et al.*, 2018).

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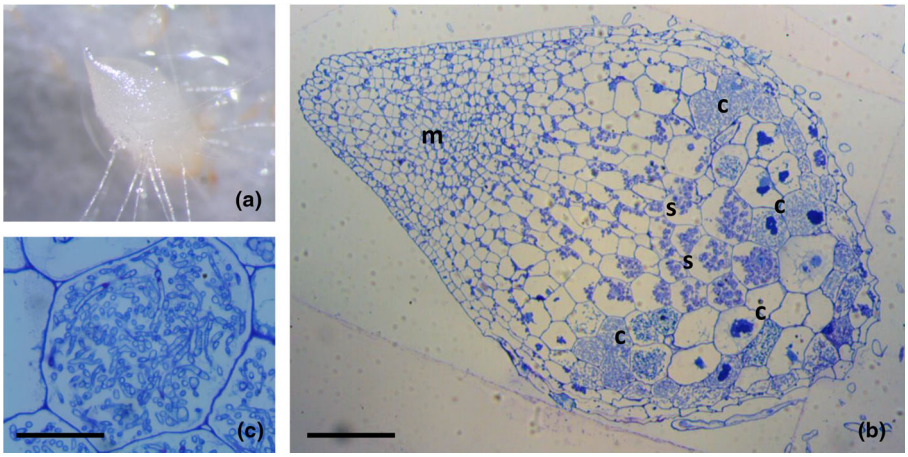

Fig. 1 Mycorrhizal protocorms of *Serapias vomeracea* 30 d after sowing with *Tulasnella calospora*. (a) Stereomicroscopic image of a protocorm, showing well-developed rhizoids. (b) Longitudinal section of a resin-embedded protocorm, showing the basal, mycorrhizal region, and the apical, uninfected region. (c) A magnification of a mycorrhizal cell containing a well-developed fungal peloton. c, coil; m, meristematic part; s, starch. Bars: (b) 250 μm; (c) 50 μm.

Thus, all orchids are mycoheterotrophs in the early life stages (initial mycoheterotrophy), and many orchid species are entirely or in part reliant on mycorrhizal fungi for carbon uptake throughout their lifecycle. Mycoheterotrophy in orchids raises several intriguing questions on the relationship between these plants and their mycorrhizal fungal symbionts (Merckx *et al.*, 2013). The behavior of orchids toward the fungal symbionts may be considered parasitic, as they exploit the fungus and take their resources. Also, the first OrM fungi identified belong to the form-genus *Rhizoctonia*, which comprises plant pathogens. Taken together, this has strongly influenced the view of OrM as an antagonistic interaction where the plant keeps an aggressive fungus under control while exploiting its resources (see Rasmussen & Rasmussen, 2009; Selosse *et al.*, 2017). This view has been challenged by gene expression studies, that indicate upregulation of plant genes known to be expressed in other endosymbioses rather than strong defense responses (Perotto *et al.*, 2014; Miura *et al.*, 2018; De Rose *et al.*, 2023b). However, many authors still consider OrM a mycorrhizal type very different, for example, from the most widespread AM symbiosis (Bucher *et al.*, 2014).

By contrast, our hypothesis is that OrM and AM are more similar than generally thought in their development and functions, especially when a broader perspective on the diversity of the AM symbiosis is considered. Commonalities between OrM and AM have been already suggested in previous papers (Dearnaley *et al.*, 2016), and here we further substantiate this view. In particular, we will focus on the development and functioning of the intracellular fungal structures at the core of these two endosymbioses by comparing the fungal coils formed in OrM with the highly branched arbuscules formed in the AM symbiosis (Fig. 2), to outline key conserved traits. We will also discuss the similarities between the intracellular fungal structures in OrM and in mycoheterotrophic plants forming AM.

The peloton: a constant feature in orchid mycorrhiza

The taxonomic relationships between orchids and their mycorrhizal fungi are extremely complex because fungal diversity in OrM is influenced by several factors, including the ontogenetic stage in the orchid life cycle and the type of tissue/organ colonized (i.e. seed embryo and protocorm or roots), the orchid trophic strategy (autotrophic or mycoheterotrophic), and environmental factors (Ventre Lespiaucq *et al.*, 2021). Despite this complexity, tightly coiled intracellular fungal hyphae (i.e. pelotons) are a constant feature of OrM and represent the most intimate contact between fungus and plant. Pelotons are formed independently from the fungal taxa involved, suggesting that the development of the microbial partner inside the host plant cells is largely, if not fully, plant-driven. As an example, *Russula* (Basidiomycetes) and *Tuber* (Ascomycetes) form ectomycorrhizal symbioses with tree species, but they have been also identified in OrM, where they form typical intracellular pelotons (Selosse *et al.*, 2004; Girlanda *et al.*, 2006). A small number of mycoheterotrophic orchids, like *Gastrodia*, form regular pelotons but also display a peculiar infection pattern in cortical cells (Rasmussen, 2002). Thus, unless the large species diversity in the Orchidaceae family (*c.* 28 000 species) will reveal a more significant and so far undescribed variation in the morphology of intracellular fungal structures, the peloton seems to be a very constant feature of OrM.

Arbuscular mycorrhiza are endosymbioses characterized by intracellular fungal structures that also represent the most intimate contact between fungus and plant, but feature more variation because two types have been described (Fig. 3), that correlated with different root colonization patterns: the *Arum*-type and the *Paris*-type (Smith & Read, 2008). In the *Arum*-type, the plant root cortex is colonized by large intercellular hyphae that give rise to highly branched terminal fungal structures (i.e. arbuscules), which lie inside the root cortical cells (Dickson *et al.*, 2007). By contrast, the *Paris*-type AM is characterized by the formation of intracellular hyphal coils that can resemble OrM pelotons (see e.g. in Yamato *et al.*, 2011), and root colonization proceeds directly from one cell to the neighboring one, not unlike OrM (Dickson *et al.*, 2007). The same plant can form hyphal coils or arbuscules depending on the AM fungal species (Kubota *et al.*, 2005) and, occasionally, hyphal coils can form fine arbuscule-like ramifications and appear to be a continuum of mycorrhizal structures ranging from *Arum* to *Paris*, depending both on plant and AM fungal species (Dickson, 2004; Karandashov *et al.*, 2004). The arbuscules in the *Arum*-type have received far more attention because most crop plants form this mycorrhizal type and therefore we will mainly refer

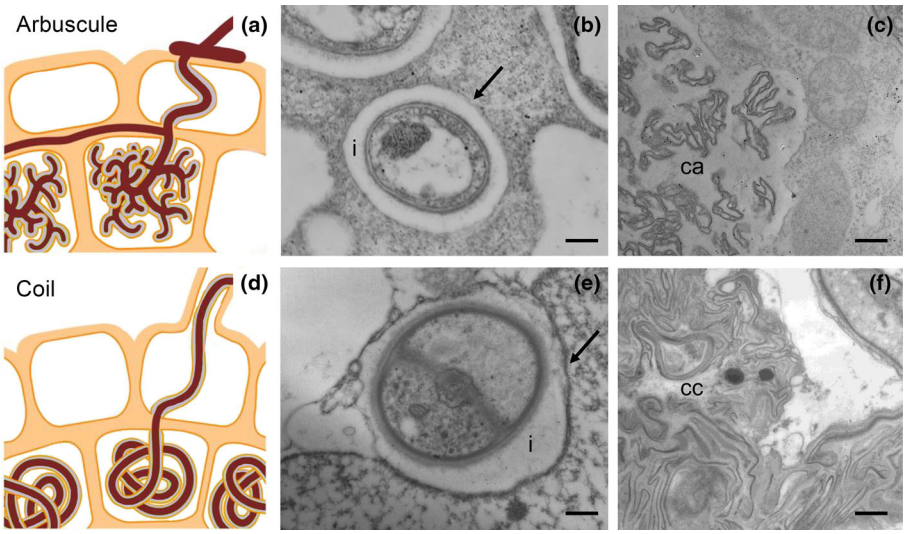

Fig. 2 Arbuscular mycorrhiza (AM) symbiosis *vs* orchid mycorrhiza (OrM) intracellular structures. (a) Scheme of the colonization by an AM fungus with the formation of an arbuscule, which is considered the key structure in the AM symbiosis. Intracellular hypha and arbuscule branches are separated from the plant cell cytoplasm by an apoplastic interface (green) surrounded by an extension of the host plasma membrane (orange). (b, c) An arbusculated cell at transmission electron microscopy showing the thin arbuscule branches with the interface compartment (b) and degenerated hyphae (c). (d) Scheme of a *peloton*, that is the typical intracellular symbiotic structure in OrM. (d, e) TEM pictures of peloton hyphae with the membrane-delimited symbiotic interface (e) and collapsed ones (f). ca, collapsed arbuscule; cc, collapsed coil; i, symbiotic interface. Arrows indicate the membrane delimiting the symbiotic interface. Bars: (a) 0.25 μm; (b) 0.4 μm; (c, d) 0.7 μm. b, modified from Balestrini *et al*. (2005) with permission; c, courtesy of Mara Novero.

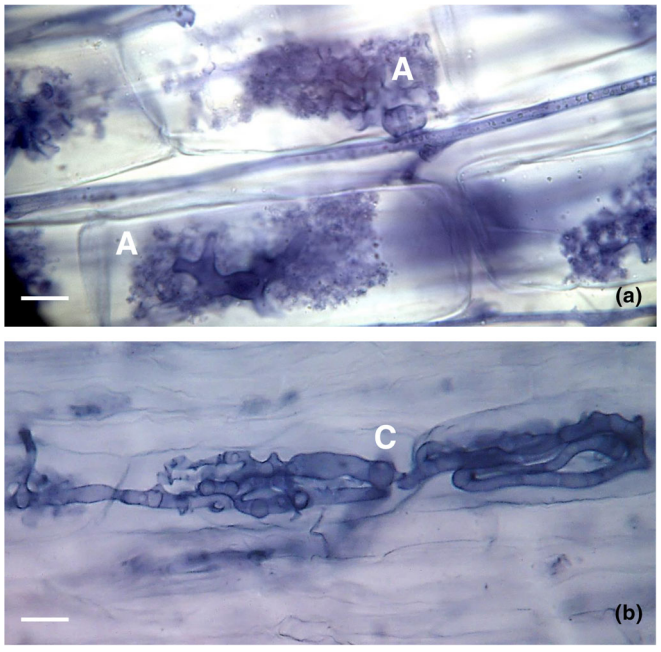

Fig. 3 *Arum*- (a) and *Paris*-type (b) arbuscular mycorrhiza (AM) morphologies. (a) Ramified intracellular arbuscules (A) in root cortical cells. (b) Intracellular hyphal coils in *Paris*-type colonized roots. (c) coil. Bars, 20 μm. Courtesy of Cristiana Sbrana.

to this AM type for comparisons with OrM pelotons. However, Brundrett & Kendrick (1990) suggested that the *Paris*-type could be as common as the *Arum*-type in natural communities, as several trees and forest herbs form *Paris*-type intracellular hyphal coils and/or arbusculate coils.

Peloton development and formation of the plant–fungus interface

The pelotons in OrM and the arbuscules in AM are both short-lived structures: they form inside the parenchyma host cell, play their functions for few days, and then collapse and are eventually digested inside the plant cell (Fig. 2). During the development of intracellular pelotons in OrM, similar to the development of arbuscules in AM (Cox & Sanders, 1974), the plant plasma membrane invaginates around the OrM fungal hyphae to envelop them and separate them from the plant cytoplasm (Peterson *et al*., 1996, 2004). During this process, a plant–fungus symbiotic interface is formed, lined by the plasma membranes of the two partners and containing plant and fungal cell wall material. Its composition has been investigated in detail both in OrM (Peterson *et al*., 1996) and in AM (Balestrini & Bonfante, 2014), showing many similarities. Plant genes involved in the development of intracellular fungal structures, as well as of the symbiotic interfaces, have been identified in AM (Guether *et al*., 2009a; Luginbuehl & Oldroyd, 2017; Ho-Plágaro & García-Garrido, 2022), whereas very limited functional molecular data are available for OrM.

In AM, the interfacial compartment contains cell wall-like material (Scannerini & Bonfante-Fasolo, 1983; Balestrini & Bonfante, 2005), suggesting that the perifungal plant membrane maintains the capacity to synthesize cell wall-related molecules. *In situ* techniques have identified the same molecular components of the peripheral cell wall in several plant–AM fungus combinations, namely β-1,4-glucans, nonesterified homogalacturonans, xyloglucans, proteins rich in hydroxyproline, and arabinogalactan proteins (Balestrini & Bonfante, 2014). This interface cell wall-like material is very thin around the fine fungal branches, and it becomes again thicker when the arbuscule collapses.

In OrM protocorms, the plant–fungus interface also contains typical plant cell wall molecules such as callose, cellulose, and pectins (Peterson *et al.*, 1996). However, these plant cell wall components were detectable around pelotons that were beginning to collapse, whereas none of the probes labeled the interface surrounding active fungal hyphae, characterized by numerous mitochondria and abundant glycogen (Peterson *et al.*, 1996). The authors hypothesized that OrM fungi may secrete enzymes able to break down plant cell wall components during the viable stages of peloton development, a hypothesis supported by the identification in OrM fungi (e.g. *Tulasnella*, *Ceratobasidium*, and *Serendipita*) of a rich repertoire of genes coding for cell wall degrading enzymes, especially cellulases (Kohler *et al.*, 2015; Miyauchi *et al.*, 2020). The role of these enzymes in symbiosis is still unclear, but their strong upregulation in germinating symbiotic seeds (Chen *et al.*, 2022) and protocorms (Kohler *et al.*, 2015; Miyauchi *et al.*, 2020) may explain the different accumulation of plant-derived cell wall material in the symbiotic interface in OrM compared with AM, as the genomes of AM fungi lack the repertoire of plant cell wall degrading enzymes (Tisserant *et al.*, 2013).

Differences have been found in the presence/absence of methylated pectins in the interface surrounding two taxonomically different OrM fungi in the same *Limodorum abortivum* roots (Paduano *et al.*, 2011), providing further support to the hypothesis that the fungal symbiont may influence the accumulation of interfacial plant cell wall components. Alternatively, the observation may suggest that the plant modifies the composition of the interface in different cells of the same root tissue depending on the fungal partner. In *L. abortivum*, transcriptomic data also showed significant downregulation and inhibition of plant pectin methylesterases (PME) in mycorrhizal roots, suggesting that the plant reduces pectin rigidity in wall and/or interface in OrM tissues (Valadares *et al.*, 2021). Unfortunately, the transcriptomic data alone cannot provide information on the activity of the enzymes and their localization on the peripheral wall or on the symbiotic interface.

The formation of the perisymbiotic plant membrane involves *de novo* membrane synthesis, and components of the machinery involved in exocytosis and vesicle targeting have been found to be necessary for the formation of the periarbuscular membrane in AM (see Luginbuehl & Oldroyd, 2017). In OrM, a general increase in the biosynthesis of membrane lipids has been reported (Valadares *et al.*, 2021) and some specific components of the exocytotic pathway have been found to be upregulated in OrM tissues. In particular, two genes coding for syntaxin 132a (SYP132a), a t-SNARE protein required for arbuscule development (Huisman *et al.*, 2016), were upregulated in mycorrhizal roots of *L. abortivum* (Valadares *et al.*, 2021) and the same was found by Zhao *et al.* (2014). Although the localization of the SYP132 proteins in OrM roots is currently unknown, they may indicate that a common exocytotic pathway is shared by AM and OrM symbioses.

Another feature shared by OrM and AM is the short-lived nature of the intracellular fungal structures formed inside the host plant cells, as both pelotons and arbuscules have a life span of a few days before eventual collapse. In AM, arbuscule development is followed by a degeneration phase in which the arbuscule and the surrounding plant membrane disappear gradually from the root cell (Floss *et al.*, 2017). The first morphological signs of arbuscule degeneration appear in the fine branches, with loss of cytoplasm and collapse of the hyphal walls (Fig. 2). In OrM, the cytoplasm of mature pelotons becomes highly vacuolated and glycogen is degraded before pelotons collapse and undergo a final lysis inside the plant cell (Peterson & Currah, 1990). A peculiar degradation pattern is found in very few mycoheterotrophic orchids, like *Gastrodia elata*, where fungal hyphae are lysed inside specific 'digestion cells' without forming peloton clumps (Rasmussen, 2002; Li *et al.*, 2020).

The plant plasma membrane remains intact around the collapsed fungal hyphae, and disassembly of pelotons in OrM and arbuscules in AM is likely mediated by the host plant cell, which remains viable throughout this process and can be recolonized by new hyphae (Smith & Read, 2008; Luginbuehl & Oldroyd, 2017). A program regulated by the MYB1 transcription factor is associated with arbuscule degeneration in AM and includes upregulation of specific hydrolases (Floss *et al.*, 2017). No information is currently available on the transcriptional program during OrM peloton degeneration.

Despite the similarities described above for the life cycle of intracellular fungal structures in OrM and AM, fungal lysis is often portrayed as a peculiarity of OrM. The digestion of collapsed fungal pelotons inside OrM cells, referred to as 'phagocytosis' in the earliest studies on OrM (see descriptions by Noël Bernard in Selosse *et al.*, 2017), has been in fact proposed as the main mechanism of nutrients transfer from the fungus to the plant by many authors, a mechanism named 'tolypophagy' (Rasmussen, 1995). It is very likely that digested fungal material is eventually taken up from the plant after peloton lysis (Kuga *et al.*, 2014), but the same could be envisaged to occur after arbuscule collapse in AM, and it is curious that the mycophagous strategy suggested for OrM (Rasmussen & Rasmussen, 2009) has not been considered as an important mechanism of nutrient transfer in AM.

Peloton functioning in nutrient transfer

As already mentioned, a peculiarity of all orchids is that the organic carbon required for seed germination and protocorm development is provided by the fungal partner, which keeps feeding partial and full mycoheterotrophs with carbon throughout their life stages. Mycoheterotrophy, in all its variegated forms, is a fundamental adaptive strategy of orchids, and the OrM has likely played an important role in orchid evolution (Těšitel *et al.*, 2018). However, this feeding strategy is not restricted to the Orchidaceae family, as > 230 mycoheterotrophic plant species are associated with AM fungi and receive carbon from the fungal partner (Merckx *et al.*, 2010; Courty *et al.*, 2011; Gomes *et al.*, 2020). Similar to green (chlorophyllous) orchids, natural stable isotope profiling has provided evidence of partial mycoheterotrophy in chlorophyllous AM species (Cameron & Bolin, 2010). Interestingly, partial mycoheterotrophy is common among plants forming AM of the *Paris*-type (Giesemann *et al.*, 2020, 2021), which is characterized by the formation of intracellular hyphal coils (Fig. 3; Smith & Read, 2008). This finding thus raises intriguing questions on

whether hyphal coils may be a precondition for the development of mycoheterotrophy (Giesemann *et al.*, 2020, 2021).

Although the natural abundance of stable isotopes can help to understand the origin and flow of elements, it provides no information on the type of molecule transferred from the fungus to the orchid host, or on the mode of nutrient transfer. As already mentioned, peloton digestion has been indicated as a major mechanism of capturing fungus-derived compounds by the host plant in OrM (Rasmussen & Rasmussen, 2009). The alternative hypothesis, and in our view more supported mechanism, is that nutrient transfer between the two OrM partners, including carbon, occurs across the expanded surface area of the symbiotic interface, similar to AM and to other plant endosymbioses. Early experiments with radioactive tracers (see Smith & Read, 2008) showed that carbon is transferred to the plant before the pelotons extensively collapsed inside OrM protocorms, with early peaks of labeled trehalose and saccharose (Smith, 1967). Intriguingly, several orchid species can grow *in vitro* on trehalose as the sole sugar source, and Ponert *et al.* (2021) reported in *Dactylorhiza majalis* an efficient trehalase-dependent metabolic pathway that enables orchids to utilize exogenous trehalose. Genomic data support this peculiar ability of orchids to utilize trehalose (Li *et al.*, 2022), and transcriptomic data further suggest that trehalose may be transferred from the fungus to the plant because genes coding for plant trehalases are significantly upregulated in OrM protocorms (Jąkalski *et al.*, 2021; Chen *et al.*, 2022) as well as in adult mycoheterotrophs (Li *et al.*, 2022). In the mycoheterotrophic orchid *G. elata*, which specifically associates with the root pathogen *Armillaria mellea*, two plant sucrose transporters may be involved in fungus-derived sucrose uptake and allocation in the orchid host (Ho *et al.*, 2021; Gebauer & Clemens, 2021), suggesting a more complex scenario. Unfortunately, in *Paris*-type AM the nature of the compound(s) transferred by the AM fungus to the host plant is currently unknown.

Mycorrhizal fungi play a major role in the uptake of macronutrients – such as phosphorus, nitrogen, and sulfur – as well as essential micronutrients. Plants have their own transporters to take up macro- and micronutrients from the soil essential for their growth, but the mycorrhizal fungal partners can greatly contribute to absorption, especially at low soil nutrient concentrations. Several plant genes encoding putative membrane transporters are upregulated both in OrM and in AM, and transfer of nutrients to the plant is thought to occur across the interface lined by the invaginated plant plasma membrane that surrounds fungal pelotons or arbuscules, respectively (Smith & Read, 2008). Nutrient transport to the host plant has been extensively investigated in the arbuscules formed by the *Arum*-type AM, thought to be the major site where mineral nutrients are exchanged for photosynthesis-derived carbon (Wipf *et al.*, 2019; Shi *et al.*, 2023). Upregulation of specific plant transporters in arbuscule-containing cells indicates that macronutrients are transferred mainly in inorganic forms, namely phosphate (Javot *et al.*, 2007), ammonium (Govindarajulu *et al.*, 2005; Guether *et al.*, 2009b), and sulfate (Casieri *et al.*, 2012; Giovannetti *et al.*, 2014).

The supply of phosphate is considered to be the main benefit of the AM symbiosis (Bucher, 2007), and the AM-specific phosphate transporter PT4 is a marker of root cells containing functional arbuscules in the *Arum*-type AM (Javot *et al.*, 2007). A phosphate transporter (*StPT3*) was expressed in coil-containing potato cells forming *Paris*-type AM, suggesting that phosphate is also transferred to the plant in this AM type (Karandashov *et al.*, 2004). Transfer of phosphorus to the host plant in OrM has been demonstrated with radiolabeled tracers by Cameron *et al.* (2007), but the actual transferred molecule remains unknown.

As compared to nitrogen and sulfur transfer in *Arum*-type AM, a different general pattern is emerging from transcriptomic studies in OrM, with both elements likely to be delivered to the host mainly as organic molecules (Fochi *et al.*, 2017a, 2017b; De Rose *et al.*, 2023a). In *Arum*-type AM, a well-supported model for nitrogen transfer is via the urea cycle, together with arginine translocation to the arbuscule (Bago *et al.*, 2001). Inside the arbuscule, arginine is broken down to release urea and eventually ammonia (Bago *et al.*, 2001), which is released to the symbiotic interface and taken up by plants (Koegel *et al.*, 2017). This model is also supported by the upregulation of plant ammonium transporters in arbuscule containing cells (Gomez *et al.*, 2009; Guether *et al.*, 2009b).

Orchids receive large amounts of ^{15}N-enriched compounds from their fungal partners, resulting in a very high nitrogen content in the orchid tissues, especially in mycoheterotrophic species (Hynson *et al.*, 2013). Kuga *et al.* (2014) demonstrated transfer of ^{15}N-labeled nitrogen to *Spiranthes sinensis* protocorms colonized by *Ceratobasidium* sp. using secondary ion mass spectrometry (SIMS). Transcriptomic studies in OrM protocorms demonstrate that, unlike AM fungi forming *Arum*-type symbioses, genes involved in the urea cycle and arginine breakdown are not upregulated in intracellular pelotons formed by the OrM fungus *Tulasnella calospora* (Fochi *et al.*, 2017a). Moreover, plant ammonium transporters were not significantly upregulated in symbiosis, whereas some amino acid transporters were upregulated in mycorrhizal protocorms, including the lysine histidine transporter 1 (LHT1) (Fochi *et al.*, 2017a; Miura *et al.*, 2018), suggesting that nitrogen is transferred to the plant likely as amino acids. Expression of the LHT1 transporter was strongly upregulated also in OrM roots (Zhao *et al.*, 2014; Valadares *et al.*, 2021). Laser microdissection and RNA extraction from subpopulations of mycorrhizal and nonmycorrhizal cells from *Serapias vomeracea* protocorms showed that these amino acid transporters were expressed mainly in mycorrhizal cells containing active pelotons (Fochi *et al.*, 2017b), confirming the hypothesis that nutrient transfer in OrM mainly occurs from an active peloton across an intact symbiotic interface. Measurement of ^{15}N enrichment and nitrogen content in the hyphal coils and plant tissues of two *Paris*-type mycoheterotrophic AM species (Gomes *et al.*, 2023) similarly suggested uptake of fungus-derived amino acids by the plant, although no information is available on the expression of plant membrane transporters in AM mycoheterotrophs and ^{15}N enrichment in plant tissues was not found in all mycoheterotrophic AM families (Gomes *et al.*, 2020).

Sulfur nutrition has received less attention, but uptake in *Arum*-type AM plants is suggested to occur as sulfate and to involve mycorrhiza-induced plant sulfate transporters (SULTR) found to be expressed in cortical cells containing fungal arbuscules

(Giovannetti *et al.*, 2014). Once taken up from the plants, incorporation of sulfur in sulfurylated organic compounds requires prior reduction of the absorbed sulfate through the sulfur assimilation pathway (Kopriva *et al.*, 2019).

In OrM, recent experiments with isotope labeling coupled with secondary ion mass spectrometry (SIMS) demonstrate ^{34}S transfer from an isolate of the OM fungus *Tulasnella calospora* to mycorrhizal protocorms of its host plant *Serapias vomeracea* (De Rose *et al.*, 2023a). Transcriptomic studies on the same OrM model system revealed a strong upregulation of the fungal sulfur assimilation pathway in the pelotons, whereas the plant sulfur assimilation pathway was not regulated (De Rose *et al.*, 2023a). These results, together with the fact that plant plasma membrane-associated SULTRs were not significantly upregulated in mycorrhizal protocorms, suggest that sulfur may be transferred to the plant in a reduced organic form in OrM, like sulfur-containing amino acid or a small peptide such as glutathione (De Rose *et al.*, 2023a). Intriguingly, SIMS revealed a linear ratio between the labeled nitrogen and sulfur incorporated in the plant nuclei of mycorrhizal protocorms, suggesting that these two elements may be translocated at the same rate, or part of the same translocated molecule.

Reduction of sulfate and nitrate is an energy-demanding process that normally occurs in the chloroplasts of photosynthetic plants. In this respect, translocation of organic molecules containing already reduced nitrogen and sulfur could be an adaptive strategy in orchids and an advantage for achlorophyllous orchid protocorms, that receive both carbon and reduced macronutrients from the fungus, packed in the same molecule(s). The same would be true for mycoheterotrophic plants in general.

Conclusions

Endomycorrhiza is a particularly fascinating association because of the high degree of integration between the plant and the fungal partner, with the two most widespread endomycorrhizal types being AM and OrM, found in *c.* 72% and 10% of flowering plant species, respectively (Brundrett & Tedersoo, 2018; Genre *et al.*, 2020). During evolution, orchids have maintained in their genomes a 'symbiotic toolkit' first established and highly conserved in plants that host AM fungi (Vigneron *et al.*, 2018). Some genes of this common symbiotic signaling pathway required for arbuscule development in AM have been also found in the orchid genomes (Radhakrishnan *et al.*, 2020), and although further studies are needed to define potential roles in the control of peloton development, they suggest a conserved pathway.

Notable differences can be found, in the morphology and functioning in nutrient exchange, between the intracellular OrM fungal pelotons and the arbuscules in *Arum*-type AM. By contrast, when the comparison is made between mycoheterotrophic *Paris*-type AM and OrM plants, most of these differences fade because these AM plants feature fungal coils that are able to transfer carbon to the host plant, just like OrM (Giesemann *et al.*, 2020, 2021; Gomes *et al.*, 2023).

The similar form and function of the intracellular fungal structures in *Paris*-type AM and OrM were already pointed out by Rasmussen & Rasmussen (2014), and given the significant plasticity of AM fungi while developing inside their host (Dickson, 2004), we suggest that AM coils and OrM pelotons may have been favored independently during the evolution of plants with a mycoheterotrophic strategy. An intriguing question is whether coiled fungal hyphae in AM and in OrM, together with their symbiotic interfaces, display some functional traits that are more suitable for nutrients transfer to mycoheterotrophic hosts. Interestingly, although the extensive branching in arbuscules greatly increases the surface-to-volume ratio, a comparison between the volume and surface area of intracellular arbuscules and coils in two plants respectively forming *Arum*-type and *Paris*-type AM resulted in higher values for coils (Dickson & Kolesik, 1999).

No differences were found in the expression of genes involved in nutrient transfer in tomato roots colonized by two AM fungi respectively forming *Arum*-type and *Paris*-type AM, indicating that the symbiotic exchange of nutrients between tomato and AM fungi is irrespective of AM morphotype (Tominaga *et al.*, 2022). However, gene expression data in *Paris*-type mycoheterotrophic AM plants are currently not available, to our knowledge, and we know in general little about the mechanisms of nutrient exchanges in mycoheterotrophic AM plants (Gomes *et al.*, 2020). Although it is clear that carbon and macronutrients are delivered from the AM fungal coils to the host plant, it would be very interesting to investigate their nature not only to reveal possible similarities with OrM, but also to better understand the functioning of AM structures in carbon and macronutrient transfer, which appears complex even in the well-known *Arum*-type AM (Wang *et al.*, 2017). In fact, although the translocation of macronutrients as inorganic forms seems to be preferred in AM plants featuring arbuscules, they could be also transferred to the plant as organic forms. For example, expression of a mycorrhiza inducible LHT-type transporter has been reported in arbusculated cells of *Lotus japonicum* and suggests that amino acids may be also transported across the plant–fungus interface (Guether *et al.*, 2011). Uptake of organic forms of sulfur has been also suggested in AM (Allen & Shachar-Hill, 2009).

The hypothesis of a convergent evolution of fungal structures in mycoheterotrophic mycorrhizal plants (either orchids or AM plants) must be confirmed by further studies on the relationship between *Paris*-type AM and mycoheterotrophy (Giesemann *et al.*, 2020, 2021; Murata-Kato *et al.*, 2022). If confirmed, this would imply that the trophic needs of the host plant can determine shape and functions of the intracellular structures formed by the fungal partners and would open intriguing questions on the mechanisms that enable mycoheterotrophic plants to control fungal development and to exploit its resources. This would be particularly interesting for AM fungi because of the different structures that these fungi can form inside the host cell (i.e. arbuscules, coils or arbusculate coils) and may help to better understand the dynamics in the *Arum–Paris* continuum. In this respect, laser microdissection could represent a useful tool to investigate transcriptomics or specific gene expression (e.g. genes coding for nutrient transporters) in cell-type populations containing fungal structures typical of *Arum*- or *Paris*-type AM.

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Competing interests

None declared.

Author contributions

SP and RB jointly discussed the ideas presented in the manuscript and wrote the initial draft. The two authors approved the final manuscript and equally shared the first authorship. SP and RB contributed equally to this work.

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Key words: arbuscular mycorrhiza, *Arum*-type, mycoheterotrophy, orchid mycorrhiza, *Paris*-type, peloton development.

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