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Material and Methods

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Material and methods

1. Fungal and plant material

Piriformospora indica Sav. Verma, Aj. Varma, Rexer, G. Kost & P. Franken (DSM11827, Deutsche Sammlung von Mikroorganismen und Zellkulturen, DSMZ, Braunschweig, Germany) cultures were propagated at 28°C in liquid complete medium (CM) (1) with 2% glucose and 130 rpm shaking or on solid medium with 1.5% agar. Hygromycin B (80 µg/ml) was supplemented for growth of *P. indica* transformants. For microarray experiments *P. indica* was cultivated on 1/10 PNM plates (2) for 3 days. 1/10 PNM final concentrations: 0.5 mM KNO_3, 0.367 mM KH_2PO_4, 0.144 mM K_2HPO_4, 2 mM $MgSO_4$ x H_2O, 0.2 mM $Ca(NO_3)_2$, 0.25% (v/v) Fe-EDTA (0.56% w/v $FeSO_4$ x $7H_2O$ and 0.8% w/v Na_2EDTA x $2H_2O$), 0.428 mM NaCl; pH-adjusted to 6.0 and buffered with 10 mM MES. For solid media, 0.4% (w/v) GELRITE (Duchefa) was added.

Barley seeds *(Hordeum vulgare* cv. Golden Promise, Ingrid or Ingrid *mlo*-5 mutants I22, kindly provided by Gregor Langen) were surface sterilized with 70% ethanol for 1 minute, 12% sodium hypochlorite for 1.5 hours and washed with sterile distilled water for 3 hours. Sterilized seeds were kept in the dark for 3 days on sterile wet filter paper at room temperature. For colonization studies, three-day-old barley seedlings were transferred into sterile jars containing 1/10 PNM medium and inoculated with 3 ml chlamydospore suspension (5×10^5 ml^{-1} in 0.002% TWEEN20). Incubation was performed in a Conviron phytochamber with a day/night cycle of 16/8 hours (light intensity: 110 µmol m^{-2} s^{-1}) at a temperature of 22/18°C. Tween water (0.002% TWEEN20) treated seedlings were used as control if required. Root samples were collected at different time points as described in the figure legends and carefully washed in distilled water. The first 4 cm of the roots, starting from the seed, were excised and immediately frozen in liquid nitrogen or used for microscopy. All experiments were prepared in 3 to 4 independent biological repetitions of four plants per jar.

Arabidopsis thaliana seeds (ecotype Columbia-0) were incubated for 5 min in 70% ethanol, surface sterilized for 5 min with 6% sodium hypochlorite and washed 6 times for 5 min in sterile water. After stratification for 3 days at 4°C in the dark on 1/10 PNM medium, Arabidopsis seedlings were grown for 14 days under sterile conditions in a phytochamber (Vötsch, Balingen-Frommern, Germany) at long day conditions (day: 16 h, 23°C, 350 µmol m^{-2} s^{-1}; night: 8 h, 18°C; 60% humidity). For inoculation of Arabidopsis roots with *P. indica* chlamydospores, plants of roughly the same size were first transferred to square petri dishes containing 1/10 PNM and then inoculated directly with either 5 x 10^5 *P. indica* spores per 20 seedlings or mock treated as described for barley. Root material was harvested, washed carefully with distilled water and frozen liquid nitrogen after 3, 7 and 14 days. For each time point roots from 80 to 100 plants were pooled and the experiments were performed in 3 to 4 independent biological repetitions.

Chlamydospores were collected from 3 to 4 week-old CM plates using 0.002% TWEEN20 for all described experiments.

2. Growth promotion assays

For co-culture of *A. thaliana* with *P. indica or P. williamsii* and tests for plant growth promotion, seeds were sterilized by incubation for 5 min in 70% ethanol followed by 2 min in 100% ethanol, left to dry and stratified as

described before either on a modified Hoagland's medium with four times more phosphate: 5mM KNO_3; 5mM $Ca(NO_3)_2$; 2mM $MgSO_4$; 4mM KH_2PO_4; 0.03g/L Sprint 138 iron chelate; 0,1% micronutrients solution containing 2.86g/L H_3BO_3; 1.81 g/L $MnCl_2.4H_2O$; 0.08g/L $CuSO_4.5H_2O$; 0.02 g/L 85% $MoO_3.H_2O$, based on (3) or on 1/10 PNM as described above. Media were solidified with 4g/L GELRITE (Carl Roth, Karlsruhe, Germany). For assays on the modified Hoagland's medium, plants were grown for 7 to 10 days under long day conditions (16h light, at 23°C, 85 µmol m^2 s^1; 60% humidity), and on 1/10 PNM medium plants grew for 10 days (16h light, at 23°C, 350 µmol m^{-2} s^{-1}; 60% humidity; night: 8h, 18°C) before mock-inoculation with tween water or inoculation with either *P. indica* or *P. williamsii* (when indicated) chlamydospores (5 x 10^5 ml^{-1}). The seeds that failed to germinate and the seedlings that did not look healthy were removed from the plates preceding tween water or spore application. Plants grown on modified Hoagland's were inspected for biomass 7 days post inoculation (dpi), and for seedlings grown on 1/10 PNM biomass was inspected 14 dpi. Statistical analyses of the results were performed in R 2.15.1 (4) (http://www.R-project.org/). Pairwise comparisons of the different subsets of data were performed using ANOVA followed by Tukey's HSD (honestly significant difference) on 95% family-wise confidence level. Homogeneous subsets were identified using the package multcompView (5) (http://CRAN.R-project.org/package=multcompView).

Barley growth promotion experiments with *P. indica* WT, empty vector control and RNAi strains were carried out as described in (6).

3. DNA, RNA extraction and real time qPCR analyses

DNA from 200 mg of ground fungal or plant material was isolated using the protocol of Doyle and Doyle (7). Total RNA from 200 mg of ground material was extracted using TRIzol (Invitrogen, Karlsruhe, Germany) following the manufacturer's instructions. The amount and quality of the extracted RNA were estimated by using an agarose gel and the NanoDrop-1000 Spectrophotometer (Agilent) or a 2100 Bioanalyzer (Agilent, Santa Clara, USA) for the microarray experiments. For real-time qPCR analyses, two µg of RNA were used for DNase I digestion (Fermentas) followed by cDNA synthesis (First Strand cDNA Synthesis Kit, Fermentas). Real-time qPCR analyses were performed from 10 ng DNA or cDNA mixed with the appropriate primers (Dataset S5) in 10 µl SYBRgreen Supermix (BIORAD) using the following amplification protocol: initial denaturation for 5 min at 95°C, followed by 40 cycles with 30 s at 95°C, 30 s at 59°C, 30 s at 72°C and a melt curve analysis. Relative DNA amount or relative expression and its fold change values were calculated using the $2^{-\Delta\Delta Ct}$ method (8).

4. Microarray experiments

Microarray analyses were performed with 200 ng total RNA extracted from *P. indica* inoculated barley and Arabidopsis roots at 14 dpi or from *P. indica* inoculated Arabidopsis roots at 3 dpi. As control total RNA from *P. indica* grown on 1/10 PNM-agar was used. Here, 3 days old hyphae were thoroughly scratched from the agar surface with a sterile scalpel and immediately frozen in liquid nitrogen. For the 3 dpi barley time point, data from Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/), accession number GSE31266 was used (9). For each treatment, samples from three independent biological replicates were labeled and hybridized according to Agilent's One-Color Microarray-Based Gene Expression Analysis Low Input Quick Amp Labeling protocol (version 6.5). Cye-3-labeled probes were hybridized to 8x60k custom-designed Agilent microarray chips. The microarray design was performed based on a previous 2x105k custom-designed Agilent microarray described in (9). For this, the best performing probe (BP) per *P. indica* gene was selected from the existing probes based on the following criteria: 1) Probes with a cross hybridization potential were discarded if an alternative probe for the gene could be used. If this was not possible, three probes with different cross hybridization targets were chosen and used (XP1-XP3). 2) The signal intensity of each probe was compared to its position in the gene. The probe was defined to be a BP if its signal intensity was high and its position was close to the 3' prime end of the respective gene. Thus, the BP probe for each *P. indica* gene was selected as such if it had either the strongest signal of all probes of the respective gene and was located at most 300 bp apart from the 3' prime end or if it was the closest to the 3' prime end and had at least 90% signal intensity of the strongest probe. 3) Non-uniformity outliers were detected by comparing the signal

intensity between biological replicates. The signal intensity of the best probe defined in step 2 was compared against those of its intra- and inter-array replicates. If the signal intensity ranged between +/- 1.42 of the interquantile range, it was accepted as the best probe. Alternatively, step 3 was repeated with the second (third, etc.) best probe. 4) New probes (NP) were calculated via the eArray program (earray.chem.agilent.com/earray/) for those genes that had no suitable BP probe. Control probes were additionally loaded on the chip as described before (9). Microarray image files were analyzed using Agilent's Feature Extraction software v. 10.5 which calculates for each spot a background corrected signal intensity value (gProcessedSignal) that was used for further analysis. For statistical analysis of the raw data, the R environment (www.r-project.org; version 2.15.1) including the Bioconductor package 'Limma' was used. In summary, raw data were standardized by quantile normalization, intensity values from replicate probes were averaged, log2-ratios between experiments were calculated and Student's t-statistic applied to test for significance. Quality and suitability of the applied statistics was estimated by generating density and MA plots. The degree of variability between the experiments was shown by principle component analysis (Figure S15). Selection of differentially expressed genes was based on a fold change of 2 and a false discovery rate-adjusted significance level (adj. p-value) of less than 0.05. Expression data from all experiments are stored in the GEO database (accession number GSE47775). Microarray data were verified by quantitative real-time PCR (qRT-PCR) as described previously (9) from three biologically independent kinetics for each host (time points: 3, 7 and 14 dpi for Arabidopsis; 3, 5, 10 and 14 dpi for barley).

5. Enrichment analysis

To identify significantly enriched gene ontology (GO) terms from the performed microarray experiments the Gene Ontology Enrichment Analysis Software Toolkit (GOEAST) was used (omicslab.genetics.ac.cn/GOEAST/index.php) with settings for customized microarray platform. GO's were assigned to *P. indica* genes by using the Blast2GO suite (version 2.6.0) (10). Significantly higher expressed genes in Arabidopsis roots colonized by *P. indica* at 14 dpi compared to barley roots colonized by *P. indica* at 14 dpi (and *vice versa*) were analyzed using the recommended parameter settings. Datasets S4a and S4b, summarizing all enriched GO terms (Arabidopsis: GO_AT14up, Barley: GO_HV14up), were prepared from the GOEAST outputs.

6. Confocal laser microscopy

Cell viability

Root colonization and Arabidopsis/barley epidermal and cortex cells viability were analyzed by confocal microscopy. Colonized roots were stained for 10 min with 10 µg/ml WGA-AF488 (Molecular Probes, Karlsruhe, Germany) to visualize fungal structures. Membranes were stained with 3 µM FM4-64 (Probes, Karlsruhe, Germany) for 5 min. Root samples were imaged with a TCS-SP5 confocal microscope (Leica, Bensheim, Germany) using an excitation at 488 nm for WGA-AF488 and detection at 500–540 nm. Excitation of FM4-64 was performed at 633 nm and detection at 650–690 nm.

Maximum projection and serial slices of barley root material

Root segments of *Hordeum vulgare* were fixed in FPA (formalin (37%): propionic acid (> 99%): ethanol (50%), 0.5:0.5:9) for three days and subsequently stored in 70% ethanol for further use. For microtomy the root segments were dehydrated in an ascending ethanol series and embedded in UnicrylTM (British Biocell Int.). For serial slices, sequential series of 4 µm longitudinal and transversal sections (Leica Supercut 2065) were stained with toluidine blue O (1 g toluidine blue O + 1 g sodium tetraborate in 100 ml distilled H_2O) and mounted in Corbit balsam. Pictures were taken with a Leica DMRB or TCS-SP5 microscope, equipped with a digital camera (Moticam 2300). For maximum projections the root of barley was stained with acid fuchsine and depicted by confocal laser scanning microscopy using an Argon blue light laser (488 nm) as described in (11). The z-stack was used to compute a Maximum Projection (LCS Version 2).

7. Yeast complementation assay

The ammonium import function of PiAmt1 (PIIN_02036) was verified by yeast complementation analysis. For this, full length cDNA of *PiAMT1* was cloned into the *Not*I site of the pDR195 vector (kindly provided by Dr. Mike Guether, Karlsruhe Institute of Technology, KIT) with primers PiAMT1_SC_notI_F and PiAMT1_SC_notI_R (Dataset S5) and the resulting plasmid was then transformed by heat shock into the ura-AMT-defective yeast (*Saccharomyces cerevisiae*) strain 31019b; ΔΔΔmep1;2;3. Growth complementation assays were performed on solid YNB medium (Yeast Nitrogen Base w/o N, Bacto) supplemented with 2% glucose and 1 mM or 5 mM $(NH_4)_2SO_4$ as sole nitrogen source.

8. RNAi vector construction and *P. indica* transformation

To perform RNAi silencing experiments, a 570 bp fragment of the *PiAMT1* (PIIN_02036) cDNA was amplified by PCR with primers PiAMT1_RNAi_ecoRV_F and PiAMT1_RNAi_ecoRV_R (Dataset S5) and then inserted in the *Eco*RV site of the convergent dual promoter vector pPiRNAi (6). PCR reactions were performed using a proof reading Pfu Polymerase (Promega). The vector pPiRNAi-AMT1 and empty vector control were sequenced and subsequently transformed into *P. indica* by PEG-mediated transformation as described in (6). Putative transformants generated from three independent transformations were transferred onto new CM plates with 80 µg/ml hygromycin B. Cloning was performed using T4 ligase (NEB), Antarctic phosphatase (NEB) and *E. coli* Top10 cells. Vector sequencing was done by Eurofins MWG Operon. The success of transformation was confirmed by southern blot and the efficiency of silencing was verified by qPCR experiments. All RNAi strains were analyzed for reduced growth on minimal medium with ammonium as sole nitrogen source as described in the next chapter. Selected silenced strains with a reduced growth phenotype on minimal medium supplemented with ammonium as sole nitrogen source and with a normal growth phenotype on CM were chosen for *in planta* assays.

9. Ammonium feeding test of *P. indica* transformants

P. indica transformants harboring the RNAi construct were analyzed for ammonium uptake by using wild-type and empty vector transformed strains as controls. Ten µl of chlamydospores suspension with 10-fold serial dilutions starting from 5×10^5 ml^{-1} were inoculated onto YNB medium (Yeast Nitrogen Base w/o N, Bacto) supplemented with 2 mM NH_4Cl (Roth) for 14 days. The experiments were carried out in three replicates.

10. Measurment of caspase activity in roots

Barley and Arabidopsis roots were inoculated with *P. indica* WT, RNAi strains, empty vector controls or mock treated. The first 4 cm of the roots, starting from the seed, were excised and immediately frozen in liquid nitrogen from plant samples harvested at 3, 5, 10 and 14 dpi. One hundred mg ground roots were extracted with a buffer containing 100 mM sodium acetate (pH 5.5), 100 mM NaCl, 1 mM EDTA, 2mM DTT and 1mM phenylmethylsulfonyl fluoride. To measure caspase activities, 100 µM fluorogenic VPE substrate (Ac-ESEN-MCA, Peptide Institute) was added to the root extracts. Fluorescence intensities were measured at 465 nm after excitation at 360 nm using a fluorescence microplate reader (TECAN Safire). The emzyme activity was caculated as the increasing unit of fluorescent intensity compared to the buffer+substrate control after 60 minutes incubation.

11. Preparation and assay of samples for amino acid concentration

Plant material was incubated for 60 minutes at 80°C in 80% ethanol and thereafter centrifuged for 10 minutes at 14000 rpm and 4°C. Supernatant were evaporated to dryness, re-suspended in purest water and used for HPLC analysis. Prior to HPLC analysis samples were derivatized using a fluorescing reagent AQC (6-aminoquinolyl-N-hydroxysuccinimidylcarbamat). 3 mg of self-made AQC (IPK, Germany) was dissolved in 1 ml acetonitrile and incubated exactly for 10 minutes at 55°C. The prepared reagent was stored at 4°C and used up to four weeks. For derivatization of the sample 0.01 ml of the prepared reagent solution was used for each sample which contained 0.8

ml of a buffer (0.2 M boric acid, pH 8.8) and 0.01 ml of the supernatant. Separation of soluble amino acids was performed on a newly developed UPLC-based method using Ultra pressure reversed phase chromatography (AcQuity H-Class, Waters GmbH, Germany). UPLC system consisted of a quaternary solvent manager, a sample manager-FTN, a column manager and a fluorescent detector (PDA eλ Detector). The separation was carried out on a C18 reversed phase column (ACCQ Tag Ultra C18, 1.7 µm, 2.1x100 mm) with a flow rate of 0.7 ml per min and duration of 10.2 min. The column was heated at 50°C during the whole run. The detection wavelengths were 266 nm for excitation and 473 nm as emission. The gradient was accomplished with four solutions prepared from two different buffers purchased from Waters GmbH (eluent A concentrate and eluent B for amino acid analysis, Germany). Eluent A was pure concentrate, eluent B was a mixture of 90% LCMS water (The Geyer GmbH, Germany) and 10 % eluent B concentrate, eluent C was pure concentrate (eluent B for amino acid analysis) and eluent D was LCMS water (The Geyer GmbH, Germany). The column was equilibrated with eluent A (10%) and eluent C (90%). The gradient was produced as indicated in the table below.

Retention time in min	Eluent A (%)	Eluent B (%)	Eluent C (%)	Eluent D (%)
0	10	0	90	0
0.29	9.9	0	90.1	0
5.49	9	80	11	0
7.10	8	15.6	57.9	18.5
7.30	8	15.6	57.9	18.5
7.69	7.8	0	70.9	21.3
7.99	4	0	36.3	59.7
8.59	4	0	36.3	59.7
8.68	10	0	90	0
10.2	10	0	90	0

12. Phylogeny

Phylogenetic relations of ammonium transporters from *Piriformospora indica*, *Saccharomyces cerevisiae* and *Hebeloma cylindrosporum* were calculated using seaview (version 4) (12). Protein alignment was generated by MUSCLE (v3.8.31) (13) and phylogenetic tree by PHYML (v3.0) (14) using standard settings. An approximate *likelihood-ratio* test (aLRT) was performed to calculate branch support.

Legends for the supplementary figures

Figure S1. Host-dependent expression profiles of *in planta*-induced *P. indica* genes encoding intracellular (A) and secreted (B) proteins at 3 and 14 days post inoculation (dpi) during Arabidopsis (PI_AT) and barley (PI_HV) colonization, calculated versus PNM-control.

Figure S2. Heatmaps showing normalized, background-corrected log2 signal intensity values of 305 hydrolytic enzymes (left) and 252 transporters (right) as previously classified (9). Columns represent biological independent replicates of *P. indica* colonized barley roots 3 (PI_HV_3) and 14 dpi (PI_HV_14), *P. indica* colonized Arabidopsis roots 3 (PI_AT_3) and 14 dpi (PI_AT_14) and *P. indica* on 1/10 PNM-agar alone 3dpi (PI_PNM_3). Signal intensities (gProcessedSignal) from Agilent's feature extraction software (v10.5) were quantile normalized and intra-array replicates were averaged using R (www.r-project.org; version 2.15.1). Only those genes are shown for which a best performing probe could be designed (see microarrays section in material and methods for details). Data from the time point PI_HV_3 is from Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/), accession number GSE31266.

Figure S3. Barley root colonized by *P. indica* 30 dpi. A colonized epidermal cell with larger hyphae and thinner hyphae in the cortex cells are visible. Hyphal contact with the endodermis layer results in autoflorescence which is visible as a diffuse white signal on the host cell wall. For maximum projections the root of barley was stained with acid fuchsine and depicted by confocal laser scanning microscopy using an Argon blue light laser (488 nm) as described in (11).

Figure S4. Growth promotion effect on Arabidopsis plants ecotype Col-0 colonized by *P. indica* or by the closely related sebacinoid fungus *P. williamsii* (2). (A-C) Box-plots of the fresh weight of *A. thaliana* plants grown in the presence or absence of *P. indica* or *P. williamsii*. In all 3 experiments, treatment had a significant effect on the mean fresh weight of the plants (plot A: ANOVA, $F_{2,74}$=64.45, p<0.001; plot B: ANOVA, $F_{2,28}$=44.63, p<0.001; plot C: ANOVA, $F_{2,48}$=14.07, p<0.001). Treatment with *P. indica* chlamydospores resulted in a significant increase on the mean fresh weight of the plants in all treatments (Tukey's HSD, p<0.001), but treatment with *P. williamsii* chlamydospores had no significant effect (A, C) or a small but significant effect on the mean fresh weight in comparison with the mock-treatment (B) (Tukey's HSD for plot A: p= 0.51; for plot B: p=0.012; for plot C: p=0.985). (D) Box-plot of the fresh weight of *A. thaliana* plants grown in the presence or absence of *P. indica* on 1/10 PNM. The mean fresh weight of *P. indica* treated plants was significantly increased compared to that of mock-treated plants (two-sided t-test, unpaired, equal variance: t = -3.04, df = 48, p-value < 0.01). In all plots, boxes not sharing a letter are significantly different (based on Tukey'sHSD). Culture media (HO: modified Hoagland's; PNM: 1/10 PNM), plant age (dps = days post-sowing) and co-culture times on the respective plant media (dpi = days post inoculation) are indicated in the upper left corner of each plot. (E) Representative plates containing sets of 14-day old plants 7 days after mock-treatment with tween water (left) or inoculation with either *P. indica* (center) or *P. williamsii* (right) chlamydospores.

Figure S5. *Piriformospora indica* biotrophic broad invasive hyphae (white asterisk) in Arabidopsis epidermal cell (A) 10 and (B) 21 dpi. In contrast to extracellular hyphae, IH are not stainable with WGA-AF488 (green) due to the presence of a plant-derived membrane. (C) After cooking with KOH for 5 minutes and infiltrating WGA for 10 minutes the hyphae of *P. indica* can be stained, indicating that chitin is still present in the cell wall of the fungus.

Figure S6. Gene Ontology Enrichment Analyses for the GO category biological process performed using GOEAST (http://omicslab.genetics.ac.cn/GOEAST/index.php). Shown is the graphical output for the significantly enriched GO terms, as well as their relationships in the whole GO hierarchy, among the *P. indica* genes induced during colonization of Arabidopsis compared to barley at 14 dpi. Data displayed in this graphic show enrichment for genes involved in tRNA aminoacylation for protein translation, amino acid biosynthesis, glycolysis, tetracycline transport and mitochondrion organization.

Figure S7. Gene Ontology Enrichment Analyses for the GO category biological process performed using GOEAST (http://omicslab.genetics.ac.cn/GOEAST/index.php). Shown is the graphical output for the significantly enriched GO terms among the *P. indica* genes induced during colonization of barley compared to Arabidopsis at 14 dpi. Data displayed in this graphic show enrichment for genes involved in chitin catabolic process, L-arabinose metabolic process, xylan and cellulose catabolic processes and carbohydrate transport.

Figure S8. Analyses of *P. indica* ammonium transporter Amt1. (A) Expression of *PiAMT1* is ammonium depletion responsive. *P. indica* was pre-grown on CM for one week (T_0) and then transferred to YNB medium supplemented with different concentrations of ammonium: without nitrogen (w/o N); with 0.5 mM $(NH_4)_2SO_4$; with 2 mM $(NH_4)_2SO_4$; with 10 mM $(NH_4)_2SO_4$. Mycelium was collected after 12 and 24 hours and immediately frozen in liquid nitrogen prior to RNA extraction and cDNA synthesis for real time qPCR analyses. (B) PiAmt1 is related to high affinity ammonium transporters. Phylogenetic relationships between the deduced amino acid sequence of *P. indica* Amt1 and Amt2 and *Hebeloma cylindrosporum* and *Saccharomyces cerevisiae* ammonium transporters/permeases. Sequences were obtained from the GenBank database. ScMEP2, HcAmt1, HcAmt2 are

functionally characterized as high affinity ammonium transporters, whereas ScMEP1, ScMEP3 and HcAmt3 are low affinity ammonium transporters (15-16). (C) Yeast complementation assay for the high affinity ammonium transporter PiAmt1. Growth of ammonium uptake-deficient yeast strain ΔΔΔmep1;2;3 transformed with the empty control plasmid pDR195 or with pDR195::PiAmt1 on YNB medium supplemented with 5 mM or 1 mM $(NH_4)_2SO_4$ as the sole nitrogen source. Shown are serial dilutions of yeast cell suspensions ranging from 1 to $1x10^{-3}$. Experiments were performed in three independent biological repetitions. (D) Predicted topological structure of the *P. indica* Amt1.

Figure S9. Analyses of selected *PiAMT1* RNAi strains and controls *in planta*. (A-B) Relative expression of the *P. indica* high affinity ammonium transporter *AMT1* compared to *PiTEF* during colonization of barley and Arabidopsis at 14 days post inoculation (dpi) in the wild type (WT), empty vector controls (EV) and RNAi strains (Amt9 and Amt28). (C) Relative expression of the *P. indica* ammonium transporter *PiAMT2* compared to *PiTEF* during colonization of barley at 14 days post inoculation (dpi) in the wild type (WT), empty vector controls (EV) and RNAi strains (Amt9 and Amt28). Error bars represent SE of the mean from three independent biological repetitions. No compensatory upregulation of *PiAMT2* was observed in the *PiAMT1* RNAi strains. (D-E) Relative abundance of fungal *PiTEF* transcripts compared to plant *UBI* transcripts in barley and Arabidopsis roots colonized by *P. indica* wild type (WT), empty vector controls (EV) and RNAi strains (Amt9 and Amt28) at 14 dpi. Silencing of *PiAMT1* results in a significant increased colonization of barley roots compared to WT and EV controls (t-test $P<0.05$). Error bars represent SE of the mean from three independent biological repetitions. *P. indica* colonized Arabidopsis and barley plants were grown on 1/10 PNM medium.

Figure S10. Host-dependent expression profiles of *in planta*-induced *P. indica* genes encoding ABC transporters identified using the TCDB (http://www.tcdb.org/) and Blast2Go as described in (9).

Figure S11. Southern blot and colonies phenotype of the *P. indica* ammonium-uptake deficient RNAi strains. (A) Southern blot analysis of *P. indica* WT and EV controls and RNAi strains. Genomic DNA from seven days old *P. indica* cultures grown on CM was extracted and digested overnight with *Sac*I (NEB). The digested DNA was separated on 0.8% TAE agarose gel for 3 h at 80V. Blotting was performed using the DIG-labeling and detection kit following the manufacturer's instructions (Roche Biochemicals). Hybridization was performed with a HYG probe. (B) Colonies phenotype of *P. indica* WT and EV controls and RNAi strains on YNB medium supplemented with 10 mM NH_4Cl as sole nitrogen source. Plates were supplemented with 1% glucose and buffered with MES pH 5.6. Ten microliters of chlamydospores suspension with 10-fold serial dilutions starting from 5×10^5 ml^{-1} were used for the growth assay. The experiments were carried out in three replicates. Representative photographs were taken at 7 days after inoculation.

Figure S12. Barley growth promotion experiment with *P. indica* WT, empty vector control and RNAi strains. Plants were grown as described in (6) and harvested at 30 days post inoculation. (A) Length of leaves in cm. (B) Leaves dry weight in grams. (C) Root dry weight (in grams) in colonized or non-colonized barley plants. Error bars represent standard errors of the mean (n=20). Asterisks represent significant differences (ANOVA, $P< 0.01$). (D) Growth promotion in representative non-colonized (left) and colonized barley plants by *P. indica* WT (middle) and RNAi strain Amt28 (right).

Figure S13. Concentrations of free amino acids in barley and Arabidopsis roots (4 cm below the seed). (A) Concentrations of the most abundant free amino acids in the roots of barley non-colonized or colonized by *P. indica* WT and RNAi strain Amt28 at 7 and (B) 14 dpi. Error bars represent SEM from three independent biological repetitions with two to three technical repetitions. (C) Concentrations of the most abundant free amino acids in roots of Arabidopsis non-colonized and colonized by *P. indica* WT and RNAi strain Amt28 at 7 and (D) 14 dpi. Error bars represent SEM from four independent biological repetitions. *T-test $P<0.05$, **T-test $P<0.01$.

Figure S14. Morphology of *P. indica* hyphae stained with WGA-AF488 grown on minimal medium containing (A) nitrate $NaNO_3$ 2mM, (B) asparagine Asn 1mM, or (C) glutamine Gln 1mM as sole nitrogen source. (D) Average of hyphal size on different nitrogen sources. (E-F) Enlarged, multilobed hyphae from *P. indica* grown on asparagine.

Figure S15. Principle component analysis (PCA) performed using R (www.r-project.org; version 2.15.1) with normalized, background-corrected log2 signal intensity values. Shown are three main principle components (90.06% of the total variability) in a x-y- (PC1 vs PC2) and z-y- (PC3 vs PC2) projection. The PCA shows that the whole transcriptome of *P. indica* colonizing barley at 14 dpi (PI_HV_14) is most different from the other conditions supporting the overall switch of the fungal transcriptome to a different feeding state. The second highest variation is shown for *P. indica* colonizing Arabidopsis at 3 dpi (PI_AT_3).

Dataset S1. Significantly differentially regulated genes from microarray data. AT, *Arabidopsis thaliana*; PNM, plant minimal medium; HV, *Hordeum vulgare*; dpi, days post inoculation.

Dataset S2. Expression data for the DELD protein family.

Dataset S3. Top20 induced *P. indica* SSPs during colonization of Arabidopsidis and barley roots.

Dataset S4. Enrichment analysis performed using GOEAST for upregulated genes.

Dataset S5. List of primers used in this study.

References

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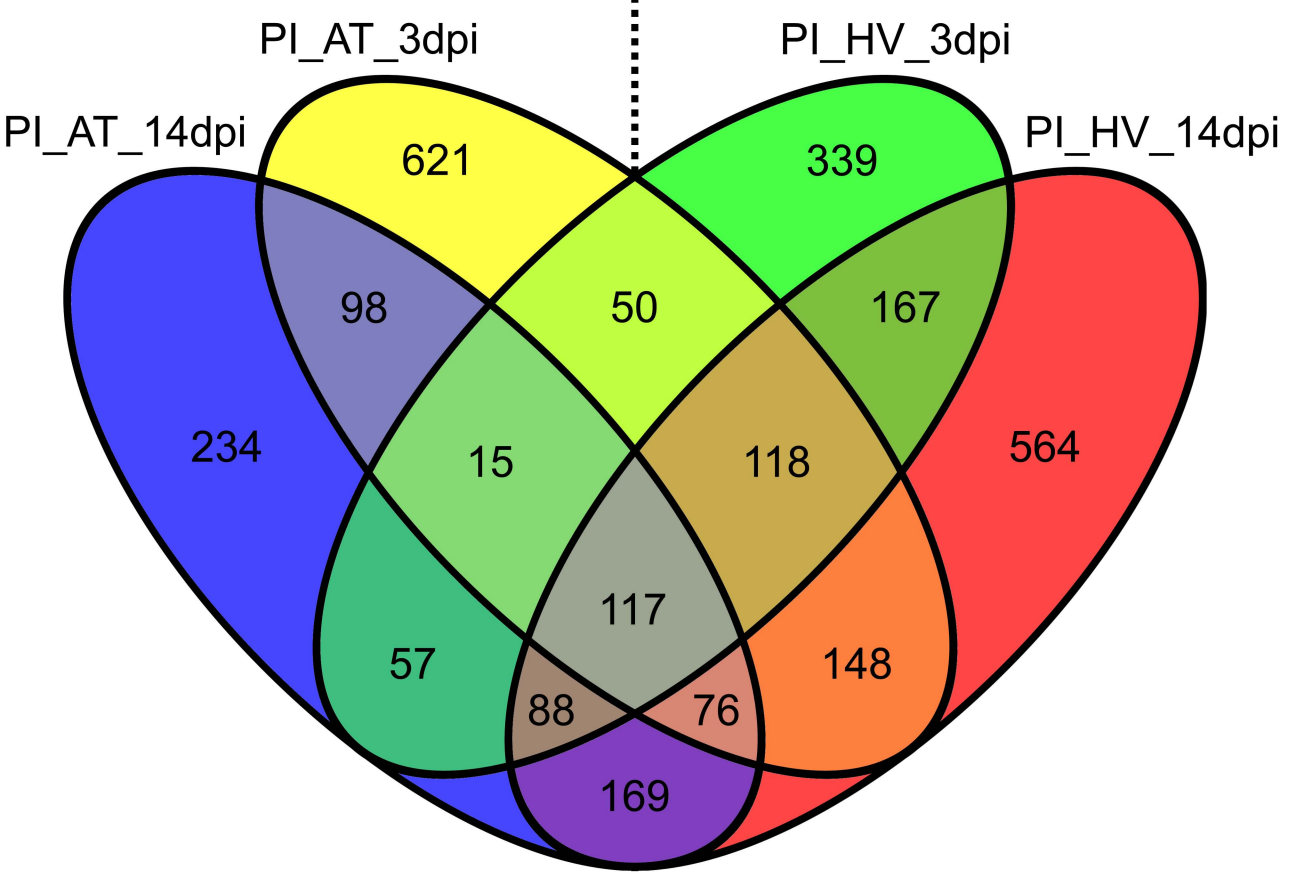
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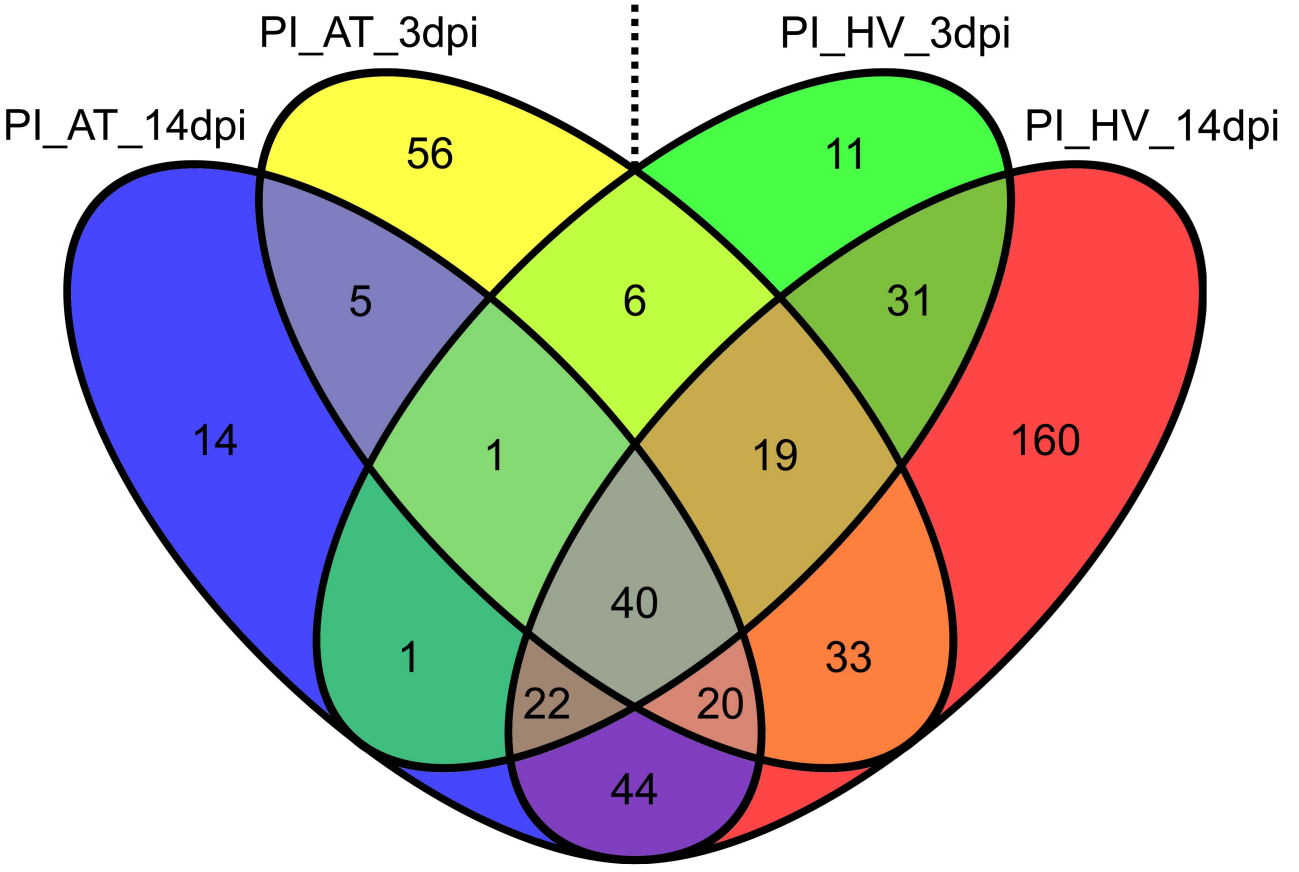
B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PI_HV_14dpi
56
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1
19
160
40
1
33
22
20
44

Hydrolytic enzymes

PIIN_07381 – Pep_S10
PIIN_07955 – GH_63
PIIN_03660 – GH_43
PIIN_02711 – GH_3
PIIN_05222 – GH_16
PIIN_07657 – Pep_S10
PIIN_09757 – GH_17
PIIN_02992 – Pep_M36
PIIN_06333 – GH_3
PIIN_00523 – GH_61
PIIN_01338 – GH_2
PIIN_01915 – Pep_A22B
PIIN_04005 – Lip_3
PIIN_05197 – Pep_M20
PIIN_05303 – Pep_M28
PIIN_09079 – Pep_M24
PIIN_05721 – GH_31
PIIN_04767 – GH_20
PIIN_09068 – Lip_3
PIIN_04140 – Pep_C14
PIIN_06296 – GH_45
PIIN_00602 – Pep_S8
PIIN_01084 – GH_35
PIIN_05614 – GH_47
PIIN_06730 – Pep_C2
PIIN_09677 – GH_28
PIIN_04885 – Pep_M28
PIIN_04006 – Pep_S8
PIIN_07390 – Lip_3
PIIN_01848 – GH_31
PIIN_10057 – GH_10
PIIN_04931 – Pep_M28
PIIN_00112 – Pep_M14
PIIN_07416 – GH_3
PIIN_01377 – Pep_S28
PIIN_01553 – GH_3
PIIN_09820 – Pep_M22
PIIN_06486 – GH_16
PIIN_01429 – Pep_M48
PIIN_00558 – Pep_C14
PIIN_00201 – GH_3
PIIN_01065 – Pep_M28
PIIN_02637 – Pep_M28
PIIN_04690 – Pep_S9
PIIN_01956 – Pep_M50
PIIN_02586 – GH_31
PIIN_08390 – Pep_M19
PIIN_03463 – Pep_M28
PIIN_00510 – Pep_M1
PIIN_02208 – Pep_M24
PIIN_08957 – GH_38
PIIN_07182 – Pep_M48
PIIN_08089 – Pep_S10
PIIN_03591 – Lip_3
PIIN_08021 – GH_16
PIIN_01067 – GH_cc
PIIN_01066 – GH_cc
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PIIN_04017 – Pep_S8
PIIN_09568 – Pep_M24
PIIN_03747 – Pep_C2
PIIN_04090 – Pep_M16
PIIN_07555 – Pep_M43
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PIIN_00907 – GH_17
PIIN_03987 – Pep_M28
PIIN_04950 – Pep_M16
PIIN_05468 – Pep_S8
PIIN_08933 – GH_9
PIIN_06875 – Pep_S10
PIIN_06381 – Pep_M14
PIIN_06825 – GH_88
PIIN_04585 – GH_72
PIIN_03555 – Pep_S24
PIIN_08458 – Pep_M24
PIIN_05500 – GH_71
PIIN_04538 – Pep_M18
PIIN_04253 – Pep_M16
PIIN_09322 – Pep_C65
PIIN_07002 – Pep_M3
PIIN_01606 – Pep_S9
PIIN_00352 – Pep_M49
PIIN_07104 – Pep_S8
PIIN_07492 – Pep_S8
PIIN_02120 – Lip_3
PIIN_07598 – Pep_S8
PIIN_01609 – GH_16
PIIN_05720 – Pep_C1B
PIIN_08245 – GH_32C
PIIN_09219 – GH_16
PIIN_02492 – Pep_S8
PIIN_04941 – Pep_M28
PIIN_08783 – GH_61
PIIN_01142 – Pep_C13
PIIN_07537 – Pep_C14
PIIN_02239 – Pep_S8
PIIN_03352 – Pep_M24
PIIN_06716 – Pep_S10
PIIN_06093 – Pep_M20
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PIIN_03534 – Pep_M24
PIIN_04500 – Pep_C14
PIIN_07484 – Pep_M41
PIIN_05476 – Pep_M36
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PIIN_00425 – Pep_M36
PIIN_07263 – Pep_M16
PIIN_09498 – Pep_M16
PIIN_07540 – GH_31
PIIN_04111 – GH_3
PIIN_06561 – GH_16
PIIN_09069 – Pep_M24
PIIN_04392 – GH_35
PIIN_01644 – Pep_C48
PIIN_08494 – Pep_M13
PIIN_00505 – Pep_C54
PIIN_01906 – GH_47
PIIN_00565 – Pep_M28
PIIN_07289 – GH_16
PIIN_08971 – GH_47
PIIN_04940 – Pep_S9
PIIN_04279 – Pep_M24
PIIN_00291 – Pep_S24
PIIN_02334 – Pep_S8
PIIN_02672 – GH_47
PIIN_07868 – Pep_S9
PIIN_05846 – GH_81
PIIN_04571 – Lip_GDSL
PIIN_00117 – Pep_C14
PIIN_06711 – Pep_M24
PIIN_01736 – Pep_M41
PIIN_04529 – Lip_3
PIIN_02391 – GH_16
PIIN_01518 – Pep_M3
PIIN_09666 – Pep_M28
PIIN_05392 – Pep_M48
PIIN_10656 – GH_15
PIIN_07954 – Pep_S28
PIIN_02985 – Lip_GDSL
PIIN_01415 – Pep_M22
PIIN_08427 – GH_16
PIIN_07096 – GH_16
PIIN_03199 – Pep_C48
PIIN_03542 – GH_18
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PIIN_03746 – Pep_C2
PIIN_001159 – Pep_C50
PIIN_04273 – GH_71
PIIN_03998 – GH_10
PIIN_05280 – Pep_C2
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PIIN_01484 – GH_61
PIIN_03654 – GH_88
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PIIN_04134 – Pep_C14
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PIIN_01931 – Pep_M43
PIIN_06651 – Pep_M43
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PIIN_05605 – Pep_M20
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PIIN_06172 – Lip_GDSL
PIIN_04136 – Pep_C14
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PIIN_10086 – Pep_C14
PIIN_00863 – Pep_S66
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PIIN_01929 – GH_88
PIIN_00621 – GH_28
PIIN_07603 – GH_18
PIIN_00976 – Pep_C78
PIIN_01867 – GH_88
PIIN_09685 – GH_12
PIIN_09022 – GH_61
PIIN_00485 – GH_30
PIIN_07105 – GH_61
PIIN_05859 – GH_11
PIIN_10181 – GH_53
PIIN_09982 – GH_10
PIIN_03995 – GH_10
PIIN_07750 – Lip_3
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PIIN_06155 – GH_61
PIIN_07320 – Lip_GDSL
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PIIN_03996 – GH_10
PIIN_03975 – GH_61
PIIN_06164 – Lip_GDSL
PIIN_04374 – Pep_S8
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PIIN_07749 – Lip_3
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PIIN_08199 – GH_61
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PI_HV_14d1
PI_HV_14d3
PI_HV_14d2
PI_HV_3d1
PI_HV_3d2
PI_AT_14d3
PI_AT_14d2
PI_AT_14d1
PI_PNM_3d3
PI_PNM_3d1
PI_PNM_3d2
PI_AT_3d2
PI_AT_3d3
PI_AT_3d1

Transporters

PIIN_05314 – carbohydrate
PIIN_04449 – metal, mitochondrion
PIIN_05146 – metal, mitochondrion
PIIN_06807 – toxin efflux, ABC
PIIN_02728 – unclassified
PIIN_03445 – toxin efflux, ABC
PIIN_03569 – unclassified
PIIN_04975 – phosphate
PIIN_05763 – metal, mitochondrion
PIIN_08131 – phosphate
PIIN_00903 – toxin efflux, ABC
PIIN_06445 – toxin efflux, ABC
PIIN_09242 – toxin efflux, ABC
PIIN_01269 – metal, mitochondrion
PIIN_01840 – metal, mitochondrion
PIIN_04961 – toxin efflux, ABC
PIIN_01525 – toxin efflux, ABC
PIIN_05758 – metal, mitochondrion
PIIN_09556 – toxin efflux, ABC
PIIN_00568 – unclassified
PIIN_06297 – toxin efflux, ABC
PIIN_01427 – metal, mitochondrion
PIIN_09879 – toxin efflux, ABC
PIIN_05745 – toxin efflux, ABC
PIIN_06569 – toxin efflux, ABC
PIIN_08230 – toxin efflux, ABC
PIIN_07148 – toxin efflux, ABC
PIIN_05160 – carbohydrate
PIIN_03256 – toxin efflux, ABC
PIIN_02272 – nitrogen
PIIN_02036 – nitrogen
PIIN_10069 – toxin efflux, ABC
PIIN_03336 – nitrogen
PIIN_02165 – toxin efflux, ABC
PIIN_03850 – nitrogen
PIIN_06654 – nitrogen
PIIN_07466 – nitrogen
PIIN_01015 – unclassified
PIIN_00660 – nitrogen
PIIN_02982 – nitrogen
PIIN_04361 – carbohydrate
PIIN_02682 – unclassified
PIIN_04233 – toxin efflux, ABC
PIIN_07354 – unclassified
PIIN_00045 – unclassified
PIIN_02520 – metal, mitochondrion
PIIN_07285 – unclassified
PIIN_06871 – nitrogen
PIIN_01424 – nitrogen
PIIN_03500 – unclassified
PIIN_06188 – toxin efflux, ABC
PIIN_04545 – nitrogen
PIIN_06154 – carbohydrate
PIIN_01003 – unclassified
PIIN_03020 – unclassified
PIIN_00318 – metal, mitochondrion
PIIN_06359 – toxin efflux, ABC
PIIN_01405 – carbohydrate
PIIN_06540 – metal, mitochondrion
PIIN_03345 – toxin efflux, ABC
PIIN_00612 – carbohydrate
PIIN_00583 – toxin efflux, ABC
PIIN_09601 – toxin efflux, ABC
PIIN_09575 – toxin efflux, ABC
PIIN_03848 – unclassified
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PIIN_03420 – metal, mitochondrion
PIIN_00579 – toxin efflux, ABC, etc
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PIIN_03843 – toxin efflux, ABC
PIIN_02738 – carbohydrate
PIIN_01740 – unclassified
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PIIN_08798 – metal, mitochondrion
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PIIN_08285 – toxin efflux, ABC
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PIIN_00838 – unclassified
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PIIN_03282 – metal, mitochondrion
PIIN_04428 – toxin efflux, ABC, etc
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PIIN_08471 – toxin efflux, ABC
PIIN_09797 – toxin efflux, ABC
PIIN_01095 – toxin efflux, ABC
PIIN_06464 – carbohydrate
PIIN_06105 – metal, mitochondrion
PIIN_00716 – unclassified
PIIN_04441 – carbohydrate
PIIN_02023 – nitrogen
PIIN_08191 – unclassified
PIIN_09569 – toxin efflux, ABC
PIIN_06700 – metal, mitochondrion
PIIN_00305 – toxin efflux, ABC
PIIN_07196 – nitrogen
PIIN_00866 – phosphate
PIIN_00428 – nitrogen
PIIN_07677 – nitrogen
PIIN_00334 – carbohydrate
PIIN_00105 – carbohydrate
PIIN_04701 – unclassified
PIIN_06029 – toxin efflux, ABC
PIIN_01799 – carbohydrate
PIIN_03367 – carbohydrate
PIIN_05137 – carbohydrate
PIIN_00918 – carbohydrate
PIIN_07404 – carbohydrate
PIIN_01861 – carbohydrate
PIIN_02129 – carbohydrate
PIIN_08633 – carbohydrate
PIIN_01298 – carbohydrate
PIIN_02191 – carbohydrate
PIIN_05331 – nitrogen
PIIN_06701 – nitrogen
PIIN_01868 – nitrogen
PIIN_03369 – carbohydrate
PIIN_08090 – carbohydrate
PIIN_04898 – carbohydrate
PIIN_06425 – metal, mitochondrion
PIIN_01320 – unclassified
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PIIN_04496 – metal, mitochondrion
PIIN_03275 – nitrogen
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PIIN_00335 – carbohydrate
PIIN_04851 – toxin efflux, ABC
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PIIN_02479 – metal, mitochondrion
PIIN_03410 – nitrogen
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PIIN_09208 – toxin efflux, ABC
PIIN_08071 – nitrogen
PIIN_05934 – toxin efflux, ABC
PIIN_11202 – toxin efflux, ABC
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PIIN_08116 – metal, mitochondrion
PIIN_07110 – metal, mitochondrion
PIIN_00378 – phosphate
PIIN_10372 – phosphate
PIIN_07986 – unclassified
PIIN_08515 – metal, mitochondrion
PIIN_03118 – metal, mitochondrion
PIIN_08405 – metal, mitochondrion
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PIIN_10480 – nitrogen
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PIIN_02855 – carbohydrate
PIIN_06341 – phosphate
PIIN_01718 – carbohydrate
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PIIN_03652 – metal, mitochondrion
PIIN_04373 – nitrogen
PIIN_09813 – unclassified
PIIN_00937 – unclassified
PIIN_05130 – toxin efflux, ABC
PIIN_00463 – metal, mitochondrion
PIIN_03207 – carbohydrate
PIIN_04341 – toxin efflux, ABC
PIIN_04343 – toxin efflux, ABC
PIIN_02020 – toxin efflux, ABC
PIIN_09801 – toxin efflux, ABC
PIIN_08284 – toxin efflux, ABC
PIIN_07338 – toxin efflux, ABC
PIIN_03658 – toxin efflux, ABC
PIIN_03409 – nitrogen
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PIIN_03408 – nitrogen
PIIN_09315 – toxin efflux, ABC
PIIN_03368 – carbohydrate
PIIN_04623 – toxin efflux, ABC
PIIN_10126 – carbohydrate
PIIN_06024 – toxin efflux, ABC
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PIIN_05175 – unclassified
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PIIN_07680 – nitrogen
PIIN_03178 – nitrogen
PIIN_00336 – carbohydrate
PIIN_08586 – carbohydrate
PIIN_08219 – unclassified
PIIN_05070 – unclassified
PIIN_05065 – unclassified
PIIN_03609 – nitrogen
PIIN_07138 – unclassified
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PIIN_09869 – toxin efflux, ABC
PIIN_11329 – toxin efflux, ABC
PIIN_05797 – metal, mitochondrion
PIIN_01819 – nitrogen
PIIN_00638 – toxin efflux, ABC
PIIN_06030 – toxin efflux, ABC

PI_HV_14d3
PI_HV_14d1
PI_HV_14d2
PI_AT_14d3
PI_AT_14d2
PI_AT_14d1
PI_HV_3d1
PI_HV_3d2
PI_PNM_3d3
PI_PNM_3d1
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PI_AT_3d3
PI_AT_3d1
PI_AT_3d2


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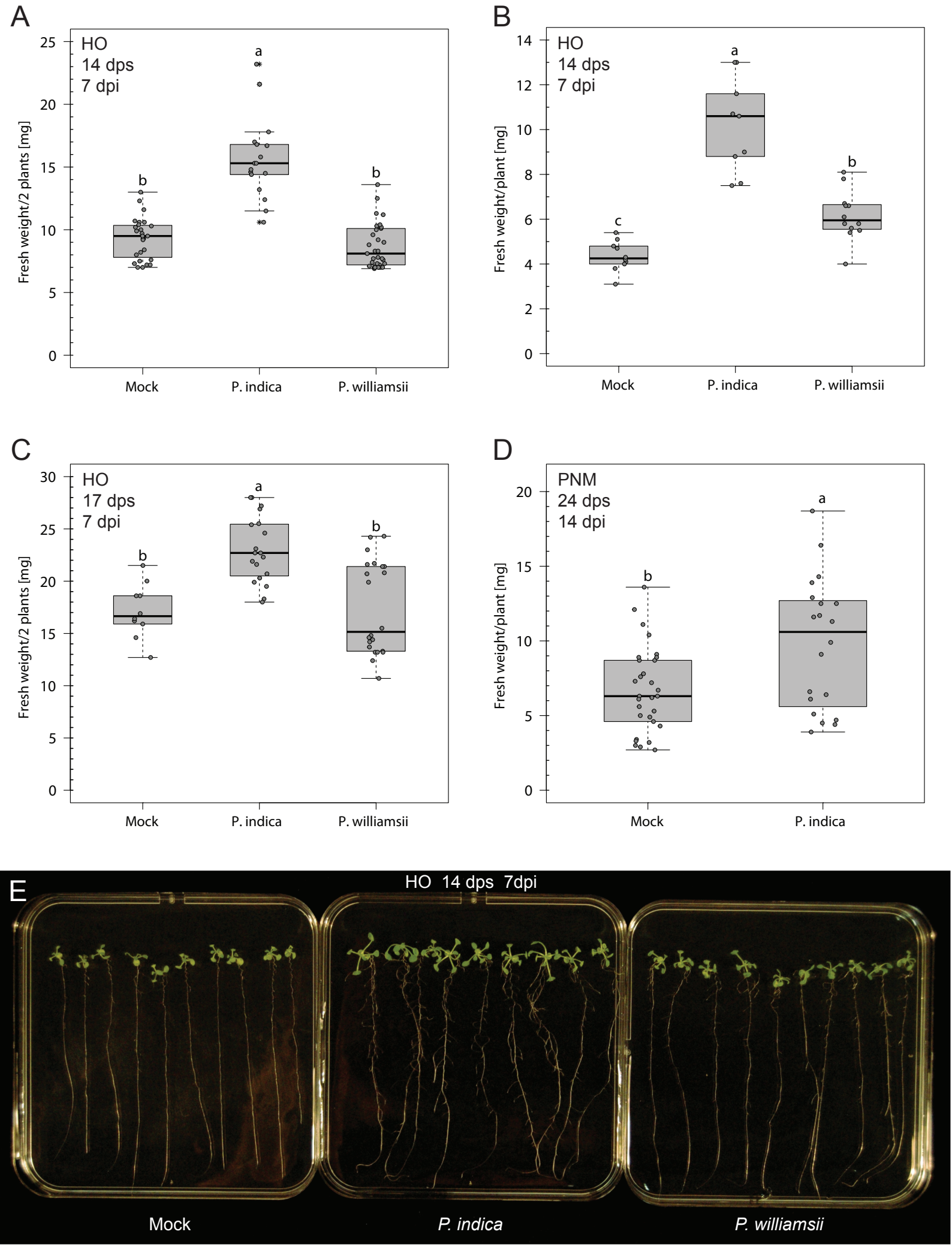
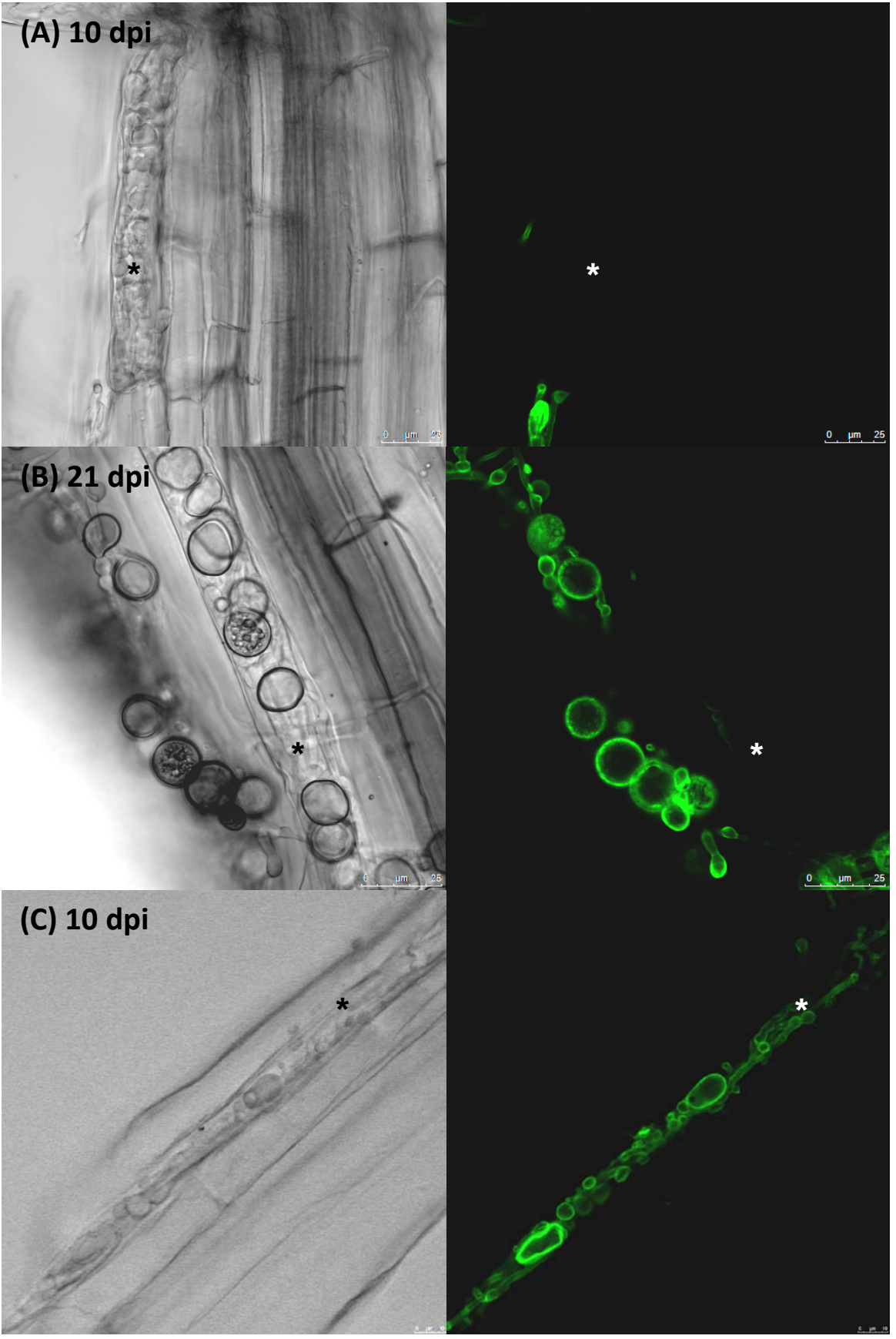
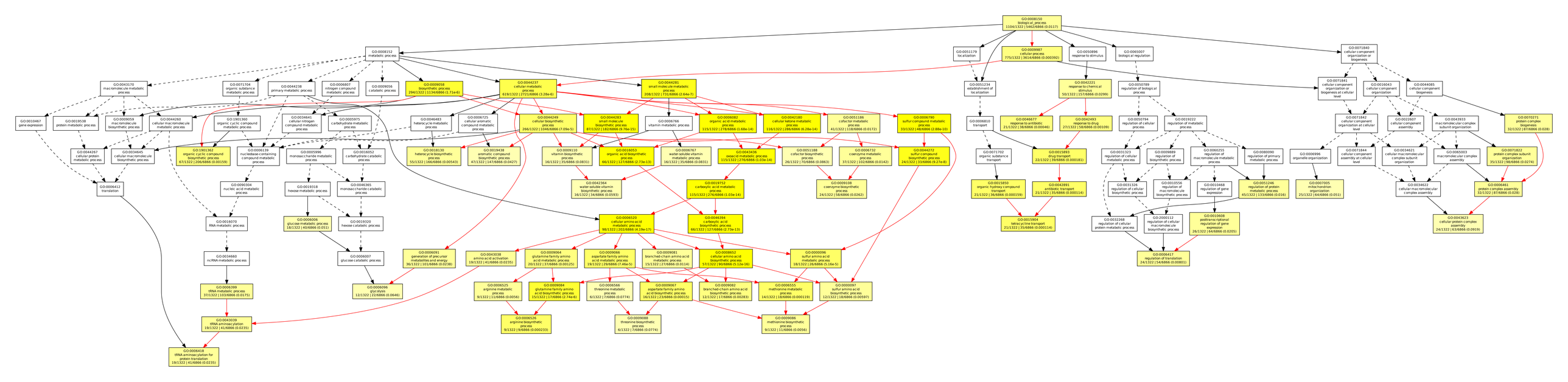
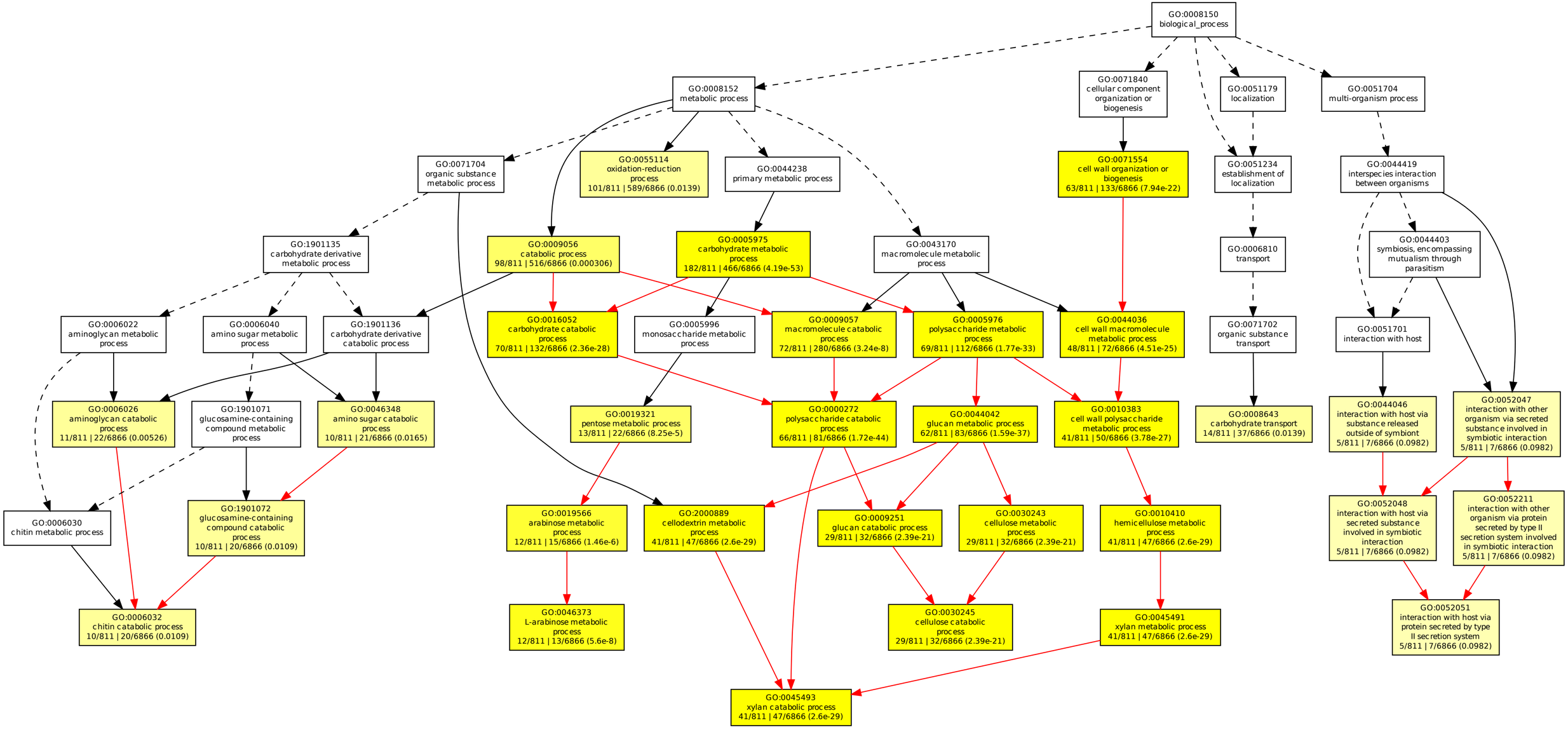
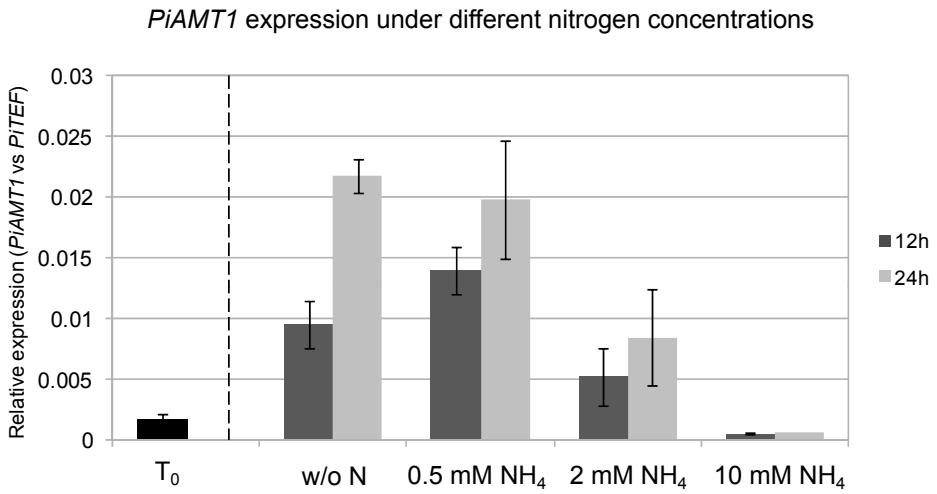

A
HO
14 dps
7 dpi
Fresh weight/2 plants [mg]
0
5
10
15
20
25
a
b
b
Mock
P. indica
P. williamsii
B
HO
14 dps
7 dpi
Fresh weight/plant [mg]
0
2
4
6
8
10
12
14
c
a
b
Mock
P. indica
P. williamsii
C
HO
17 dps
7 dpi
Fresh weight/2 plants [mg]
0
5
10
15
20
25
30
b
a
b
Mock
P. indica
P. williamsii
D
PNM
24 dps
14 dpi
Fresh weight/plant [mg]
0
5
10
15
20
b
a
Mock
P. indica
E
HO 14 dps 7dpi
Mock
P. indica
P. williamsii


(A) 10 dpi
(B) 21 dpi
(C) 10 dpi

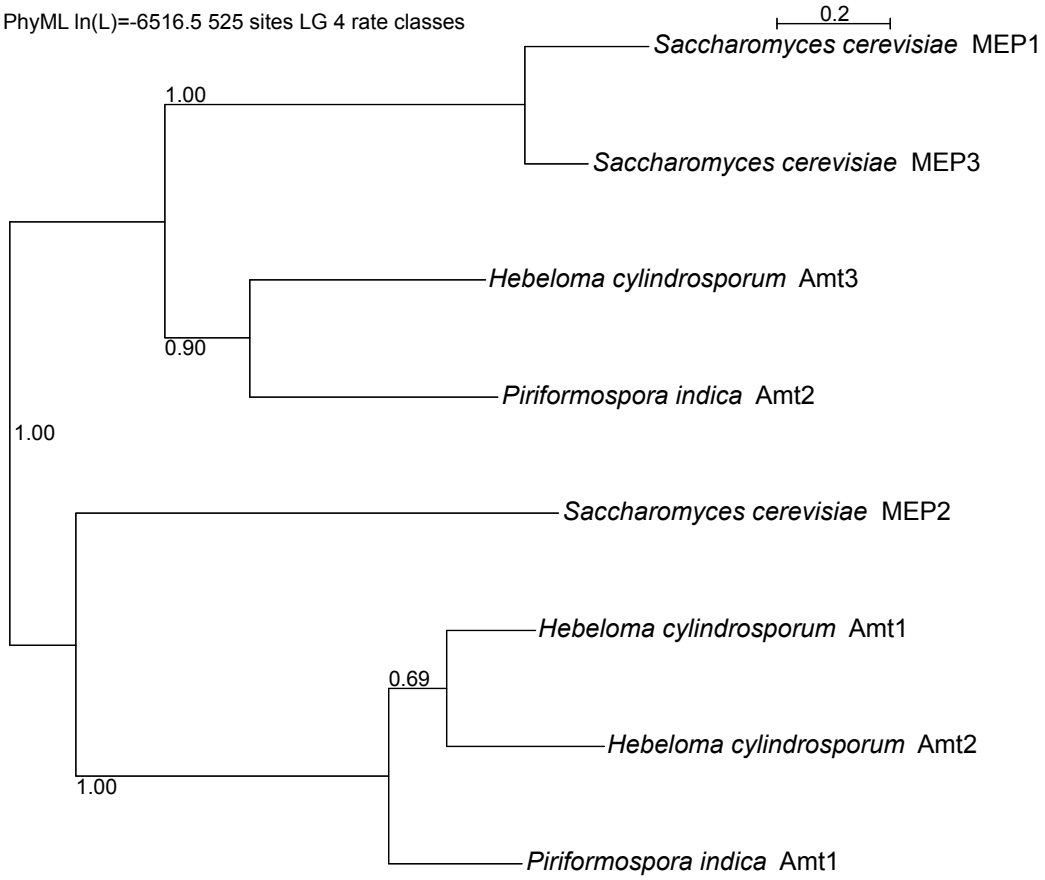

GO:0008150
biological_process
1104/1322 | 5462/6866 (0.0117)
GO:0008152
metabolic process
GO:0051179
localization
GO:0009987
cellular process
775/1322 | 3614/6866 (0.000392)
GO:0050896
response to stimulus
GO:0065007
biological regulation
GO:0071840
cellular component organization or biogenesis
GO:0043170
macromolecule metabolic process
GO:0071704
organic substance metabolic process
GO:0044238
primary metabolic process
GO:0006807
nitrogen compound metabolic process
GO:0009056
catabolic process
GO:0009058
biosynthetic process
294/1322 | 1134/6866 (1.71e-6)
GO:0044237
cellular metabolic process
619/1322 | 2721/6866 (3.28e-6)
GO:0044281
small molecule metabolic process
208/1322 | 731/6866 (2.64e-7)
GO:0051234
establishment of localization
GO:0042221
response to chemical stimulus
50/1322 | 157/6866 (0.0299)
GO:0050789
regulation of biological process
GO:0071841
cellular component organization or biogenesis at cellular level
GO:0016043
cellular component organization
GO:0044085
cellular component biogenesis
GO:0010467
gene expression
GO:0019538
protein metabolic process
GO:0009059
macromolecule biosynthetic process
GO:0044260
cellular macromolecule metabolic process
GO:1901360
organic cyclic compound metabolic process
GO:0034641
cellular nitrogen compound metabolic process
GO:0051186
cofactor metabolic process
41/1322 | 118/6866 (0.0172)
GO:0006790
sulfur compound metabolic process
33/1322 | 86/6866 (2.88e-10)
GO:0006810
transport
GO:0046677
response to antibiotic
21/1322 | 36/6866 (0.00046)
GO:0042493
response to drug
27/1322 | 58/6866 (0.00109)
GO:0071842
cellular component organization at cellular level
GO:0022607
cellular component assembly
GO:0043933
macromolecular complex subunit organization
GO:0070271
protein complex biogenesis
32/1322 | 87/6866 (0.028)
GO:0044267
cellular protein metabolic process
GO:0034645
cellular macromolecule biosynthetic process
GO:1901362
organic cyclic compound biosynthetic process
67/1322 | 206/6866 (0.00159)
GO:0006139
nucleobase-containing compound metabolic process
GO:0005996
monosaccharide metabolic process
GO:0016052
carbohydrate catabolic process
GO:0018130
heterocycle biosynthetic process
55/1322 | 166/6866 (0.00543)
GO:0019438
aromatic compound biosynthetic process
47/1322 | 147/6866 (0.0427)
GO:0009110
vitamin biosynthetic process
16/1322 | 35/6866 (0.0831)
GO:0016053
organic acid biosynthetic process
66/1322 | 227/6866 (2.73e-13)
GO:0006767
water-soluble vitamin metabolic process
16/1322 | 35/6866 (0.0831)
GO:0043436
oxoacid metabolic process
115/1322 | 276/6866 (1.03e-14)
GO:0051188
cofactor biosynthetic process
26/1322 | 70/6866 (0.0863)
GO:0006732
coenzyme metabolic process
37/1322 | 102/6866 (0.0142)
GO:0044272
sulfur compound biosynthetic process
24/1322 | 63/6866 (9.27e-8)
GO:0071702
organic substance transport
GO:0015893
drug transport
22/1322 | 39/6866 (0.000181)
GO:0031323
regulation of cellular metabolic process
GO:0009889
regulation of biosynthetic process
GO:0060255
regulation of macromolecule metabolic process
GO:0080090
regulation of primary metabolic process
GO:0007005
mitochondrion organization
25/1322 | 64/6866 (0.051)
GO:0034621
cellular macromolecular complex subunit organization
GO:0065003
macromolecular complex assembly
GO:0071822
protein complex subunit organization
35/1322 | 98/6866 (0.0274)
GO:0006412
translation
GO:0042364
water-soluble vitamin biosynthetic process
16/1322 | 34/6866 (0.0593)
GO:0019752
carboxylic acid metabolic process
115/1322 | 276/6866 (1.03e-14)
GO:0009108
coenzyme biosynthetic process
24/1322 | 58/6866 (0.0262)
GO:0015850
organic hydroxy compound transport
21/1322 | 36/6866 (0.000159)
GO:0042891
antibiotic transport
21/1322 | 35/6866 (0.000114)
GO:0015804
tetracycline transport
21/1322 | 35/6866 (0.000114)
GO:0032268
regulation of cellular protein metabolic process
GO:0010556
regulation of macromolecule biosynthetic process
GO:0010468
regulation of gene expression
GO:0051246
regulation of protein metabolic process
45/1322 | 133/6866 (0.016)
GO:0034622
cellular macromolecular complex assembly
GO:0006461
protein complex assembly
32/1322 | 87/6866 (0.028)
GO:0016070
RNA metabolic process
GO:0006520
cellular amino acid metabolic process
98/1322 | 320/6866 (4.19e-17)
GO:0046394
carboxylic acid biosynthetic process
66/1322 | 227/6866 (2.73e-13)
GO:2000112
regulation of cellular macromolecule biosynthetic process
GO:0010608
posttranscriptional regulation of gene expression
26/1322 | 64/6866 (0.0205)
GO:0043623
cellular protein complex assembly
24/1322 | 63/6866 (0.0919)
GO:0034660
ncRNA metabolic process
GO:0006091
generation of precursor metabolites and energy
36/1322 | 103/6866 (0.0238)
GO:0043038
amino acid activation
19/1322 | 41/6866 (0.0235)
GO:0009064
glutamine family amino acid metabolic process
20/1322 | 37/6866 (0.00125)
GO:0009066
aspartate family amino acid metabolic process
19/1322 | 29/6866 (7.46e-5)
GO:0009081
branched-chain amino acid metabolic process
15/1322 | 27/6866 (0.0114)
GO:0008652
cellular amino acid biosynthetic process
57/1322 | 90/6866 (5.12e-16)
GO:0000096
sulfur amino acid metabolic process
18/1322 | 26/6866 (5.16e-5)
GO:0006417
regulation of translation
24/1322 | 54/6866 (0.00801)
GO:0006399
tRNA metabolic process
37/1322 | 103/6866 (0.0175)
GO:0006525
arginine metabolic process
6/1322 | 11/6866 (0.0056)
GO:0009084
glutamine family amino acid biosynthetic process
15/1322 | 17/6866 (2.74e-6)
GO:0006566
threonine metabolic process
6/1322 | 7/6866 (0.0774)
GO:0009067
aspartate family amino acid biosynthetic process
16/1322 | 23/6866 (0.000015)
GO:0009082
branched-chain amino acid biosynthetic process
12/1322 | 17/6866 (0.00283)
GO:0006555
methionine metabolic process
14/1322 | 18/6866 (0.000119)
GO:0000097
sulfur amino acid biosynthetic process
12/1322 | 18/6866 (0.00597)
GO:0006006
glucose metabolic process
18/1322 | 40/6866 (0.051)
GO:0019318
hexose metabolic process
GO:0043365
monosaccharide catabolic process
GO:0019320
hexose catabolic process
GO:0006007
glucose catabolic process
GO:0006096
glycolysis
12/1322 | 22/6866 (0.0646)
GO:0043039
tRNA aminoacylation
19/1322 | 41/6866 (0.0235)
GO:0006418
tRNA aminoacylation for protein translation
19/1322 | 41/6866 (0.0235)
GO:0006526
arginine biosynthetic process
9/1322 | 9/6866 (0.000233)
GO:0009088
threonine biosynthetic process
6/1322 | 7/6866 (0.0774)
GO:0009086
methionine biosynthetic process
9/1322 | 11/6866 (0.0056)


GO:0008150
biological_process
GO:0008152
metabolic process
GO:0071840
cellular component organization or biogenesis
GO:0051179
localization
GO:0051704
multi-organism process
GO:0071704
organic substance metabolic process
GO:0055114
oxidation-reduction process
101/811 | 589/6866 (0.0139)
GO:0044238
primary metabolic process
GO:0071554
cell wall organization or biogenesis
63/811 | 133/6866 (7.94e-22)
GO:0051234
establishment of localization
GO:0044419
interspecies interaction between organisms
GO:1901135
carbohydrate derivative metabolic process
GO:0009056
catabolic process
98/811 | 516/6866 (0.000306)
GO:0005975
carbohydrate metabolic process
182/811 | 466/6866 (4.19e-53)
GO:0043170
macromolecule metabolic process
GO:0006810
transport
GO:0044403
symbiosis, encompassing mutualism through parasitism
GO:0006022
aminoglycan metabolic process
GO:0006040
amino sugar metabolic process
GO:1901136
carbohydrate derivative catabolic process
GO:0016052
carbohydrate catabolic process
70/811 | 132/6866 (2.36e-28)
GO:0005996
monosaccharide metabolic process
GO:0009057
macromolecule catabolic process
72/811 | 280/6866 (3.24e-8)
GO:0005976
polysaccharide metabolic process
69/811 | 112/6866 (1.77e-33)
GO:0044036
cell wall macromolecule metabolic process
48/811 | 72/6866 (4.51e-25)
GO:0071702
organic substance transport
GO:0051701
interaction with host
GO:0006026
aminoglycan catabolic process
11/811 | 22/6866 (0.00526)
GO:1901071
glucosamine-containing compound metabolic process
GO:0046348
amino sugar catabolic process
10/811 | 21/6866 (0.0165)
GO:0019321
pentose metabolic process
13/811 | 22/6866 (8.25e-5)
GO:0000272
polysaccharide catabolic process
66/811 | 81/6866 (1.72e-44)
GO:0044042
glucan metabolic process
62/811 | 83/6866 (1.59e-37)
GO:0010383
cell wall polysaccharide metabolic process
41/811 | 50/6866 (3.78e-27)
GO:0008643
carbohydrate transport
14/811 | 37/6866 (0.0139)
GO:0044046
interaction with host via substance released outside of symbiont
5/811 | 7/6866 (0.0982)
GO:0052047
interaction with other organism via secreted substance involved in symbiotic interaction
5/811 | 7/6866 (0.0982)
GO:0006030
chitin metabolic process
GO:1901072
glucosamine-containing compound catabolic process
10/811 | 20/6866 (0.0109)
GO:0019566
arabinose metabolic process
12/811 | 15/6866 (1.46e-6)
GO:2000889
cellodextrin metabolic process
41/811 | 47/6866 (2.6e-29)
GO:0009251
glucan catabolic process
29/811 | 32/6866 (2.39e-21)
GO:0030243
cellulose metabolic process
29/811 | 32/6866 (2.39e-21)
GO:0010410
hemicellulose metabolic process
41/811 | 47/6866 (2.6e-29)
GO:0052048
interaction with host via secreted substance involved in symbiotic interaction
5/811 | 7/6866 (0.0982)
GO:0052211
interaction with other organism via protein secreted by type II secretion system involved in symbiotic interaction
5/811 | 7/6866 (0.0982)
GO:0006032
chitin catabolic process
10/811 | 20/6866 (0.0109)
GO:0046373
L-arabinose metabolic process
12/811 | 13/6866 (5.6e-8)
GO:0030245
cellulose catabolic process
29/811 | 32/6866 (2.39e-21)
GO:0045491
xylan metabolic process
41/811 | 47/6866 (2.6e-29)
GO:0052051
interaction with host via protein secreted by type II secretion system
5/811 | 7/6866 (0.0982)
GO:0045493
xylan catabolic process
41/811 | 47/6866 (2.6e-29)

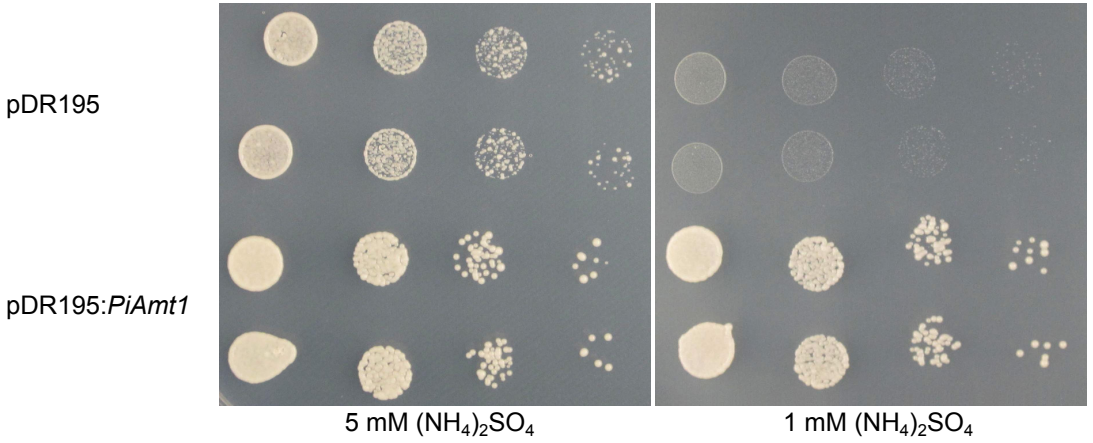
A


PiAMT1 expression under different nitrogen concentrations
Relative expression (PiAMT1 vs PiTEF)
0.03
0.025
0.02
0.015
0.01
0.005
0
12h
24h
T0
w/o N
0.5 mM NH4
2 mM NH4
10 mM NH4

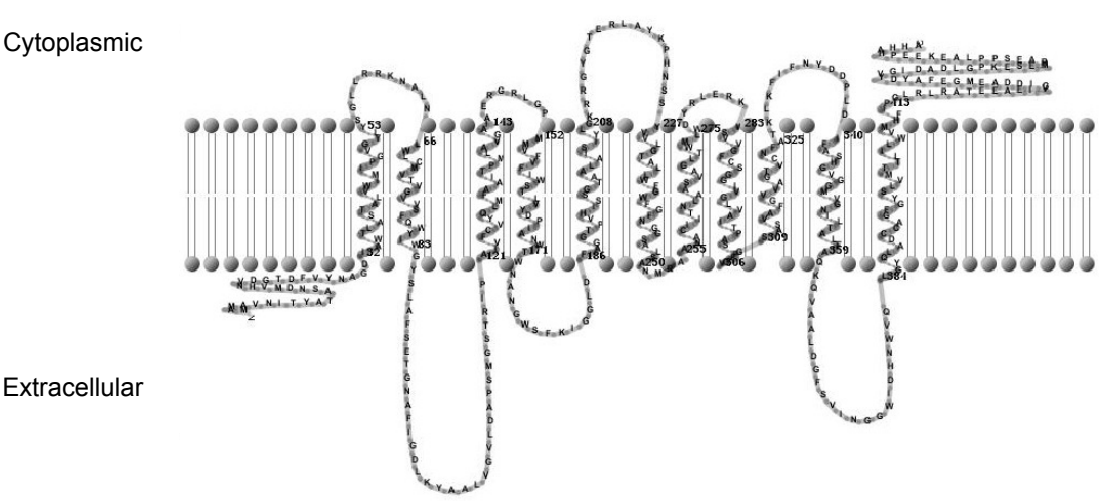
B


PhyML ln(L)=-6516.5 525 sites LG 4 rate classes
0.2
Saccharomyces cerevisiae MEP1
Saccharomyces cerevisiae MEP3
Hebeloma cylindrosporum Amt3
Piriformospora indica Amt2
Saccharomyces cerevisiae MEP2
Hebeloma cylindrosporum Amt1
Hebeloma cylindrosporum Amt2
Piriformospora indica Amt1
1.00
0.90
1.00
0.69
1.00

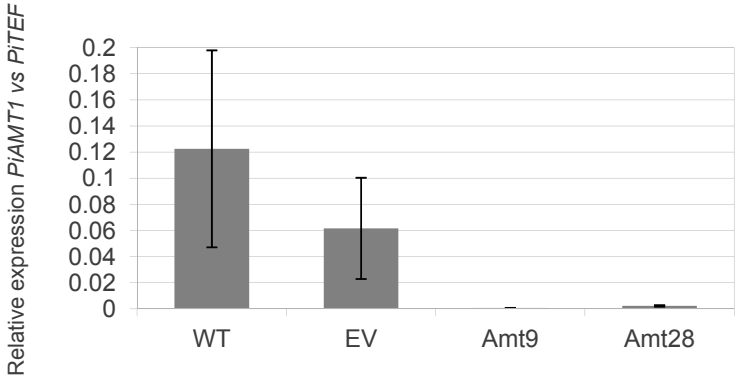
C


pDR195
pDR195:PiAmt1
5 mM (NH4)2SO4
1 mM (NH4)2SO4

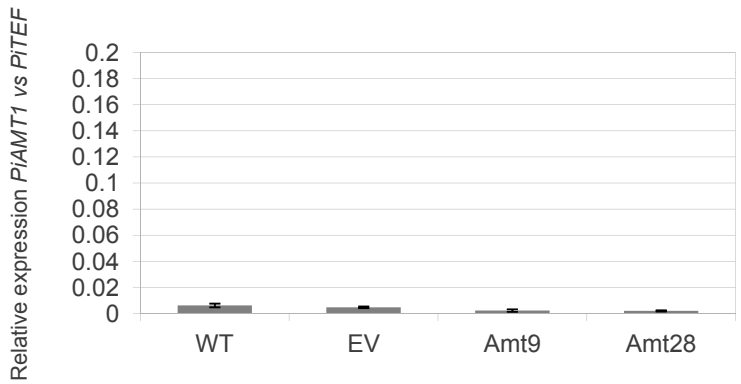
D


Cytoplasmic
Extracellular

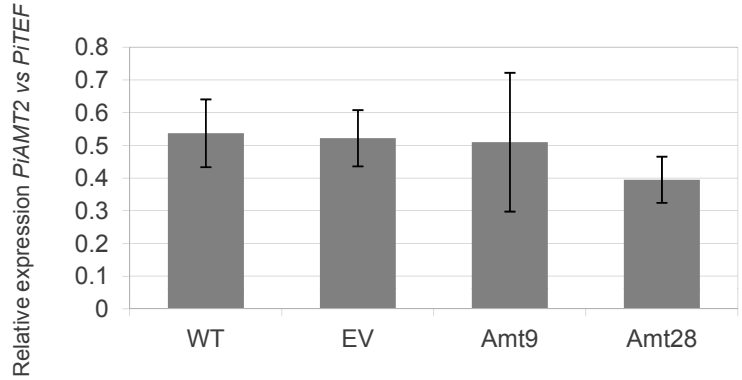
A


PiAMT1 expression in *H. vulgare* 14 dpi
Relative expression *PiAMT1 vs PiTEF*
0.2
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
WT
EV
Amt9
Amt28

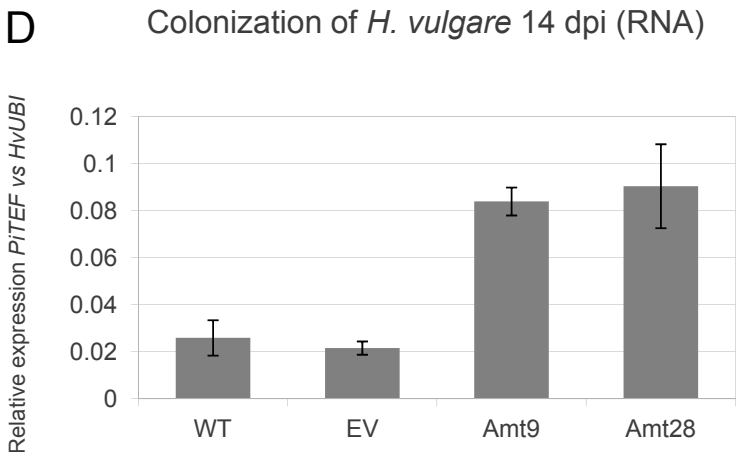
B


PiAMT1 expression in *A.thaliana* 14 dpi
Relative expression *PiAMT1 vs PiTEF*
0.2
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
WT
EV
Amt9
Amt28

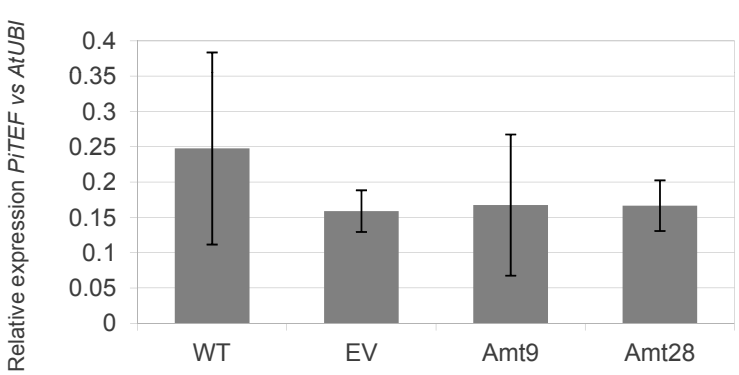
C

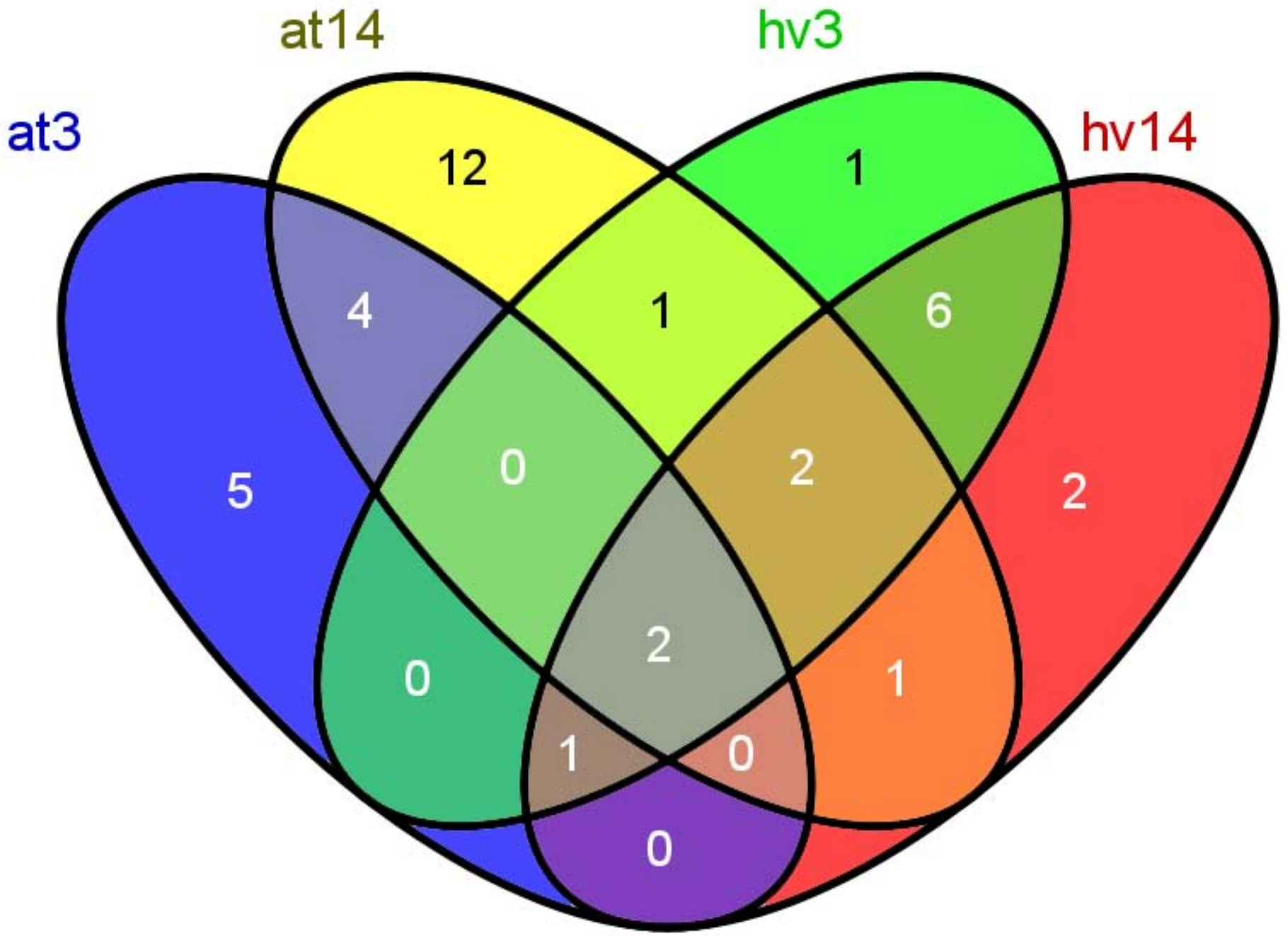

PiAMT2 expression in *H. vulgare* 14 dpi
Relative expression *PiAMT2 vs PiTEF*
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
WT
EV
Amt9
Amt28

D


Colonization of *H. vulgare* 14 dpi (RNA)
Relative expression *PiTEF vs HvUBI*
0.12
0.1
0.08
0.06
0.04
0.02
0
WT
EV
Amt9
Amt28

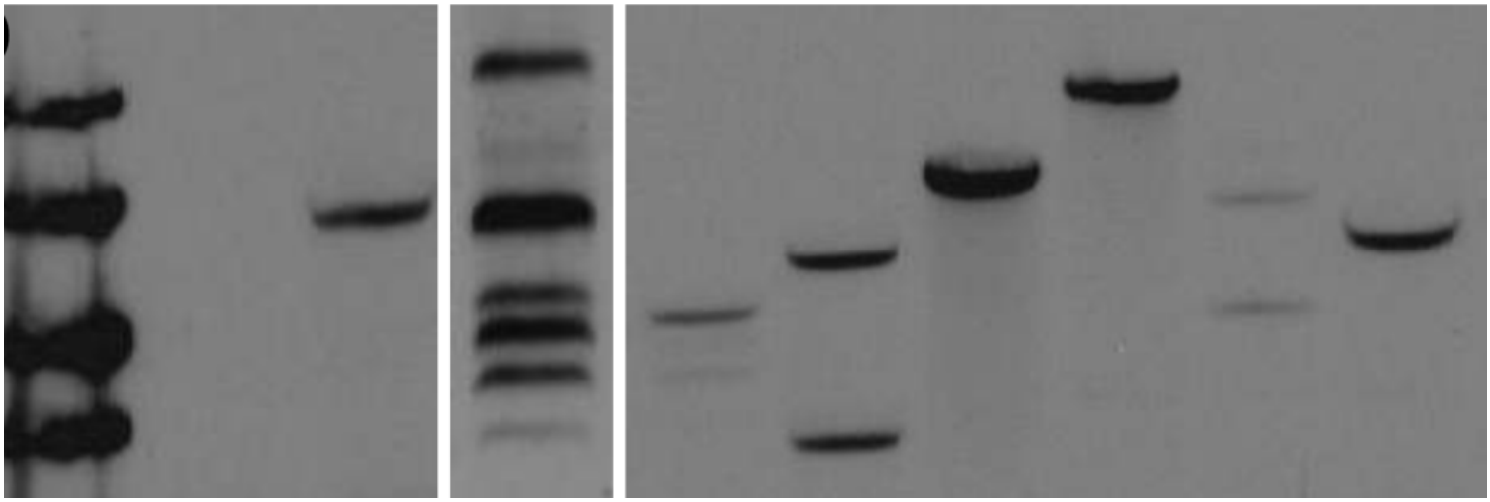
E


Colonization of *A. thaliana* 14 dpi (RNA)
Relative expression *PiTEF vs AtUBI*
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
WT
EV
Amt9
Amt28


at14
hv3
at3
hv14
12
1
4
1
6
5
0
2
2
0
2
1
1
0
0

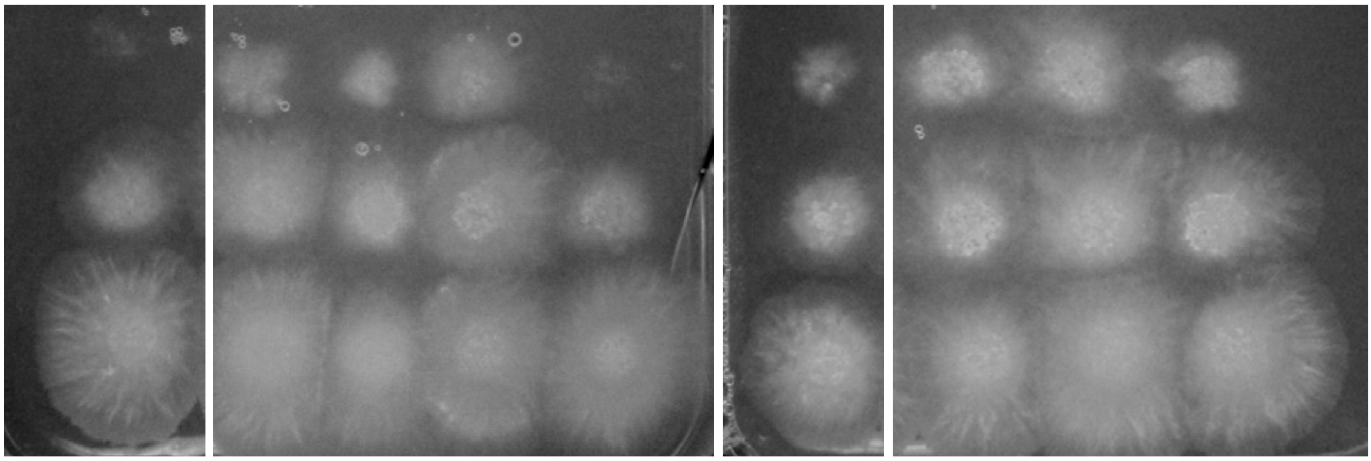
A

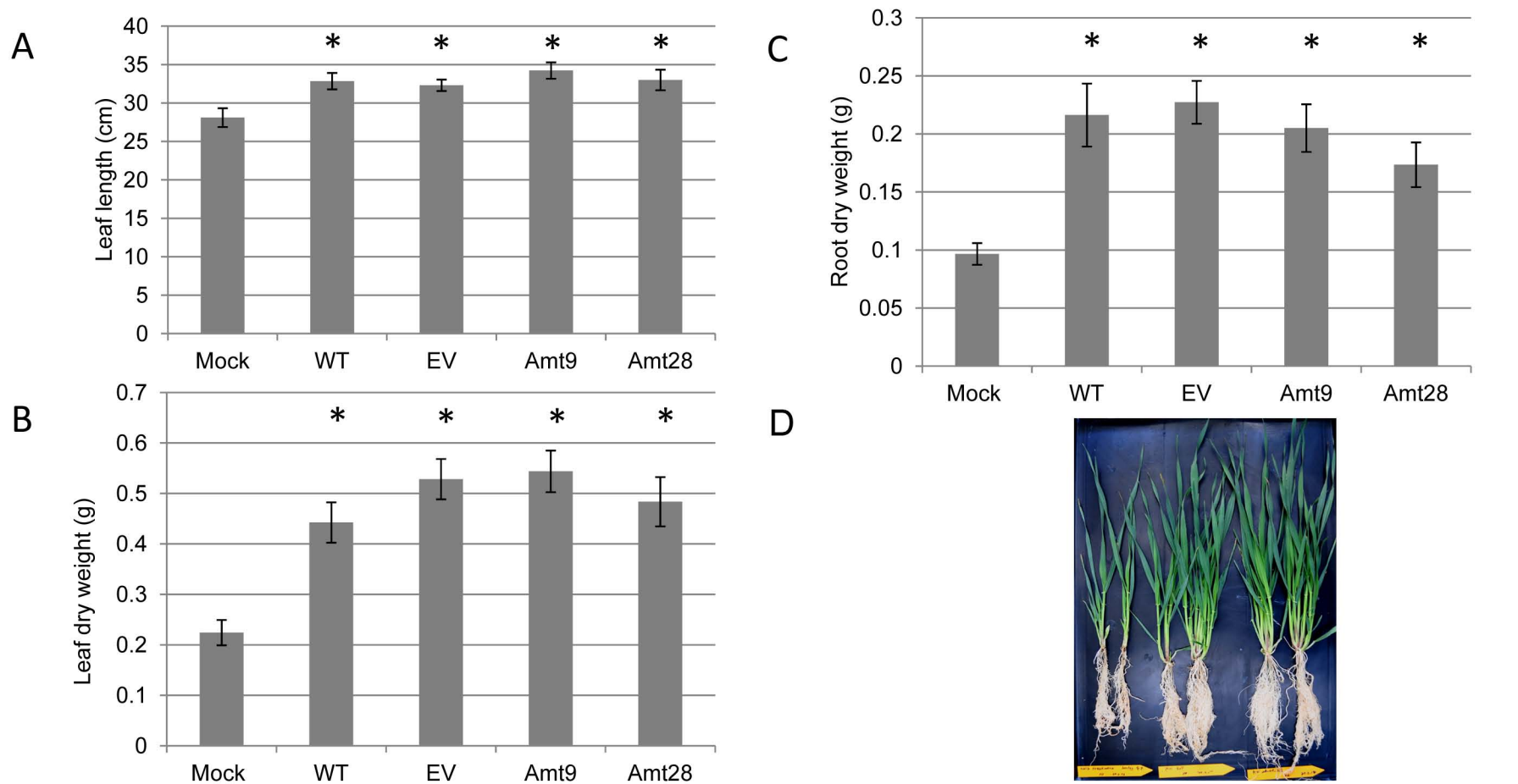
Southern blot

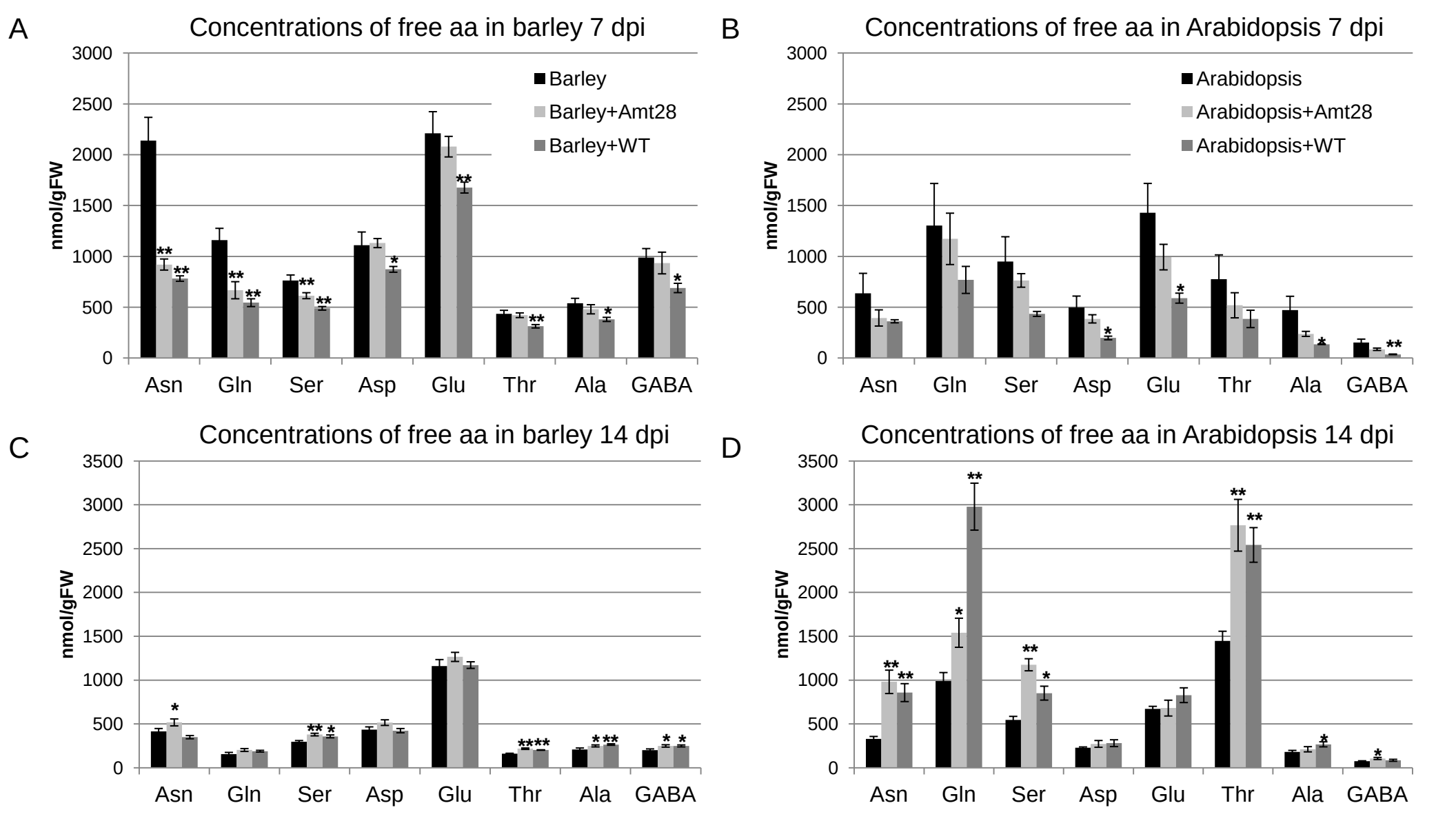


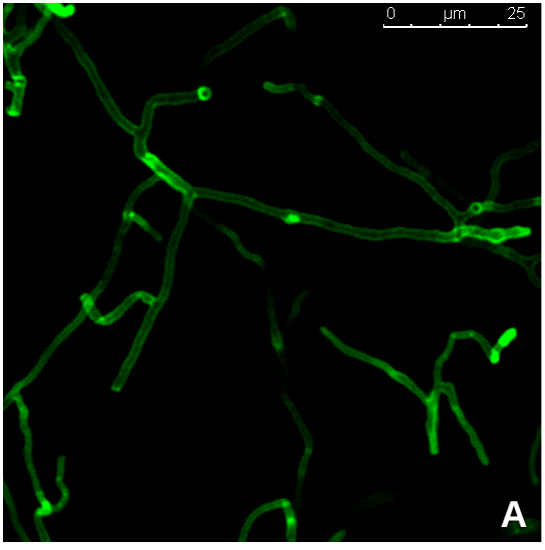
B

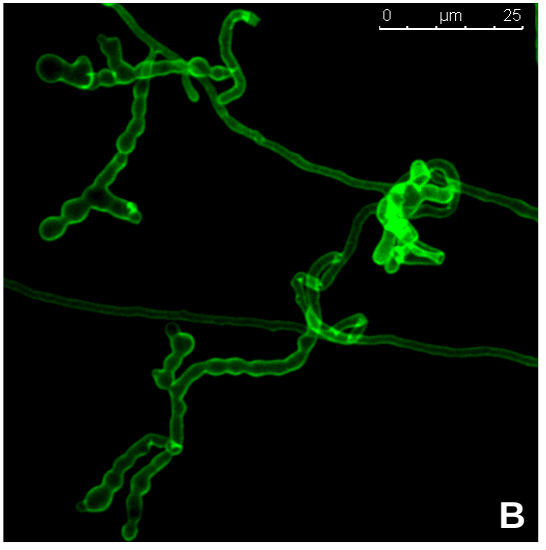
P. indica strains on YNB + 10 mM ammonium

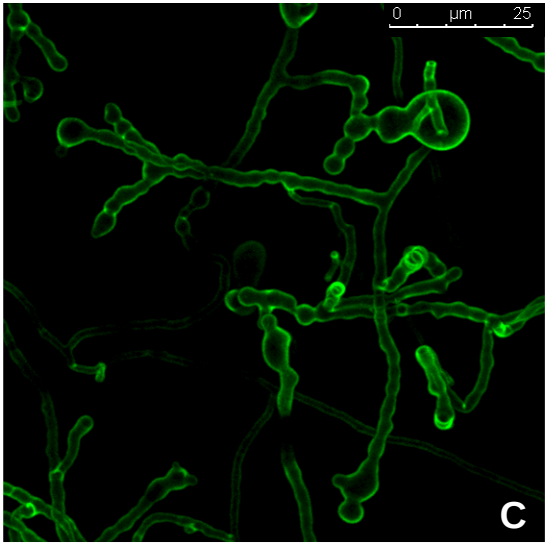


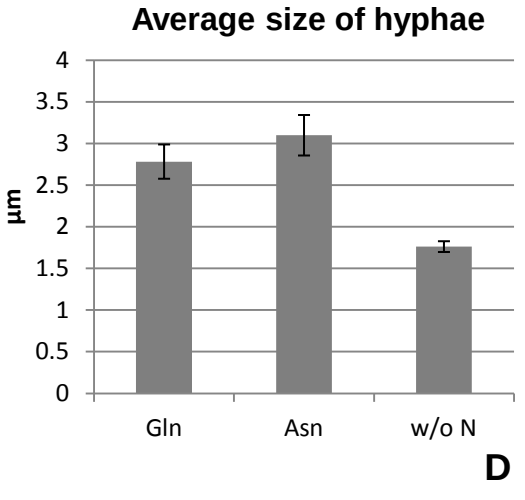

A
Leaf length (cm)
40
35
30
25
20
15
10
5
0
*
*
*
*
Mock
WT
EV
Amt9
Amt28
B
Leaf dry weight (g)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
*
*
*
*
Mock
WT
EV
Amt9
Amt28
C
Root dry weight (g)
0.3
0.25
0.2
0.15
0.1
0.05
0
*
*
*
*
Mock
WT
EV
Amt9
Amt28
D

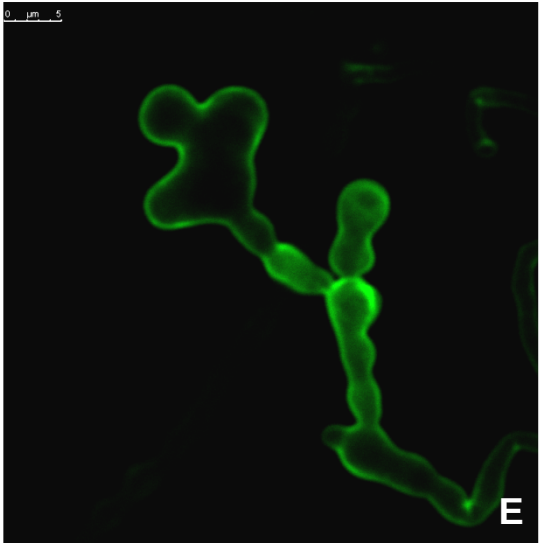

A
Concentrations of free aa in barley 7 dpi
nmol/gFW
Barley
Barley+Amt28
Barley+WT
Asn
Gln
Ser
Asp
Glu
Thr
Ala
GABA
B
Concentrations of free aa in Arabidopsis 7 dpi
Arabidopsis
Arabidopsis+Amt28
Arabidopsis+WT
C
Concentrations of free aa in barley 14 dpi
D
Concentrations of free aa in Arabidopsis 14 dpi

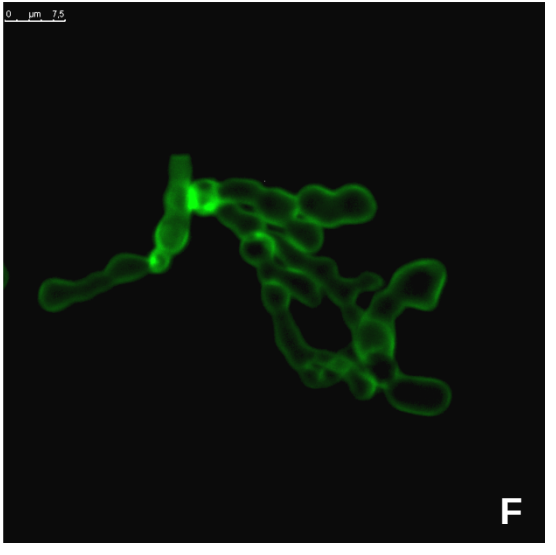

0 µm 25
A

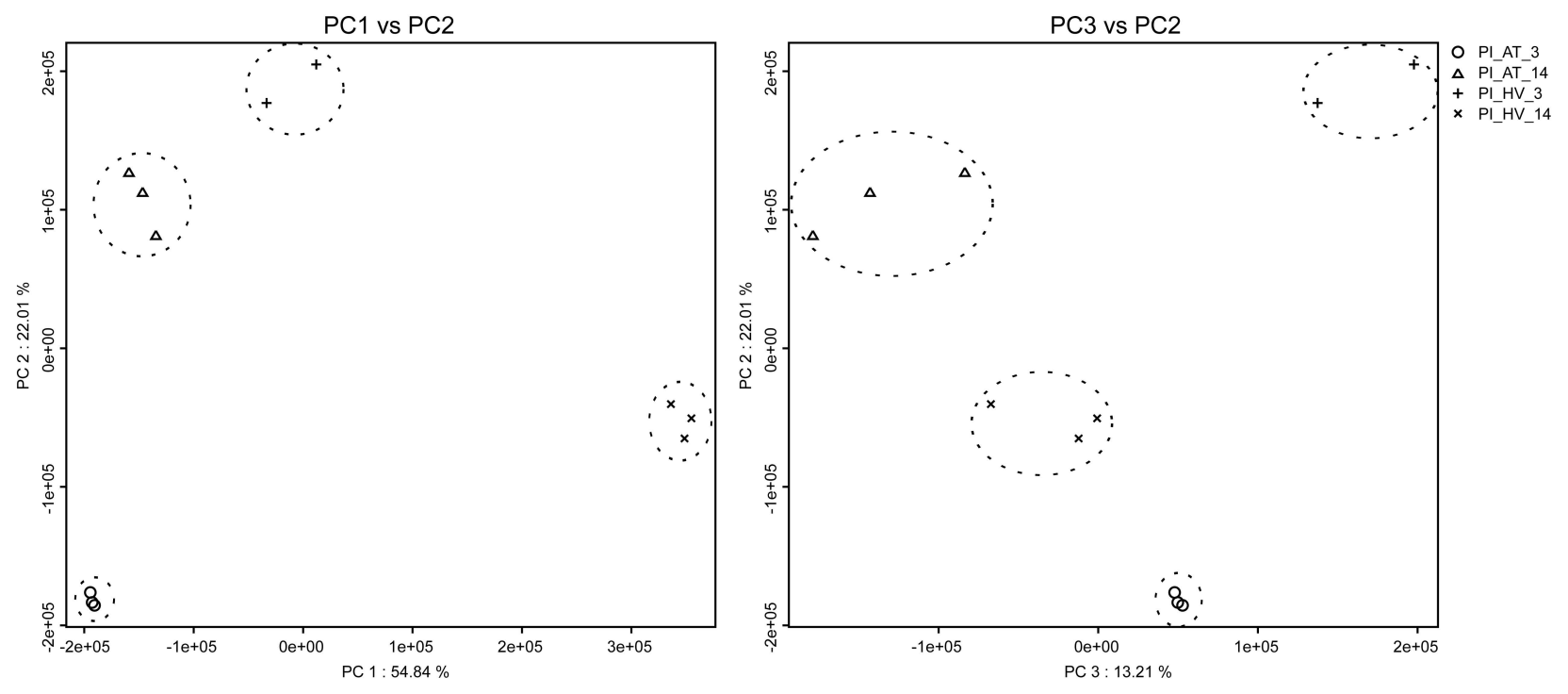

0 µm 25
B


0 µm 25
C


Average size of hyphae
µm
4
3.5
3
2.5
2
1.5
1
0.5
0
Gln
Asn
w/o N
D


E


F


PC1 vs PC2
PC 1 : 54.84 %
PC 2 : 22.01 %
-2e+05
-1e+05
0e+00
1e+05
2e+05
3e+05
PC3 vs PC2
PC 3 : 13.21 %
PI_AT_3
PI_AT_14
PI_HV_3
PI_HV_14