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To trade in the field: the molecular determinants of arbuscular mycorrhiza nutrient exchange

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Abstract

Traditionally, the most popular sentences used to describe the arbuscular mycorrhizal symbiosis sound like: "AM fungi form one of the most widespread root symbioses, associating with 80% of land plants. In this symbiosis, the fungus provides the plant host with mineral nutrients, especially phosphate, receiving in turn carbohydrates." In the last years, the mycorrhiza research field has witnessed a big step forward in the knowledge of the physiology and the mechanisms governing this important symbiosis, that helped plants colonizing the lands more than 400 MYA. The huge expansion of the -omics studies produced the first results on the fungal side, with genomes and transcriptomes of AM fungi being published. In parallel, the need for more sustainable agricultural practices has boosted the research in the field of the plant symbioses, with the final aim of improving plant productivity employing symbiotic microbes as bioinoculants. Beside all the other (positive) effects that mycorrhizal fungi exert on plants, the nutrient exchange is considered as the keystone, and the core mechanism governing this symbiosis. This review will focus on the molecular determinants underneath this exchange, both on the fungal and the plant side. Coming back to the sentence that claims this symbiosis as based on phosphate provided to the plant in return to carbohydrate, we will find that some concepts of this view still stand, while some others have been partly revolutionized.

Keywords: Arbuscular mycorrhizal fungi, Nutrient exchange, Phosphate, Symbiosis

Introduction

Arbuscular mycorrhizal fungi belong to the basal fungal phylum of Glomeromycota [1]. They are obligate biotrophs that associate with plant roots forming the mycorrhiza. The establishment of such symbiosis follows a finely tuned pattern that starts in the soil with the exchange of molecular signals produced by both the sides of the interaction [2]. Once a host is found, the fungus enters the plant root with a mechanism strictly regulated by both the partners. The functional core of this symbiosis is represented by the arbuscule, a complex, highly branched structure formed by the fungus intracellularly, and surrounded by a plant membrane called periarbuscular membrane (PAM) [3]. Here, the nutrient exchange between plant and fungus occurs. Outside the root, the fungus forms a net of extraradical hyphae that take up nutrients extending the portion of soil that the plant can reach with its own roots (Fig. 1).

The rules that govern this exchange of nutrients are complex, and should be viewed in the context of two symbionts that of course are not interacting alone, but also face a plethora of diverse biotic and abiotic stimuli in natural conditions. Furthermore, many reports have shown that the mycorrhizal outcome in terms of growth response can vary considerably, ranging from positive, to neutral to even negative [4, 5], and that among the AMFs, some have been described as more collaborative while some others less [6]. At the moment the bases of such variability have not been completely elucidated, even if the researchers already did big steps forward to assess the molecular determinants of the nutrient exchange, giving important clues on the factors acting as main regulators. All these data are in fact instrumental to draw the connection between the molecular and

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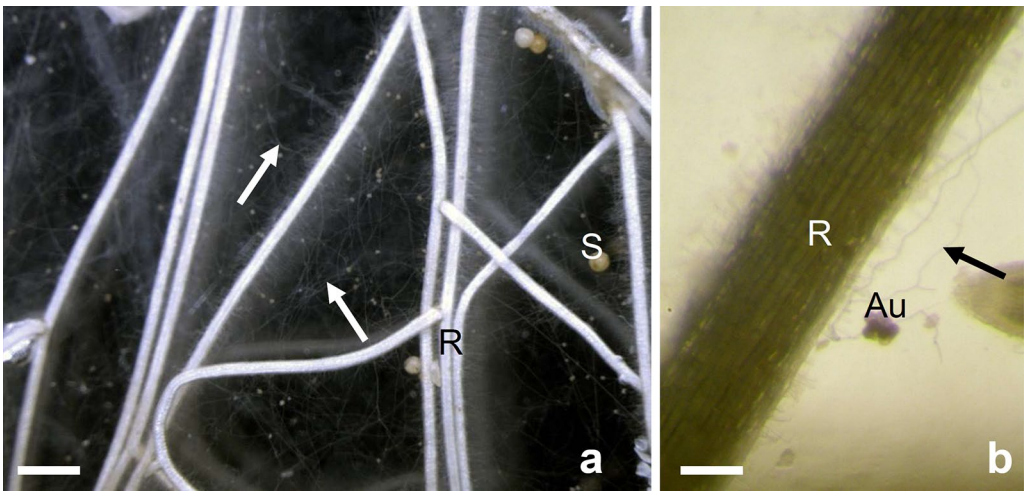

Fig. 1 The interaction between *Lotus japonicus* roots and the arbuscular mycorrhizal fungus *Gigaspora margarita*. **a** The *L. japonicus* roots (R) are surrounded by a dense net (arrows) of *G. margarita* extraradical hyphae that give rise to newly formed fungal spores (S). **b** A detail of the contact between a *L. japonicus* root (R) and *G. margarita* hyphae decorated by an auxiliary cell cluster (Au). Bars correspond to 1.3 mm in a and 140 µm in **b**

the eco-physiological level of the mycorrhizal symbiosis functioning.

Major recent breakthroughs in the AM biotrophy, as the discovery of the fungal dependency on host fatty acids, represented a real paradigm shift, and stimulated the researchers to construct an updated scenario of the plant–fungal exchanges to integrate the new findings. Although both carbon and mineral nutrition in the AM symbiosis have been exhaustively reviewed by many Authors (as, for example, Casieri et al. [7], Garcia et al. [8], Shi et al. [9], Wang et al. [10]), the aim of this review is to provide the reader with a "handy guide" through the current view of the symbiotic transportome.

The first part of the story: the fungus provides the plant host with mineral nutrients

The improvement of plant phosphate nutrition by AM fungi has been extensively studied over the years. The transfer of phosphate from fungi to plant hosts has been demonstrated in the 90s, when *Trifolium* plants were mycorrhized in a two-compartment system in which radiolabeled P was added to the compartment accessible to the AM fungus only [11]. AM fungi provide plants with phosphate via an indirect pathway, called "the AM pathway", that parallels "the direct pathway" where roots directly take up phosphate from the soil [12]. In mycorrhizal plants, a considerable part (up to 70%) of the overall phosphate uptake can be acquired via the AM pathway [13]. Expression of the direct phosphate transporter genes in non-mycorrhizal plants is regulated by the phosphate starvation signaling pathway; while in AM-colonized plants, the direct pathway can be modulated independent of the phosphate status, as the result of the interplay with the AM pathway. It has been demonstrated that colonization by AM fungi reduces the direct root phosphate uptake locally, but without affecting it in distant non-colonized roots [14].

The main plant actors of the AM pathway are specific phosphate transporters, which have been identified in different plant species, including *Medicago truncatula*, *Oryza sativa* and *Lotus japonicus* [15–19]. These PHT1 family transporters show a specific pattern of expression in response to AM fungal colonization, being exclusively expressed in response to AMFs and localized at the interface of the two symbionts, in the PAM [15].

The best characterized mycorrhiza-inducible *PHT1* gene is *MtPT4* from *M. truncatula*. It has been detected in arbusculated cells, and immunolocalization assays suggested its exclusive presence in the PAM [15, 16]. Other PHT1 family transporters are present that are likely involved in the direct phosphate acquisition pattern. In *M. truncatula MtPT1*, *MtPT2*, *MtPT3*, *MtPT5* and *MtPT6* belong to PHT1 family and, though with different specific patterns, their expression in mycorrhizal roots is generally reduced [20–22]. In rice, the genes *OsPT2* and *OsPT6* likely involved in the direct pathway are down-regulated by the AM symbiosis, but this repression is missing in the mutant line that lacks the mycorrhiza-responsive phosphate transporter *OsPT11* [13].

The AM-inducible phosphate transporters are considered as good markers for the mycorrhizal status, since their transcripts specifically accumulate in response to fungal colonization [23–25]. Interestingly, Sawers et al. [26] showed that the mycorrhizal outcome in terms of

growth response of maize plants better correlates with the abundance of the extraradical mycelium than with the accumulation of the mycorrhiza-inducible phosphate transporter *ZmPT6*. This might indicate that the fungal ability of exploring the surrounding soil matters more than the amount of transporters expressed at the PAM. Very recently, a further mycorrhiza-inducible P transporter, *ZmPt9*, has been characterized in maize [27] Intriguingly, *ZMPT9* seems to localize in the cytoplasm, and ZmPt9-overexpressing hairy roots displayed a dramatic reduction of AMF colonization [27].

The environmental phosphate level is a key regulator of AM symbiosis. When plants are grown at high phosphate concentration, the AM colonization is drastically reduced, with a response that depends on the plant species considered [28, 29]. Moreover, the lack of functioning of AM-inducible transporters impairs the arbuscules formation [15, 17, 19, 30]; these data suggest a role for phosphate transporters (or alternatively for the phosphate transfer itself) in the signaling pathway that determines the establishment of a successful AM colonization. A similar role for phosphate has been suggested by Yang et al., who showed that a mycorrhiza-responsive phosphate transporter from rice did not display a clear role in phosphate transfer but was requested for the correct arbuscule formation [13]. On this line, Volpe et al. [19] demonstrated that the expression of the mycorrhiza-inducible *PT4* from *M. truncatula* and *L. japonicus* was not restricted to the PAM but also present in the root tips of non-colonized plants. The authors suggested these PTs might act as transceptors, i.e., proteins with a dual role, in phosphate transport and in the sensing of the phosphate status.

On the fungal side, some phosphate transporters appear to be responsible for the first step of the symbiotic phosphate transport. They have been described on the basis of transcriptomic and genomic data: *GmosPT* from *Funneliformis mosseae*, *GvPT* from *Diversispora epigaea*, *GiPT* from *Rhizophagus intraradices*, and one from *Gigaspora margarita* [31–34]. These PTs are all expressed in the extraradical mycelium, where they likely mediate the phosphate uptake from the soil. *GmosPT* from *Funneliformis mosseae* (formerly *Glomus mosseae*) and *GigmPT* from *Gigaspora margarita* expression have been recorded also in the intraradical hyphae, where they are supposed to be active in the phosphate re-uptake from the periarbuscular space [32, 35, 36]. The inactivation of *GigmPT* by host-induced gene silencing impaired arbuscule development, corroborating the view that phosphate sensing might also play a role in the establishment of a functional symbiosis, possibly entailing a role for PTs as transceptors also on the fungal side. Upon phosphate uptake from the soil, its internal levels have to be strictly regulated to allow the accumulation and the transfer to the plant host of high amounts of phosphate without disturbing the fungal homeostasis. The acknowledged model includes the phosphate polymerization into polyphosphate (polyP) and its storage in the fungal vacuoles, from which it can be further released, thanks to the activity of vacuolar polyphosphatases, and then exported to the cytosol through a vacuolar exporter [37]. The molecular determinants of AM phosphate homeostasis are not clearly identified so far. Recently, SPX domain-containing proteins have been widely recognized as main players in the regulation of phosphate homeostasis. The SPX domain acts by allowing the binding of the regulatory protein with inositol polyphosphates (InsPs), thus modulating its activity in a phosphate-dependent manner [38]. Recent mining of the published genomic and transcriptomic data from AMFs detected the presence of genes encoding for SPX domain-containing proteins and for InsPs metabolic enzymes [37, 39]. Some of them have been found to be regulated upon polyP formation and in the response to high phosphate concentrations. Strikingly, the *R. irregularis* genome also revealed the presence of genes encoding for SPX-containing proteins characterized so far only in plants, such as the *Arabidopsis thaliana SPX1*, and *NLA* genes, both involved in the maintenance of plant phosphate homeostasis [40, 41]. Taken together, these data suggest that a regulatory mechanism based on SPX domain-containing proteins and InsPs metabolism might have specifically evolved in AMF to meet the double need of managing the transfer to the plant of massive amounts of phosphate and finely tuning at the same time the fungal phosphate homeostasis.

Although the phosphate transfer surely covers the lion's share, the relevance of nitrogen uptake in the AM symbiosis has been also disclosed more recently, with an important role played both for plant nutrition and for the regulation of the symbiosis functioning itself. In the soil, inorganic nitrogen is present as nitrate (NO_3) and ammonium (NH_4^+), and AMF possess specific transporters for both the N forms. In *Rhizophagus irregularis,* three sequences refer to ammonium transporters, and one nitrate transporter has been identified [42]. The transcriptome assembly of *Gigaspora margarita*, an AM fungus belonging to a different order as the model species *R. irregularis*, confirmed a similar equipment in nitrate/ammonium transporters, being the respective genes well expressed in all the considered fungal life stages [34]. The expression of *R. irregularis* ammonium transporter *GintAMT1* has been demonstrated to be induced under low environmental NH_4^+ conditions [43], while the nitrate transporter is induced by the presence of NO_3^- [44]. When NO_3^- is taken up by AMF, it is reduced to nitrite by a nitrate reductase and then converted into NH_4^+ by a

nitrite reductase. The latter (originated by NO_3^- reduction or directly taken up by ammonium transporters) is then assimilated into amino acids following two pathways: the NAD(P)-glutamate dehydrogenase or the glutamine synthetase–glutamate synthase (GS-GOGAT) pathway. The GS-GOGAT pathway generates arginine that represents the most abundant amino acid in the extraradical mycelium of AMF. Arginine is then transferred through the hyphae to the intraradical mycelium [45], where it is broken down into urea and ornithine. Finally, NH_4^+ is produced from urea via the urease activity, and then released in the symbiotic interface. This fungal ability to take up and transfer N is mirrored by the presence of specific plant transporters: several AM-inducible ammonium transporters have been in fact identified in different species such as *Lotus japonicus*, *Glycine max* (soybean), and *Medicago truncatula*. In *L. japonicus*, the NH_4^+ transporter *LjAMT2;2* is exclusively expressed in the mycorrhizal roots, and preferentially in arbusculated cells [46]. Similarly, in soybean, a specific expression of an ammonium transporter has been detected in arbusculated cortical root cells [47].

AM fungi can also acquire organic N from the soil [48] (Table 1). An amino acid permease, *GmosAAP1*, has been characterized from *Funneliformis mosseae* (formerly *Glomus mosseae*) as expressed in the extraradical mycelium and induced by high levels of organic N [49]. Recently, a dipeptide transporter from *R. irregularis*, *RiPTR2*, has also been described [50]. Its expression profile indicates responsiveness to diverse environmental cues when the fungus grows symbiotically, both in intra- and extraradical compartments. On the plant side, in *Lotus japonicus,* a high-affinity amino acid transporter (*LjLHT1.2*) has been identified and characterized as preferentially expressed in arbusculated cells. The authors suggested for this transporter a possible role in the re-uptake and the recycling of amino acids from the plant–fungal symbiotic interface [51].

In more recent times, multiple evidences demonstrated that a complex interplay occurs between nitrogen and phosphate homeostasis, both at the level of nutrient acquisition and sensing, eventually regulating also the symbiosis establishment and functioning. A simultaneous low phosphate and low nitrogen soil condition dramatically increases the extent of AM colonization [52], and N starvation is partially overruling the negative effect that high soil phosphate availability exerts on mycorrhization [53]. A striking demonstration of such an interconnection has been provided by Breuillin-Sessoms et al. [54]. They observed that in the *M. truncatula* pt4 mutant, the premature arbuscule degeneration due to the lack of the P transporter is averted when plants are kept under nitrogen starvation. This compensatory effect is lost in the double mutant pt4,amt2;3. Moreover, no functional role in the NH4 + transport could be demonstrated for *MtAMT2;3*. The authors, thus, concluded that *AMT2;3* in *M. truncatula* represents a keystone in the signaling cross-talk between phosphate and nitrogen metabolism, with an active role in sensing/signaling more than in nutrient transport [54].

Sulphur (S) is an essential macronutrient for plants, but its role in the arbuscular mycorrhizal symbiosis has been poorly investigated so far. The demonstration that AM fungi can take up both organic and inorganic S and transfer it to the plant partner only came in recent times [55, 56] (Table 1). Mycorrhizal colonization has been demonstrated to positively impact plant sulphur nutrition, with an effect particularly relevant under low environmental S conditions [56–58]. Both *Lotus japonicus* and *Medicago truncatula* possess sulphate transporters (*LjSultr1;2* and *MtSultr1;2*, respectively) that respond to mycorrhizal symbiosis [18, 57]. *LjSultr1;2* has been demonstrated to be strongly activated in arbuscule-containing root cells [58], being at the same time also involved in the sulfate uptake directly from soil. A recent microarray study of *M. truncatula* root and leaf responses to S starvation combined with colonization with the AM fungus *R. irregularis* showed that transcriptional changes directly linked to a sulphate-deficiency status were less dramatic in mycorrhizal *versus* non-mycorrhizal plants [59]. Whether and to which extent mycorrhiza-mediated S uptake can interplay with the sensing and transport of the other nutrients, and whether the S transporters can also have a role in the regulation of the symbiosis itself are still a matter of research.

Potassium (K) is perhaps the most neglected macronutrient in the AM symbiosis. Yet, several reports indicate that the mycorrhizal status results in an improved K nutrition, and this has been observed in different plant–AM fungus associations [60–62] (Table 1). Several types of plant K transporters have been characterized, such as Trk (transporter of K), HAK (high-affinity K uptake) and SKC (Shaker-like channels), but their role in the AM symbiosis [63] has not been investigated so far [63]. A few exceptions are represented by a putative HAK transporter found as strongly induced in mycorrhizal *L. japonicus* roots [18] and a SKC-like channel of maize which resulted up-regulated by AM colonization under salt stress [64]. On the fungal side, several sequences annotated as putative SKC and HAK are present in the genomes of the sequenced AM fungi (see for example those available at the JGI MycoCosm portal, https://genome.jgi.doe.gov/programs/fungi/index.jsf), but more focused research is needed to elucidate their possible role in the symbiotic K uptake and transfer [8].

Table 1 List of the transporters from different host plants and AM fungi cited in this review

Nutrient	Plant transporter name	Plant species	References
Phosphorus	MtPT4	*Medicago truncatula*	Harrison et al. [15] Javot et al. [16] Volpe et al. [19]
	MtPT1		Bucher [20] Liu et al. [21] Grunwald et al. [22]
	MtPT2		
	MtPT3		
	MtPT5		
	MtPT6		
	OsPT2	*Oryza sativa*	Yang et al. [13]
	OsPT6		
	OsPT11		
	ZmPT6	*Zea mays*	Sawers et al. [26]
	ZmPT9		Liu et al. [27]
	LjPT4	*Lotus japonicus*	Volpe et al. [19]
Nitrogen	LjAMT2;2	*Lotus japonicus*	Guether et al. [46]
	LjLHT1.2		Guether et al. [51]
	GmAMT4.1	*Glycine max*	Kobae et al. [47]
	MtAMT2;3	*Medicago truncatula*	Breuillin-Sessoms et al. [54]
Sulphur	LjSultr1;2	*Lotus japonicus*	Guether et al. [18]
	MtSultr1;2	*Medicago truncatula*	Casieri et al. [57]
Water	LjNIP1	*Lotus japonicus*	Giovannetti et al. [70]
	LjXIP1		
	ZmTIP1;1	*Zea mays*	Barzana et al. [72]
	ZmTIP1;2		
Arsenic			
Zinc			
Iron			
Sugars	MtSUTs	*Medicago truncatula*	Doidy et al. [96]
	MtSucS1		Baier et al. [98]
	MtSut2		Kafle et al. [104]
	MtSUT4-1		
	MtSWEET12		
	MtSWEET15c		
	MtSWEET15d		
	GmSWEET6	*Glycine max*	Zhao et al. [105]
	GmSWEET15		
	StSWEET1a	*Solanum tuberosum*	Manck-Götzenberger et al. [103]
	StSWEET1b		
	StSWEET7a		
	StSWEET12a		
Lipids	LjCBX1	*Lotus japonicus*	Xue et al. [123]
	MtWRI5a	*Medicago truncatula*	Jiang et al. [24]
Nutrient	**Fungal transporter name**	**Fungal species**	**References**
Phosphorus	GmosPT	*Funneliformis mosseae*	Benedetto et al. [32]
	GvPT	*Diversispora epigaea*	Harrison et al. [31]
	GiPT	*Rhizophagus irregularis*	Fiorilli et al. [13]
	GigmPT	*Gigaspora margarita*	Salvioli et al. [34]

Table 1 (continued)

Nutrient	Fungal transporter name	Fungal species	References
Nitrogen	GintAMT1	*Rhizophagus irregularis*	López-Pedrosa et al. [43]
	GmosAAP1	*Funneliformis mosseae*	Cappellazzo et al. [49]
	RiPTR2	*Rhizophagus irregularis*	Belmondo et al. [50]
Sulphur			
Water	*RiAQPF2*	*Rhizophagus irregularis*	Chitarra et al. [69]
Arsenic	RiArsAB	*Rhizophagus irregularis*	Maldonado-Mendoza and Harrison [84]
	RiMT-11		Gonzalez-Chavez et al. [83]
Zinc	*GintZnT1*	*Rhizophagus irregularis*	González-Guerrero et al. [85]
Iron	*RiFRE1*	*Rhizophagus irregularis*	Tamayo et al. [87]
	RiFTR1-2		
Sugars	RiMST2	*Rhizophagus irregularis*	Helber et al. [107]
	RiMST5		Ait Lahmidi et al. [108]
	RiMST6		
Lipids			

Considering the symbiotic nutrient flow in a broader sense, the water transport is also worth to be mentioned. Plant water homeostasis is mediated at the cellular level by specific channels called aquaporins (AQPs, [65]; Table 1). AQPs belong to a large protein family further grouped into five sub-families (see Wang et al. [10] for a review). Mycorrhizal plants have been shown to take advantage of an improved water flow from the soil, with a better tolerance to (mild) drought conditions (see Balestrini et al. [66] for a review), and this effect has been linked to a modulation of the plant AQPs [67–69].

To cite an example, in *L. japonicus,* two AQP genes (*LjNIP1* and *LjXIP1*) have been demonstrated to be induced by mycorrhization [70]. Interestingly, laser microdissection experiments demonstrated that transcripts of one of these AQPs specifically accumulated in arbuscule-containing cells [70].

AM fungi also appear to modulate their AQP genes during the symbiosis. The transcript profiles of two *R. irregularis* AQP genes showed an activation in arbuscule-containing maize root cells [71]. On the same line, the expression of the *R. intraradices RiAQPF2* gene in tomato plants subjected to drought showed a significantly up-regulation [69].

Beside water transport, AQPs are also involved in the translocation of small molecules as ammonia, urea and glycerol, and this function might also play a role in the mycorrhizal symbiosis [10]. As an example, Barzana et al. [72] analyzed the expression of the maize AQPs in roots under diverse experimental conditions, and found that two of them, namely *ZmTIP1;1* and *ZmTIP1;2*, were up-regulated upon mycorrhization [72]. In maize, most of the AQPs belonging to the TIP subfamily, including ZmTIP1;1 and ZmTIP1;2, have been demonstrated to transport NH3 and urea [25, 73]: taken together, these data point to a fine-tuned interplay between symbiotic mineral nutrition and water flow.

Beyond the mineral nutrition: the dual role of the AM symbiosis in plant metal ions uptake

Some metals such as iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), molybdenum (Mo) and nickel (Ni) play an important role in plant nutrition as essential micronutrients (Table 1). They are required in minimal amounts by the organisms, but become toxic when present at high concentrations, thus polluting soils and water. In this respect, plants have remarkable abilities to scavenge heavy metals and tolerate them at relatively high concentrations, with some species acting as hyperaccumulator employed in phytoremediation strategies for the recovery of polluted soils [74]. Mycorrhizal plants exposed to high environmental heavy metal concentrations exhibited a wide spectrum of behaviors ranging from hyper accumulation to a reduction of the uptake, also including neutral responses (see Shi et al. [9] for a review). Early reports showed that zinc uptake in maize was positively affected by AM fungi, with an increase of plant growth parameters [75]. In addition, AM fungi can be acclimatized to high heavy metal concentrations, mitigating in turn their accumulation in plants, following a mechanism that likely involves the binding and immobilization of metals on the mycelium surface [76–78]. Unfortunately, this tolerance was shown to dramatically decrease being even got lost when the acclimatized fungal strain grew in heavy metal-free substrate, compromising in turn the fungal ability to confer tolerance to the plant host [79].

Very recently, the possibility to assess the elemental composition of living organisms at the -omics level (referred as ionomics) has allowed the simultaneous and quantitative analysis of 19 ions including metal ones in leaves and roots of maize with and without inoculation with the AM fungus *F. mosseae* [80]. This analysis indicated that a cluster of elements was positively affected by mycorrhization in roots, including Ca, Na, Mo, P, Rb, S and Sr, while the content of some metals such as Cd, Co, Cu, Mn, Ni and Zn was reduced. In the leaves, the influence of AM colonization on the ion profile was different but still evident, with Al, As, Co, Fe, Na, Ni and P increased, while Mn and Zn were decreased as already evidenced in roots [80]. Recent findings demonstrate that the AM symbiosis can modulate the expression of genes that play crucial roles in the plant heavy metal accumulation and detoxification processes. In *Festuca arundinacea*, the AM fungus *F. mosseae* led to an induction of ABC transporters and metallothionein transcripts under high nickel concentrations [81], and the inoculation with a fungal consortium that included *R. irregularis* increased the transcription of *Solanum lycopersicum* phytochelatin synthase, metallothionein and NRAMP (natural resistance-associated macrophage protein) genes in polluted soils [82].

On the fungal side, the metal ion homeostasis has been poorly investigated so far. Current data suggest that AM fungi respond to high metal concentrations by regulating the expression of genes dealing with their transport and metabolism. The exposure of *R. irregularis* to high arsenate concentrations led to the up-regulation of the two components of the *RiArsAB* arsenite efflux pump and of a methyltransferase (*RiMT-11*) in the fungal mycelium [83, 84]. Putative fungal transporters have been characterized: GintZnT1 from the extraradical mycelium of *R. irregularis*, with a predicted function in the fungal zinc homeostasis [85] and RintABC1, putatively involved in heavy metal detoxification [86].

Tamayo et al. [87] performed a careful data mining on the *R. irregularis* genome assembly to retrieve and characterize in silico the copper, iron and zinc transporter genes. The same authors went more in detail characterizing the key components of the reductive pathway of Fe assimilation in *R. irregularis*, namely the ferric reductase (*RiFRE1*) and the high-affinity Fe permeases (*RiFTR1-2*) [88]. Expression data of those genes in the fungal mycelium and complementation assays of yeast mutants indicate their fine-tuning in dependence of the fungal life stages and of the external Fe availability, suggesting that Fe homeostasis in AMF is tightly regulated. On the ecological point of view, the iron uptake from the surrounding environment has important implications for immunity, preventing pathogens invasion on one hand and being also involved in beneficial plant–microbe interactions on the other [89]. Interestingly, comparative transcriptomics of the AM fungus *G. margarita* colonized or not by an obligate intracellular bacterium, showed that one of the genes more up-regulated by the bacterial presence is actually an iron transporter [34]. Taken together, these data strengthen the vision that the regulation of iron homeostasis might represent a relevant mechanism enabling AM fungi to cope with bacteria in the rhizosphere.

Does the plant reward the fungus only with sugars?

Early reports showed that sugars can be transported from the plant host to the fungus in the AM symbiosis [90, 91] (Table 1). Mycorrhizal colonization increases the root sink strength, with up to 20% of photosynthates transferred to the fungus [92]. Consistently, AM plants often display an increased photosynthesis [93, 94], that seems not only to sustain the fungal metabolism but also to correlate with an increase in plant biomass [95]. Plants have different families of sucrose transporters (SUTs) that can be involved in the sugar transfer to the colonized roots: in *M. truncatula,* the expression profiles of *MtSUTs* are finely tuned by the presence of the fungal symbiont [96], and the three sucrose transporters from tomato are also up-regulated in roots colonized by *Funneliformis mosseae* [97]. *M. truncatula* antisense lines for the biosynthetic enzyme sucrose synthase (*MtSucS1*) in roots displayed an abnormal mycorrhizal phenotype, with an impairment of plant growth under phosphate limitation, a reduced mycorrhization and relevant alterations in the morphology and life span of the arbuscules [98]. These traits were mirrored by a reduced expression of plant genes markers for the AM symbiosis, pointing to a central role of plant-derived sugars in the mycorrhiza establishment and functioning [98].

In the roots, the sucrose unloaded from the phloem or newly synthesized is thought to be cleaved into monosaccharides by plant invertases. Monosaccharides are the most likely sugar forms transferred to the fungal symbiont: consistently, plant monosaccharides transporters (*MSTs*) are finely regulated in roots upon mycorrhizal colonization [99–101]. Recently, a new class of sucrose and monosaccharide exporters has been characterized that likely operates the plant sugar efflux in both pathogenic and symbiotic interactions [102]. These so-called SWEET transporters have been also linked to the AM symbiosis, since a recent paper highlighted a transcriptional induction of some of them in arbusculated cells from potato plants [103]. Recent findings strongly suggest that sugar transporters can operate at the molecular level the "reward mechanism" described by Kiers et al. [6], which postulated that plants can modulate the symbiotic C allocation to reward the most collaborative

symbionts. Kafle et al. [104] provided an elegant demonstration employing the split-root system and $^{13}CO_2$ labeling to dissect a tripartite association of *M. truncatula* with the nodule-forming rhizobacterium *Ensifer meliloti* and the AM fungus *R. irregularis*. By modulating the symbiotic nutrient access and the plant nutritional status, they demonstrated that plants under N demand preferentially allocated organic C to the nodulated root half, but this flux was more balanced when the AM fungus also had access to an exogenous N source. Interestingly, some specific isoforms of the *SUT* and *SWEET* transporters showed expression patterns that nicely followed the plant C partitioning: the expression levels of *MtSUT2* and *MtSUT4-1* positively correlated with the C allocation to the symbiotic partners, and *MtSWEET12*, *MtSWEET15c*, and *MtSWEET15d* were up-regulated in the mycorrhizal roots when the fungus had access to a N source, but were down-regulated when the host plant was not under N starvation. Another recent research analyzed the transcriptional responses of soybean roots colonized with more or less cooperative (in terms of their ability to promote plant growth) AM fungi, and found that two *SWEET* genes (*GmSWEET6* and *GmSWEET15*) and one sugar invertase (Glyma.17G227900) were exclusively induced when the roots were colonized with more cooperative AMF species [105].

To parallel the activation of plant sugar transporters, on the fungal side, a few actors have been demonstrated to take part in the symbiotic sugar uptake [106, 107]. In particular, *RiMST2* from *R. irregularis* is expressed in the intraradical fungal structures, and its silencing affects both arbuscule morphology and the extent of the mycorrhization [107].

Recently, two further fungal sugar transporters (*RiMST5* and *RiMST6*) have been found to be expressed also in the extraradical mycelium, being involved in the direct uptake from the soil [108]. Both these are monosaccharide transporters, and *RiMST6* has been characterized as a glucose-specific, high-affinity H^+ co-transporter. However, the contribution of the non-symbiotic sugar uptake in AMF has not been clarified yet. Our survey of the *G. margarita* transcriptome highlighted the expression of fungal sugar transporters also in the pre-symbiotic stages of the fungal life cycle. Furthermore, potato mutant defective for the *SUT* gene did not display an impaired mycorrhizal phenotype [109]. Taken together, these evidences indicate that, though the sugar flux in the AM symbiosis is not questioned, this mechanism seems not to represent the keystone of the AMF strict biotrophy. To justify this peculiar lifestyle, the fungus should be dependent on its host for some essential (nutritional) factors. At the molecular level, this might be due to the lack of expression of some crucial genetic determinant in the asymbiotic phase, or alternatively to the absence of the coding potential for an essential pathway. The availability of genomic data on the first sequenced AMF *R. irregularis* followed in the last year by other species and genera [42, 110–113] allowed to reveal that AMF do not possess the genes encoding the fungal type I Fatty acid Synthase (FASI). Some very recent researches well characterized at the molecular level the dynamics of such a fatty acid auxotrophy, and clarified that lipids are likely transferred from the plant host to the fungus at the symbiotic interface (Table 1). First of all, a number of fatty acid- and lipid-biosynthesis genes were found to be up-regulated in arbusculated roots, including a specific acyl-ACP thioesterase (*FatM*) and a glycerol-3-phosphate acyl transferase (*RAM2*) required for the symbiosis [101, 114–116]. *L. japonicus fatm* mutant lines showed a reduced shoot phosphate content attributable to an impaired symbiotic functionality, and biochemical analyses evidenced a decrease of the mycorrhiza-specific phospholipids and an alteration of the fatty acid profile [117]. Also, mutations in *FatM*, *RAM2* and another FA biosynthetic gene called *DIS* (encoding a β-keto-acyl-ACP synthase I) resulted in an impaired mycorrhization, with alteration of the arbuscule morphology [115, 116, 118, 119]. The lack of a specific ABC transporter that localizes at the symbiotic interface (STR-STR2) displays a phenotype very similar to that of the AM-specific lipid biosynthesis mutants: this transporter represents a plausible candidate to operate the lipid flow from the plant to the fungus [116, 118, 120]. The next step has been the demonstration that a transfer of lipids from the plant to the fungus actually takes place, and this has been provided by different research groups following distinct approaches on *Lotus japonicus* and *Medicago truncatula* [116, 118, 121]. Taken all these data together, the current model for lipid transfer from the plant to the AM fungus includes an induction of fatty acid biosynthesis in the colonized roots, with 16:0 fatty acids produced by DIS and released by FatM. Then, RAM2 transfers the newly generated FAs to a glycerol moiety to produce 16:0 monoacylglycerols (MAG). This lipidic molecule is then transported through the PAM by the STR–STR2 transporter, and taken up by the fungus with a mechanism that remains still unknown. The AMF can, thus, use these symbiotic 16:0 MAGs directly for energy production or in anabolic processes, modifying the FA structure by means of FA active enzymes as elongases and desaturases encoded by the fungus itself.

Recent findings shed some lights on the regulatory mechanisms that orchestrate the plant symbiotic responses in terms of nutrient exchange.

The RAM1 transcription factor has been identified as an early regulator of the mycorrhiza-specific reprogramming, activating on the one hand genes involved in the

transfer of FAs to the fungus [121] and the AM-specific phosphate transporter PT4 on the other [122]. Following different strategies to screen for transcription factors that could bind the promoters of mycorrhiza-inducible genes, two very recent researches identified elements acting downstream RAM1, namely LjCBX1 in *Lotus japonicus* [123] and MtWRI5a in *Medicago truncatula* [124]. LjCBX1 binds the conserved cis-regulatory motif "CTTC" enriched in mycorrhiza-regulated genes as well as an AW-box motif present in the promoters of glycolysis and fatty acid biosynthesis genes. Accordingly, the authors showed that LjCBX1 can activate the transcription of FA metabolic genes as well as of the *L. japonicus* PT4 [123]. The *M. truncatula* MtWRI5a transcription factor has also been shown to bind the AW-box motif present in the promoter of the fatty acid ABC transporter STR, as well as the phosphate transporter MtPT4, enhancing their expression [124]. On the contrary, hairy roots of *M. truncatula* wri5a mutants showed an impaired arbuscule formation [124]. These two WRINKLED1-like transcription factors seem to represent key elements in the regulation of the symbiotic bidirectional nutrient exchange, and well fit into an updated the scenario of the "reciprocal rewards" in the AM symbiosis [6], that also accounts for the central role played by FAs beside sugars.

Conclusions

The nutrient exchange has surely been the more extensively studied aspect of the arbuscular mycorrhizal symbiosis. Yet, recent findings demonstrated that the scenario depicted in many years of research was far to be conclusive, and that much work is still needed to clarify the mechanics and the implications underneath this flow of nutrients. In particular, some important milestones have been recently placed:

- In the fungus-to-plant direction, the relevant role of the transfer of nutrients other than P and N has been brought to light, as well as the intricate network of connections that orchestrates the regulation of the nutrient exchange as a whole;
- In the plant-to-fungus direction, recent compelling results requested a real paradigm shift that shook up the mainstream bulk of knowledge: beside sugars, lipids are also transferred from the plant to AMFs, and their transfer might represent the key of the fungal obligate biotrophy.

The advancements made in the deciphering of this multifaceted scenario are extremely meaningful for the mycorrhiza scientific community. Nonetheless, they are also instrumental to the implementation of the mycorrhizal symbiosis into agronomical practices aimed at improving the health and productivity of crop plants.

Abbreviations

AMF: arbuscular mycorrhizal fungi; PAM: periarbuscular membrane.

Authors' contributions

ASdF wrote the manuscript; MN collaborated to the text redaction, provided the Figure and formatted the whole text. Both authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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