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Abbreviations: Pi, phosphate; PHR1, phosphate starvation response 1; PT, phosphate transporter; AM, arbuscular mycorrhiza; TF, transcription factor

Abstract

The arbuscular mycorrhizal-induced LjMAMI gene is phylogenetically related to GARP transcription factors involved in Pi-starvation responses such as AtPHR1. The gene is strongly upregulated in arbusculated cells from mycorrhizal plants and in root meristems, irrespectively of the fungal presence. A further expression analysis revealed a similar expression pattern for LjPT4, considered a marker gene for mycorrhizal functionality. Here we show that the LjPT4 promoter contains two conserved cis-acting elements typically found in Pi-starvation induced Pi transporters. One of these is strongly related to the binding site of AtPHR1, suggesting a direct regulation of LjPT4 by LjMAMI. The expression of both genes in non-mycorrhizal tissues leads to the hypothesis that these symbiosis-inducible genes are also involved in Pi starvation responses in root meristems in an AM-independent manner.

The arbuscular mycorrhizal (AM) symbiosis is the most widespread symbiotic interaction between land plants and fungi. The formation of the arbuscules, i.e., extensive branched fungal hyphae into plant cortical cells, triggers a deep reprogramming of the plant cell transcriptome and cell organization, allowing the nutrient exchanges between the two partners. Notwithstanding the relevance of unraveling the molecular basis of this interaction, factors controlling the transcriptional changes of plant arbusculated cells are still unknown. In this context, many transcriptomic analyses of mycorrhizal roots from different plant species have been performed. [1-3] Some recent independent works revealed that one of the most upregulated genes of plant arbusculated cells is a putative MYB transcription factor (TF), but to date no clues were proposed for its function.[4,5] We recently characterized an arbuscular-induced MYB-gene of Lotus japonicus.[6] Our phylogenetic analysis showed that this protein was related to a group of constitutive expressed TFs that trigger phosphate (Pi) starvation responses, such as Arabidopsis AtPHR1. According to its putative role as a TF, the protein was localized in the nucleus of two heterologous systems and also in L. japonicus roots under the control of the endogen promoter. Interestingly, GUS staining and quantitative reverse transcriptase PCR (qRTPCR) revealed that gene transcripts were found not only in arbusculated cells but also in root meristems (Fig. 1, left column). In this region gene expression was independent of the presence of the fungus and did not change according to Pi concentrations. For this reason we called the gene LjMAMI (meristem and arbuscular mycorrhiza induced). A functional role of LjMAMI gene in the root meristems was revealed by its downregulation in root explants and in composite plants, where a high deficiency in root growth and branching was observed in LjMAMI silenced lines in comparison to the controls (Fig. 1, right column). This effect was observed exclusively in the absence of the fungus, since the fungal presence rescued root growth as in the controls. Neither RNAi nor overexpressing lines showed any impairment in their mycorrhization capacities. These results suggest a clear-cut relationship between root development, AM symbiosis and Pi assimilation. We also discovered that the marker of arbuscular functionality, LjPT4, is expressed in root meristems as well as LjMAMI. However, differently from LjMAMI, its expression is specifically induced by Pi starvation. A bioinformatic analysis of the LjPT4 promoter allowed us to detect the presence of two conserved cis-elements (Fig. 2). One of these is a sequence strongly similar to P1BS, i.e., the binding site of AtPHR1 which is present in the promoter region of PHR1-induced genes such as At4 and AtIPS1.[7] More recently, it has been shown that the same sequence is also found in the promoter region of AM-induced Pi transporters of many plants, such as the PT4 genes from tomato and tobacco. In this last case another recurrent DNA motif, named MYCS, is often present and can be found in the promoter of LjPT4 as well. Both the P1BS and the MYCS motifs have been shown to be necessary for the proper

induction of AM responsive Pi transporters.[8] Coherently with this hypothesis, the same motifs are also present in the promoter of LjPT3, another Pi-transporter induced by the AM symbiosis,[9] even if the distance between the two sequences is far more pronounced than usually observed. LjPT1 and LjPT2 are other two genes which are involved in Pi transport but are not induced by AM symbiosis.[9] Interestingly, the P1BS and the MYCS motifs resulted to be absent in their promoter regions. Up to date the TFs that recognize these sequences have not been identified. The phylogenetic affinity, between PHR1 and LjMAMI, together with the overlap of the expression profile of LjMAMI and LjPT4 raises the hypothesis that LjMAMI could be one of the factors controlling the expression of LjPT4. Our work revealed that at least two genes which are strongly upregulated in arbuscules are also expressed in root meristems, either constitutively, in the case of LjMAMI, or in Pi starvation conditions, in the case of LjPT4. The expression of LjMAMI has also a high impact on root morphology and might trigger the expression of LjPT4 in Pi depletion. In addition to their peculiar cellular organization (nucleus with a central position, abundance of organelles like Golgi bodies, endoplasmic reticulum, mitochondria, vacuoles with reduced size, membrane proliferation and cell wall deposition), another strong point in common between arbusculated cells and root meristems is that both cell types are specialized in the sensing of nutrients. In particular, Pi starvation is known to enhance root growth at the tip level,[10] and is also a condition that favors the AM symbiosis.[11] It is therefore tempting to speculate that LjMAMI and LjPT4 are implicated in the control of nutrient assimilation in both root cortex and tips, but that two different, tissue-specific regulatory mechanisms have evolved. In the case of arbusculated cells, other transcription factors are probably functionally redundant and responsible for the correct arbuscule formation and functioning even when LjMAMI is downregulated. In the case of root meristems, still unknown downstream targets are expected to link the expression of LjMAMI to the root architecture. More insights will come with the identification of LjMAMI target genes and with more accurate gene expression analyses at root tips.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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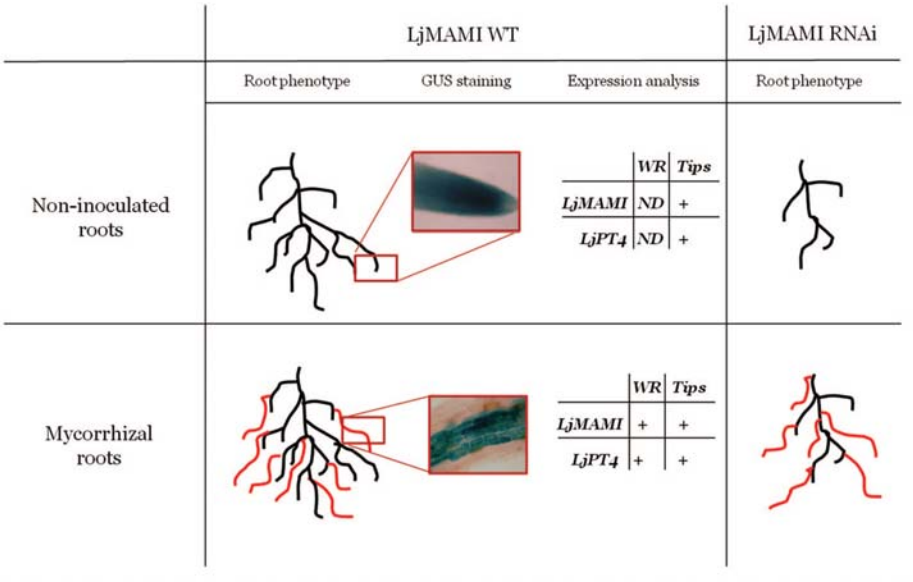

Figure 1. Schematic representation of LjMAMI expression profile and related phenotypes of WT Lotus japonicus plants and RNA i lines in the presence and in the absence of the mycorrhizal fungus. The gene expression is induced in both root meristems (upper left panel) and arbusculated cells (bottom left panel). When LjMAMI is downregulated both root growth and branching are impaired (upper right panel) but the fungus keeps its ability of inducing root growth (bottom right panel). Expression analysis was performed by qRT-PCR. WR, whole roots; ND, not detected. Red roots represent the increased root growth induced by the fungus.

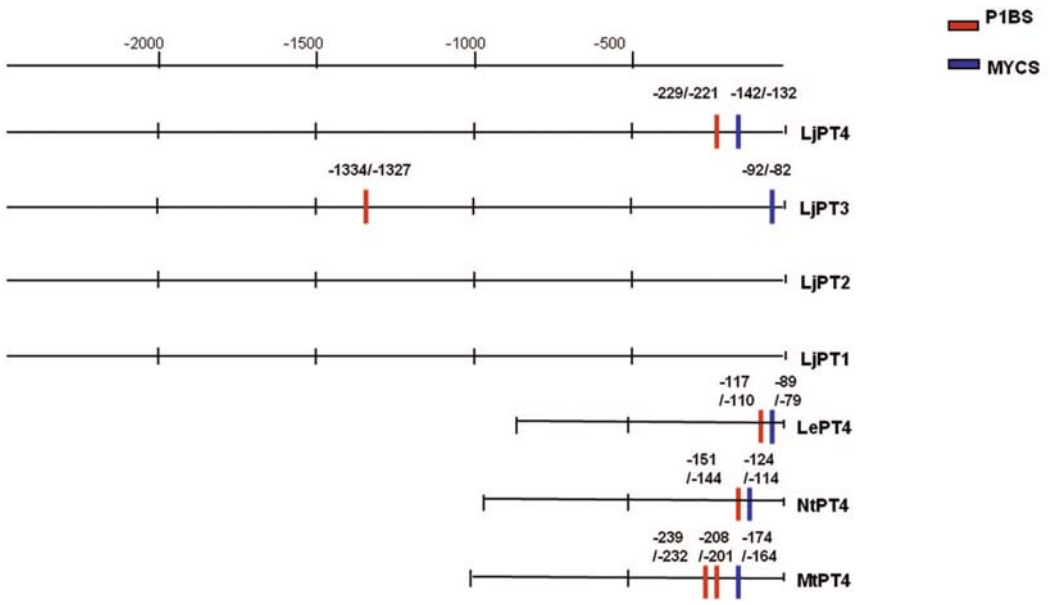

Figure 2. Putative cis-regulatory elements in the promoter of LjPT1-4 genes. The P1BS site, GNATATNC , and the mycorrhiza TF binding sequence “MYCS”, NTTCTT GTTCN , (Chen et al., 2011) were screened in the promoter region of LjPT genes using Dna-pattern matching analysis (http://rsat.ulb.ac.be/rsat/). Both elements were identified only in the promoter region of LjPT3 and LjPT4, the arbuscular mycorrhiza-inducible PTs genes. On the contrary, they were absent in the promoter of LjPT1 and LjPT2, the genes that are not induced during AM symbiosis. Other PT4 genes of Medicago, (MtPT4), tobacco (NtPT4) and tomato (LePT4) are also reported as analyzed in Chen et al., 2011.