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# Review: Arbuscular mycorrhizas as key players in sustainable plant phosphorus acquisition: An overview on the mechanisms involved*

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**This paper is dedicated to the memory of our brilliant and enthusiastic colleague, Professor José Miguel Barea, who recently passed away. José Miguel was a very smart, cheerful and generous person. His human and scientific legacy will continue through his impact on those who knew him.*

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**Abstract**

Phosphorus (P) is a poorly available macronutrient essential for plant growth and development and consequently for successful crop yield and ecosystem productivity. To cope with P limitations plants have evolved strategies for enhancing P uptake and/or improving P efficiency use. The universal 450-million-yr-old arbuscular mycorrhizal (AM) (fungus-root) symbioses are one of the most successful and widespread strategies to maximize access of plants to available P. AM fungi biotrophically colonize the root cortex of most plant species and develop an extraradical mycelium which overgrows the nutrient depletion zone of the soil surrounding plant roots. This hyphal network is specialized in the acquisition of low mobility nutrients from soil, particularly P. During the last years, molecular biology techniques coupled to novel physiological approaches have provided fascinating contributions to our understanding of the mechanisms of symbiotic P transport. Mycorrhiza-specific plant phosphate transporters, which are required not only for symbiotic P transfer but also for maintenance of the symbiosis, have been identified. The present review provides an overview of the contribution of AM fungi to plant P acquisition and an update of recent findings on the physiological, molecular and regulatory mechanisms of P transport in the AM symbiosis.

*Keywords:* arbuscular mycorrhiza, arbuscular mycorrhizal fungi, phosphate transporter, phosphorus nutrition, phosphorus signalling

*Abbreviations:* arbuscular mycorrhizal (AM), inorganic phosphorus (Pi), phosphorus (P)

52 **Table of Contents**

53

## 1. Introduction

Phosphorus (P) is one of the most important nutrients for plant growth and development, as it is a central component of nucleic acids and phospholipids. Plants preferentially absorb P as inorganic phosphate (Pi), a limiting ion in many environments due to its low solubility and mobility in soil. Since the rate of Pi absorption by roots is much higher than its diffusion rate in the soil solution, a Pi-depletion zone surrounding the root system is generated. To cope with Pi limitation, plants have evolved a suit of adaptive strategies oriented towards increasing Pi acquisition and use efficiency. These strategies involve extensive root branching, increase in root hairs length and/or solubilisation of soil Pi through organic acid and phosphatase secretion, processes that are orchestrated by a series of genetically controlled Pi sensing and signalling pathways [1]. Another widespread strategy engaged by plants to overcome P deficiency is the formation of a mutualistic symbiotic interaction, referred as arbuscular mycorrhiza, with some soil-borne fungi belonging to the subphylum Glomeromycotina within the phylum Mucoromycota [2]. Arbuscular mycorrhizal (AM) fungi are obligate biotrophs that colonize the root cortex and develop an external mycelium that overgrows the soil surrounding plant roots. This extraradical mycelia, which has been found to be 10 to 40 fold more extensive than roots [3] and whose length may range from 10 to 22 meter per plant depending on the host plant [4], function as an additional absorptive surface area for the plant increasing its capacity to forage nutrients beyond the Pi depletion zone. This hyphal network represents, therefore, an adaptation strategy to increase the supply of mineral nutrients to the plant [5]. Although the main benefit of the AM symbiosis is an improved plant P status, root colonization by AM fungi often results in enhanced uptake of other nutrients in the soil, such as nitrogen, copper and zinc [5–7].

The AM symbiosis emerged approximately 450 million years ago coinciding with the emergence of early land plants. It is accepted that the primitive roots developed associated to AM fungi and coevolved with them to build the root system, commonly mycorrhizal, of extant vascular plants [8]. The establishment of AM symbiosis, therefore, represents an important innovation for plant adaptation, and can be envisaged as the first mechanism evolved by the plant to cope with low Pi availability in natural ecosystems. That is why AM fungi were suggested to play a key role in land colonization by plants. This plant-fungus association has proven to be an evolutionary successful strategy, since more than 80% of all terrestrial plant species live in symbiosis with AM fungi [9]. However, the benefits for the plant are not free of charge and the plant transfers fixed carbon, sugars and lipids, to the AM fungi, which are obligate biotrophs [10–13].

Development of the symbiosis starts with the exchange of signalling molecules between both symbionts: plant-derived strigolactones are perceived by AM fungi [14], which in return produce a mixture of chito- and lipooligosaccharides [15,16], the so-called "*Myc factors*", that are perceived by the plant leading to the expression of key genes and tightly programmed cellular events. The outcome is fungal colonization of the root, a process that culminates with the formation in cortical cells of differentiated, highly branched and tree-shaped structures called arbuscules, the sites where nutrient exchange between the plant and the fungus takes place. During the past decade, a focus in research has been to identify the plant genetic programme controlling symbiosis development and nutrient exchange between symbionts. Many of the molecular players required for root colonization and functioning of the AM symbiosis have been identified and extensively reviewed [17–20]. In this review we summarize our current knowledge of symbiotic Pi transport and discuss major gaps in our understanding of this process.

115 For clarity, we have maintained in the manuscript the binomial nomenclature of AM
116 fungi reported in the quoted papers.
117
118 **2. Symbiotic Pi transport**
119
120 Mycorrhizal plants have two potential pathways for P uptake, a direct pathway
121 by root epidermal cells and root hairs and a mycorrhizal pathway via the AM fungal
122 symbiont (Fig. 1). Symbiotic P uptake involves the acquisition of Pi from the soil
123 solution by the extraradical mycelia and the subsequent translocation to the root and
124 transfer to the plant cells. Whatever the pathway, plants and fungi take up P as
125 negatively charged Pi ions, which possess thermodynamically problems since the cell
126 membrane has an inside negative electric potential and the concentration inside the cell
127 is about 1000 times higher than in the soil solution. Therefore, Pi transport across the
128 plasma membrane requires metabolic energy and involves high-affinity Pi transporters.
129
130 *2.1. Fungal Pi transporters*
131
132 The first AM fungal Pi transporter, GvPT, was described in 1995 in *Glomus*
133 *versiforme* [21], a homolog of the *Saccharomyces cerevisiae* Pho84p. By using a
134 heterologous system, *GvPT* was shown to encode a high-affinity Pi transporter (Km 18
135 μM) that transports Pi via proton-coupled symport. *GvPT* was expressed primarily in
136 the extraradical mycelia, suggesting a role in Pi uptake from the soil solution.
137 Cotransport of $H^+$/Pi by a high affinity transporter requires the activity of a plasma
138 membrane $H^+$-ATPase. Genes encoding the $H^+$-ATPases generating the proton-motive
139 force driving the uptake of Pi across the membrane of the extraradical mycelia have

been identified in *G. mosseae* and *R. irregularis* [22,23]. However, the role of these porteins in Pi uptake could not be verified due to the lack of stable transformation systems for AM fungi. Afterwards, orthologous of GvPT were identified in *Rhizophagus irregularis* (*GintPT*), *Glomus mosseae* (*GmPT*) and *Gigaspora margarita* (*GigmPT*). Surprisingly, these transporters were found to be not only expressed in the extraradical mycelium, but also in the arbuscules where the Pi flux is expected to be directed towards the plant cell [24–28]. Although the primary purpose of these fungal Pi transporters may be Pi uptake from the soil solution, a secondary purpose may be to control the amount of Pi delivered to the plant, by competition between efflux and influx of Pi in the symbiotic interface. Interestingly, the *G. margarita* Pi transporter has recently been shown to be required for development of the symbiosis, as inactivation of *GigmPT* by using a host-induced gene silencing approach led to fungal growth arrest and impaired arbuscule development [27]. It was proposed that Pi retrieved from the interfacial matrix acts as a signal that controls fungal growth and metabolism and that GigmPT is a transceptor that is involved both in Pi uptake and Pi sensing [27].

Recent advances in genomics and transcriptomics of AM fungi is enabling to get a better picture of the mechanisms of Pi acquisition and metabolism. In addition to the high-affinity Pi transporters that are homologs of the *S. cerevisiae* Pho84p, the genome of *R. irregularis* contains homologs of the $Na^+$/Pi symporter Pho89p and of the low-affinity vacuolar Pi transporter Pho91p, being all of them expressed in spores, extraradical and intraradical mycelia [29]. Functional characterization of the full complement of the Pi transporters is needed to understand their specific role in the different fungal structures.

*2.2. Fungal Pi metabolism and translocation*

Once in the cytosol, Pi is transformed into ATP in the mitochondria and then polymerized into polyphosphate, lineal polymers of three to thousands of Pi residues connected by high-energy bonds, in the vacuoles [30]. It has been suggested that polyphoshate is synthesized by the vacuolar transporter chaperon complex (VTC), as the expression of the *Rhizophagus sp.* orthologs of the yeast *VTC1* and *VTC4* genes required for polyphosphate biosynthesis is up-regulated upon Pi application to the extraradical mycelia [31]. The negative charges of polyphosphate are neutralized by inorganic cations given that Pi supply to the extraradical mycelia triggered a near-synchronous and equivalent accumulation of polyphosphate and of $Na^+$, $K^+$, $Ca^{2+}$ and $Mg^{2+}$ [31]. Polyphosphate is then translocated from the extraradical to the intraradical mycelia via cytoplasmic streaming and/or along a motile tubular vacuole system [32,33]. Cytoplasmic streaming is likely driven by water flow mediated by a fungal plasma membrane aquaporin, as suppression of host transpiration and knockdown of the *Rhizophagus clarus* aquaporin *RcAQP3*, which is highly expressed in the intraradical mycelia and mediates water transport across the plasma membrane, decelerated polyphosphate translocation [34]. Mechanisms involved in Pi efflux from the arbuscules represent a significant gap in our knowledge. Since the chain length of polyphosphate is shorter in mycorrrhizal roots than in the extraradical mycelium, it is expected that localized hydrolysis by fungal polyphosphatases leads to high Pi concentrations in the arbuscules, facilitating efflux [31,35,36]. Once polyphosphate is hydrolysed, the intraradical mycelium-expressed vacuolar Pi transporter PHO91 might mediate Pi export to the cytosol [29]. Then, Pi transfer to the periarbuscular space should occur through as an as-yet-unidentified efflux protein. A possible candidate for Pi release is

190 the orthologous of the yeast plasma membrane Pho89p that mediates bidirectional Pi
191 transport. As an alternative model, it has been proposed that a plasma membrane VTC
192 complex polymerizes cytosolic Pi into polyphosphate and exports it to the
193 periarbuscular interface, which will be then hydrolysed by a plant acid phosphatase
194 [37]. Further studies are required to uncover the mechanisms of polyphosphate
195 breakdown and Pi efflux from the arbuscules.
196
197 *2.3. Plant Pi uptake*
198
199 *2.3.1. The periarbuscular membrane*
200 Symbiotic P uptake by a mycorrhizal plant occurs at a specialized interface
201 formed in arbuscule-colonized cortical cells. Arbuscules are always enveloped in a
202 plant-derived plasma membrane, the periarbuscular membrane, which separates the
203 fungal hyphae from the host cytoplasm. The interface between the fungus and the host
204 includes, therefore, the periarbuscular membrane, the fungal cell wall and the fungal
205 plasma membrane. Between the fungal cell wall and the periarbuscular membrane there
206 is a narrow compartment, the periarbuscular space or interface matrix, containing
207 amorphously structured plant cell wall material. Formation of the periarbuscular
208 membrane involves the *de novo* synthesis of membranes and recent research has shown
209 that periarbuscular membrane deposition is achieved via polarized exocytosis [18,38].
210 Although biogenesis of the periarbuscular membrane is not fully understood, some key
211 players have recently been identified. Among them a specific subset of the exocytotic
212 vesicle-associated membrane proteins [38], a symbiosis-specific splice variant of the
213 tSNARE protein SYP132, the EXO70I subunit of the exocyst complex [39] and a plant-
214 specific protein VAPYRIN that physically interacts with EXO70I [40–42]. The

215 periarbuscular membrane is continuous with the plasma membrane, but represents a
216 specialized membrane domain with symbiosis-specific features that contains a unique
217 complement of proteins. In the model plant *Medicago truncatula* one of these proteins
218 is the well-characterized Pi transporter MtPT4 that mediates uptake of fungus-delivered
219 Pi [43,44]. Pumplin and coworkers (2012), by expressing MtPT4 and other plasma
220 membrane proteins from promoters active at different phases of the symbiosis,
221 demonstrated that trafficking of these transporters occurs by default. It was shown that
222 proper targeting into the periarbuscular membrane is achieved by precise temporal
223 regulation of gene expression, coincident with arbuscule formation. This is coupled with
224 a transient reorientation of the secretory pathway, favouring fusion with developing
225 periarbuscular membrane rather than with the plasma membrane, and with changes in
226 the protein cargo entering the secretory system of the arbuscule-colonized cortical cell
227 [45].
228
229 *2.3.3. Mycorrhiza-induced transporters*
230 Putative Pi transporters mediating the acquisition of the Pi delivered by the
231 fungus to the periarbuscular space were initially identified by their specific- or
232 increased-expression in mycorrhizal roots relative to non-mycorrhizal plants. The first
233 AM-induced Pi transporters were described in potato [46], *M. truncatula* [43] and rice
234 [47]. Afterwards, similar proteins were identified and characterized in different plant
235 species [48–52]. They are homologs to the yeast pho84 and belong to the Phosphate
236 transporter 1 (Pht1) family of the plant $H^+$/Pi symporters. The well-characterized low-
237 affinity Pi transporter MtPT4 of *M. truncatula* [43] and its orthologous in rice OsPT11
238 [53] and in *Astragalus sinicus* AsPT4 [50] localize in arbuscule-colonized cortical root
239 cells and, more specifically, at domains of the periarbuscular membrane surrounding the

fine branches of the arbuscule. Reverse genetic approaches have shown that MtPT4, OsPT11 and AsPT4 are essential for symbiotic Pi uptake, as their mutation results in significantly lower shoot P content and in polyphosphate accumulation in the arbuscules. Interestingly, mutation or silencing of these transporters leads to premature arbuscule degeneration and to symbiosis abortion, which indicates that Pi plays a regulatory role in the symbiosis. In the absence of symbiotic Pi delivery, the plant might reduce carbon flow to the periarbuscular interface, preventing, therefore, fungal development.

Recently, an additional role for MtPT4 and its *Lotus* ortholog LjPT4 in root architecture responses to low Pi has been suggested, as they are expressed in root tips of non-mycorrhizal plants at low Pi and their knockout impairs early root branching responses to low Pi and the expression of Pi-starvation marker genes [54]. It was suggested that these transporters could act as transceptors in the non-mycorrhizal Pi uptake pathway that act upstream of the Pi-sensing machinery [54]. However, their mechanisms of action remain to be elucidated.

Orthologous of MtPT4, OsPT11 and AsPT4 are present in mono- and dicotyledonous plant species and belong to subfamily 1 of the Pht1 family, a subfamily that only contains AM-induced genes. In addition to these subfamily 1 Pi transporters, most plants have at least one additional Pi transporter, such as rice OsPT13 [53], *A. sinicus* AsPT1 [50], potato StPT3 [48] and tomato LetPT3 [25], that are phylogenetically dispersed within the Pht1 family. These not subfamily 1 orthologs are also expressed in cortical cells colonized by arbuscules, although some, such as StPT3 of tobacco and PhPT3 of petunia, are constitutively expressed in roots but significantly up-regulated in AM roots [48,51]. Interestingly, functional analysis of the phylogenetic distant AsPT1 and StPT3 in yeast indicates that they are high-affinity Pi transporters

while the members of subfamily 1 characterized so far are low-affinity transporters. It was proposed that the presence in arbuscule-colonized cells of non-orthologous Pi transporters with different affinities for Pi could enable symbiotic Pi transport over a range of periarbuscular Pi concentrations [48]. However, this hypothesis has to be tested and genetic analyses of OsPT13 and AsPT1 reveal that they are not required for symbiotic Pi uptake.

Most plants have a single Pi transporter gene in subfamily 1. However, members of the Solanaceae, such as potato, tomato and petunia, have two paralogs (PT4 and PT5), among which PT4 is the only AM-specific one [48]. Although both paralogs are expressed in arbuscule-containing cells, the PT5 ones are also expressed in non-colonized cortical cells [25]. The findings that the tomato LePT3, LePT4 and LePT5 transporters are simultaneously expressed in arbuscule-containing cells [25,55], that symbiotic Pi transport was not affected in a null allele of the tomato LePT4 and that the expression of the other mycorrhiza-inducible transporters of tomato LePT3 and LePT5 remained unchanged in the mutant line suggest that there might exist functional redundancy between the three mycorrhiza-associated Pi transporters [48].

Since the Pht1 transporters are $Pi/H^+$ symporters, symbiotic Pi uptake across the periarbuscular membrane requires generation of a proton gradient by a $H^+$-ATPase. Up-regulation of $H^+$-ATPase genes has been reported in mycorrhizal roots of several plant species [56–58]. In tobacco, two $H^+$-ATPase genes (*pma2* and pma4) were found to be up-regulated in mycorrhizal roots and expressed both in the root meristem and in arbuscule-colonized cortical cells [56]. However, in *M. truncatula* a single isoform, HA1, was specifically expressed in cortical cells containing arbuscules [58,59]. The HA1 isoforms of *M. truncatula* and rice have been shown to be essential to generate the proton gradient required for Pi uptake by the Pi transporters localized in the

290 periarbuscular membrane, as disruption of the genes in *Mtha1-2* and *Osha1-2* mutants
291 leads to impaired Pi transport via the mycorrhizal pathway [60]. Interestingly, these
292 mutants displayed the same mycorrhizal phenotype than the *M. truncatula* PT4 mutants,
293 that is, a reduced colonization level and stunted arbuscules, which supports the idea that
294 symbiotic Pi transfer is required for maintenance of the symbiosis.
295
296 **3. Contribution of symbiotic Pi uptake to plant P nutrition**
297
298 Physiological approaches using radioactively labelled Pi to trace the relative
299 contribution of direct and AM pathways to plant P nutrition have demonstrated that the
300 contribution of the mycorrhizal pathway varies from a small percentage to nearly all
301 plant P and that Pi uptake via the fungal pathway reduces the contribution of the direct
302 root pathway [6,61]. Therefore, during AM symbiosis the plant changes its Pi
303 acquisition strategy and favours Pi acquisition from the AM fungus over acquisition
304 through its epidermal cells. These physiological studies have shown that the absolute
305 quantity of P obtained through the AM pathway correlates better with the abundance of
306 extraradical hyphae than with the percentage of fungal colonization [62,63].
307 Interestingly, genotypic variation in growth response to *R. irregularis* and in symbiotic
308 Pi uptake of six maize lines positively correlated with the length of the extraradical
309 mycelium [64].
310 The contribution of the fungal pathway depends on the plant and fungal species
311 involved in the association. In species that do not respond positively to AM colonization
312 in terms of growth and P nutrition, the majority of the Pi taken by the plant is acquired
313 via the mycorrhizal pathway [53,65]. In an extreme case, *R. irregularis* was shown to
314 provide 100% P to tomato plants [61]. In these cases, the mycorrhizal pathway is

315 “hidden” and can dominate plant P uptake. Therefore, the mycorrhizal P uptake is not
316 just a simple addition to direct P uptake and the contributions of the AM pathway
317 cannot be determined by subtracting total P in non-mycorrhizal plants from total P in
318 mycorrhizal plants grown under the same conditions.
319 Molecular analyses support the interplay between the mycorrhizal and direct P
320 uptake pathways, as the Pi-starvation inducible transporters that are expressed in the
321 epidermal cells are down-regulated in mycorrhizal roots [46,53,66]. Down-regulated Pi
322 transporters have been described, among other plant species, in potato [48], *M.*
323 *truncatula* [67], rice [47], *A. sinicus* [50] and maize [64]. However, in most cases the
324 plant retains some level of P acquisition through the direct pathway, as not all root Pi
325 transporters are inhibited in a mycorrhizal root, as it is the case of *OsPT3*, *OsPT5* and
326 *OsPT8* from rice [47] and *PhPT*1 and *PhPT2* from petunia [51]. Given that the AM
327 down-regulated Pht1 transporters are frequently P sensitive, suppression of the direct
328 uptake in a mycorrhizal root could be due to the increased P status of the plant.
329 Alternatively, changes in the contribution of the direct pathway could result from direct
330 signalling/interactions between the plant and the fungus. To try to distinguish between
331 these possibilities, the distal and local effects of AM colonization on direct Pi were
332 determined by uptake using both physiological and molecular approaches in wild-type
333 and mt*pt4* mutant *M. truncatula* plants grown in a split-root system [68]. It was found
334 that AM fungi reduce the direct root Pi uptake activity locally but not in a distal and
335 non-colonized part of the root. Since the mt*pt4* mycorrhizal roots behaved mostly like
336 non-mycorrhizal despite being colonised, it was suggested that a major factor driving
337 the effects of AM colonization on direct root Pi uptake was the increased P content of
338 the colonized roots. However, more work using different plant and fungal species is
339 required to understand the mechanisms of the interplay between direct and mycorrhizal

pathways.

## 4. Regulation of symbiotic Pi uptake

The mycorrhizal Pi uptake pathway is often reduced at high soil P concentrations, which could be attributed to the general observation that AM colonization is inhibited by a high Pi supply [69–71]. However, regulation of symbiotic P transport is controlled at least partially by the host plant, as at high P, despite sufficient root colonization, inhibition of the mycorrhizal Pi uptake pathway is accompanied by down-regulation of the mycorrhiza-inducible Pi transporter genes [65,72].

Analysis of the promoter regions of the mycorrhiza-induced Pi transporters in several plant species has shown the presence of two conserved cis-elements: P1BS that is common to many promoters of P starvation-induced genes and MYCS (CTTC, mycorrhiza transcription factor binding) that is over-represented in mycorrhiza-induced genes [73,74]. Functional analysis of these promoters showed that both elements are essential for activation of the mycorrhiza-responsive P transporters [74]. Therefore, regulation of the mycorrhiza-inducible Pi transporters integrates signals derived from the plant P status and from mycorrhizal colonization. Currently, very little is known about the transcription factors controlling the expression of symbiotic Pi transporters, but indirect evidence suggests that RAM1 participates in the transcriptional activation of the mycorrhiza-induced Pi transporters, as in the *M. truncatula* and petunia *ram1* mutants the mycorrhiza-specific *PT4* gene was unresponsive to colonization [75,76]. However, induction of *PT4* by RAM seems to be dependent on the plant species, as in *L. japonicus* RAM1 is not required for the activation of *PT4* [77].

The specific expression of the symbiotic Pi transporters in arbuscule-colonized cells led to the suggestion that a cell autonomous signal involved in arbuscule development activates their expression [55]. The signal has been identified as lysophosphatidylcholine, a common component of both plant and fungal membranes, as it was shown to elicit the expression of the mycorrhiza-inducible Pi transporters *StPT3*, *StPT4*, *LePT4* and *LjPT4* in non-mycorrhizal plants of potato, tomato and *L. japonicus*, respectively [72,78].

Obviously the fungal transporters expressed in the extraradical mycelium are also involved in the mycorrhizal P uptake pathway. Expression of these transporters is regulated in response to external Pi levels, being induced at limited levels and repressed at high Pi concentrations [24,26,28,50], which could explain the reduction of the mycorrhizal P uptake pathway observed at high Pi levels. Transcriptional regulation of the *R. irregularis* and *G. margarita* Pi transporters *GintPT* and *GigmPT* positively correlates with the Pi transport capability of the ERM. The plant P status also affects the expression levels of the Pi transporters expressed in the ERM, which suggests that Pi uptake by the extraradical mycelium is also regulated by a plant signal [28]. A carbon signal could also regulate Pi uptake efficiency by the extraradical mycelium, as the expression of the fungal Pi transporters and Pi transfer to the plant increases with sucrose availability [27].

## 5. Pi signalling in AM symbiosis

High Pi levels have long been recognised to have a negative impact on AM development [70,79,80]. Since Pi is the major nutrient provided by the fungus, suppression of AM by high Pi levels could be interpreted as an energy-saving negative

390 feedback mechanism of the plant to limit carbon transfer to the obligate AM fungus
391 under conditions at which the plant can obtain an optimal nutrient supply without the
392 symbiosis.

393 AM suppression by Pi is systemic and depends on the nutritional status of the
394 shoot. In split root experiments, in which only one half of the root system was supplied
395 with high Pi, a suppression of AM colonization was observed in the whole root system
396 [70,80]. Foliar Pi application was also shown to inhibit AM colonization [81]. These
397 observations suggest a scenario in which shoot Pi level generates a mobile signal that
398 travels to the root to alter root physiology and to regulate, therefore, AM colonization.
399 Members of the miR399 family, key players in systemic Pi-starvation signalling [82],
400 have been proposed as signalling molecules in the regulation of AM by Pi, as the
401 expression of some miR399 family members was increased in leaves of mycorrhizal
402 plants [83,84]. Given that miR399s target the PHO2 ubiquitin E2 conjugase that
403 mediates the degradation of proteins required for Pi starvation responses [85] and that
404 transcript levels of *MtPho2* and of a PHO2-dependent P-starvation marker remained
405 low in *M. truncatula* mycorrhizal roots [83], it was suggested that increased expression
406 of miR399 is needed to keep Pi-starvation responses high to allow continuous
407 colonization, despite the high shoot P levels of the mycorrhizal plants. However, it
408 seems that other mechanisms are involved given that miR399 overexpression failed to
409 restore AM colonization at high Pi levels [83].

410 The negative impact of Pi on the symbiosis could also occur at the early stages
411 of AM establishment. The biosynthesis of strigolactones, molecules exuded by the plant
412 into the rhizosphere that stimulate presymbiotic AM fungal growth, is inhibited by high
413 Pi levels [86]. However, reduced strigolactone exudation is not the main reason for AM
414 suppression at high Pi, since the exogenous supply of the synthetic strigolactone GR24

415 did not restore mycorrhizal colonization levels at high Pi [70,80]. It is also unlikely that
416 receptor availability at the epidermis limits colonization at high Pi, as the nuclear $Ca^{+2}$-
417 spiking that occurs in epidermal cells in response to hyphopodia formation or to
418 germinating spore exudates is not inhibited at high Pi levels [87]. AM repression by Pi
419 could be also regulated by a cross-talk between Pi and phytohormone signalling, given
420 that AM development is regulated by several phytohormones [88] and that the
421 biosynthesis of some of them is regulated by Pi [89]. For example, gibberellins
422 negatively regulate AM development and, accordingly, the DELLA proteins, which are
423 repressors of gibberellin formation, are required for AM formation [90] and an
424 accumulation of DELLA proteins and reduced levels of gibberellins have been found in
425 Pi starved *Arabidopsis* plants [91]. However, assessing whether DELLA overexpression
426 can revert AM repression by Pi is needed to determine the role of gibberellins in the Pi
427 control of the symbiosis.
428 Pi itself can also act as a local signal that triggers reprogramming of the cortical
429 cells for maintaining the fungus, as symbiotic P import is essential for arbuscule
430 dynamics and progression of colonization. Mutation or silencing of a plant mycorrhiza-
431 specific or a fungal Pi transporter leads not only to suppression of symbiotic Pi transfer
432 but also to a reduction of colonization and premature death of arbuscules [27,44,53].
433 The lack of Pi flow across the symbiotic interface might prompt the plant to reject the
434 fungus to avoid parasitism. It has been proposed that Pi can act as a local, cell-
435 autonomous signal that triggers accommodation and maintenance of the arbuscule by
436 the host cell [27,44,50,53,55]. This hypothesis is supported by the observation that the
437 rice and *Astragalus* Pi transporters OsPT13 and AsPT1, both expressed in the
438 periarbuscular membrane, are required for arbuscule maintenance but not for symbiotic
439 Pi transport. This indicates that these transporters are important for Pi sensing and it has

been suggested that the Pi transporters expressed at the periarbuscular membrane could act as transceptors, proteins that can have both a receptor and transporter function. Interestingly, premature arbuscule degeneation due to the loss of symbiotic Pi transport in the *M. truncatula Pt4* mutant is supressed when the plant is grown under low nitrogen conditions, indicating that not only Pi but also nitrogen can act as a signal to support arbuscule survival in a cell-autonomous fashion [92]. The *M. truncatula* ammonium transporter AMT2.3 was found to be essential for suppression of the arbuscule degeneration phenotype of the pt4 mutant under low nitrogen conditions [93]. As AMT2.3 did not display ammonium transport activity in yeast complementation assays, it was proposed that it could act as a receptor. Recently, the *G. margarita* Pi transporter GigPT1, that is expressed both in the extraradical and intraradical mycelium, has been shown to be a transceptor, indicating that Pi sensing is also important for the fungus [27]. These findings indicate that a flow of nutrients across the symbiotic interface is required to sustain arbuscule within the cortical cell. This nutrient flow might be a mechanism to distinguish AM fungi from less beneficial colonizers.

## 6. Concluding remarks and perspectives

Research over the past few years has enhanced our understanding of the mechanisms of Pi transport in AM. Pi flow in a mycorrhizal root can include Pi uptake through the epidermis and through the symbiotic fungus. In most cases, some Pi is delivered via the mycorrhizal pathway, but in others, the plant receives its entire Pi through the fungus. On the plant side, the Pi transporters mediating Pi flow through the mycorrhizal pathway have been identified and they are useful markers for a functional mycorrhiza. On the fungal side, the Pi transporters involved in acquisition have been

465 also characterized, but further studies are required to understand the specific roles of the
466 full complement of the fungal Pi transporters. A new function, as Pi sensors, has
467 emerged for the fungal and AM-inducible plant Pi transporters. Yet, despite these
468 advances, a full understanding of the regulatory mechanisms of symbiotic Pi flow
469 remains to be achieved. In the future work, it would be of interest to identify the fungal
470 players mediating Pi release from the arbuscules and to go further on the understanding
471 of the mechanisms controlling the amount of Pi transferred to the plant, the interplay
472 between direct and mycorrhizal pathways and the role of Pi and other nutrients in
473 regulating the maintenance of the symbiosis. On the fungal side, despite the difficulties
474 for the genetic manipulation of AM fungi, recent genome sequencing and the
475 development of host-induced and virus-induced gene silencing techniques of AM fungal
476 genes will accelerate our knowledge of Pi metabolism and transport in the arbuscule.
477 Improved technologies will allow getting a comprehensive picture of symbiotic Pi
478 transport and the corresponding regulatory network. A deeper knowledge of these issues
479 will be crucial to understand differences in the symbiotic efficiency of different AM
480 fungi and for a better exploitation of the AM symbiosis in sustainable agricultural
481 practices. This will be also important under the current scenario of climate change, in
482 which plants are exposed to more extreme environmental stresses and where soil P
483 availability and plant use P efficiency decrease. Given that Pi fertilization increases
484 plant stress tolerance and productivity, and that AM fungi not only increase plant
485 nutrition but also plant tolerance to multiple stresses, the optimized application of AM
486 fungi in sustainable agriculture will be crucial not only for developing more P-efficient
487 farming systems but also to counteract the negative impacts of climate change.
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**Conflict of interest**

None declared

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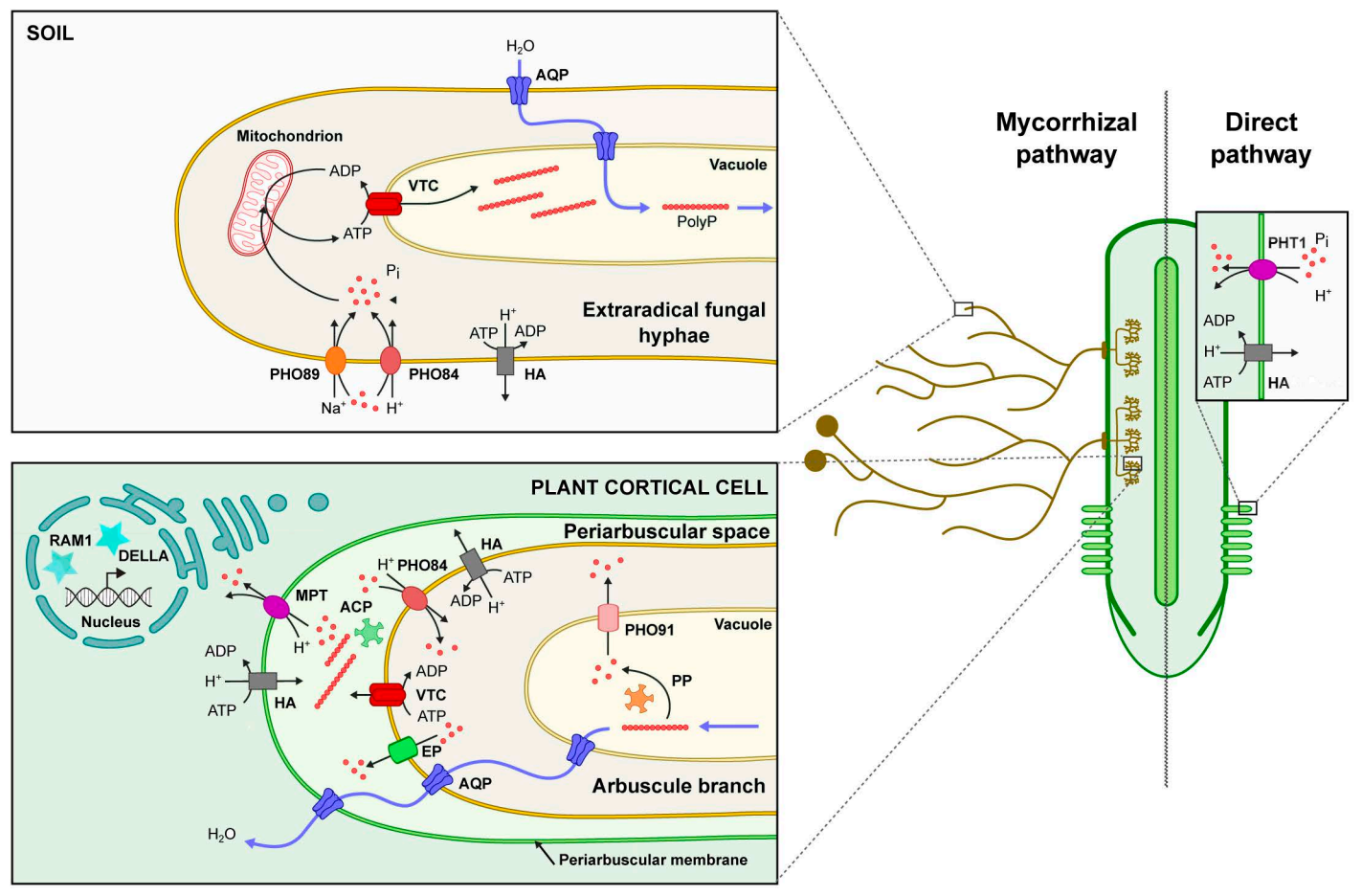
**Fig. 1. Schematic representation of the Pi uptake pathways in a mycorrhizal root.** Direct Pi uptake by root epidermal cells is mediated by the activities of a Pi transporter of the Pht1 family and a $H^+$-ATPase (HA). Mycorrhizal Pi uptake starts with Pi acquisition by the extraradical hyphae through a $H^+$/Pi symporter (PHO84) driven by a $H^+$-ATPase (HA), or through a $Na^+$/Pi symporter (PHO89). Once in the cytosol, Pi is likely transformed into ATP in the mitochondria and then polymerized into polyphosphate by the vacuolar transporter chaperon complex (VTC). Polyphophate is translocated to the intraradical mycelium by cytoplasmic streaming, likely driven by water flow mediated by an aquaporin (AQP). In the arbuscules, polyphosphate is hydrolysed by a fungal polyphosphatase (PP) and Pi is then exported to the cytosol through a vacuolar Pi transporter (PHO91). Once in the fungal cytosol, Pi is either released into the periarbuscular space by an as-yet-unidentified efflux protein (EP) or polymerized into polyphosphate through a plasma membrane VTC complex that will be hydrolysed by a plant acid phosphatase (ACP) in the periarbuscular space. Finally, plant cells take up Pi from the periarbuscular interface by a mycorrhiza-inducible Pi transporter (MPT) located at the periarbuscular membrane, which is transcriptionally regulated by the transcription factors RAM1 and DELLA. Fungal Pi uptake transporters are also expressed in the arbuscules, where they act as transceptors.


SOIL
H2O
AQP
Mitochondrion
ADP
ATP
VTC
Vacuole
PolyP
Pi
Extraradical fungal hyphae
H+
ATP ADP
HA
PHO89
PHO84
Na+
H+
Mycorrhizal pathway
Direct pathway
PHT1
Pi
H+
ADP
H+
ATP
HA
PLANT CORTICAL CELL
Periarbuscular space
RAM1
DELLA
Nucleus
MPT
ACP
H+ PHO84
HA
ATP
ADP H+
PHO91
Vacuole
PP
ADP
H+
ATP
HA
ADP
VTC
ATP
EP
AQP
Arbuscule branch
H2O
Periarbuscular membrane