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How does phosphate status influence the development of the arbuscular mycorrhizal symbiosis?

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Most terrestrial plant roots form mutualistic symbiosis with soil-borne arbuscular mycorrhizal fungi (AMF), a characteristic feature of which is nutrient exchange between the two symbiotic partners. Phosphate (Pi) is the main benefit the host plants acquired from the AMF. It has long been a common realization that high Pi supply could suppress the AMF development. However, the direct molecular regulatory mechanisms underlying this plant directed suppression are lacking. Here we reviewed the recent work providing the evidences that high Pi supply induces transcriptional alteration, leading to the inhibition of AMF development at different stages of AM symbiosis, and gave our view on potential cross-talk among Pi starvation, AM as well as phytohormone signaling.

Phosphorus (P) is a critical macro-nutrient for plant growth and development, and is often a limiting factor in many soils due to its poor mobility and availability. Therefore, plants have evolved a suit of adaptive strategies to overcome P deficiency, among which forming a mutualistic symbiotic interaction with arbuscular mycorrhizal fungi (AMF) belonging to the phylum Glomeromycota is one of the most striking responses.[1] AMF are a class of ancient habitants on earth, aging more than 400 million years. Their main contribution to ecosystem is the transfer of water and mineral nutrients, in particular phosphate (Pi), to their host plants (>80% terrestrial vascular plants).[2]

The establishment of a functional symbiotic system goes through a series of steps involving physiological, morphological and accompanying molecular alterations for both symbionts, thereby a complex signaling network is required.[2] In the past decades, an emerging number of components essential for arbuscular mycorrhiza (AM) development ranging from small signaling molecules to diverse protein-coding genes as well as microRNA (miR) genes have been discovered, whereas much has to be learned to get a better understanding of the entire signaling network.[3-10] More recently, several reports focused on the molecular mechanisms responsible for the inhibition of AM development by high level Pi supply.[11-13]

In this mini-review, we summarized the current understanding for the molecular mechanisms of Pi as repressor for the AMF symbiosis development, which is still largely unknown and needs substantial further investigations.

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Establishment of a Functional AM Symbiosis: From Presymbiotic Stage to Late Symbiotic Stage

AMF are obligate biotrophs, namely they are not capable of complete their life cycles independently without forming symbiotic interaction with host plants. AMF spores germinate in the soil under certain conditions with proper temperature and humidity. They cease hyphae growth and even die (nutrients in the spores are depleted) if no plant roots were encountered.[2] Thus, an efficient sensing mechanism for searching for plant roots is required. In fact, many lines of evidences showed that signal intercourse happens early at presymbiotic stage when there is no physical contact between the two symbionts.[2,14] Recently, signaling molecules involved in this presymbiotic signaling have been elucidated. Strigolactones, a novel class of phytohormones, released by plant roots activate AMF growth[15,16] and at the mean time fungal lipochitooligosaccharides stimulate root growth and branching,[17] resulting in increased chance for direct contact.

Once the hyphae make direct contact with the root epidermis, a structure named hyphopodium (also known as appressorium) through which the fungi enter the root is formed on the surface of epidermal cells, followed by continuous intraradical dichotomous branching of the hyphae and forming highly coiled hyphae or arbuscules within the root cortical cells. In arbuscule-containing cells, the morphology of the plant plasma membrane changes dramatically and forms a peri-arbuscular membrane surrounding the outline of the arbuscule.[2,14] The interface between the arbuscule and the peri-arbuscular membrane is the main space for nutrient exchange. This nutrient exchange is the feature for the late stage of a functional AM symbiosis.[2,14]

It is well known that Pi level is negatively correlated with AM development, and that the inhibition of high Pi supply to AM development is more likely driven by the host plants, but not the fungi.[11-14,18] While the knowledge about whether the inhibition by high Pi supply happens at a certain stage or multiple stages

during AM development is just emerging. Up to date, it has been shown that high Pi level affect AM development at both early stage and late stage.[11-14]

High Pi Supply Affects Presymbiotic Signaling and Hyphopodium Formation by Inhibiting Strigolactone Biosynthesis

Strigolactones, a group of terpenoid lactones, have recently turned out to be another type of plant hormone crucial for plant shoot branching and stimulating parasitic weeds and presymbiotic AM growth.[15,16,19-22] It stimulates spore germination and cell proliferation of AMF by activating mitochondria.[16] In Pi-replete plants, production of bioactive strigolactones is dramatically reduced compared with that in Pi-starved plants, concurring with the decreased susceptibility of plants to AMF. In Pi-sufficient supplied pea roots, the association with two different AM fungi was almost completely abolished prior to the formation of hyphopodia.[13] Previous experiments showed that hyphopodium formation is specific to the epidermal cells[2,23-25] which are closest to the external environment and might be the first to sense fluctuations of nutrient availability (Pi deprivation in this case). Consequently, an epidermal cell-specific mechanism for suppressing hyphopodium formation could be expected and need to be further investigated. It should be noted that increase of Pi supply level does not always completely diminish the formation of plant root association with AMF. There was about 70% or 20% AMF colonization in the Pi sufficient roots of *Medicago truncatula* and three solanaceous species (tobacco, tomato and petunia), respectively.[9,11-13,26] This discrepancy might be explained as different sensitivity to high Pi or diverse regulation in response to high Pi of different plant species, while the mechanisms responsible for this difference remain to be established. Nonetheless, it is certain that high Pi acts as a negative regulator for presymbiotic processes of AM symbiosis.

In a very recent transcriptomic study, the genes encoding enzymes for the biosynthesis of carotenoid and strigolactone are found downregulated upon high level Pi supply.[11] Given that strigolactones are derived from carotenoid cleavage, it is likely that Pi inhibits presymbiotic signaling partially, if not completely, by suppressing the expression of these genes. In addition, two novel half-size ABC (ATP Binding Cassette) transporters of *Medicago truncatula* belonging to the ABCG subfamily have been discovered functioning as a heterodimer and essential for arbuscule development in AM symbiosis.[27] Under non-mycorrhizal condition, these two ABC transporter genes are expressed in vascular tissues, while in mycorrhizal roots, they localize to the periarbuscular membrane. They are predicted to be responsible for the export of strigolactones out of cortical cells to the arbuscule apoplastic space.[27] It might be postulated that strigolactones are also required for endosymbiotic stage, although a direct evidence for the transport activity of these two transporters is lacking.

In the report by Balzergue et al. (2011), the supplementation of GR24, an analog of strigolactone, to Pi-replete mycorrhizal plants did not restore the AM symbiosis,[13] indicating that the suppression of AM development by high Pi involves pre-symbiotic signaling molecules other than strigolactones or high Pi affects the symbiosis at other stage(s).

Role of AM Responsive Phosphate Transporter (PT) Genes in Repression of AM Development by High Pi and their Regulation

Two Pi uptake pathways have been proposed in mycorrhizal plants, namely direct uptake pathway (DUP) and mycorrhizal uptake pathway (MUP). The former is mediated by the non-mycorrhiza-regulated Pht1 members, while the latter by AM-induced or -specific PTs.[28,29] In some plant species, MUP could be the dominant one.[28] Since the first report on *StPT3*, a *PT* gene of *Solanum tuberosum* that is regulated by AM symbiosis,[30] many AM-enhanced or -specific *PT* genes have been isolated and functionally characterized in both monocots and dicots afterwards.[18,31-37] Among these AM-related *PT* genes, *LjPT3* of *Lotus japonicus* and *MtPT4* of *Medicago truncatula* were shown to be indispensable for AM symbiosis.[34,36] Knockdown and/or loss-of-function of *LjPT3* and *MtPT4* caused suppressed symbiosis,[34,36] demonstrating Pi transport is a requirement for the AM symbiosis. It could be speculated that plant may sense the external Pi level and adopt an active strategy inhibiting AM hyphae development within the root to reduce extra carbohydrate consumption. Since sucrose transport from shoots to roots through phloem is enhanced upon Pi starvation and prior to Pi starvation responses,[38,39] and is required for the subsequent hydrolysis into hexose and sustaining AMF growth,[14] one possible explanation for this strategy might be decrease of sucrose transport from shoots to roots when Pi is sufficient inside the plant.

Many *PT* genes belonging to the *Pht1* family are predominantly or exclusively expressed in roots and are Pi starvation inducible.[40] Their Pi starvation-induced expression is rapidly repressed by Pi re-supply (1–2 days or even sooner).[37,41,42] A large proportion of these *PT* genes, possess P1BS (PHR1 Binding Sequence) cis-element in their putative promoter region and have been proved to be the direct target of PHRs (Phosphate Starvation Response), a conserved central regulator controlling a number of Pi starvation responses, or be upregulated by PHR overexpression,[12,43,44] suggesting that like many other PSI (Phosphate Starvation Induced) genes, *PT* genes are also an integral part of the central Pi starvation signaling pathway. In a very recent work, our group isolated the promoter region of six AM-induced or -specific *PT* genes from solanaceous species, and found that all the promoter fragments isolated as well as some other orthologous genes (*StPT3*, *StPT4*, *LePT4*, *MtPT4*, *OsPT11*, *OsPT13*) contain at least one copy of P1BS motif in their promoter region.[12] We have demonstrated that P1BS is essential for the AM-induced or -specific expression, confirming that these AM-related (AMR) *PT* genes are under the control of PHRs.[12] However, unlike other PSI genes upregulated by PHRs, Pi starvation alone is not sufficient to trigger their strong expression in response to AM colonization. MYCS (Mycorrhiza Transcription Factor Binding Sequence), a novel cis-elements, was found to be also required for AM-induced or -specific expression of the AMR *PT* genes.[12] Consequently, it is possible that MYCS and P1BS

along with the transcription factor binding to them are responsible for the upregulation of the AMR *PT* genes, and absence of either of the two would deprive the strength of their native promoters.[12] This is further supported by a latest report that even for one of the PSI genes, *IPS1* from Arabidopsis, P1BS acts in concert with another cis-element.[45] MYCS is present in the promoter region of AMR *PT* genes from both solanaceous species and leguminous species, but not rice,[12] consistent with the previous realization that although the morphology and physiology of AM symbiosis are quite similar among diverse plant species, the underlying molecular regulatory mechanism might be different.[4]

Lyso-phosphatidylcholine (LPC) extracted from mycorrhizal roots was able to activate the expression of AMR *PT* genes under non-mycorrhizal condition, indicating that it is a late signal in the AM symbiosis.[46] It is noteworthy that the expression of *StPT3* triggered by LPC was mainly restricted to the region behind the root tip.[46] This is not completely overlap with the expression pattern observed under regular AM colonization condition. The possible reasons could be that LPC is not the only molecule for AMR *PT* expression, and other unknown stimulant(s) might be required. Similar to the case in GR24, roots of Pi-replete plants are insensitive to LPC,[13] demonstrating that high Pi suppression of AMR *PT* genes is also dominant. Besides, whether LPC is derived from plant roots or AMF remains to be uncovered.

High Pi Suppresses AM Development Systemically and AM-Related Systemic Signals are Blocked by High Pi

Long distance signaling between shoot and root has been demonstrated important for plant Pi homeostasis.[47-50] Split-root experiments showed that the inhibition effect of high Pi on AM development, which is at least partially mediated by suppressing the expression of genes encoding enzymes for carotenoid and strigolactone biosynthesis and AM induced PTs, could be systemic.[11,13] This highlighted the requirement of shoot-root communications for AM symbiosis.

MiRs are probable candidates for systemic signaling, since many of them have been detected in great abundance in phloem sap.[51] The first Pi starvation-responsive miR characterized, miR399, is well-known as a long distance signaling molecule regulating plant homeostasis.[48,49] Branscheid et al. (2010) reported that the transcript level of some of the primary miR399s (pri-miR399) further increased upon AM colonization in Medicago leaves,[9] suggesting that an AM colonization-derived signal was responsible for the increase of miR399 transcription in leaves. Moreover, the abundance of mature miR399s were elevated in Pi-depleted mycorrhizal roots of both Medicago and tobacco plants, which were transported through phloem from shoots. It was postulated that the increased mature miR399s suppress the expression and activity of PHO2 in mycorrhizal roots. Because the upregulation of pri-miR399s was not observed in the leaves of Pi-replete mycorrhizal plants, while the AMF inoculation decreased only to ~70% as compared with Pi-depleted mycorrhizal Medicago plants,[9] it is apparent that high Pi was also capable to inhibit the putative AM colonization-derived signal, although this signal and the subsequent events triggered by it (phloem transport of mature miR399 to roots) might not be necessary for AM colonization.

In addition to miR399, four novel miRs were all positively regulated by Pi deprivation in Arabidopsis.[52-54] Whether they are also involved in the AM signaling in a similar way as miR399 or in unknown fashions remains to be determined. In tomato, several groups of miRs showed altered expression upon either P nutrition or AMF colonization or both,[10] further supporting that miRs are components of the complex signaling network of Pi starvation and AM colonization. However, the roles of them associated with their target genes are widely unknown and needs further functional characterization.

Potential Cross-Talk among Pi, AM and Phytohormone Signaling

Establishment of AM symbiosis is a complex and highly regulated process involving a number of factors, among which plant hormones also play roles. Up to date, the reports focused on figuring out the correlation between AM development and phytohormones other than strigolactones are very limited, although several phytohormones, including abscisic acid (ABA), ethylene, jasmonic acid and auxin, have already been implicated.[14,55-59]

The biosynthesis of some of the phytohormones or plant sensitivity to them is increased during Pi starvation, which is reversible by repletion of Pi.[38] For example, Pi-starved plants are hypersensitive to auxin, and both the in planta concentration and transport of it enhanced.[38,60,61] A typical response of plant root system to Pi deprivation is a reduction in primary root length and an increased in root hair density and lateral root formation.[1,38] Moreover, Pi availability was found to alter lateral root development by modulating auxin sensitivity via a mechanism involving TIR1 auxin receptor.[60] Notably, lateral roots which are favored sites for AMF colonization are also induced upon AM colonization. In a maize *lrt1* mutant, in which lateral root formation is impaired in primary and seminal roots, increased lateral roots formation was also obvious under Pi-replete condition.[2,62,63] Furthermore, plant endogenous strigolactone could interact with auxin to accelerate outgrowth of lateral root primordial under Pi starvation, while supplementation of GR24 to roots of Pi-replete plants suppressed lateral root development.[64] Altogether, these prompted us to explore the mechanisms underlying the complex cross-talk between the signaling of Pi, AM and auxin as well as other phytohormones.

Conclusions and Perspectives

Based on the current knowledge about the role of high Pi in AM symbiosis, we postulated here that high Pi affects the symbiosis at early stage when there is no direct contact between host plant roots and AMF and also at late stage when a stable functional symbiotic association is established. The inhibition is largely mediated by suppressing the expression of AM-related genes, which would subsequently change the physiological processes and reduce the susceptibility of plants to AMF.

In the future work, it would be of interest and importance for the researchers to go further on these aspects with regarding to the

mechanisms for the inhibition of high Pi on AM development as well as the transcriptional regulation of the genes involved: (1) the mechanisms leading to the different responses of diverse mycorrhizal plant species to high Pi supply; (2) the genes responsible for the cross-talk between Pi signaling and other signalings (e.g., phytohormone signalings); (3) PHR independent transcription factor(s) binding to the MYCS elements (dicot species) and unknown cis-elements (monocot species) on promoters of AMR *PT* genes.

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