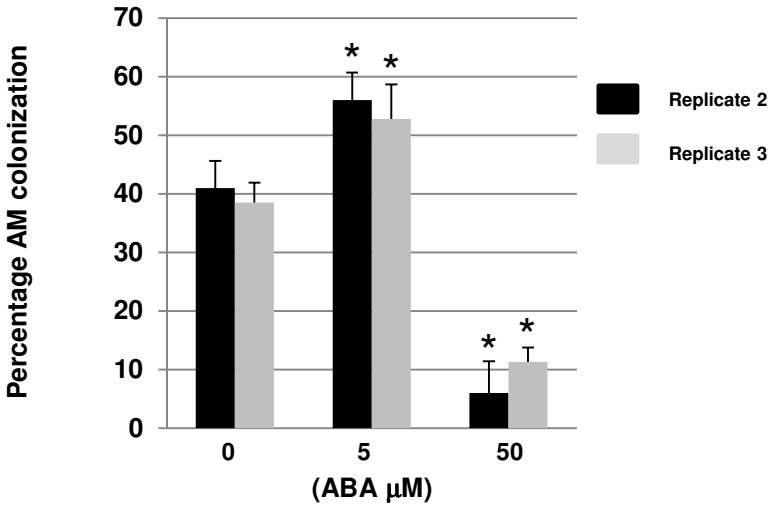
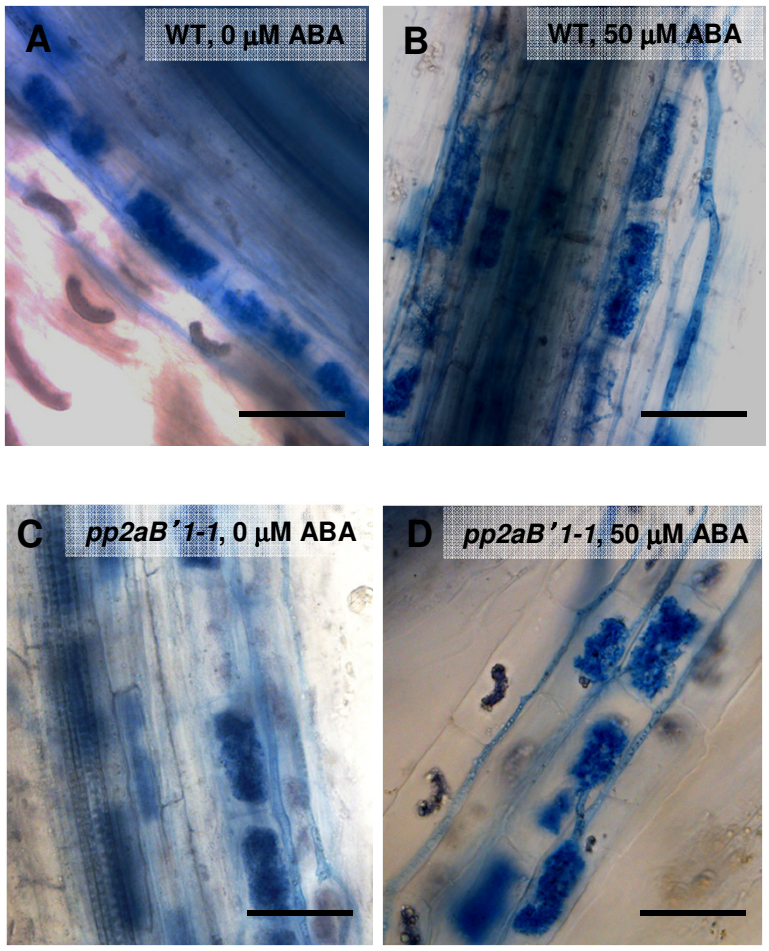


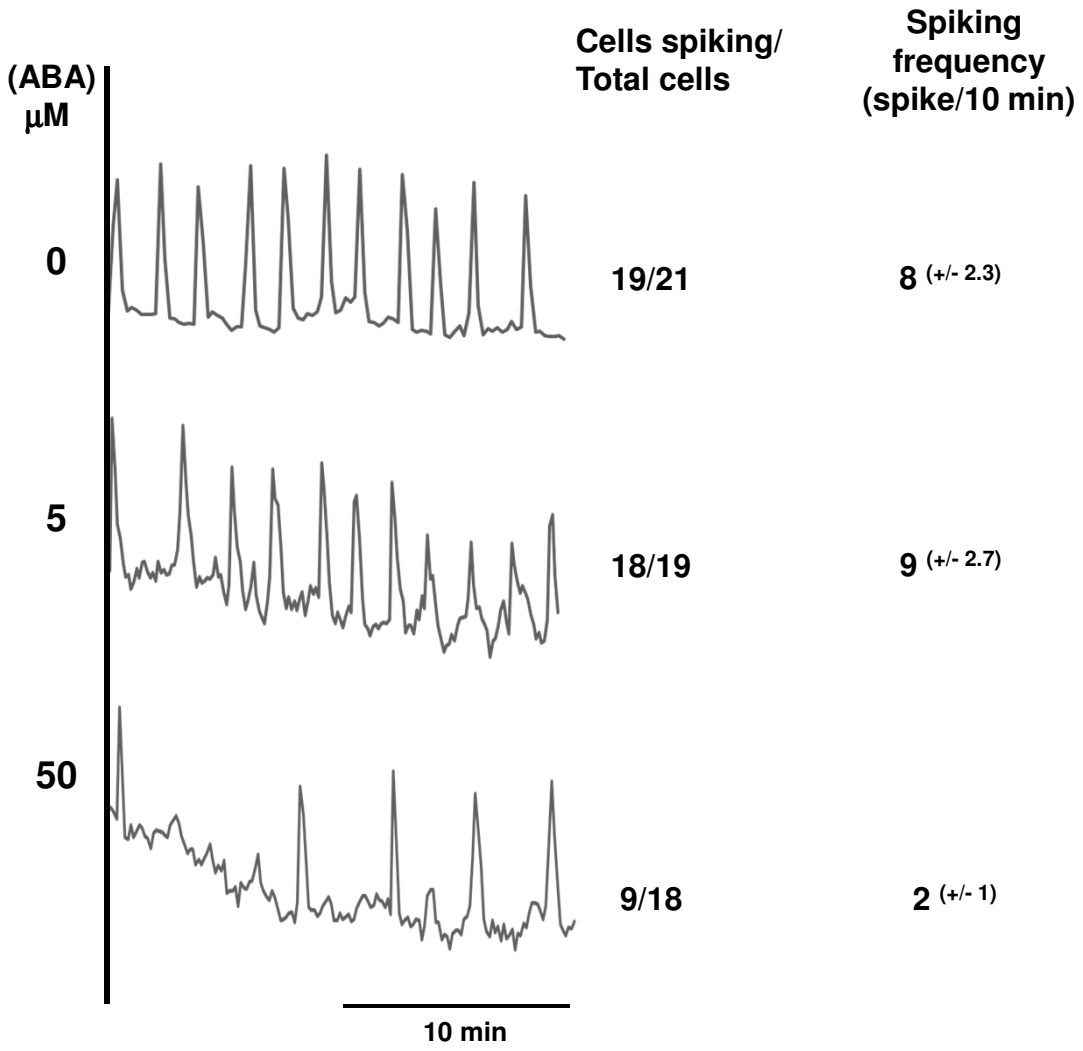
Supplemental Figure 1. Quantification of AM colonization in wild type after 4 weeks inoculation with *R. irregularis* and external application of 0, 5 or 50 µM ABA.

Biological replicates 2 and 3. Values are means ± SE (n=10 for each replicate). Asterisks indicate significant differences between the ABA treated roots (5 and 50 µM ABA) and the control (0 µM ABA), Student's *t* test; $P \leq 0.01$.



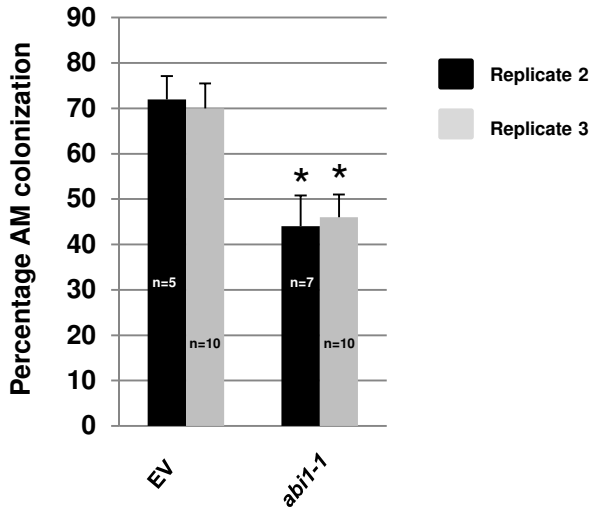
Supplemental Figure 2. Arbuscule formation in wild type and *pp2aB´1* is not altered by external application of 50 µM ABA.

Bright field image of arbuscules formed in wild type and *pp2aB´1-1* roots 4 weeks after *R. irregularis* inoculation. The plants were watered with 0 µM ABA (A) and (B), or with 50 µM ABA (C) and (D). The fungal structures are ink stained. Bar= 49 µm.

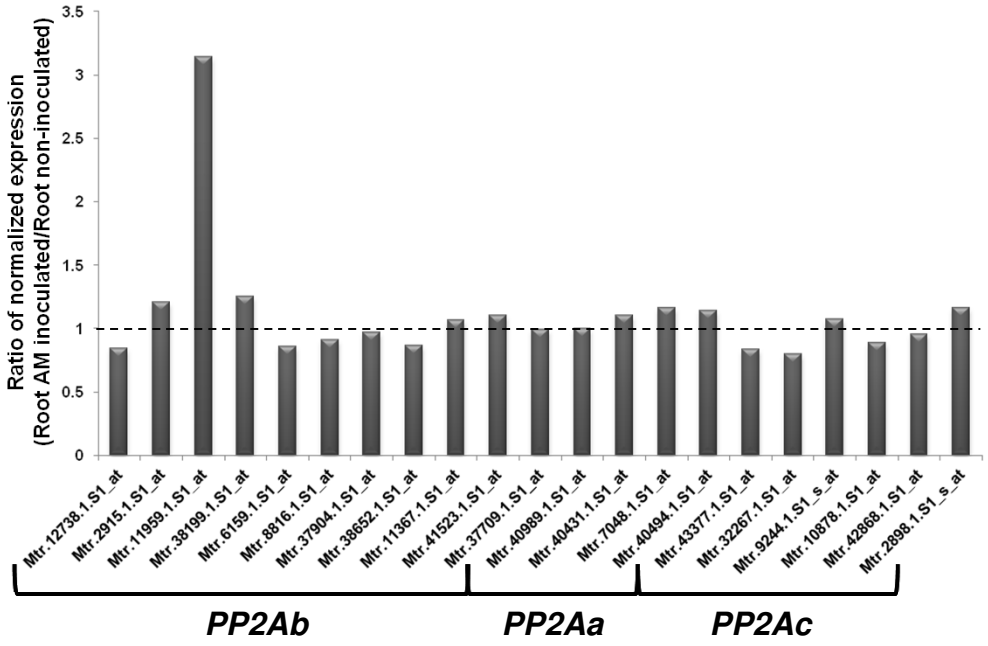


Supplemental Figure 3. 50 µM ABA impaired NS-LCO-induced calcium spiking.

Calcium spiking in response to 10^{-5} M non-sulfated Myc LCO was assessed in plants grown on BNM medium supplied with 0, 5 or 50 µM ABA. The numbers indicate the numbers of cells showing calcium spiking, relative to the total number of cells analysed and the frequency of spikes within 10 min including the standard deviation in brackets.

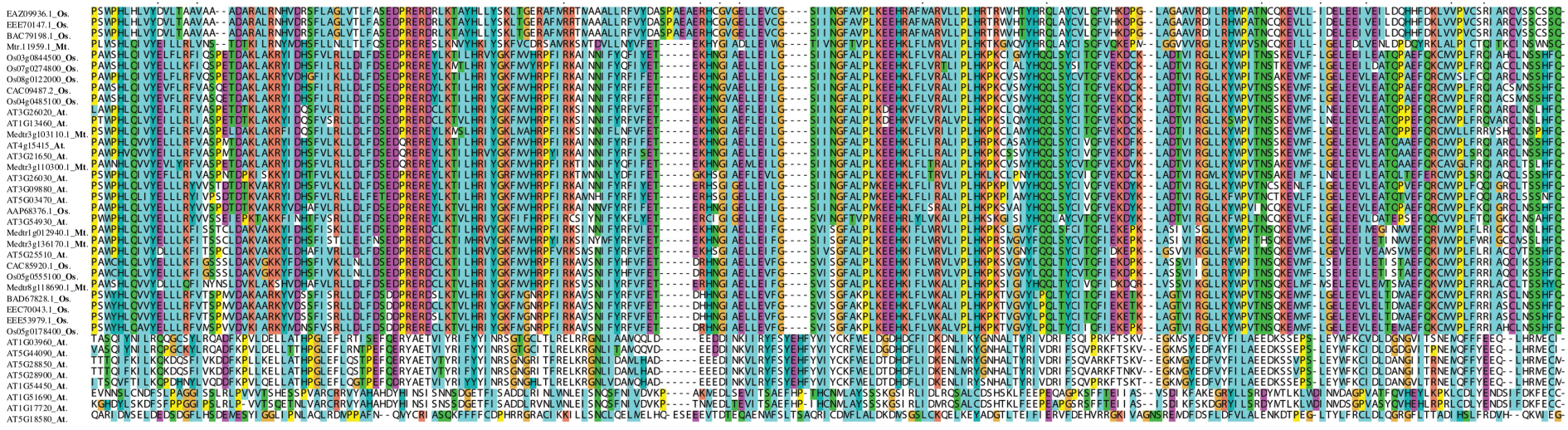


Supplemental Figure 4. AM colonization of transformed roots expressing the *A. thaliana* dominant-negative allele of *abscisic acid insensitive 1-1 (abi1-1)* in wild type (WT) at 7 weeks post inoculation. Biological replicates 2 and 3. Values are means ± SE with number of plants (n) as indicated. The asterisks indicate significant difference from the wild type transformed with the empty vector (EV), (Student's *t* test; $P \leq 0.01$).



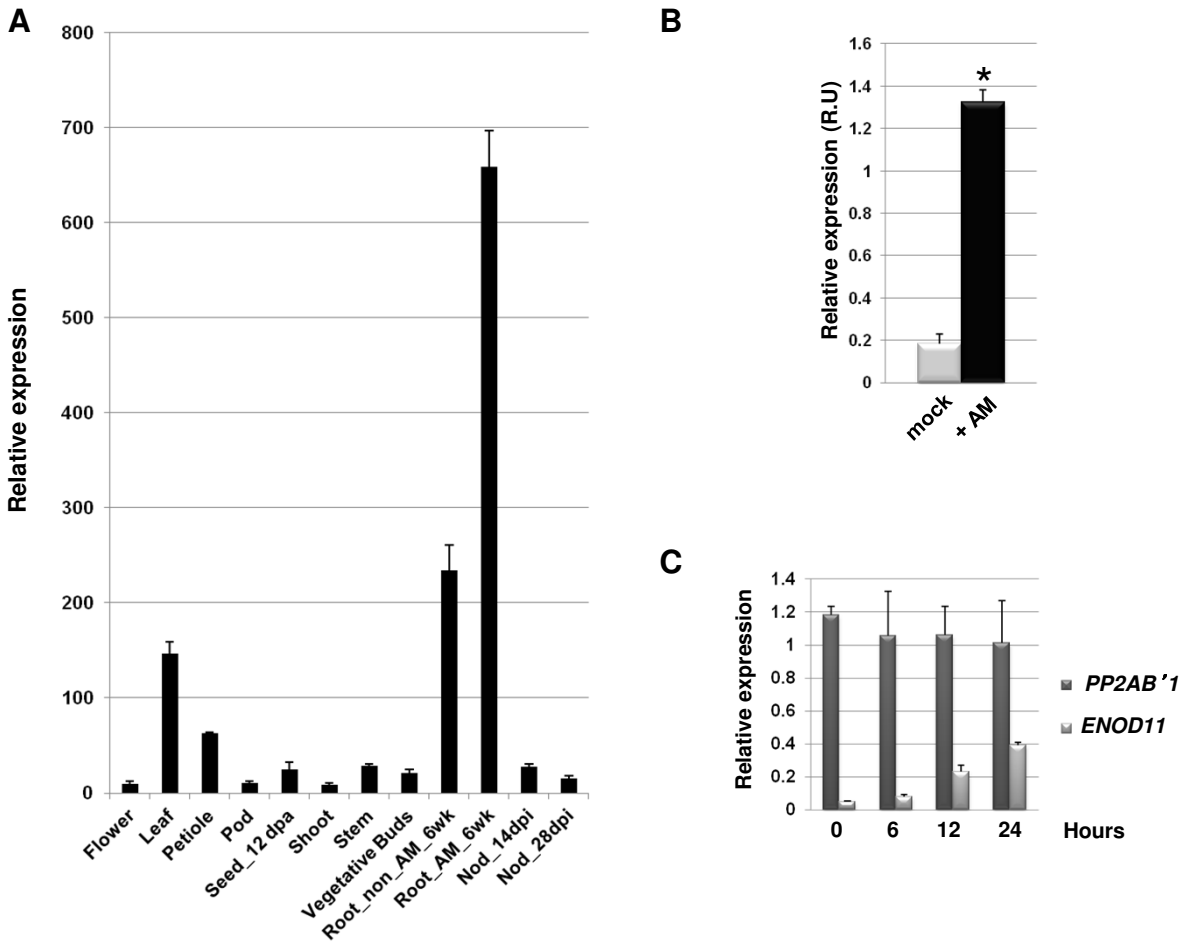
Supplemental Figure 5. Expression analyses of *M. truncatula* Ser/Thr protein phosphatase *PP2A* a, b and c units.

Expression analyses based on publically available microarray data from *M. truncatula* gene expression atlas (MtGEA; http://mtgea.noble.org/v3/). The ratio of normalized average expression of each probe was assessed in roots after 6 weeks of *R. irregularis* inoculation versus non inoculated roots, both samples supplied with 20 µM phosphate. Probe set numbers correspond to unique GenBank identifiers in Table S2.



Supplemental Figure 6. Alignment of PP2AB, B' and B'' proteins from *M. truncatula*, *A. thaliana* and *O. sativa.*

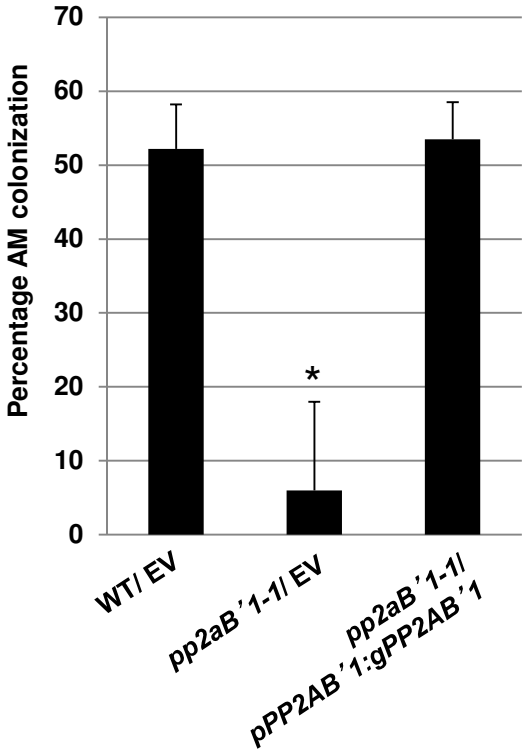
Multiple sequence alignment was performed using the Clustalw (version 1.83) program and edited using Bioedit sequence alignment editor. The alignment is coloured according to amino acid physicochemical properties. The alignment was used to generate the phylogenetic tree in Figure 2. GenBank/UniProt accessions and species acronyms are indicated to the left of the sequences.



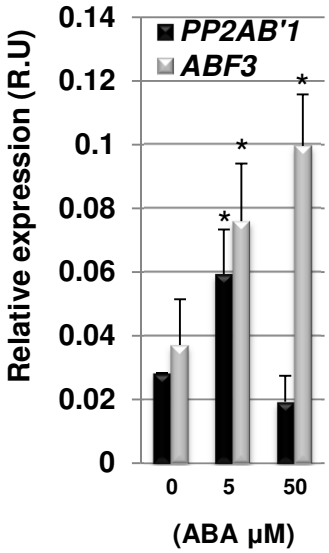
Supplemental Figure 7. Regulation of *PP2AB´1* expression by arbuscular mycorrhiza (AM)

A, Microarray analyses of *PP2AB´1* expression. Normalized expression of *PP2AB´1* probe (Mtr.11959.1.S1_at) in different tissues including seeds excised from pods 12 days after pollinisation (Seed_12 dpa), wild type roots after 6 weeks inoculation with *R. irregularis* (Root_AM_6wk) and nodules after 14 and 28 days post inoculation with *S. meliloti* (Nod_14dpi and Nod_28 dpi). Data from MtGEA; http://mtgea.noble.org/v3/. B, Quantitative RT-PCR to monitor *PP2AB'1* expression in wild type *M. truncatula* roots infected with *R. irregularis* (AM) (80% root length colonized) or non inoculated (Mock) after 4 weeks. The expression is normalized to *polyubiquitin* (*UBI*) level. R.U; Relative Unit. Values are means ± SE from three biological replicates. Asterisk indicates statistical significance relative to the mock samples (Student's *t* test; $P \leq 0.01$).

C, QRT-PCR analyses of *PP2AB'1* expression in response to 10^{-8} M Nod factor treatment after 6, 12, and 24 hours. *ENOD11* expression is used as a marker for Nod factor induction. Expression is normalized to *UBI* levels. Mean values and SD derived from three biological replicates.

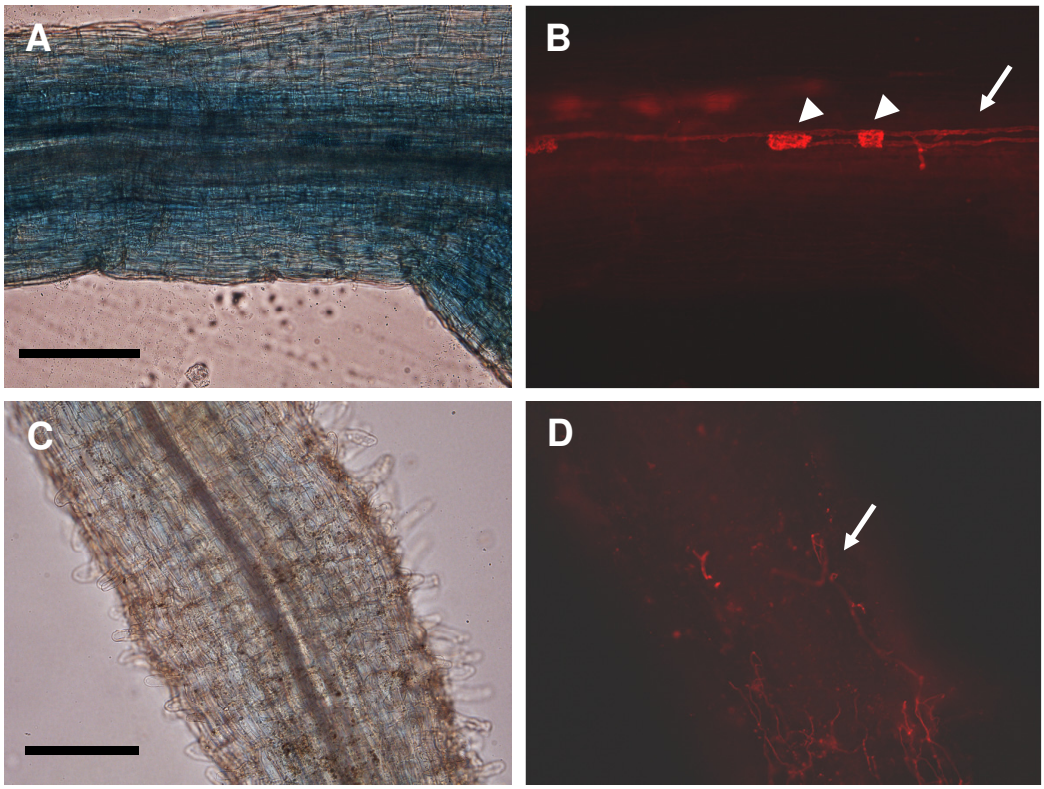


Supplemental Figure 8. AM colonization of *pp2aB'1-1* transformed roots complemented with *PP2AB'1* genomic sequence driven by its own promoter (*pPP2AB'1:gPP2AB'1)* or empty vector (EV), and wild-type (WT) transformed roots with the empty vector (EV) at 5 weeks post inoculation with *R. irregularis*. Values are means ± SE (n=10). Asterisks indicate statistical significance relative to the WT/EV control (Student's *t* test; $P \leq 0.0001$).



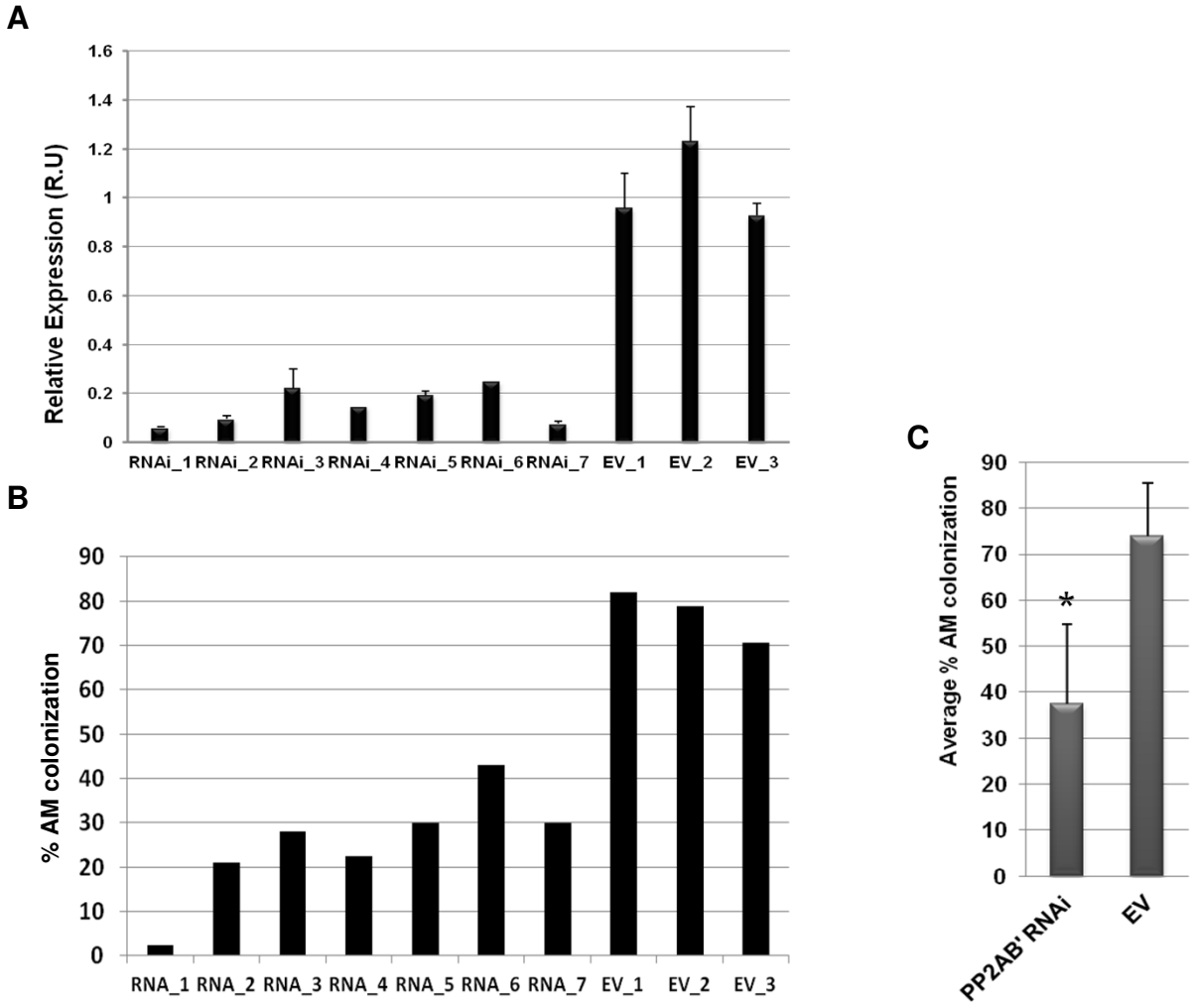
Supplemental Figure 9. *PP2AB'1* expression is modulated by ABA in the absence of arbuscular mycorrhiza.

Quantitative RT-PCR to monitor *PP2AB'1* and *ABF3* expressions in roots watered with 0, 5 or 50 µM ABA. Asterisks indicate statistical significance relative to the wild type watered with 0 µM ABA (Student's *t* test; $P \leq 0.01$). Expression is normalized to *EF-1α*, R.U; relative unit.



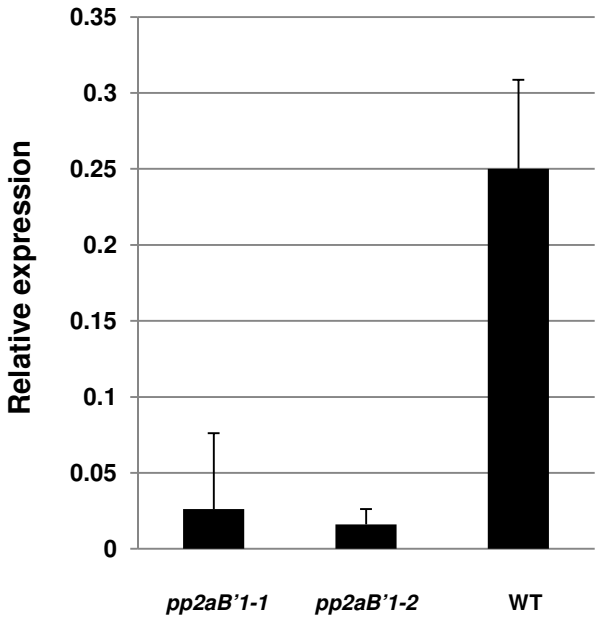
Supplemental Figure 10. 50 µM ABA impaired *PP2AB´1* expression in mycorrhized roots.

A and C, Promoter GUS activity in *A. rhizogenes* transformed roots expressing *PP2AB´1* promoter-*UidA* fusions in roots inoculated with *R. irregularis* for 4 weeks and water with 0 µM ABA (A) or 50 µM ABA (C). B and D, Fluorescent microscopy images of the corresponding bright field image (A) and (C) respectively. *R. irregularis* is stained with WGA-Alexafluor 594 which fluoresces red. Scale bar = 100 µm. White arrow, fungal hyphae; arrow head, arbuscule.



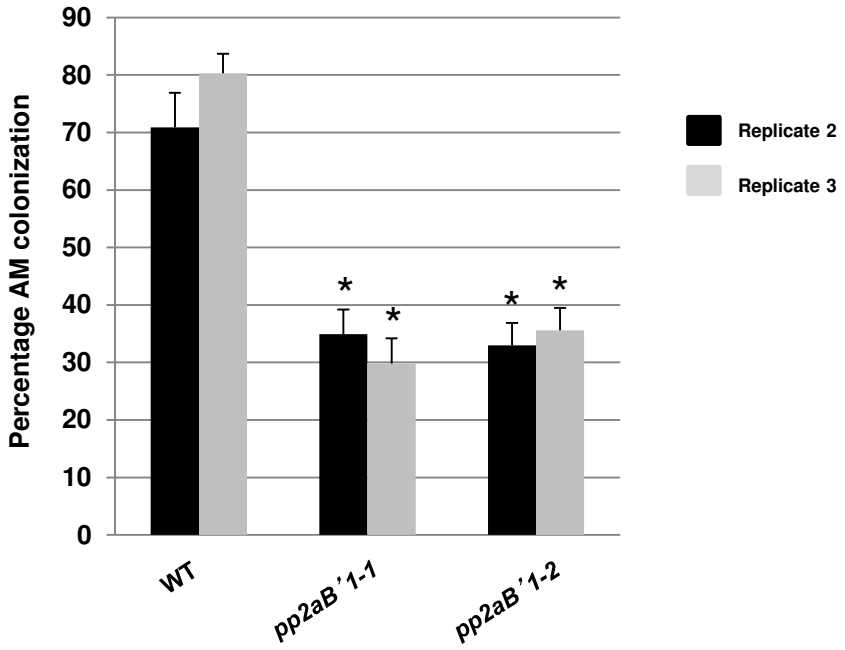
Supplemental Figure 11. Downregulation of *PP2AB´1* impairs AM colonization.

A, Relative expression of *PP2AB´1* in seven individual transgenic root systems expressing an RNA interference construct targeting *PP2AB´1* (RNAi 1 to 7) or the empty vector (EV 1 to 3). The expression is significantly silenced in comparison to the EV control for each silenced root system (Value are mean ±SD of three technical replicates, Student's *t* test ; P<0.05). Expression is normalized to *EF1-α* levels. B, Percentage of AM colonization in the seven individual transgenic roots (A) expressing the RNAi construct targeting *PP2AB´1* or the empty vector (EV) 5 weeks after *R. irregularis* inoculation. The reduced level of AM colonization in individual plants correlates with the silencing level of *PP2AB'1*, Pearson correlation coefficient r=0.9. C, Average of AM colonization in transgenic roots expressing the RNAi construct targeting *PP2AB´1* or the empty vector (EV) 5 weeks after *R. irregularis* inoculation. Mean and SD values were calculated for 12 transgenic root systems expressing *PP2AB´1* RNAi and 10 expressing the empty vector. Asterisk indicates significant differences to control level (Student's *t* test ; P<0.05).

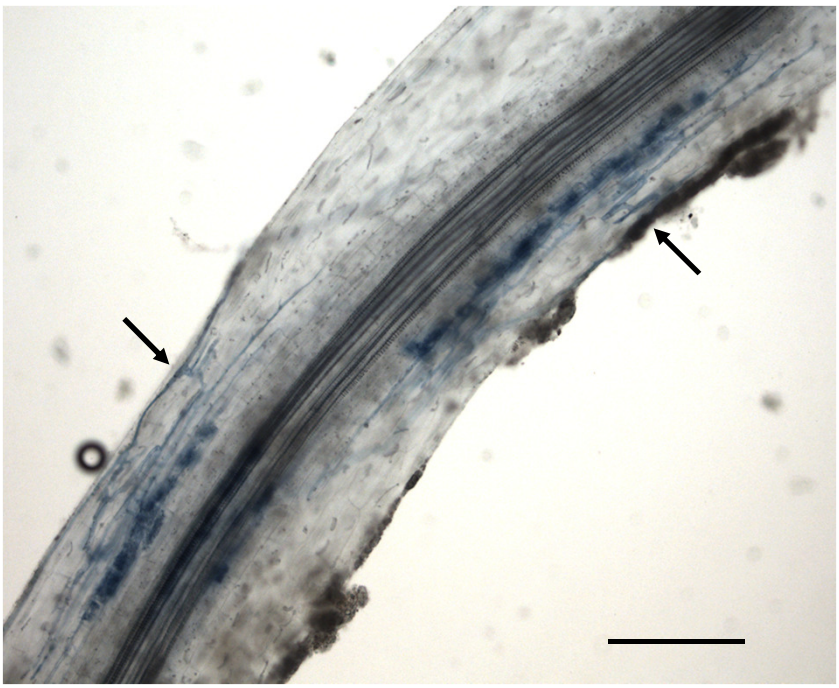


Supplemental Figure 12. QRT-PCR analyses of *PP2AB'1* expression in WT and mutant alleles *pp2aB'1-1* and *pp2aB'1-2.*

Expression is normalized to *EF1-α* levels. Mean values and SD derived from three biological replicates.

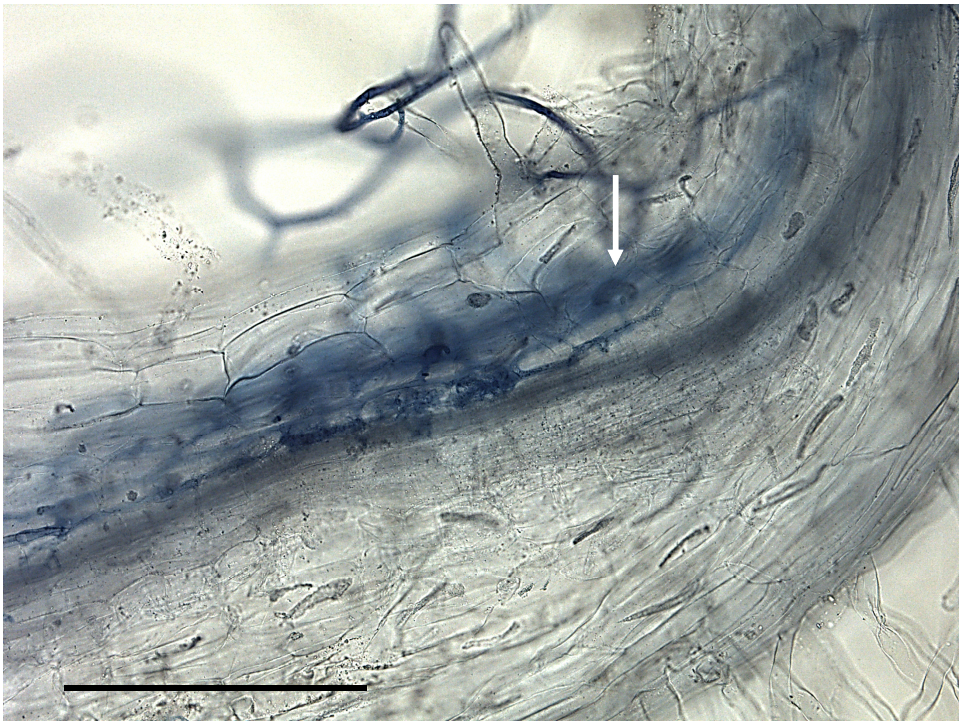


Supplemental Figure 13. AM colonization of WT and *pp2aB'1* roots at 6 weeks post inoculation with *R. irregularis*. Values are means ± SE (n=8). The colonization between WT and *pp2aB'1* mutant lines differs significantly as indicated by asterisks (Student's *t* test, $P \leq 0.0001$).



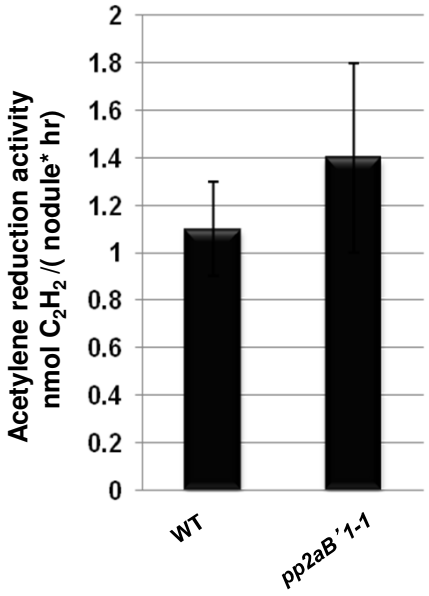
Supplemental Figure 14. AM fungus propagation in *pp2aB′1-1* mutant roots after 6 days post inoculation with *R. irregularis* spores.

The picture illustrates two penetration events (black arrow) with arbuscules. The AM fungus is stained with ink. Scale bar = 147 µm

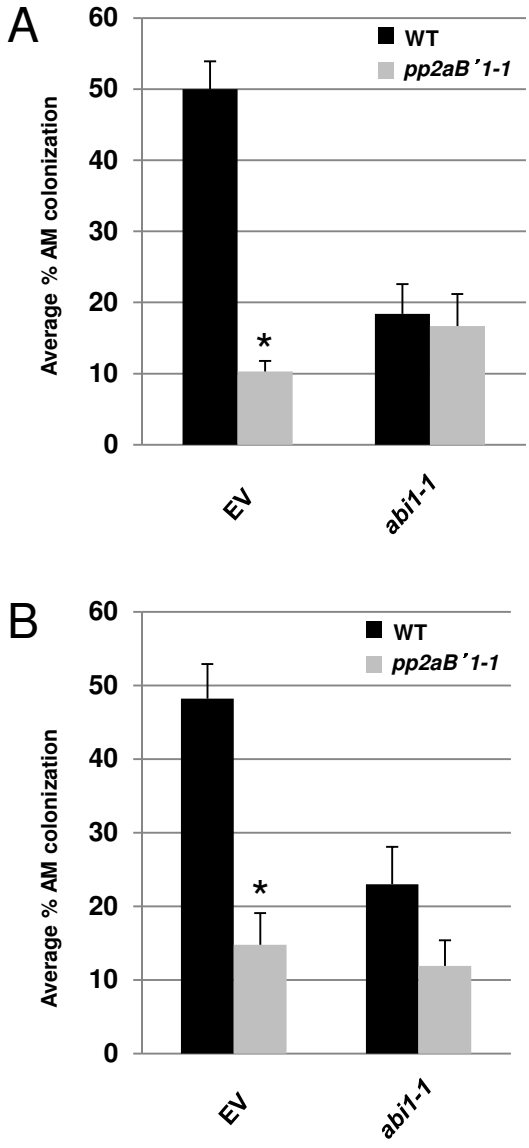


Supplemental Figure 15. Enlargement of Figure 1D showing AM fungus propagation in *pp2aB´1-1* mutant roots 6 days post inoculation with *R. irregularis* spores.

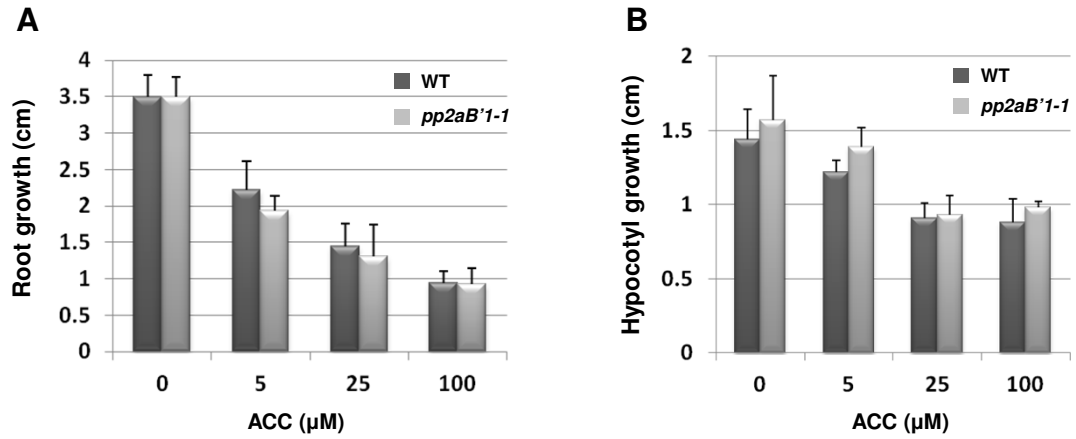
The picture illustrates a penetration event with one vesicle (white arrow) and arbuscules. The AM fungus is stained with ink. Scale bar = 200 µm



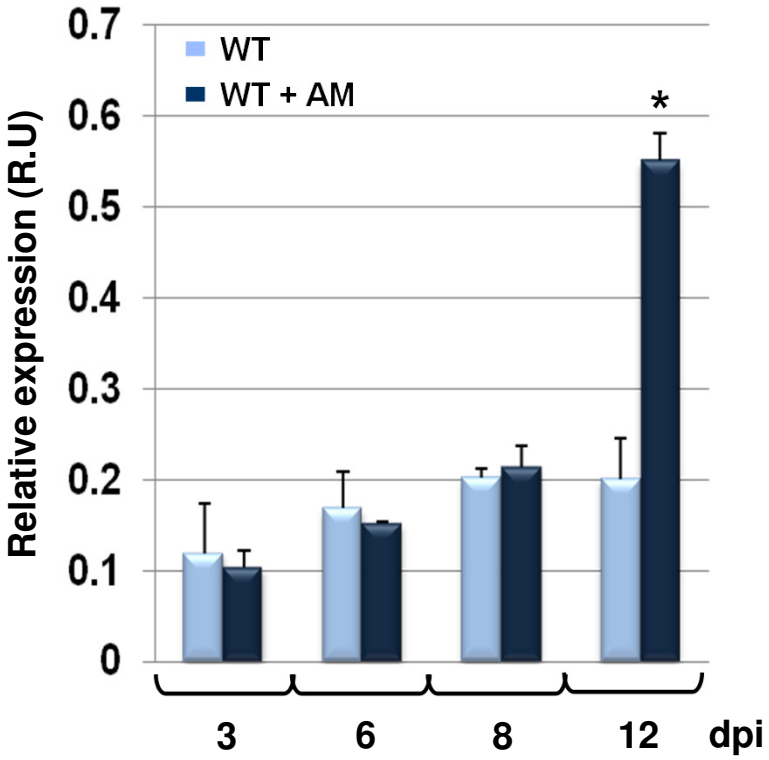
Supplemental Figure 16. Quantification of nitrogenase activity responsible for N_2-fixation using reduction of actelylene to ethylene. Values are means ± SD from 10 plants.



Supplemental Figure 17. Quantification of AM colonization in *pp2aB'1-1* mutant transformed with *abi1-1* or empty vector (EV) at 5 weeks post inoculation with *R. irregularis*. A, Biological replicate 2. B, Biological replicate 3. Values are means ± SE with n=8. Asterisks indicate significance in comparison to the WT, Student's *t* test ; $P \leq 0.001$)

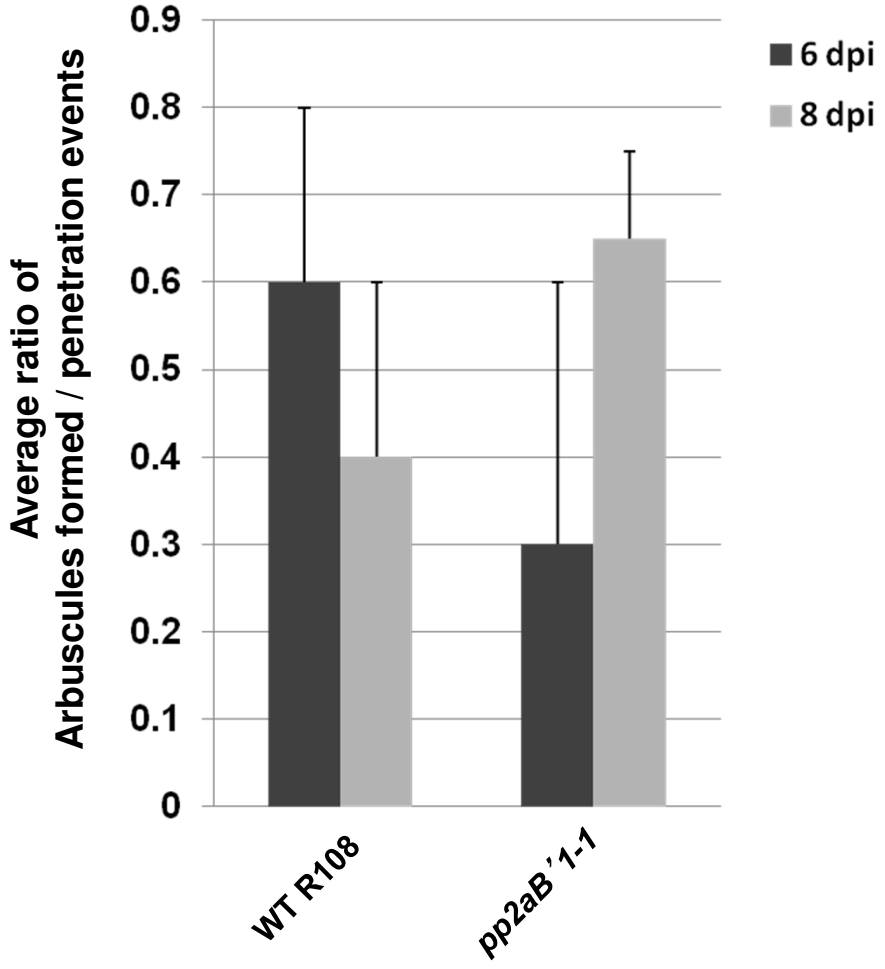


Supplemental Figure 18. Sensitivity of wild-type (WT) and *pp2aB'1-1* seedlings to exogenous 1-aminocyclopropane carboxylic acid (ACC). A**,** Root growth response of *pp2aB'1-1* and WT to ACC after 5 days. Error bar represents standard deviation, n=11. B, Hypocotyl growth response of *pp2aB'1-1* and WT to ACC after 5 days. Error bar represents standard deviation, n=11.

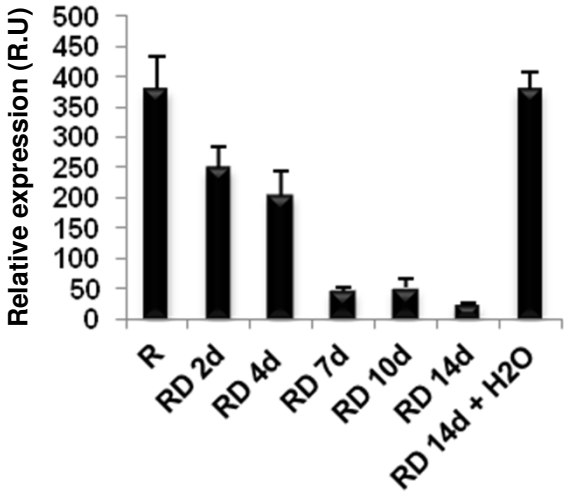


Supplemental Figure 19. Expression of *PP2AB´1* during fungal propagation.

Quantitative RT-PCR to monitor *PP2AB'1* expression in the wild type *M. truncatula* roots infected with *R. irregularis* (WT+AM) and non inoculated roots. The expression is normalized to *EF-1a* level. The level of AM colonization of the WT over time is presented in Figure 7. Asterisk indicates statistical significance relative to the wild type non inoculated roots (Value are mean ± SD from three biological replicates, Student's *t* test; $P \leq 0.01$).



Supplemental Figure 20. Average ratio of arbuscules formed per penetration event at 6 and 8 days post inoculation. Mean value and SD derived from three biological replicates.



Supplemental Figure 21. Drought stress represses *PP2AB´1* expression.

PP2AB´1 expression in roots (R) or in roots after 2, 4, 7, 10 and 14 days of drought stress (RD), and in roots after 14 days drought stress and re-watered 1 day (RD 14d + H2O). (*PP2AB´1* Probe: Mtr.11959.1.S1_at, MtGEA; http://mtgea.noble.org/v3/).