ABSTRACT

Plants are sessile organisms and are therefore dependent on complex interactions with their environment, including symbiosis with soil microorganisms. Arbuscular mycorrhiza (AM) is the most widespread root endosymbiosis between plants and glomeromycete fungi. Formation of the symbiosome, which is the site where bi-directional exchange of nutrients and metabolites takes place, helps the plant to improve uptake of water and mineral nutrients like phosphorus. In the symbiosome, phosphorus uptake in form of orthophosphate (Pi) into the host plant is mediated by membrane proteins from the Pht1 family. Many plant mycorrhiza-inducible Pht1 family members have been studied. Still, little is known about their transcriptional regulation. Previous work identified putative regulatory elements within the promoter of the AM-inducible Pht1 transporter gene *StPT3* from potato. Serial *StPT3* promoter deletion studies together with phylogenetic footprinting including numerous mycorrhiza-inducible Pi transporter genes suggested a regulatory function of an 8 bp *cis*-element which was named *CTTC* element.

In the present study, the *CTTC* element was shown to be necessary and sufficient for AM- and P-regulated transcription response in *Solanum tuberosum* and *Lotus japonicus*. Further, two putative transcription factors (TF) from the potato AP2/ERF and GRAS family were identified to be able to bind specifically to the *CTTC* element in the yeast system. In roots, nuclear (AP2/ERF) or cytoplasmic (GRAS) localization was shown. Both TFs were expressed in leaves and roots. Nevertheless, the GRAS-TF exhibited significantly increased RNA levels in roots upon AM-colonization and a repression upon Pi-supply. The AP2/ERF-TF showed an opposite transcriptional regulation, suggesting a complementary role of both TFs in AM- and P-regulated gene response.

Most of the AM-inducible Pht1 members studied previously showed presence of the Pi-starvation element *P1BS* in close proximity to the *CTTC* element. It was thus hypothesized that *P1BS* is involved in transcriptional activation of mycorrhizal genes at low Pi conditions in cooperation with *CTTC*. The combination of *CTTC*- and *P1BS*-element were used for scanning the lotus genome in order to identify candidate genes involved in AM-symbiosis. Further, quantitative RT-PCR combined to laser micro-dissection experiments led us to identify a trafficking-

involved SNARE protein (VTI12), enhanced upon colonization and expressed in arbusculated cells. Further, we could show that VTI12 localizes to the ER and Golgi as well as to small punctuated structures. Analysis of *vti12* mutants generated by RNAi revealed an AM-phenotype at the level of arbuscule morphology. Taken together, one can conclude that VTI12-mediated subcellular trafficking is important in *Lotus* AM-symbiosis.