Abscisic Acid Promotion of Arbuscular Mycorrhizal Colonization Requires a Component of the PROTEIN PHOSPHATASE 2A Complex[1][W][OPEN]

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Legumes can establish intracellular interactions with symbiotic microbes to enhance their fitness, including the interaction with arbuscular mycorrhizal (AM) fungi. AM fungi colonize root epidermal cells to gain access to the root cortex, and this requires the recognition by the host plant of fungus-made mycorrhizal factors. Genetic dissection has revealed the symbiosis signaling pathway that allows the recognition of AM fungi, but the downstream processes that are required to promote fungal infection are poorly understood. Abscisic acid (ABA) has been shown to promote arbuscule formation in tomato (*Solanum lycopersicum*). Here, we show that ABA modulates the establishment of the AM symbiosis in *Medicago truncatula* by promoting fungal colonization at low concentrations and impairing it at high concentrations. We show that the positive regulation of AM colonization via ABA requires a PROTEIN PHOSPHATASE 2A (PP2A) holoenzyme subunit, PP2AB'1. Mutations in *PP2AB'1* cause reduced levels of AM colonization that cannot be rescued with permissive ABA application. The action of PP2AB'1 in response to ABA is unlinked to the generation of calcium oscillations, as the *pp2aB'1* mutant displays a normal calcium response. This contrasts with the application of high concentrations of ABA that impairs mycorrhizal factor-induced calcium oscillations, suggesting different modes of action of ABA on the AM symbiosis. Our work reveals that ABA functions at multiple levels to regulate the AM symbiosis and that a PP2A phosphatase is required for the ABA promotion of AM colonization.

Plants have evolved ingenious strategies to cope with environmental challenges, including partnerships with other organisms in mutualistic symbioses (Thompson, 2005). The arbuscular mycorrhizal (AM) symbiosis is one of the most abundant mutualistic interactions (Smith and Read, 2008) and is formed between more than 80% of terrestrial plant species and fungi in the phylum Glomeromycota (Schüßler et al., 2001; Smith and Read, 2008). This mutualistic symbiosis evolved over 450 million years ago (Remy et al., 1994), which attests to a remarkable selective advantage for both symbiotic partners. As obligate symbionts, AM fungi rely on carbon provided by their plant hosts to complete their life cycle. In exchange, the fungi not only improve the mineral nutrition (particularly phosphate) of the partner but also enhance protection against pathogens as well as drought tolerance (Boomsma and Vyn, 2008; Smith and Read, 2008; Hao et al., 2012; Jung et al., 2012).

Over the past decade, tremendous progress has been made in understanding how the AM symbiosis is established; it is initiated from phosphate-deprived plants that secrete strigolactones to stimulate AM metabolism, spore germination, and hyphae branching within the vicinity of the root (Akiyama et al., 2005; Besserer et al., 2006; Kretzschmar et al., 2012). The AM fungus releases mycorrhizal factors, including sulfated lipochitooligosaccharides and nonsulfated lipochitooligosaccharides (NS-LCOs; Maillet et al., 2011) and chitooligosaccharides (Genre et al., 2013), which are recognized by the plant host to activate the common symbiosis signaling pathway (Harrison, 2012), leading to calcium oscillations in the nuclear region (Kosuta et al., 2008; Chabaud et al., 2011; Sieberer et al., 2012). Following its recognition by the host plant, the AM fungus invades the root epidermal cells and grows through the root cortex, where it initiates highly branched hyphae, the so-called arbuscules in inner root cortical cells. Arbuscules are believed to be the main site for nutrient exchange between the two partners (Harrison, 2012), and this is supported by phosphate transporters such as PHOSPHATE TRANSPORTER4 (PT4) that are localized to the peri-arbuscular membrane (Javot et al., 2007; Pumplin et al., 2012). The arbuscule is a transient structure (Alexander et al., 1989; Javot et al., 2007), and mutation of *PT4* leads

to its premature senescence, indicating plant regulation of the process in response to the degree of phosphate released (Javot et al., 2007). Mycorrhization is also regulated via a microRNA, miR171h, and it is thought that this regulation may prevent overcolonization of the roots (Liu et al., 2011; Lauressergues et al., 2012).

Abscisic acid (ABA) is a key abiotic stress signal, and previous studies have demonstrated a role for ABA in the regulation of AM infection (Herrera-Medina et al., 2007). In an ABA biosynthetic mutant of tomato (*Solanum lycopersicum*), *sitiens* (Taylor et al., 1988; Harrison et al., 2011), AM colonization, arbuscule formation, and functionality are impaired (Herrera-Medina et al., 2007), despite a residual amount of ABA maintained in this mutant (Herde et al., 1999). These results highlight that ABA positively regulates processes associated with the arbuscular mycorrhiza (Herrera-Medina et al., 2007; Martín-Rodríguez et al., 2011). However, this effect was at least in part explained by an indirect effect of the *sitiens* mutation on ethylene signaling (Herrera-Medina et al., 2007; Martín-Rodríguez et al., 2011). In contrast to a positive role during AM colonization, ABA acts as a negative regulator during the root nodule symbiosis (Suzuki et al., 2004; Ding et al., 2008). At the root epidermis, ABA was shown to inhibit early signaling such as nodulation factor-induced calcium spiking, early gene expression, and bacterial infection (Ding et al., 2008). In the root cortex, ABA negatively regulates nodule initiation in response to cytokinin (Ding et al., 2008). In addition, it is believed that ABA also negatively regulates nitrogen fixation (Tominaga et al., 2009). Although AM and root nodule symbioses share a common signaling pathway, the opposite regulation by ABA implies different effects on symbiosis-specific signaling pathways and/or developmental processes. However, at this stage, we cannot discriminate whether this difference in symbiotic responses simply reflects differential effects for ABA in different plant species; for instance, in *Medicago truncatula*, ABA promotes lateral root emergence, while in Arabidopsis (*Arabidopsis thaliana*), it impairs lateral root emergence (Liang and Harris, 2005; De Smet et al., 2006; Laplaze et al., 2007).

Major progress over the last few years has unraveled the earliest events of ABA perception, involving the ABA receptors (PYRABACTIN RESISTANCE [PYR]/REGULATORY COMPONENT OF ABSCISIC ACID RECEPTOR [RCAR]), type 2C protein phosphatases (PP2Cs), and SUCROSE NONFERMENTATION-RELATED PROTEIN KINASE2 (SnRK2; Cutler et al., 2010; Hauser et al., 2011; Santiago et al., 2012). The PYR/RCAR receptors bind ABA and inhibit PP2C activity, removing an inhibition of SnRK2 that phosphorylates numerous downstream targets such as ion channels, transcription factors, and NADPH oxidase (Kulik et al., 2011), and regulates the expression of ABA-responsive genes such as basic leucine zipper transcription factors or ABA-responsive element-binding factors (Finkelstein and Lynch, 2000; Hoth et al., 2002). Downstream of ABA perception, genetic studies have revealed additional protein phosphatases involved in ABA signaling in the PP2A class (Cutler et al., 2010). In contrast

to PP2Cs, which are magnesium- and manganese-dependent monomeric enzymes, PP2As are holoenzymes composed of three subunits: a catalytic subunit, c, that complexes with two regulatory units, a and b (Farkas et al., 2007). Arabidopsis has five, three, and 17 PP2Ac, PP2Aa, and PP2Ab, respectively (Farkas et al., 2007). Among these, the Arabidopsis mutant *rcn1* (for designated roots curl in naphthylphthalamic acid), impaired in a *PP2Aa*, is insensitive to ABA, suggesting that RCN positively regulates ABA signaling. This is in contrast to members of the PP2C class of protein phosphatases that are negative regulators of ABA signaling (Kwak et al., 2002; Pernas et al., 2007). So far, little is known about the function of the PP2Ab subunit, and nothing has been investigated in relation to its role in ABA signaling (Farkas et al., 2007; Leivar et al., 2011). The b subunit, which is further classified into B, B′, and B′′ subfamilies, is believed to determine the substrate specificity and the subcellular localization of the PP2A holoenzymes (Matre et al., 2009).

The previous work on ABA during AM colonization focused on a mutant defective in ABA biosynthesis, which had off-target effects on ethylene levels (Herrera-Medina et al., 2007; Martín-Rodríguez et al., 2011). In order to clarify the role of ABA during AM colonization, we targeted components of ABA signaling and show that at least some components of ABA signaling are involved in the promotion of AM colonization. Our work reveals that a PP2A protein complex is likely associated with the promotion of AM colonization and that at least one member of this complex is transcriptionally activated by ABA and during AM colonization.

RESULTS

ABA Modulates AM Colonization in *M. truncatula*

ABA has been shown to regulate AM colonization in tomato, and we tested the effect of ABA treatment on AM colonization in *M. truncatula*. External application of 5 μM ABA significantly enhanced AM colonization, while 50 μM ABA reduced AM colonization but did not impair arbuscule formation (Fig. 1A; Supplemental Figs. S1 and S2). The ABA marker gene *ABA binding factor3* (*ABF3*; Hoth et al., 2002) was induced upon these ABA treatments, implying appropriate perception of ABA (Fig. 1B). These results suggest that ABA can modulate AM colonization in a concentration-dependent manner, where high concentrations of ABA impair AM colonization, in contrast to low concentrations, which promote the AM symbiosis. We previously reported that ABA modulates nodulation factor-induced calcium spiking (Ding et al., 2008). Therefore, we questioned whether the modulation of AM colonization in an ABA concentration-dependent manner occurred via regulation of the symbiotic signaling pathway leading to calcium oscillations. The role of ABA in mycorrhizal factor-induced calcium spiking was assessed using an *M. truncatula* line stably transformed with the calcium reporter cameleon YC3.6. The plants were grown on medium with 0, 5, or 50 μM ABA
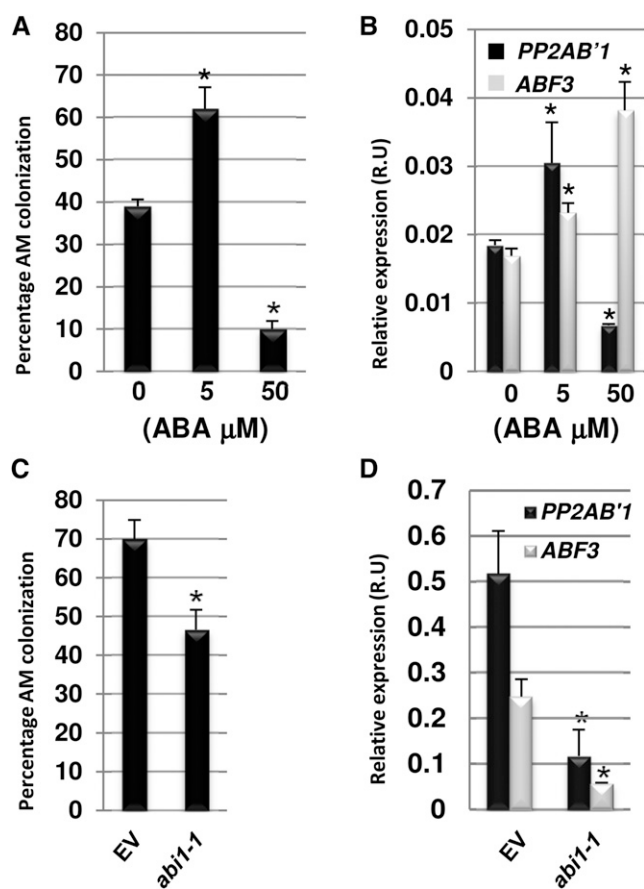
Figure 1. ABA modulates AM colonization and *PP2AB′1* expression. A, Quantification of AM colonization in the wild type after 4 weeks of inoculation with *R. irregularis* and external application of 0, 5, or 50 μM ABA. Values are means ± SE (*n* = 16) from one biological replicate (for biological replicates 2 and 3, see Supplemental Fig. S1). Asterisks indicate significant differences between the ABA-treated roots (5 and 50 μM ABA) and the control (0 μM ABA; Student's *t* test; *P* ≤ 0.01). B, Quantitative RT-PCR to monitor *PP2AB′1* and *ABF3* expression in AM colonized roots watered with 0, 5, or 50 μM ABA. Asterisks indicate statistical significance relative to the untreated control (Student's *t* test; *P* ≤ 0.01). R.U, Relative units. C, Transformation of the Arabidopsis dominant-negative allele of *abi1-1* in the wild type leads to a decrease of *R. irregularis* colonization at 7 weeks after inoculation. Values are means ± SE (*n* = 10 [empty vector] and *n* = 14 [*abi1-1*]) from one biological replicate (for biological replicates 2 and 3, see Supplemental Fig. S4). The asterisk indicates a significant difference from the wild type transformed with the empty vector (EV; Student's *t* test; *P* ≤ 0.01). D, Quantitative RT-PCR to monitor *PP2AB′1* expression in roots transformed with *abi1-1*. *ABF3*, up-regulated in response to ABA, was used as an ABA marker. *PP2AB′1* and *ABF3* expression was significantly down-regulated in roots expressing *abi1-1* in comparison with the empty vector. Asterisks indicate significant differences relative to the empty vector control (Student's *t* test; *P* ≤ 0.01). Expression is normalized to *EF-1α* in B and D.

and analyzed for mycorrhizal factor (NS-LCO)-induced calcium spiking (Maillet et al., 2011). We found that calcium spiking was not altered in plants grown on 5 μM ABA (Supplemental Fig. S3), but at 50 μM ABA, only 50% of cells displayed calcium spiking, and this calcium

spiking had a reduced frequency in comparison with plants treated with 5 μM ABA or untreated with ABA (Supplemental Fig. S3). These results show that permissive ABA concentrations do not influence NS-LCO-induced calcium spiking, in contrast to higher concentrations of ABA, which can impair NS-LCO activation of the symbiotic signaling pathway and AM colonization.

To further test the function of ABA during AM colonization of *M. truncatula*, we used a dominant negative allele of a *PP2C* from Arabidopsis. PP2Cs sit at the core of the ABA receptor complex, and the Arabidopsis *abscisic acid insensitive1-1* (*abi1-1*) allele, mutated in a PP2C, dominantly suppresses ABA signaling in Arabidopsis as well as other plant species (Hagenbeek et al., 2000; Gampala et al., 2001; Wu et al., 2003; Ding et al., 2008). We overexpressed Arabidopsis *abi1-1* in *M. truncatula* roots via *Agrobacterium rhizogenes*-mediated gene transfer (Boisson-Dernier et al., 2001) and assessed the effect on *Rhizophagus irregularis* colonization (Fig. 1C; Supplemental Fig. S4). Roots expressing *abi1-1* showed a 30% reduction of AM colonization in comparison with roots expressing the empty vector (Fig. 1C; Supplemental Fig. S4). Arabidopsis *abi1-1* transformation suppresses *ABF3* expression (Fig. 1D), implying that *abi1-1* does indeed block ABA signaling.

In a previous study, we identified the *M. truncatula sensitivity to abscisic acid-1* (*sta-1*) mutant, which is impaired in ABA modulation of the root nodule symbiosis due to a hypersensitivity to ABA during nodule organogenesis in the root cortex (Ding et al., 2008). In the absence of ABA treatment, *sta-1* was hypercolonized by AM fungi (Fig. 5, B and C), supporting the role of ABA in promoting AM colonization. Combining our results with external ABA application, *abi1-1* transformation, and the effect of the *sta-1* mutation on AM colonization, we conclude that ABA positively regulates AM colonization in *M. truncatula*, and this is consistent with previous work in tomato (Martin-Rodriguez et al., 2010; Martín-Rodríguez et al., 2011). However, high concentrations of ABA impair the symbiosis signaling pathway in the root epidermis in a manner analogous to its effect on nodulation factor signaling (Ding et al., 2008), and this is likely to lead to the impairment of AM colonization that we observed upon external application of high concentrations of ABA.

A *PP2AB′1* Induced upon *R. irregularis* Infection and Regulated upon ABA Treatment

The finding that ABA promotes AM colonization led us to question whether there were ABA signaling components specific to the AM symbiosis. Interestingly, a component of the PP2A holoenzyme, the *Medicago sativa* PP2Ab expressed in roots, is induced by ABA (Tóth et al., 2000), suggesting that the PP2A holoenzymes might be involved in ABA signaling in *Medicago* spp. Since the PP2A subunits are induced by the conditions associated with their effect (Ariño et al., 1993; Casamayor et al., 1994), we explored the expression of the *PP2Aa*,

PP2Ab, and *PP2Ac* gene families during mycorrhizal colonization using the *M. truncatula* gene expression atlas (Benedito et al., 2008). None of the *PP2Aa* and *PP2Ac* genes were strongly induced in mycorrhized roots, but one *PP2Ab* gene was highly induced during mycorrhizal colonization (Supplemental Fig. S5). This mycorrhiza-induced *PP2Ab* is part of the *PP2AB'* subfamily (Fig. 2; Supplemental Fig. S6). This gene, whose probe set is *Mtr11959.1*, was not induced upon nodulation factor treatment or in mature nodules (Supplemental Fig. S7, A and C). This *M. truncatula PP2AB'* has three rice (*Oryza sativa*) homologs but no closely related Arabidopsis homolog (Fig. 2). We chose to name it *PP2AB'1*.

In order to confirm the gene expression atlas data, we checked the induction levels of *PP2AB'1* using quantitative reverse transcriptase (qRT)-PCR (Supplemental Fig. S7B) and found induction upon AM colonization (Supplemental Figs. S7B). To further validate this, we monitored the expression of the *PP2AB'1* promoter driving *GUS* (note that this promoter region was used in complementation studies; Fig. 4D; Supplemental Fig. S8). *PP2AB'1* showed constitutive expression in all root cell layers (Fig. 3, A and B). In correlation with the quantitative RT-PCR analysis (Supplemental Fig. S7B), AM colonization led to stronger *pPP2AB'1-GUS* expression, and this enhancement occurred in all root cell layers (Fig. 3, C and D), including cortical cells containing arbuscules or not (Fig. 3, E and F).

The presence in the putative promoter region of *PP2AB'1* of three abscisic acid-responsive elements (ABREs; Fig. 4A) strongly suggests that *PP2AB'1* expression is regulated via ABA signaling. To test whether ABA modulates *PP2AB'1* expression at the concentrations promoting or impairing AM colonization, we applied 0, 5, and 50 μM ABA in the absence of AM colonization (Supplemental Fig. S9). *PP2AB'1* expression was up-regulated by 5 μM ABA, but in contrast to *ABF3* expression, which increased upon rising ABA concentrations, the expression of *PP2AB'1* was down-regulated with 50 μM ABA treatment (Supplemental Fig. S9). *PP2AB'1* expression was also down-regulated in roots transformed with *abi1-1*, which impairs ABA signaling (Fig. 1D). The modulation of *PP2AB'1* expression by ABA was further confirmed in AM-inoculated roots, with an up-regulation
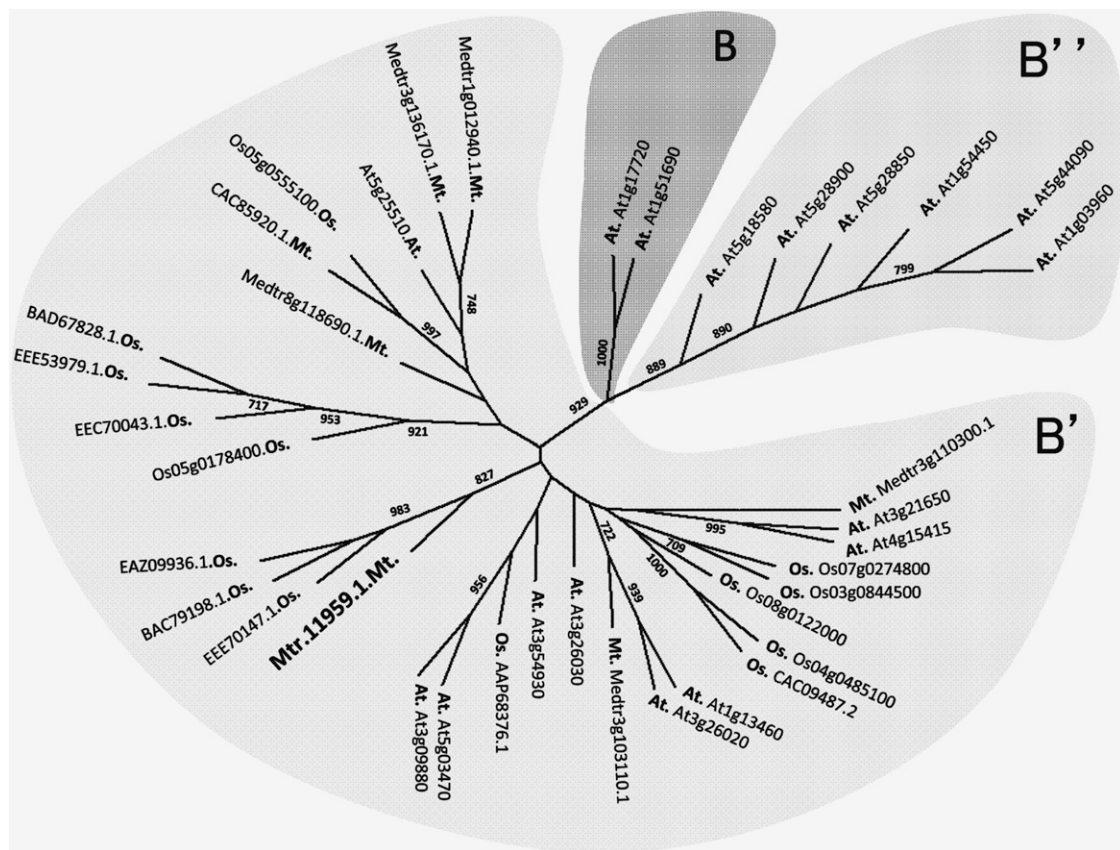

Figure 2. Mtr.11959.1 is a regulatory B' subunit of the Ser/Thr phosphatase type 2A family. The unrooted phylogenetic tree was calculated from the alignment presented in Supplemental Figure S4, including the amino acid sequences from the B, B', and B'' regulatory subunits of the Ser/Thr phosphatases type 2A of Arabidopsis (At.), rice (Os.), and *M. truncatula* (Mt.). Branch labels indicate bootstrap values (*n* = 1,000) of the consensus tree. Low bootstrap values are not shown. Labels at the branch tips include unique identifiers (GenBank/Uniprot) followed by the species designation; for Mtr.11959.1, the GenBank identifier is KC859637.
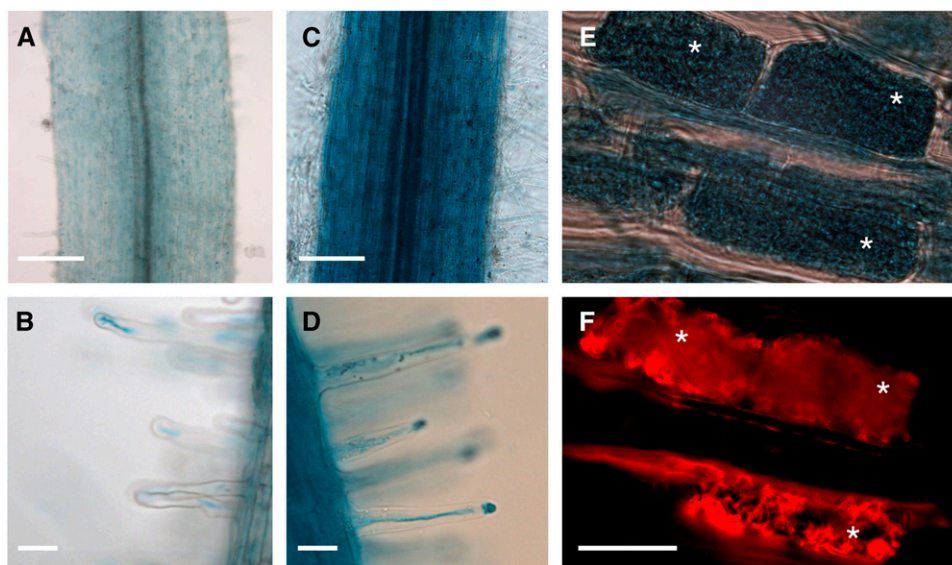
Figure 3. Expression analysis of the *PP2AB′1* promoter in AM colonized roots. Promoter GUS activity in *A. rhizogenes*-transformed roots expressing the *PP2AB′1* promoter-β-glucuronidase fusion was assessed 4 weeks after inoculation with *R. irregularis* in C to F and without *R. irregularis* in A and B. F shows a fluorescence microscopy image of the corresponding bright-field image in E showing *R. irregularis* stained with wheat germ agglutinin Alexa Fluor 594, which fluoresces red. White asterisks indicate cortical cells containing arbuscules. Bars = 250 μm (A and C), 50 μm (B and D), and 20 μm (F).

upon 5 μM ABA and a down-regulation upon 50 μM ABA (Fig. 1B). The down-regulation of *PP2AB′1* following 50 μM ABA application was further confirmed by analysis of the *PP2AB′1* promoter driving *GUS* (Supplemental Fig. S10). Taken together, the similarity between the expression pattern of *PP2AB′1* in response to ABA and *R. irregularis* infection and the AM colonization levels upon ABA treatments suggests that *PP2AB′1* may be a new regulator of ABA signaling during the AM symbiosis.

PP2AB′1 Mutations Impair AM Colonization

To test for a role of *PP2AB′1* during AM colonization, we generated an RNA interference (RNAi) construct targeting *PP2AB′1*. Transgenic roots of *M. truncatula* were obtained by *A. rhizogenes*-mediated gene transfer (Boisson-Dernier et al., 2001). Roots showing red fluorescence (the *Discosoma* spp. red fluorescent protein gene *DsRed* was included in the transfer DNA) were retained and inoculated with *R. irregularis*. After 5 weeks of inoculation, the AM colonization on the RNAi *PP2AB′1* roots, whose *PP2AB′1* expression was impaired (Supplemental Fig. S11A), was reduced significantly by 50%, in comparison with roots transformed with the empty vector (Supplemental Fig. S11, B and C). This observation suggests that *PP2AB′1* positively regulates AM colonization.

To further validate the function of *PP2AB′1*, transposable element of *Nicotiana tabacum cell type1* (*Tnt1*) mutagenized lines (Tadege et al., 2008) of *M. truncatula* were used for PCR-based reverse genetic screening (Cheng et al., 2011), and this identified two independent mutant lines, *pp2aB′1-1* (NF3036) and *pp2aB′1-2* (NF584), harboring *Tnt1* insertions in *PP2AB′1* exons at positions 692 and 1,379 bp, respectively (Fig. 4A). Both lines were predicted to be null mutants, as evidenced by the fact that no mRNA was detectable for *PP2AB′1* in the mutant lines (Fig. 4B; Supplemental Fig. S12). Following *R. irregularis* inoculation, we observed

a reduction of 50% AM root length colonization in both *pp2aB′1-1* and *pp2aB′1-2* compared with the wild type (Fig. 4C; Supplemental Fig. S13). However, fungal structures typical of the AM symbiosis, including hyphae, arbuscules, and vesicles, were all observed (Supplemental Figs. S14 and S15). This reduced colonization in *pp2aB′1-1* was complemented by introducing the genomic sequence of *PP2AB′1* driven by 3 kb of its own promoter region (Fig. 4D; Supplemental Fig. S8). Although *PP2AB′1* expression was not enhanced upon nodulation factor or *Sinorhizobium meliloti* treatment (Supplemental Fig. S7, A and C), we investigated whether *PP2AB′1* had a role in the root nodule symbiosis. After 7 d of inoculation with an *S. meliloti* strain expressing β-galactosidase (*LacZ*), infection threads formed on the mutant line *pp2aB′1-1* at a frequency similar to that in the wild type (Fig. 4F). Moreover, 19 d post inoculation (dpi) with *S. meliloti*, nodules formed on *pp2aB′1-1* at the same rate as on the wild type (Fig. 4E) and nitrogen fixation occurred at an equivalent level (Supplemental Fig. S16). Altogether, these results demonstrate that *PP2AB′1* is required for appropriate AM colonization and is not required for nodulation in *M. truncatula*.

AM Colonization Mediated by ABA Is Dependent on *PP2AB′1*

We would predict that *PP2AB′1* functions downstream of ABA perception by the ABA receptor complex. To test this, we assessed the effect of ABA treatment and *abi1-1* on mycorrhizal colonization in *pp2aB′1-1*. Transformation with *abi1-1* (Fig. 5A; Supplemental Fig. S17) and treatment with ABA (Fig. 5B) had no impact and did not rescue the AM colonization levels of *pp2aB′1-1*, indicating that ABA regulation of AM colonization is completely dependent upon *PP2AB′1*. We propose that the regulation of *PP2AB′1* by ABA signaling allows ABA promotion of
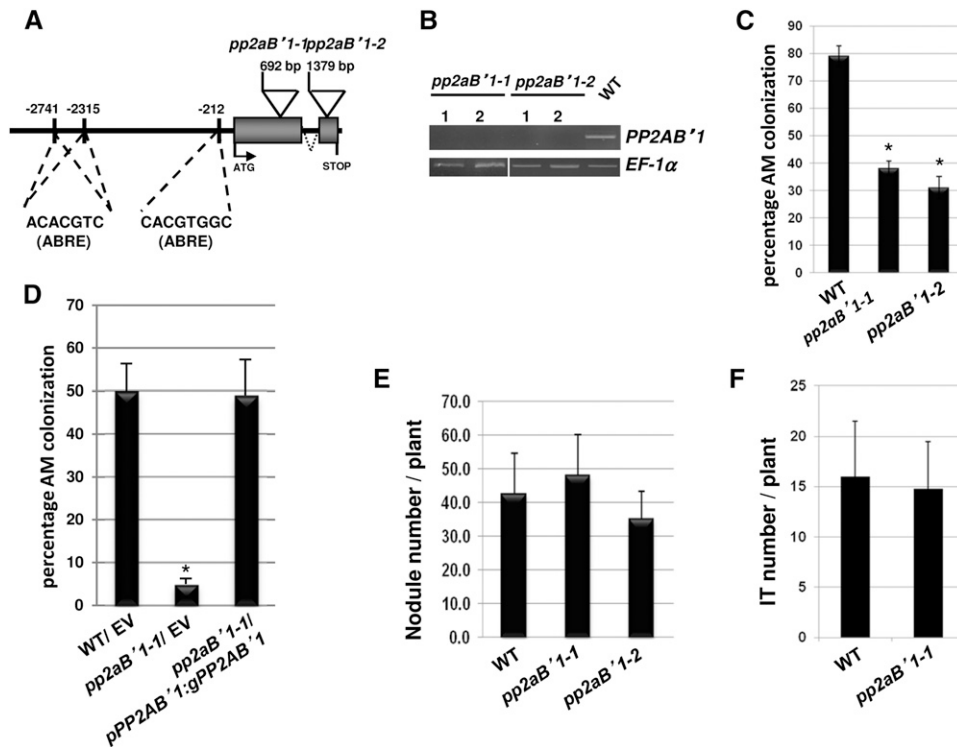
Figure 4. *pp2aB′1* mutants are impaired in AM colonization. A, Structure of the *PP2AB′1* gene showing the positions of the retrotransposon *Tnt1* insertions of the *pp2aB′1-1* and *pp2aB′1-2* mutant alleles. Three ABREs are predicted in the promoter of *PP2AB′1* as indicated. B, RT-PCR to detect the accumulation of full-length *PP2AB′1* transcript (1,290 bp) in mRNA from roots of two homozygous plants identified for *pp2aB′1-1* and *pp2aB′1-2* mutant alleles and in the wild type (WT). *EF-1α* expression was used as a constitutive control. C, AM colonization of wild-type and *pp2aB′1* roots at 6 weeks after inoculation with *R. irregularis*. Values are means ± SE (*n* = 10) from one biological replicate (for biological replicates 2 and 3, see Supplemental Fig. S13). The colonization between the wild type and *pp2aB′1* mutant lines differs significantly, as indicated by asterisks (Student's *t* test; *P* ≤ 0.0001). D, AM colonization of *pp2aB′1-1* transformed roots complemented with the *PP2AB′1* genomic sequence driven by its own promoter (*pPP2AB′1:gPP2AB′1*) or empty vector (EV) and wild-type transformed roots with the empty vector at 5 weeks after inoculation with *R. irregularis*. Values are means ± SE (*n* = 10) from one biological replicate (for biological replicate 2, see Supplemental Fig. S8). The asterisk indicates statistical significance relative to the wild-type/empty vector control (Student's *t* test; *P* ≤ 0.0001). E, Nodule quantification on the wild type and the two *pp2aB′1* mutant alleles at 19 dpi with *S. meliloti* 2011 (optical density = 0.001). Values are means ± SD (*n* = 30) from three biological replicates. F, Infection thread (IT) number per wild type and *pp2aB′1-1* mutant plant formed 7 dpi with an *S. meliloti* strain expressing *LacZ*. Values are means ± SD (*n* = 18) from two biological replicates.

mycorrhizal colonization. The mechanism of ABA modulation may simply be through the promotion of *PP2AB′1* expression, and notably, the induction of *PP2AB′1* reflects ABA impact on AM colonization (compare Fig. 1, A and B).

PP2AB′1 Modulates ABA Sensitivity in Leaves and Seeds

PP2Aa and PP2Ac subunits have been shown to promote ABA signaling in a variety of tissues (Kwak et al., 2002; Pernas et al., 2007). To assess whether *PP2AB′1* had a specific AM function, we assessed the *pp2aB′1* mutants for lateral root emergence, seed germination, and water loss from the leaf, all of which are controlled by ABA (Finkelstein et al., 2002; Liang et al., 2007). By growing the plants under different concentrations of ABA, we observed no significant difference between the wild type and *pp2aB′1-1* for lateral root emergence (Fig. 6A), but *pp2aB′1* mutants were hypersensitive to ABA during seed

germination at higher ABA concentrations (Fig. 6B). Furthermore, leaves of *pp2aB′1-1* are less susceptible to water loss when excised from the plant (Fig. 6C), suggesting either a reduced stomatal opening or increased protection of cells from dehydration; both processes are promoted by ABA (Yamaguchi-Shinozaki and Shinozaki, 2006; Okamoto et al., 2013). We conclude that *PP2AB′1* functions in ABA promotion of seed germination, regulation of water loss from leaves, and ABA promotion of AM colonization but not in the ABA regulation of lateral root emergence or the root nodule symbiosis.

Previous analyses suggest that the plant hormone ethylene increases AM epidermal infection in *M. truncatula* (Penmetsa et al., 2008) or fungal spread in tomato roots (Martín-Rodríguez et al., 2011) and that the *sitiens* mutant that affects ABA biosynthesis modifies AM colonization at least in part due to the effect of the mutation on ethylene levels (Martín-Rodríguez et al., 2011). One possible explanation for the mycorrhizal defects in the *pp2aB′1* mutant is
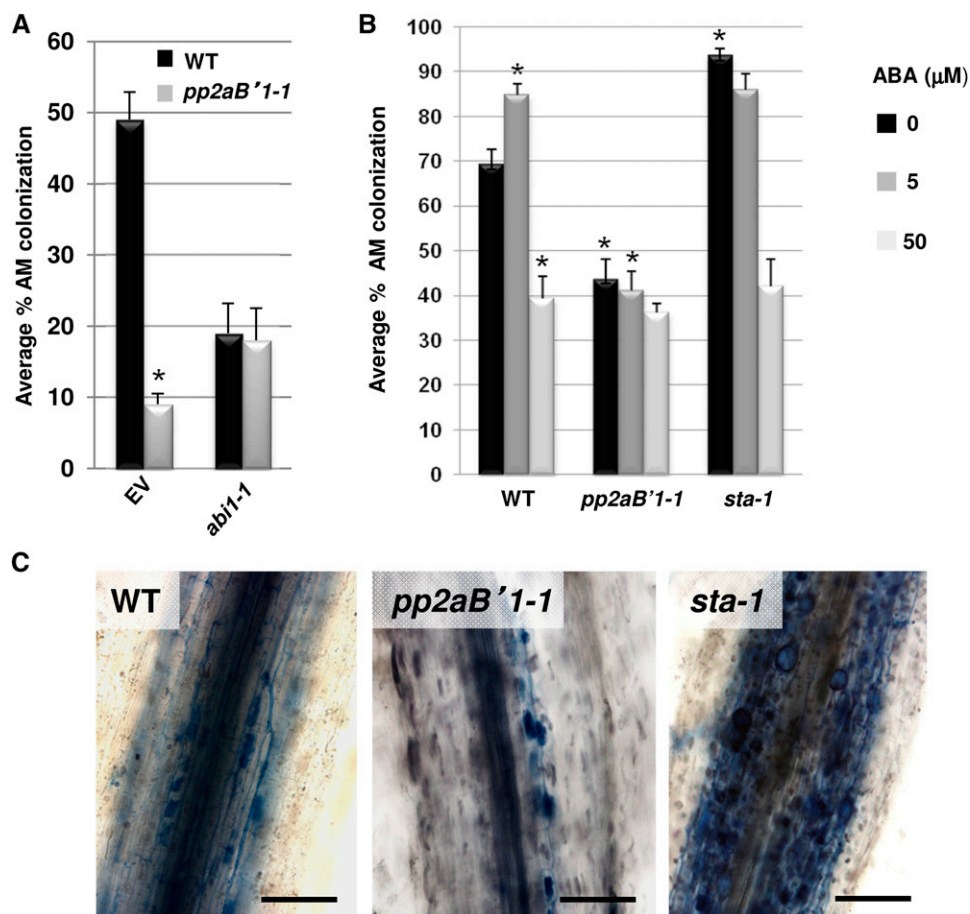
Figure 5. *PP2AB'1* acts downstream of ABA biosynthesis and perception during AM colonization. A, Quantification of AM colonization in the *pp2aB'1-1* mutant transformed with *abi1-1* or empty vector (EV) at 5 weeks after inoculation with *R. irregularis*. Expression of *abi1-1* impaired AM colonization in the wild type (WT) similarly to *pp2aB'1-1* expressing *abi1-1*. Values are means ± SE (*n* = 10) from one biological replicate (for biological replicates 2 and 3, see Supplemental Fig. S17). The asterisk indicates significance in comparison with the wild type (Student's *t* test; $P \leq 0.001$). B, External application of ABA does not restore AM colonization in the *pp2aB'1* mutant. AM colonization is shown in *pp2aB'1-1* and *sta-1* mutants watered with 0, 5, or 50 μM ABA for 4 weeks; the colonization is observed 4 weeks after *R. irregularis* inoculation. Asterisks indicate significant differences compared with the wild type watered with 0 μM ABA or with the wild type with the same ABA regime (Student's *t* test; $P < 0.001$). Values are means ± SE (*n* = 12). C, Bright-field images of wild-type as well as *pp2aB'1-1* and *sta-1* roots inoculated with *R. irregularis* for 4 weeks and watered with 0 μM ABA. The fungal structures are ink stained (blue coloration). Bars = 70 μm.

a role for this gene in ethylene responses. To test this, we assessed whether the *pp2aB'1* mutant is perturbed in ethylene responses by measuring the hypocotyl and root length of wild-type and *pp2aB'1* seedlings in the presence of various concentrations of 1-aminocyclopropane carboxylic acid (ACC). The *pp2aB'1-1* mutant displays all aspects of the ethylene response, with reduction of root and hypocotyl length in a dose-dependent manner (Supplemental Fig. S18). This indicates that *PP2AB'1* does not function in the plant's response to ethylene.

pp2aB'1 Mutants Are Impaired in AM Propagation

PP2AB'1 and ABA signaling could function at many different stages of the AM interaction, leading to promotion of the association. To follow the mycorrhizal

fungal progression, we undertook a detailed analysis of *pp2aB'1* mutants during colonization by *R. irregularis* (Fig. 7A). One-week-old seedlings were inoculated with germinated *R. irregularis* spores, and at 3, 6, and 8 dpi, plants were evaluated for the number of AM penetration events into epidermal cells (Fig. 7B) and AM penetration events into underlying cortical cells leading to arbuscules and vesicles (Fig. 7, C and D; Supplemental Figs. S14 and S15). The total root length colonization at 12 dpi was also scored (Fig. 7E). We monitored *PP2AB'1* expression in the wild type over time (Supplemental Fig. S19) and found the induction of *PP2AB'1* correlated with high AM colonization (Fig. 7E). At 3 dpi, the levels of fungal penetration were too low for a confident assessment of differences between the wild type and the mutants. However, penetration of AM into the mutant epidermal cells was
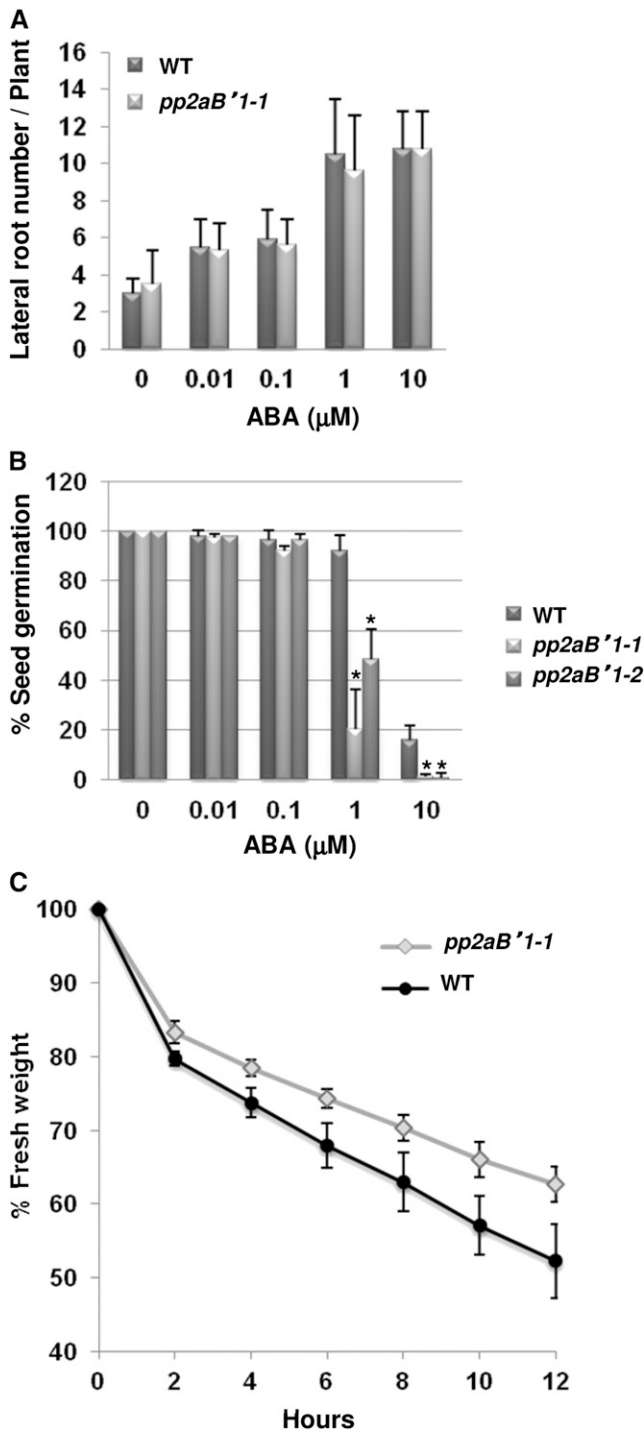
Figure 6. *PP2AB'1* regulates ABA responses in leaves and seeds. A, The number of lateral roots formed in *pp2aB'1-1* is similar to that in the wild type (WT) in response to ABA treatment. Values are means ± SD from three biological replicates (*n* = 30). B, The germination of *pp2aB'1* mutant seeds is hypersensitive to ABA. Values are means ± SD from three biological replicates. Asterisks indicate significant differences in comparison with the wild type (Student's *t* test; *P* ≤ 0.01; *n* = 300). C, The fresh weight of detached leaves was used to measure relative water loss over time. *pp2aB'1-1* shows slower water loss than the wild type. Values are means ± SD from three biological replicates.

observed, meaning that mutation in *PP2AB'1* does not block epidermal entry. But at 6 and 8 dpi, the *pp2aB'1* mutants showed clear reductions in the density of AM penetration of epidermal cells and reductions in the levels of arbuscules and vesicles (Fig. 7A). If we consider a ratio of epidermal penetration events to arbuscules (Supplemental Fig. S20), it is equivalent between the wild type and the *pp2aB'1-1* mutant. This suggests that a primary defect of the fungal propagation is during the penetration of epidermal cells, and once the fungus has initiated infection, these infection events effectively progress to cortical colonization with resultant arbuscules and vesicles. To confirm the AM fungal colonization levels observed in the *pp2aB'1* mutants, we checked the expression of the mycorrhizal marker gene *PT4* (Fig. 7F). Consistent with the density of arbuscules formed, *PT4* expression was impaired in the *pp2aB'1-1* mutant (Fig. 7F). These results suggest that PP2AB'1 plays a role in AM propagation by promoting the infection of epidermal cells, but it also functions in growth of the fungus through the root cortex. We assessed whether *PP2AB'1* regulates the early signaling pathway required for the generation of nucleus-localized calcium spiking in response to mycorrhizal factors. Both the sulfated and nonsulfated mycorrhizal factors (NS-LCO) induced calcium spiking in root hair cells in the *pp2aB'1-1* mutant at a frequency similar to that in wild-type plants (Fig. 7G). These results demonstrate that *PP2AB'1* is not required for early signaling but acts downstream of calcium spiking to promote AM penetration of the root epidermis and colonization of the root cortex.

DISCUSSION

Phytohormones are known to mediate the AM symbiosis (Hause et al., 2007), with strigolactones involved in presymbiotic signaling (Akiyama et al., 2005; Besserer et al., 2006; Kretzschmar et al., 2012; Lauressergues et al., 2012) and ethylene postulated to increase AM infection but not cortical colonization of *M. truncatula* (Penmetsa et al., 2008). In tomato, ABA was shown to have a direct effect on arbuscule formation and functionality as well as an indirect effect on plant susceptibility to AM fungi by inhibition of ethylene (Herrera-Medina et al., 2007; Martin-Rodriguez et al., 2010; Martín-Rodríguez et al., 2011). In this study, we identify a new component of the ABA pathway, *PP2AB'1*, which is required for AM colonization and whose expression is controlled by ABA. The effect of external ABA application on the promotion of AM colonization was entirely dependent on the presence of *PP2AB'1*, implying that the positive action of ABA on AM colonization is predominantly via *PP2AB'1*. Moreover, in comparison with the tomato *sitiens* mutant (Herrera-Medina et al., 2007), *pp2ab'1* mutants show neither an arbuscule defect nor impairment in the ethylene response. This observation implies that the regulation of AM colonization by ABA via *PP2AB'1* occurs in a different pathway to that of *SITIENS*, which appears to be involved in the ABA regulation of arbuscule development.
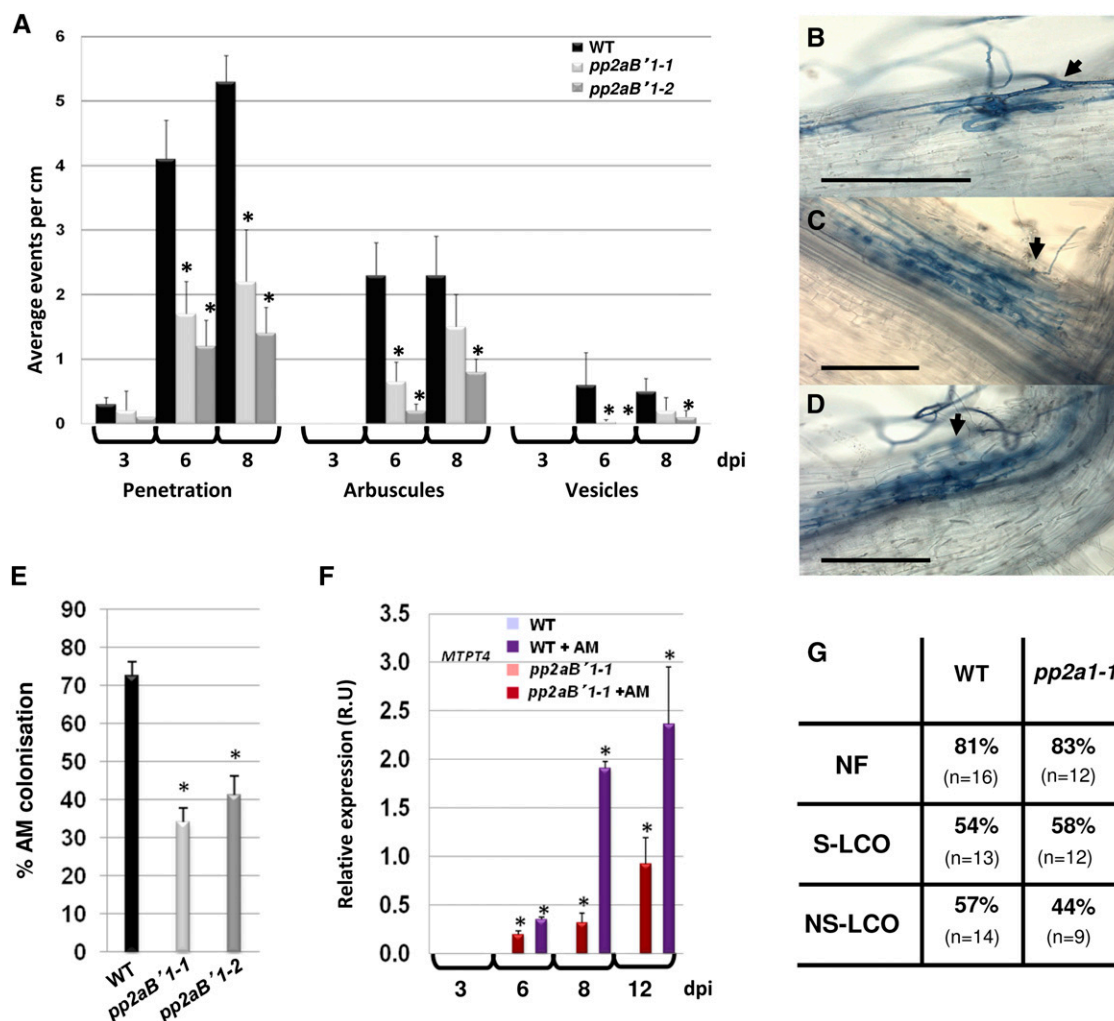
Figure 7. *PP2AB′1* is required for AM penetration and propagation. A to E, AM propagation analysis 3, 6, 8, and 12 dpi with germinating *R. irregularis* spores infecting wild-type (WT) and *pp2aB′1* mutant roots. A, The numbers of penetration events with intraradical mycelium (B), penetration events with cortical cells containing arbuscules (C), and penetration events with cortical cells containing arbuscules and vesicles (D; Supplemental Fig. S10) were quantified per cm of root. B to D, Bright-field images show the propagation of the fungus infecting the *pp2aB′1-1* mutant 6 dpi. Black arrows show the fungal penetration. Bars = 200 µm. The AM fungus is ink stained. E, Percentage of AM root length colonization with *R. irregularis* germinating spores 12 dpi. F, Quantitative RT-PCR to monitor *MtPT4* expression in wild-type *M. truncatula* roots infected with *R. irregularis* (WT + AM) and in *pp2aB′1-1* during fungal propagation (A–E). The expression is normalized to *EF-1α* level. Values are means ± SE from three biological replicates. Asterisks indicate significant differences from the wild type (A and E) or from the control noninoculated roots (the wild type and *pp2aB′1-1*) in F (Student's *t* test; $P \leq 0.01$; $n = 24$). G, Calcium spiking measurements in the wild type and *pp2aB′1-1* mutants in response to 10^{-9} M nodulation factor (NF), 10^{-8} M sulfated lipochitooligosaccharides (S-LCO), and 10^{-6} M NS-LCO. The percentage of cells spiking is indicated, and n = number of cells recorded.

However, this difference may also reflect subtle species-specific differences in the function of ABA during the regulation of AM colonization.

Although the level of ABA in mycorrhized *M. truncatula* roots has not been investigated, previous reports suggest that AM fungi induce ABA accumulation in the colonized roots of soybean (*Glycine max*) and maize (*Zea mays*; Danneberg et al., 1992; Meixner et al., 2005). This accumulation could be due to endogenous ABA production but may also derive from the fungus (Esch et al., 1994). Therefore, in a hypothetical

scenario, ABA accumulation in response to AM colonization leads to the induction of *PP2AB′1* expression, which contains three ABREs within its promoter region. While the induction of *PP2AB′1* is likely important for its function, there must be a basal level of this protein that is necessary for AM colonization and that may be induced at constitutive ABA levels, since we observed a phenotype in the *pp2aB′1* mutants at time points prior to the induction of *PP2AB′1*. Notably, *PP2AB′1* showed constitutive expression throughout the root prior to fungal colonization.

PP2As form heteromeric complexes; thus, we assume that PP2AB′1 functions in a complex with PP2Aa and PP2Ac subunits. The PP2Aa and PP2Ac components must be constitutively expressed, as we found no members of these gene families that were induced upon AM colonization. The mutant analysis indicates that *PP2AB′1* predominantly functions at the root epidermis. Interestingly, a role for a PP2Ac at the root epidermis has been described in tomato, in which silencing of a *PP2Ac* leads to enhanced resistance to fungal pathogens (He et al., 2004). The PP2Ab family is believed to determine the substrate specificity of the PP2A holoenzymes, in contrast to the PP2Ac subunit, which is responsible for the catalytic activity. Thus, the PP2AB′ part of a holoenzyme complex might target a protein that is then dephosphorylated by a PP2Ac. The protein(s) targeted might be either required for or act as a negative regulator of AM colonization downstream of the common signaling pathway. However, it remains to be proven that PP2AB′1 does indeed act in a functional protein phosphatase complex.

ABA is also a regulator of the association with nitrogen-fixing rhizobial bacteria (Ding et al., 2008); however, this mode of action does not involve *PP2AB′1*. ABA modulates rhizobial colonization via suppression of the symbiotic signaling pathway but also via suppression of the cytokinin activation of nodule organogenesis (Ding et al., 2008). In common with the action of ABA on the rhizobial interaction, ABA suppressed the NS-LCO activation of calcium oscillations, and this probably explains the negative effect of higher concentrations of ABA on AM colonization. However, unlike the rhizobial interaction, lower concentrations of ABA positively regulate AM colonization. This opposing effect of different ABA concentrations on AM colonization may be explained if one considers a temporal process of fungal colonization. At the initial stages of the interaction, the presence of the fungus activates the symbiotic signaling pathway, which may lead to the promotion of ABA levels. These in turn induce *PP2AB′1* expression, which facilitates AM colonization. Perhaps under prolonged exposure to AM fungi, sufficient levels of ABA accumulate that ultimately suppress the symbiotic signaling pathway, and this may be associated with down-regulating early signaling in cells already colonized by the fungus. Alternatively, stressful conditions that promote ABA production may block the symbiotic signaling pathway and thus inhibit the earliest stages of the AM interaction. Consistent with this, drought stress in *M. truncatula* suppresses *PP2AB′1* expression (Supplemental Fig. S21), and this may be associated with the inhibition of AM colonization under certain plant stresses.

In Arabidopsis, ABA-dependent SnRK2 activity is controlled by a panel of positive and negative regulatory components (Fujita et al., 2011). As such, the SnRK2-interacting calcium sensor impairs SnRK2 activity in a calcium-dependent manner (Bucholc et al., 2011). The ABA regulation of cellular processes is intrinsically connected to calcium. Thereby, ABA can increase the cellular calcium sensitivity (Kim et al., 2010) as well as modulate the expression of similar genes containing ABA-calcium-responsive elements, notably ABREs (Whalley et al., 2011). In this regard, it is notable that symbiosis signaling involves calcium oscillations, and these may directly impact aspects of ABA signaling. Thus, the processes of symbiosis and ABA signaling may be more closely intertwined than we had imagined previously.

AM fungi activate the symbiotic signaling pathway of plants, and this is required to allow fungal colonization of the root. We propose that PP2AB′1 acts within a holoenzyme to dephosphorylate target protein(s) that are associated with mycorrhizal infection. As the absence of *PP2AB′1* leads to a decrease in mycorrhizal infection and does not impair mycorrhizal lipochitooligosaccharide or nodulation factor-induced calcium spiking, possible targets are likely specific to AM colonization, functioning downstream of the common signaling pathway. ABA signaling appears to play an important positive role in AM colonization, and this function is predominantly via the promotion of the PP2A holoenzyme complex. The fact that ABA signaling promotes AM colonization but suppresses rhizobial colonization may be significant for discriminating between these different symbionts.

MATERIALS AND METHODS

Phylogenetic Analysis

Protein sequences similar to MtPP2AB′1 were retrieved by protein BLAST using the *Medicago truncatula* version 3.5 database, the Arabidopsis (*Arabidopsis thaliana*) database, and the rice (*Oryza sativa*) database. Multiple sequence alignments were performed using the ClustalW (version 1.83) program (Thompson et al., 1994) and edited using the BioEdit sequence alignment editor (Hall, 1999). The final alignment used for phylogenetic analysis is shown in Supplemental Figure S4. Phylogenetic analysis was performed with Phylip programs (version 3.69; Felsenstein, 2004). A distance matrix method employing the Jones-Taylor-Thornton model was used to compare the sequences, and a tree was built using the neighbor-joining clustering method (Saitou and Nei, 1987). One thousand bootstrapped data sets were used to indicate the confidence of each tree clade. The unrooted neighbor-joining tree was generated using PhyloDraw version 0.8 (Graphics Application Laboratory, Pusan National University).

Identification of *PP2AB′1* Sequence

Full-length *PP2AB′1* complementary DNA was obtained by RT-PCR using SuperScriptIII (Invitrogen) and 3′ and 5′ RACE methods using the GENE Racer Kit (Invitrogen) according to the manufacturer's instructions. The primers were designed on the available *Mtr11959.1* EST and are listed in Supplemental Table S1. PCR and nested PCR were performed with the DNA polymerase Phusion (New England Biolabs) using primers P1/GeneRacer 5′ primer and P2/GeneRacer 5′ nested primer for the 5′ RACE and P3/oligo(dT) primers and P3/GeneRacer 3′ primer for 3′ RACE. Fragments obtained were subsequently cloned into the pENTR Zero Blunt Topo vector (Invitrogen) and sequenced. Full-length *PP2AB′1* complementary DNA or genomic sequences were amplified using primers P4/P5.

Gene Expression Analyses

To monitor gene expression, RNAs were extracted from root tissue with the Plant RNeasy Kit (Qiagen). RNAs were treated with TURBO DNA-free (Ambion), and 500 ng of RNA was retrotranscribed using SuperScriptII reverse transcriptase (Invitrogen). Gene expression was monitored by SYBR Green-based quantitative PCR on a Bio-Rad thermocycler using gene-specific primers. For the reactions, *ELONGATION FACTOR-1α* (*EF-1α*) or *Ubiquitin* expression was used for normalization. The primers used (P15–P28) are listed in Supplemental Table S1.

Identification of Homozygous *pp2aB′1* Mutant Lines

DNA was extracted with the DNeasy 96 Plant Kit (Qiagen). Genotyping of segregating seedling populations was performed by PCR using the following primer combinations: *Tnt1* insertions were identified with P6/P7 for *PP2AB′1-1* and P6/P9 for *PP2AB′1-2*, and the wild type was identified with P6/P8. RT-PCR to amplify *PP2AB′1* mRNA was performed using primers P4/P5. The primers used are listed in Supplemental Table S1.

Agrobacterium rhizogenes-Mediated Gene Transfer

The *A. rhizogenes*-mediated gene transfer was performed according to Boisson-Dernier et al. (2001) using *A. rhizogenes* strain AR1193 or ARquaI (Stougaard et al., 1987). The *A. rhizogenes* strain AR1193 was transformed with the constructs generated as follows. The RNAi *PP2AB′1* construct was produced by amplification of a 105-bp fragment of *PP2AB′1* EST with the primers P10/P11. The amplified product was subcloned into pENTR/D-TOPO (Invitrogen) and subsequently into the RNAi vector pK7GWIWG2D(II)R as described previously (Capoen et al., 2011) by gateway reaction using the LR clonase mix (Invitrogen). The construct pPP2AB′1:gPP2AB′1 used to complement the *pp2aB′1* mutant was generated by amplification of the 3-kb promoter region of *PP2AB′1* and its genomic sequence with the primers P12/P13. The amplified product was subcloned into pDONR207 (Invitrogen) by gateway reaction using the BP clonase mix (Invitrogen) and subsequently transferred into the vector pK7RD v/o 35S as described previously (Groth et al., 2010) by gateway reaction using the LR clonase mix (Invitrogen). The pPP2AB′1:GUS construct was generated by amplification of the 3-kb upstream region of gPP2AB′1 with the primers P12/P14, cloned into pDONR207 by gateway reaction using the BP clonase mix (Invitrogen) and subcloned into the vector pKGWFS7 (Karimi et al., 2002) by gateway reaction using the LR clonase mix (Invitrogen). The *A. rhizogenes* strain ARquaI was transformed with the construct containing *ABI1-1* that was described previously (Ding et al., 2008). The primers are listed in Supplemental Table S1.

Plant Growth Conditions and AM and Nodulation Assays

Plants were grown in controlled-environment rooms at 22°C (80% humidity, 16-h photoperiod, and 300 μmol m^{-2} s^{-1}). To monitor AM root length colonization, plants were grown in sterilized Terragreen/Sand (Oil-Dri) and inoculated with *Rhizophagus irregularis* (Endorize) to the ratio Terragreen:sand:spore-containing substrate 5:5:1. To monitor AM root length colonization in response to ABA or in overexpressing *ABI1-1* transformed roots, plants were grown in sterilized Terragreen/Sand (Oil-Dri) to a ratio of 1:1 and inoculated with 500 *R. irregularis* spores (Symplanta). To reduce variation in AM colonization, biological replicates were initiated 4 d apart using the same fungal inoculum and grown under the same conditions. When treated this way, we found little variance between biological replicates. Another adaption that reduced variance between experiments was to increase the number of wild-type plants in each experiment. At different time points after inoculation, we screened colonization levels in wild-type plants and only initiated the full experiment when a sufficient level of colonization had occurred in wild-type plants. This reduced variance between different experiments, although we were not able to perform this prior control when performing experiments with *A. rhizogenes*-transformed roots. The fungal structures were stained in acidic ink as follows: roots were cleared in 10% (w/v) KOH for 15 min at 95°C, washed three times in water, and subsequently stained in acidic ink (5% [v/v] ink and 5% [v/v] acetic acid) for 4 min at 95°C. The AM root length colonization was quantified using the grid intersect method (Giovanetti and Mosse, 1980). To monitor the development of AM symbiosis on the *pp2aB′1* mutant and wild-type ecotype R108, 7-d-old plants grown on buffered nodulation medium (BNM) plates (Ehrhardt et al., 1992) were planted in sterilized Terragreen/Sand (Oil-Dri) with 800 *R. irregularis* spores from soil substrate exposed to chive (*Allium schoenoprasum*) roots for 8 weeks. Plants were harvested at 3, 6, 8, and 12 dpi and acidic ink stained as described previously. For the 3-, 6-, and 8-d inoculations, the root length was measured and the infection progression was evaluated with the Leica DM6000 microscope. For nodulation assays, 1-week-old plants were grown in Terragreen/Sand (Oil-Dri) to a ratio of 1:1 and inoculated with approximately 4 mL of *Sinorhizobium meliloti* 2011 diluted with water to optical density at 600 nm = 0.001. Nodules were scored after 19 d.

GUS and Wheat Germ Agglutinin Staining

Transformed roots were stained for GUS activity with 5-bromo-4-chloro-3-indolyl-β-D-glucuronide for 3 h at 37°C in the dark according to Jefferson et al.

(1987) and fixed. AM fungal structures were subsequently stained with 0.2 μM mL^{-1} wheat germ agglutinin Alexa Fluor 594 conjugate (Invitrogen) for 6 h (Ivashuta et al., 2005). The Leica DM6000 microscope was used for inspection and documentation.

Acetylene Reduction Assay

Nitrogenase activity was determined by assaying acetylene reduction. Acetylene reduction of nodulated root systems was measured in 2-mL sealed vials in the presence of 10% acetylene. After 2 h of incubation, 10 μL of acetylene was injected in a Photovac 10S Plus gas chromatograph to assess ethylene production. Samples were compared with a standard curve generated against a 5 μL L^{-1} ethylene standard, and nitrogenase activity was expressed as acetylene reduction assay units: ethylene products/(time [h] × nodule numbers).

ABA Activation of Lateral Root Formation

Wild-type ecotype R108 and mutant seeds were scarified, surface sterilized, and sown on 0.8% (w/v) water-agar medium. Seeds were cold treated at 4°C in the dark for 4 d and then moved to room temperature in the dark to germinate for 24 h. Germinated seeds were transferred to BNM (Ehrhardt et al., 1992) supplied with different concentrations of ABA (Sigma; A1049). The lateral roots that developed were scored after 3 weeks of growth in controlled-environment rooms at 23°C (16-h photoperiod and 300 μmol m^{-2} s^{-1}).

ABA Inhibition of Seed Germination

Wild-type ecotype R108 and mutant seeds were scarified, surface sterilized, and sown on BNM supplemented with different concentrations of ABA (Sigma; A1049). Seeds were cold treated at 4°C in the dark for 4 d and then moved to room temperature in the dark. Germination was assessed after 24 h.

Water Loss Assay

Water loss was measured from detached leaves of wild-type ecotype R108 and mutants. For each biological replicate, 10 leaves from 4-week-old plants grown in controlled-environment rooms at 22°C (80% humidity, 16-h photoperiod, and 300 μmol m^{-2} s^{-1}) were cut and weighed over time. The assay was performed at room temperature (approximately 23°C) under dim-light conditions with 70% relative humidity. The water loss was calculated as the percentage fresh weight at each time point.

Root Growth Response to ACC

Root growth response and hypocotyl growth were assessed in wild-type R108 and mutant *pp2aB′1-1*. For each biological replicate, 10 plants were grown on BNM (Ehrhardt et al., 1992) supplemented with 0, 5, 25, or 100 μM ACC (Sigma; A3903). The lengths of the roots and hypocotyls were measured after 5 d.

Calcium Spiking Analyses

For analysis of mutants, we microinjected root hair cells with calcium-responsive dyes as described (Wais et al., 2000). Micropipettes were pulled from filamented capillaries on a pipette puller (model 773; Campden Instruments). These were loaded with Oregon Green dextran (10,000 molecular weight; Molecular Probes) and Texas Red dextran (10,000 molecular weight; Molecular Probes). Injections were performed using iontophoresis with currents generated from a cell amplifier (model Intra 767; World Precision Instruments) and a stimulus generator made to our specifications (World Precision Instruments). Cells were analyzed on an inverted epifluorescence microscope (model TE2000; Nikon) using a monochrometer (model Optoscan; Cairns Research) to generate specific wavelengths of light. During image capture, the image was split using the Optosplit (Cairn Research), and each image passed through a filter for either Oregon Green or Texas Red emissions prior to exposure on the CCD chip (model ORCA-ER; Hamamatsu). Data were analyzed using Metaflor (Universal Imaging).

Sequence data from this article can be found in the GenBank/EMBL data libraries under accession number KC859637.

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Figure S1. Quantification of AM colonization in wild type after 4 weeks of inoculation with *R. irregularis* and external application of 0, 5, or 50 μM ABA.

Supplemental Figure S2. Arbuscule formation in the wild type and *pp2aB'1* is not altered by external application of 50 μM ABA.

Supplemental Figure S3. ABA (50 μM) impaired NS-LCO-induced calcium spiking.

Supplemental Figure S4. AM colonization of transformed roots expressing the Arabidopsis dominant-negative allele of *abi1-1* in the wild type at 7 weeks post inoculation.

Supplemental Figure S5. Expression analyses of *M. truncatula* Ser/Thr protein phosphatase *PP2A* a, b, and c units.

Supplemental Figure S6. Alignment of PP2AB, B', and B'' proteins from *M. truncatula,* Arabidopsis, and rice.

Supplemental Figure S7. Regulation of *PP2AB'1* expression by AM.

Supplemental Figure S8. Complementation of *pp2aB'1-1* mutant.

Supplemental Figure S9. *PP2AB'1* expression is modulated by ABA in the absence of arbuscular mycorrhiza.

Supplemental Figure S10. ABA (50 μM) impaired *PP2AB'1* expression in mycorrhized roots.

Supplemental Figure S11. Downregulation of *PP2AB'1* impairs AM colonization.

Supplemental Figure S12. QRT-PCR analyses of *PP2AB'1* expression in WT and mutant alleles *pp2aB'1-1* and *pp2aB'1-2*.

Supplemental Figure S13. AM colonization of WT and *pp2aB'1* roots at 6 weeks post inoculation with *R. irregularis*.

Supplemental Figure S14. AM fungus propagation in *pp2aB'1-1* mutant roots after 6 dpi with *R. irregularis* spores.

Supplemental Figure S15. AM fungus propagation in *pp2aB'1-1* mutant roots 6 dpi with *R. irregularis* spores.

Supplemental Figure S16. Quantification of nitrogenase activity responsible for N_2 fixation using reduction of actelylene to ethylene.

Supplemental Figure S17. Quantification of AM colonization in *pp2aB'1-1* mutant transformed with *abi1-1* or empty vector.

Supplemental Figure S18. Sensitivity of wild-type and *pp2aB'1-1* seedlings to exogenous ACC.

Supplemental Figure S19. Expression of *PP2AB'1* during fungal propagation.

Supplemental Figure S20. Average ratio of arbuscules formed per penetration event at 6 and 8 dpi.

Supplemental Figure S21. Drought stress represses *PP2AB'1* expression.

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