Plant genes determining compatibility with hyphal pathogens and symbionts

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Abbreviations

°C	degree(s) Celsius
Δ	delta
µ	micro
aa	amino acid
AM	Arbuscular Mycorrhiza
Amt	ammonium transporter
ANOVA	analysis of variance
bp	base pair(s)
BLAST	Basic Local Alignment Search Tool
Ca^{2+}	calcium
CCaMK	Calcium/Calmodulin dependent Kinase
cDNA	complementary DNA
CFP	Cyan Fluorescent Protein
CLSM	confocal laser scanning microscopy
co	complementation line
Col-0	Columbia-0
CSGs	common symbiosis genes
df	degree(s) of freedom
DMR	Downy Mildew Resistant
DNA	desoxyribonucleic acid
dpi	day(s) post infection/inoculation
FLS2	Flagellin-Sensing 2
fwd	forward
g	gram(s) / gravitational acceleration
g/L	gram(s) per liter
GDPC	glycin-asparagin-prolin-cystein
GFP	Green Fluorescent Protein
GG	golden gate
GPAT	Glycerol-Phosphate Acyl-Transferase
GUS	β-Glucuronidase
h	hour(s)
HCSG	homologs of common symbiosis genes
HO	Hoagland's

Hpa	*Hyaloperonospora arabidopsidis*
HR	hypersensitive response
HSD	honestly significant difference
IOS	Impaired Oomycete Susceptibility
ISR	induced systemic resistance
JA	jasmonic acid
kg	kilogram
K	potassium
KDRI	Kazusa DNA Research Institute
L	litre(s)
LRR	leucine-rich repeat
m	meter(s) / mili
M	Molar
μ	micro
min	minute(s)
mL	milliliter(s)
MLD	malectin-like domain
mm	millimeter(s)
mRNA	messenger RNA
MLO	Mildew Resistance *Locus* O
mol	mol(es)
MS	Murashige and Skoog
MST	Monosaccharide Transporter
MYB	myeloblastosis
N	nirogen
n	nano
NASC	The Nottingham Arabidopsis Stock Centre
$NH4^+$	ammonium
NIN	Nodule Inception
NO_3^-	nitrate
NUP	nucleoporin
OXI	Oxidative Signal Inducible
p	p-value
P	probability
P	phosphate

PAD4	Phytoalexin Deficient 4
PAMP	pathogen-associated molecular pattern
PCR	polymerase chain reaction
PDK	Phosphoinositide-Dependent protein Kinase
Pi	inorganic orthophosphate
PI	propidium iodide
PI-LTP	Phosphatidylinositol-Lipid Transfer Protein
PLD	Phospholipase D
PMR	Powdery Mildew Resistant
PPA	prepenetration apparatus
PR1	Pathogenesis-Related Gene 1
PSKR1	Phytosulfokine Receptor 1
PT	phosphate transporter
qRT-PCR	quantitative real-time PCR
RAM2	Required for Arbuscular Mycorrhiza 2
RLK	receptor-like kinase
rev	reverse
RIC	ROP (RHO-like GTPase of Plants)-Interactive CRIB (CDC42/RAC interactive binding)
rRNA	ribosomal RNA
ROI	region of interest
ROS	reactive oxygen species
rpm	rotations per minute
RT	room temperature
RT-PCR	polymerase chain reaction following reverse transcription
RNA	ribonucleic acid
s	second(s)
SA	salicylic acid
S.D.	standard deviation
SEH	*SEC13* Homolog
ShRK	SYMRK homologous Receptor-like Kinase
SP	signal peptide
SYM	symbiosis
SYMRK	Symbiosis Receptor-like Kinase

TAIR	The Arabidopsis Information Resource
TEF	Transcriptional Elongation Factor
TM	transmembrane domain
TX	texas red
UBI	Ubiquitin
WGA-AF	wheat germ aglutinin
WT	wild-type
X-Gluc	5-bromo-4-chloro-3-indolyl glucuronide
w/v	weight/volume
YFP	Yellow Fluorescent Protein

Species names and affiliations

Plants (Kingdom Plantae)

Full scientific name	Abbreviation	Order	Common name
Adenostyles alliariae (Gouan) A. Kern.	*A. alliariae*	Asterales	hedge-leaved Adenostyle
A. thaliana thaliana (L.). Heynh	*A. thaliana*, *A. thaliana*	Brassicales	thale cress
Brassica rapa L.	*B. rapa*	Brassicales	chinese cabbage
Hordeum vulgare L.	*H. vulgare*	Poales	barley
Lotus japonicus (Regel) K. Larsen	*L. japonicus*	Fabales	birdsfoot trefoil
Lycopersicon esculentum Mill.	L. esculentum	Sonalanes	tomato
Medicago truncatula Gaertn.	*M. truncatula*	Fabales	barrel medic
Oryza sativa L.	O. sativa	Poales	rice
Plantago lanceolata L	*P. lanceolata*	Lamiales	narrowleaf plantain
Triticum aestivum L.	*T. aestivum*	Poales	wheat
Zea mays L.	*Z. mais*	Poales	maize
Nicotiana tabacum L.	*N. tabacum*	Solanales	tobacco

Animals (Kingdom Animalia)

Full scientific name	Abbreviation	Order	Common name
Melitaea cinxia L.	*M. cinxia*	Lepdoptera	Glanville fritillary
Phaedon cochleariae Fabricius.	*P. cochleariae*	Coleoptera	mustard beetle
Oreina elongate Suffrian.	*O. elongata*	Coleoptera	alpine leaf beetle
Oreina cacaliae Schrank.	*O. cacaliae*	Coleoptera	alpine leaf beetle
Spodoptera littoralis Boisduval.	*S. littoralis*	Lepdoptera	african cotton leafworm

Fungi (Kingdom Fungi)

Full scientific name	Abbreviation	Order	Common name
Alternaria brassicae (Berk.). Sacc	*A. brassicae*	Pleosporales	Alternaria black spot
Blumeria graminis (DC.) Speer	*B. graminis*	Erysiphales	barley powdery mildew

Erysiphe orontii	*E. orontii*	Erysiphales	Brassica powdery mildew
Erysiphe cruciferarum Opiz ex L. Junell	*E. cruciferarum*	Erysiphales	Brassica powdery mildew
Fusarium graminearum Schwabe	*F. graminearum*	Hypocreales	Fusarium head blight
Glomus intraradices Schenck & Smith	*G. intraradices*	Glomerales	AM fungus
Golovinomyces orontii (Castagne) V.P. Heluta	*G. orontii*	Erysiphales	Brassica powdery mildew
Piriformospora indica Sav. Verma, Aj. Varma, Rexer, G. Kost & P. Franken	*P. indica*	Sebacinales	
Piriformospora williamsii Zuccaro & M. Weiss	*P. williamsii*	Sebacinales	
Podosphaera plantaginis (Castagne) U. Braun & S. Takam.	*P. plantaginis*	Erysiphales	powdery mildew
Rhizophagus irregularis Blaszk, Wubet, Renker & Buscot	*R. irregularis*	Glomerales	AM fungus
Uromyces cacaliae (DC) Unger.	*U. cacaliae*	Erysiphales	.rust fungus

Oomycetes (Kingdom Chromalveolata)

Full scientific name	Abbreviation	Order	Common name
Hyaloperonospora arabidopsidis (Gäum.) Göker, Riethm., Voglmayr, Weiß & Oberw.	*H. arabidopsidis*, *Hpa*	Peronosporales	downy mildew
Phytophtora infestans (Mont.) de Bary	*P. infestans*	Peronosporales	late blight
Phytophthora palmivora Butler.	*P. palmovora*	Peronosporales	bud-rot of palms

Bacteria (Kingdom Eubacteria)

Full scientific name	Abbreviation	Order
Mesorhizobium loti	*M. loti*	*Rhizobiales*
Pseudomonas syringae	*P. syringae*	Pseudomonales

List of publications

Research papers

Banhara A*, Ried MK*, Binder A, Gust AA, Höfler C, Hückelhoven R, Nürnberger T, and Parniske M (2015) Symbiosis-related genes sustain the development of a downy mildew pathogen on *A. thaliana.* Under review.

* These authors contributed equally to the work.

Banhara A, D Yi, Kühner R, Zuccaro A and Parniske M (2015) Biotrophic colonization of host roots by Piriformospora indica occurs independently of plant common symbiosis genes. Under review.

Lahrmann U, Ding Y, **Banhara A**, Rath M, Hajirezaei MR, Döhlemann S, von Wirén N, Parniske M, Zuccaro A (2013) Host-related metabolic cues affect colonization strategies of a root endophyte. Proc Natl Acad Sci USA. 110(34): 13965-70.

Alves-Ferreira M, Wellmer F, **Banhara A**, Kumar V, Riechmann JL, Meyerowitz EM (2007) Global expression profiling applied to the analysis of *A. thaliana* stamen development. Plant Physiol. 145: 747–762.

1. Summary

The majority of land plants are able to engage in root symbiosis with arbuscular mycorrhizal (AM) fungi. As member of the Brassicales, the model plant *Arabidopsis thaliana* is unable to form AM symbiosis, a fact that is correlated with a partial loss of the archaic genetic program for the intracellular accommodation of AM fungi that constitutes the common symbiosis pathway. Despite this partial loss, *A. thaliana* retained homologs of common symbiosis genes (HCSGs), raising questions on evolutionary forces that drive the maintenance of this core program on an AM-asymbiotic plant. Biotrophic microorganisms like AM fungi associates with living plant cells and often colonize them by forming specialized intracellular feeding structures that are surrounded by a plant-derived membrane, named arbuscules in symbiosis, or haustoria in pathogenic interactions. Based on structural and functional similarities between those accommodation structures, we speculated that the *A. thaliana* HCSGs might be utilized during the interaction with other biotrophic microorganisms other than AM fungi. In our work, we used two main approaches to test this hypothesis, that is, the interactions of *A. thaliana* with the biotrophic root symbiont *Piriformospora indica*, and with two biotrophic foliar pathogens: the oomycete *Hyaloperonospora arabidopsidis* (*Hpa*), and the powdery mildew fungus *Erysiphe cruciferarum*. We show that root colonization by *P. indica* is independent of the common symbiosis genes (CSGs) in the legume *Lotus japonicus* or its homologs in *A. thaliana.* Interestingly, though, the CSGs seem to have a negative role on fungal proliferation in *L. japonicus* roots, suggesting an interconnection between symbiosis signalling and plant defence or developmental programs. Colonization of *A. thaliana* leaves by *E. cruciferarum* is also independent of the HCSGs. We discovered, however, that these genes are required for haustoria development and full reproductive success of *Hpa* on *A. thaliana*, and the decreased susceptibility to the oomycete observed in the HCSG mutants occurs without constitutive or exacerbated activation of known defence responses. Together our findings reveal novel genetic commonalities in the plant program for the intracellular accommodation of biotrophic organisms in symbiosis and disease. The retention of HCSGs in the *A. thaliana* lineage calls for a selective advantage conferred by these genes, despite them supporting the colonization of an oomycete pathogen. We were therefore prompted to

investigate whether ecological conditions exist, under which *Hpa* improves host fitness.

2. Zusammenfassung

Ein Großteil der terrestrischen Pflanzen ist in der Lage Wurzelsymbiosen mit arbuskulären Mykorrhizapilzen (AM Pilze) einzugehen. Als Mitglied der Brassicales kann die Modellpflanze *A. thaliana* keine AM Symbiose ausbilden. Dies steht in Zusammenhang mit dem teilweisen Verlust eines archaischen genetischen Programms, des "common symbiosis" Signalwegs, welcher für die intrazelluläre Einnistung von AM Pilzen nötig ist. Trotz dieses teilweisen Verlusts, finden sich noch Homologe von "common symbiosis" Genen (HCSGs) in *A. thaliana*, was Fragen nach den evolutionären Triebkräften aufwirft, die für den Erhalt dieses Programmes in einer AM asymbiotischen Pflanze verantwortlich sind. Biotrophe Organismen wie AM Pilze kolonisieren Pflanzenzellen üblicherweise durch die Ausbildung von speziellen Ernährungsstrukturen, die von einer pflanzlichen Membran umschlossen sind. In der AM Symbiose werden diese Strukturen als Arbuskel bezeichnet; bei pathogenen Interaktionen spricht man von Haustorien. Aufgrund von strukturellen und funktionellen Gemeinsamkeiten zwischen diesen Strukturen, vermuteten wir, dass die *A. thaliana* HCSGs in biotrophen Interaktionen mit anderen Mikroorganismen als AM Pilzen eine Rolle spielen. In der vorliegenden Arbeit nutzten wir zwei Hauptansätze um diese Hypothese zu testen, nämlich die Untersuchung der Interaktion von *A. thaliana* mit dem biotrophen Wurzelsymbionten *Piriformospora indica* und mit zwei biotrophen Blattpathogenen: dem Oomyceten *Hyaloperonospora arabidopsidis* (*Hpa*) und dem Mehltaupilz *Erysiphe cruciferarum.* Wir zeigen, dass die Besiedlung durch *P. indica* in der Leguminose *Lotus japonicus* und in *A. thaliana* unabhängig von den "common symbiosis" Genen (CSGs) bzw. den HCSGs ist. Interessanterweise scheinen die CSGs jedoch eine negative Rolle bei der Vermehrung des Pilzes in *L. Japonicus* Wurzeln auszuüben, was auf eine Verbindung zwischen dem symbiotischen Signalweg und den pflanzlichem Abwehr- oder Entwicklungsprogrammen schließen lässt. Die Kolonisierung von *A. thaliana* Blättern durch *E. cruciferarum* ist ebenfalls unabhängig von den HCSGs. Wir stellen jedoch fest, dass diese Gene für die Haustorienentwicklung und für den maximaler Fortpflanzungserfolg von *Hpa* in *A. thaliana* nötig sind. Die beobachtete verminderte Suszeptibilität gegenüber dem Oomyceten in HCSG Mutanten geht dabei nicht mit einer konstitutiven oder verstärkten Aktivierung von Abwehrreaktionen einher.

Zusammengenommen offenbaren diese Ergebnisse neuartige genetische Gemeinsamkeiten im pflanzlichen Programm bei der Einnistung von symbiotischen und krankheitsauslösenden biotrophen Organismen. Die Tatsache, dass HCSGs im Genom von *A. thaliana* behalten wurden/konserviert sind, legt nahe, dass diese Gene nicht nur die Kolonisierung durch einen pathogenen Oomyceten unterstützen, sondern außerdem selektive Vorteile für die Pflanze bieten. Aus diesem Grund untersuchen wir, ob es ökologische Bedingungen gibt, unter denen *Hpa* die Fitness der Wirtspflanze verbessert.

3. Aims of the thesis

The main objective of this thesis was to elucidate the role of symbiosis-related genes of *Arabidopsis thaliana* and *Lotus japonicus* in biotrophic interactions with hyphal pathogens and symbionts. With that purpose we addressed the following:

1. Investigate the influence of plant genotype on root cell colonization and/or on the growth-promoting effect of the fungal endophyte *Piriformospora indica* on *A. thaliana*;
    1.1. Explore the existence of overlaps between the root-*P. indica* association and classical root symbioses with rhizobia and arbuscular mycorrhizal fungi;
    1.2. Investigate the role of HCSGs in the intracellular colonization of *A. thaliana* roots;
    1.3. Examine whether the CSGs are required for the colonization of *L. japonicus* roots by *P. indica*;
    1.4. Confirm growth promotion of *A. thaliana* (Col-0) by *P. indica* and to what extent this effect is dependent on the HCSGs.

2. Evaluate the requirement of the HCSGs for the intracellular accommodation and reproduction of the oomycetal pathogen *Hyaloperonospora arabidopsidis* (*Hpa*) in *A. thaliana* leaf epidermal cells;

3. Analyse if the HCSGs are important for colonization of *A. thaliana* leaves by the fungal pathogen *Erysiphe cruciferarum*;

4. Test whether the HCSGs are necessary for the infection of *A. thaliana* with the extracellular bacterial pathogen *Pseudomonas syringe*.

5. Exclude the role of the *A. thaliana* HCSGs as potential negative regulators of plant immunity by looking for altered defence responses on HCSG mutants upon infection with the pathogenic oomycete *Hpa*.

4. Introduction

Plants engage in biotrophic relationships with fungi from diverse taxonomic groups as well as with oomycetes (Voegele & Mendgen, 2003; Schulze-Lefert, 2004), fungal-like eukaryotic microorganisms (Margulis & Schwartz, 2002). Obligate biotrophs depend on living cells to obtain their nutrients, grow and complete their life cycle (Spanu *et al.*, 2010). The interaction of plants with symbiotic biotrophs such as arbuscular mycorrhiza (AM) fungi is an important acquisition of higher plants that is thought to have assisted them in the colonization of land (Schüßler & Walker, 2011). On the other hand, pathogenic biotrophic organisms like oomycetes, rust fungi, and powdery mildew fungi are agents of devastating diseases of crop plants (Voegele & Mendgen, 2003; Schulze-Lefert, 2004). Even though symbiotic and pathogenic relationships have opposite outcomes for the plants, their establishments share features such as overall suppression of or evasion from host immune responses (Okmen & Doehlemann, 2014; van Schie & Takken, 2014). Moreover, both pathogenic and symbiotic biotrophs form specialized intracellular infection structures (Yi & Valent, 2013) known as arbuscules in the AM symbiosis and haustoria in pathogenic interactions. Both structures are surrounded by plant-derived membranes called, respectively, periarbuscular membrane and perihaustorial membrane, which isolate the microorganisms from the plant cytoplasm. These membranes delimitate the respective periarbuscular space or the extrahaustorial matrix (Mims *et al.*, 2004; Pumplin & Harrison, 2009), which likely constitute the site of nutrient and signal exchange between the microorganisms and the plant host (Mendgen & Hahn, 2002; Voegele & Mendgen, 2003; Parniske, 2008; Bonfante & Genre, 2010; Harrison, 2012). The structural and functional similarities (Harrison, 1999; Parniske, 2000) between accommodation organs for microbes in symbiosis and disease (Figure 1) raised the hypothesis that both types of interactions rely on a shared genetic program (Parniske, 2000).

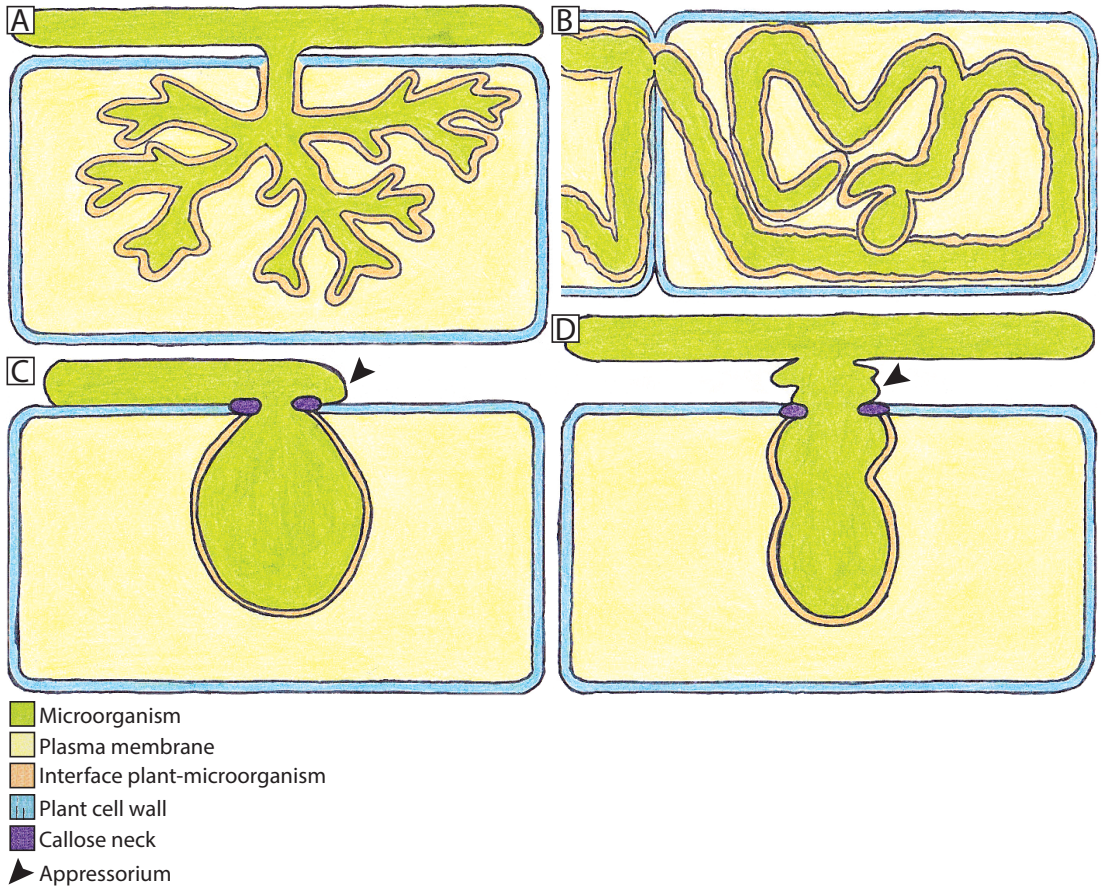

Figure 1 – Schematic representation of intracellular accommodation organs in symbiosis and disease. All accommodation organs are surrounded by a plant-derived membrane that separates them from the plant-cell cytoplasm and likely creates an environment for exchange of substances between plant and microorganisms. The highly specialized infection cell known as appressorium (arrow) (Deising *et al.*, 2000), and the callose neck (purple) that typically forms upon cell penetration during pathogenic interactions are shown. (A) The arbuscule of *Glomus sp*. inside a *Lotus japonicus* root cortical cell; (B) Hyphae of *Piriformospora indica* within a root epidermal cell from *Arabidopsis thaliana*; (C) The haustoria of an oomycete (*Hyaloperonospora parasitica*) inside an *A. thaliana* leaf mesophyll cell; (D) The haustoria of the powdery mildew fungus *Erysiphe cruciferarum* within a leaf epidermal cell of *A. thaliana*.

4.1 The arbuscular mycorrhiza symbiosis and the common symbiosis pathway

AM symbiosis is one of the most widespread types of symbiosis between plants and microorganisms. It has existed for more than 400 million years and is present in circa 80% of all terrestrial plant species (Parniske, 2008; Smith & Read, 2008). In AM

symbiosis, plants provide carbon sources to fungi of the phylum Glomeromycota, which in turn deliver water, phosphate, nitrogen, and other mineral nutrients via finely branched, tree-shaped hyphae, called arbuscules (Gutjahr & Parniske, 2013; Gutjahr, 2014). The intracellular accommodation of fungal symbionts requires extensive reprograming of the plant host cell. This process starts with the first contact between fungal hyphae and the plant roots, followed by the formation of the hyphopodium, a structure that attaches to the root surface at the fungal entry point preceding cell invasion (Gutjahr & Parniske, 2013). Even before cell penetration, hyphopodium formation activates cellular processes in which the plant cell nucleus shifts towards the point of contact with the AM fungus before moving through the plant cell vacuole. This initiates the formation of the prepenetration apparatus, a transcellular tubular cytoplasmic column that connects the nucleus of the plant cell with the fungal entry point. This course of events, which occurs in every cell preceding fungal penetration, guides the way of the fungus through the rhizodermal and the outer cortical cells (Genre *et al.*, 2005; Genre *et al.*, 2008). When the inner cortex is reached, the hypha enters the cell, where it finally branches to form the arbuscule (Gutjahr & Parniske, 2013).

The establishment of the AM symbiosis requires an ancient genetic program that is conserved among angiosperms and comprises the "common symbiosis genes" (CSGs) (Kistner & Parniske, 2002; Markmann *et al.*, 2008). In four orders of eurosids, the CSGs are also required for root nodule symbiosis with nitrogen-fixing bacteria (Kistner *et al.*, 2005; Gherbi *et al.*, 2008). In the order Fabales (legumes), the interaction occurs with bacteria generally known as rhizobia, while in the Rosales, Cucurbitales, and Fagales the symbiosis is termed Actinorhiza and characterizes the interaction with *Frankia* bacteria (Pawlowski & Bisseling, 1996). The proteins encoded by the CSGs participate in a signal transduction pathway that leads from the perception of microbe signals at the plasma membrane to the activation of symbiosis-related genes in the nucleus (Gutjahr & Parniske, 2013).

AM symbiosis formation requires calcium-spiking, periodic calcium oscillations in the nucleus and in the peri-nuclear region that constitute one of the earliest measurable plant responses during symbiotic stimulation (Ehrhardt *et al.*, 1996; Miwa *et al.*, 2006; Oldroyd, 2013). In the legume *Lotus japonicus*, the products of the CSGs Symbiosis Receptor-like Kinase (SYMRK; (Stracke *et al.*, 2002; Markmann *et al.*,

2008), the nucleoporins of the NUP107-160/NUP84 sub-complex (Alber *et al.*, 2007) NUP85, NUP133 and the SEC13 HOMOLOG1 (SEH1) NENA (Kanamori *et al.*, 2006; Saito *et al.*, 2007; Groth *et al.*, 2010), as well as the nuclear-envelope localised cation channels CASTOR and POLLUX (Adam *et al.*, 1999; Charpentier *et al.*, 2008; Venkateshwaran *et al.*, 2012), are all required for calcium-spiking generation, while the nuclear complex Calcium and Calmodulin dependent protein Kinase (CCaMK)-CYCLOPS is responsible for decoding these signals (Singh & Parniske, 2012; Singh *et al.*, 2014).

It has been speculated that the symbiotic phenotypes of *L. japonicus* mutants defective in the CSGs coding for the nucleoporins *seh1*, *nup133*, or *nup85* could be related to a decrease of POLLUX levels in the inner nuclear membrane due to impaired import caused by structural defects in the NUP107-160 subcomplex (Capoen *et al.*, 2011; Binder & Parniske, 2013). Many nucleoporins of this complex show a high rate of evolution (Bapteste *et al.*, 2005), potentially allowing for distinct functional adaptations while keeping the overall complex structure intact. This rapid evolution may be facilitated by the structural modularity of alpha-solenoid and beta-propeller domains shared by many of these nucleoporins (Hoelz *et al.*, 2011). Agreeing with this, phenotypes of individual NUP107-160 subcomplex mutants vary both in occurrence and severity depending on the organism (Gonzalez-Aguilera & Askjaer, 2012; Binder & Parniske, 2013).

The model plant *A. thaliana* lost the ability to undergo root symbiosis with AM fungi. This loss, which happened after the divergence of the Brassicales, is correlated with the absence of a set of CSGs genes, including *CYCLOPS* and *CCaMK* (Delaux *et al.*, 2014).

4.2 The *Piriformospora indica* symbiosis with plant roots

Besides AM symbiosis, plants are able to undergo root symbiosis with sebacinoid fungi, which belong to the phylum Basidiomycota and are widespread root associated filamentous fungi able to engage into ecto- and endomycorrhizal interactions (Selosse *et al.*, 2009). Members of the order Sebacinales frequently form endophytic interactions with various taxonomically unrelated hosts, displaying a generalist lifestyle (Weiss *et al.*, 2004). The experimental model for this group of fungi is the

root endophyte *Piriformospora indica*, whose association with plant roots results in a series of benefits to the host. Apart from growth promotion (Peškan-Berghöfer *et al.*, 2004; Shahollari *et al.*, 2007; Sherameti *et al.*, 2008; Camehl *et al.*, 2010; Camehl *et al.*, 2011; Hilbert *et al.*, 2012; Nongbri *et al.*, 2012; Lahrmann *et al.*, 2013; Venus & Oelmuller, 2013), plants grown in the presence of the fungus are more tolerant to abiotic (Waller *et al.*, 2005; Sherameti *et al.*, 2008; Kumar *et al.*, 2009; Ghabooli *et al.*, 2013; Jogawat *et al.*, 2013) and biotic stresses (Waller *et al.*, 2005; Deshmukh & Kogel, 2007; Stein *et al.*, 2008; Molitor *et al.*, 2011), and show increased uptake of nutrients such as phosphorus and nitrogen (Sherameti *et al.*, 2005; Kumar *et al.*, 2011).

Like AM fungi, *P. indica* is able to undergo at least part of its life cycle as a biotroph. However, in plants such as barley and *A. thaliana*, *P. indica* has a distinct, biphasic lifestyle (Jacobs *et al.*, 2011; Zuccaro *et al.*, 2011; Lahrmann & Zuccaro, 2012; Lahrmann *et al.*, 2013). After the initial biotrophic stage, during which the fungus grows within metabolically active root cells, a phase characterized by cell death and necrotrophy or saprotrophy follows. At this point, *P. indica* grows and feeds from dead root cells even though the plant host displays no obvious signs of disease (Deshmukh *et al.*, 2006; Zuccaro *et al.*, 2011; Lahrmann & Zuccaro, 2012; Qiang *et al.*, 2012). Despite the fact that *P. indica* possesses characteristics of both biotrophic and saprotrophic fungi, its lifestyle contrasts with the lifestyle of hemibiotrophic pathogens, whose saprotrophy growth is normally associated with the appearance of diseases symptoms in the host (Zuccaro *et al.*, 2011).

To date, little is known about plant genes that directly contribute to the intracellular accommodation of *P. indica* in plant roots during the biotrophic phase, with exception of the tubby-like proteins, which are necessary for normal colonization of *A. thaliana* roots by *P. indica* during this colonization stage (Reitz *et al.*, 2012; Reitz *et al.*, 2013). Tubby-like proteins participate in vesicle trafficking in mammals (Mukhopadhyay & Jackson, 2011) and, in plants, they are targeted to the plasma membrane and released upon abiotic stresses to activate intracellular signalling (Santagata *et al.*, 2001). In regards to host growth promoted by *P. indica*, a few molecules have been shown to participate in this process, such as components of the OXI1 kinase pathway (Camehl *et al.*, 2011), which is involved in plant responses to pathogens such as the oomycete *Hyaloperonospora parasitica* (Rentel *et al.*, 2004), or the *CYP79B2*, *CYP79B3*,

CYP71A13, *PAD3*, and *WRKY33* genes, all required for the synthesis of indole-3-acetaldoxime (IAOx)-derived compounds in *A. thaliana* roots colonized by *P. indica* (Nongbri *et al.*, 2012). In *A. thaliana*, IAOx is an intermediate in the biosynthesis of the hormone IAA and the phytoalexin camalexin (Mikkelsen *et al.*, 2009; Burow *et al.*, 2010), which is usually produced in response to plant pathogens (Glawischnig, 2007; Rauhut & Glawischnig, 2009). In the *cyp79b3*, *cyp79b3*, *cyp71a13*, *pad3*, and *wrky33* mutants, the fungus grows in an uncontrolled manner and the host growth promotion effect is no longer present.

4.3 Nutrient exchange in AM and *P. indica* symbioses

Even though phosphorus is indispensable for plant growth and development, it is mostly available in the soil as inorganic orthophosphate (Pi), which is readily sequestered by cations under acid conditions (Tinker & Nye, 2000), resulting in low availability of free Pi to the plants (Bieleski, 1973). In this context, AM fungi play a crucial role in plant nutrition, as they are able to take up Pi from the soil and release it to the plant (Jakobsen, 1995; Harrison, 1997; Smith & Smith, 2011). Phosphorus is quantitatively the most important mineral nutrient transported from the fungus to the plant in AM symbiosis (Smith & Read, 2008). Its uptake by the host plant requires first the assimilation of Pi by the fungus via high-affinity phosphate transporters expressed exclusively in the extraradical mycelium (Harrison & van Buuren, 1995; Maldonado-Mendoza *et al.*, 2001; Fiorilli *et al.*, 2013), where it is stored in vacuoles as polyphosphate (poly-Pi) before being transported to the intraradical hyphae (Callow *et al.*, 1978; Solaiman *et al.*, 1999; Ezawa *et al.*, 2005). Pi is then hydrolysed and translocated to the periarbuscular space, where it is taken up by cortical root cells (Javot *et al.*, 2007b). This last step involves plant transporters located at the periphery of arbuscules (Bapaume & Reinhardt, 2012). The most studied phosphate transporter specifically induced during AM symbiosis and expressed at the periarbuscular membrane is the *Medicago truncatula* MtPT4 (Harrison *et al.*, 2002). Nevertheless, phosphate transporters that are strongly upregulated upon mycorrhization and expressed in arbuscule-containing cells have been identified in other plant species as well, including potato, tomato, *L. japonicus*, and rice (Rosewarne *et al.*, 1997; Rausch *et al.*, 2001; Paszkowski *et al.*, 2002; Nagy *et al.*, 2005; Maeda *et al.*, 2006; Glassop *et al.*, 2007; Gutjahr *et al.*, 2008). The establishment of AM symbiosis depends on

nutrient availability, and high Pi concentrations in the soil can inhibit the symbiotic interaction (Breuillin *et al.*, 2010; Balzergue *et al.*, 2013; Bonneau *et al.*, 2013; Gosling *et al.*, 2013). Phosphate availability also determines the amount of carbon transferred from the plant to the fungus, which is the "carbon fee" the plant host needs to pay in exchange for the mineral nutrients obtained from the fungus and corresponds to up to 20% of the total carbon photosynthetically fixed (Bago *et al.*, 2003). In the situation of high Pi availability, the symbiosis turns out to be no longer necessary, and the carbon transfer towards the fungus, which occurs in the form of monosaccharides, is reduced (Olsson *et al.*, 2010). The sugar transporter Monosaccharide Transporter2 (MST2) from *Rhizophagus irregularis* (previously known as *Glomus intraradices* (Kruger *et al.*, 2012)) and *Glomus* sp., the largest genus of AM fungi (Schwarzott *et al.*, 2001; Schüßler & Walker, 2011), has been recently described. It participates in the transport of glucose and other hexoses to the fungus and, interestingly, has a temporal expression pattern that coincides with that of MtPT4 (Helber *et al.*, 2011).

While phosphorus nutrition during AM symbiosis has been well studied over the last decades, fewer is known about nitrogen transport to the plant, even though AM fungi improve nitrogen acquisition by the plant as well, mainly as ammonium (Villegas *et al.*, 1996; Smith & Read, 1997; Hawkins *et al.*, 2000; Toussaint *et al.*, 2004; Smith & Smith, 2011). After nitrogen is assimilated over the glutamine synthetase/glutamate synthase pathway (Jin *et al.*, 2005; Cruz *et al.*, 2007), it is transported as arginine along the fungal hyphae (Govindarajulu *et al.*, 2005; Tian *et al.*, 2010) and cleaved by arginase in the arbuscules, where it is released to the symbiotic interface and delivered to the host as ammonium ($NH4^+$) (Bago *et al.*, 2003; Govindarajulu *et al.*, 2005). In support to this model, the *R. irregularis* $NH4^+$ transporter LjAMT2;2 was identified as preferentially expressed in arbuscule-containing cells (Bonfante & Genre, 2010). In addition, two *R. irregularis* $NH4^+$ transporters were shown to act in synchrony to promote $NH4^+$ assimilation and amino acid incorporation by the plant (Lopez-Pedrosa *et al.*, 2006; Perez-Tienda *et al.*, 2011). In *Glomus mosseae*, the $NH4^+$ transporter gene *GmAMT 4.1* was specifically upregulated in arbusculated cells during $NH4^+$ transfer across the periarbuscular membrane to the host cells (Kobae *et al.*, 2010). Corroborating the importance of nitrogen levels for the maintenance of a functional symbiosis, Javot and colleagues showed that, in a *M. truncatula* mutant defective in PT4, nitrogen limitation suppressed premature arbuscule degeneration and root

colonization by AM fungi occurred normally (Javot *et al.*, 2011). Furthermore, similar to high Pi levels in the soil surrounding the roots, elevated nitrogen concentrations can reduce carbon allocation to the fungus (Olsson *et al.*, 2005). Limitation of both phosphorus and nitrogen has a cumulative effect on root colonization, highlighting the importance of these nutrients for AM symbiosis formation (Bonneau *et al.*, 2013).

As in AM symbiosis, the presence of *P. indica* in the plant root also seems to improve nitrogen and phosphorus uptake by the plant host. Recently, it has been shown that phosphate transfer is a major player in the symbiosis between *P. indica* and maize plants. A high affinity phosphate transporter from the fungus (PiPT) was upregulated in *P. indica*-inoculated plants, especially under low phosphate availability, and phosphate was transported from the fungus to the host plants, which had increased biomass compared to non-inoculated controls (Yadav *et al.*, 2010; Pedersen *et al.*, 2013). These results are in accordance to previous findings in *A. thaliana*, where *P. indica* increased host phosphate uptake by up to three-fold (Kumar *et al.*, 2011).

The role of nitrogen uptake in the *A. thaliana*-*P. indica* association has not been conclusively established, even though it was suggested that, different than AM symbiosis, in the *P. indica* symbiosis nitrogen is taken up by the plants mainly in the form of nitrate (Sherameti *et al.*, 2008). It has been revealed that inoculated *A. thaliana* and tobacco seedlings show a transfer of nitrogen from the medium (with nitrate as nitrogen source) to the aerial part, with a concomitant induction of plant enzymes related to nitrate and starch metabolism. These findings are contrary to the observation that the preferred sources of nitrogen in *P. indica* are ammonium and glutamine instead of nitrate (Zuccaro *et al.*, 2011). Supporting the latter finding, we recently identified a $NH4^+$ transporter from the fungus, PiAmt1, which is responsible for sensing nitrogen levels in barley and leading to the switch from the biotrophic to the saprotrophic phase upon nitrogen starvation (Lahrmann *et al.*, 2013).

4.4 AM fungi and *P. indica* protect plant hosts against environmental challenges

There is evidence that colonization of plant roots by *P. indica* leads to improved plant tolerance against abiotic stresses such as drought (Sherameti *et al.*, 2008; Ghabooli *et al.*, 2013), high concentrations of salt (Waller *et al.*, 2005; Jogawat *et al.*, 2013), and

reactive oxygen species (Kumar *et al.*, 2009), and heavy metals (Oelmüller *et al.*, 2009). It also protects plants against pathogens like head blight (*Fusarium graminearum*) (Deshmukh & Kogel, 2007) and the powdery mildew fungi *Blumeria graminis* f. sp. *hordei* (Waller *et al.*, 2005; Molitor *et al.*, 2011) in barley, and *Golovinomyces orontii* in *A. thaliana* (Stein *et al.*, 2008). The improved resistance of plants colonized by *P. indica* against pathogens occurs regardless of the fact that the fungus induces a widespread suppression of pathogen-associated molecular pattern (PAMP)-induced host innate immunity (Jacobs *et al.*, 2011). The resistance of *P. indica*-colonized plants against powdery mildew fungi is thought to occur by a different defence mechanism, in which the pathogen reportedly leads to induced systemic resistance (ISR) both in barley (Waller *et al.*, 2005; Stein *et al.*, 2008; Waller *et al.*, 2008) and in *A. thaliana*. Improved tolerance to abiotic stresses (Mena-Violante *et al.*, 2006; Miransari, 2010; Aloui *et al.*, 2011) such as drought stress (Aroca *et al.*, 2007) and to biotic stresses (Smith & Read, 2008) have been described for plants undergoing associations with Glomeromycota fungi as well. Besides, similar to *P. indica*, AM fungi temporarily induce plant immunity at initial stages of root colonization, while the later stages of the symbiosis are characterized by localized suppression of defence responses (Kapulnik *et al.*, 1996), thought to be mediated by effector molecules that suppress plant immunity to allow the establishment of a successful infection (de Jonge *et al.*, 2011; Kloppholz *et al.*, 2011). *P. indica* effectors were also described as key players in the formation of a thriving interaction with the plant host (Lahrmann & Zuccaro, 2012; Lahrmann *et al.*, 2013).

4.5 Different approaches to explore possible genetic commonalities of biotrophic interactions

Despite the loss of symbiosis-related genes, *A. thaliana* retained vestiges of the symbiosis program as homologs of CSGs (HCSGs), such as *POLLUX*, *SYMRK-homologous Receptor-like Kinases* (*ShRKs*), and members of the NUP107-160 subcomplex, including *SEC13* and *NUP133*.

This fact raises questions regarding the evolutionary forces driving the retention of this core program and suggests that, despite differences among *P. indica* and AM symbioses, their genetic requirements could be overlapping. The *POLLUX* ortholog in

A. thaliana has already been shown not to be required for the *P. indica*-induced growth promotion (Shahollari *et al.*, 2007). Nevertheless, the study did not exclude that the gene is required for other aspects of the *P. indica* symbiosis, such as intraradical colonization *per se*. Furthermore, the transgenic complementation of a symbiosis-defective legume mutant by expression of *AtPOLLUX* variants suggested an overall functional conservation (Venkateshwaran *et al.*, 2012). This finding, together with the fact that HCSGs have been retained in the *A. thaliana* genome, and considering the structural and functional commonalities between the intracellular accommodation organs of biotrophic microorganisms in symbiosis and disease, led us to speculate that the partial retention of an ancestral symbiotic program in *A. thaliana* could be related to a selection pressure imposed by biotrophic interactions other than AM symbiosis, such as the interaction with *P. indica* itself. The fungus grows inside living plant cells without forming any characteristic accommodation organ. Nevertheless, a plant-derived membrane envelops its hyphae during the biotrophic phase (Jacobs *et al.*, 2011; Lahrmann & Zuccaro, 2012; Lahrmann *et al.*, 2013) and possibly creates an interface between fungal and plant membrane comparable to the peri-fungal space surrounding transcellular hyphae of AM fungi, where signal and nutrient exchange is likely to take place (Bonfante & Genre, 2010).

The oomycetal pathogen *Hpa* is responsible for the downy mildew of *A. thaliana* (reviewed in (Slusarenko & Schlaich, 2003)). Though it causes neither a highly destructive disease on the plants nor is economically important, the *Hpa*-*A. thaliana* association is a particularly well-studied plant-oomycete pathogen system that was established in the laboratories in the 1990's, and has been of great importance for the identification and cloning of disease resistance genes (Slusarenko & Schlaich, 2003; Heidel & Dong, 2006; Baxter *et al.*, 2010; Coates & Beynon, 2010; Fabro *et al.*, 2011; Stassen & Van den Ackerveken, 2011), and, more recently, of compatibility factors, which facilitate the colonization of the plant host or promote disease susceptibility by biotrophs without leading to exacerbated activation of known defence responses (Huibers *et al.*, 2009). Such factors have also been shown to play a role in the association between plants (including *A. thaliana*) and powdery mildew fungi, another group of biotrophic pathogens (Adam *et al.*, 1999).

4.6 The plant immune system

Different from mammals, which present both an innate immune system as well as a somatic adaptive immune system and mobile defender cells (Litman *et al.*, 2010), plants depend exclusively on their innate immune system to defend themselves against invading pathogens. The plant innate immune system that has been classically divided into two branches (Jones & Dangl, 2006). The first layer of defence is known as PAMP (pathogen associated molecular pattern)-triggered immunity (PTI), in which transmembrane pattern recognition receptors recognize PAMPs and trigger the defence responses. However, many pathogens have evolved the so-called effector proteins, which interfere with host responses and enables them to evade PTI (Dou & Zhou, 2012). To neutralize the activity of effectors, plants evolved mechanisms to recognize effectors using resistance (*R*) genes, most of which encode nucleotide-binding leucine-rich repeat (NB-LRR) proteins (Jones & Dangl, 2006; van Schie & Takken, 2014). The *R*-gene-mediated immunity consists of a more rapid and amplified layer of defence known as ETI (effector triggered immunity) (Jones & Dangl, 2006). This arms race between plant and pathogens results in a continuous process of co-evolution: while pathogens are constantly evolving new strategies, such as new effectors, to evade or supress plant immune responses, the plants continuously expand their receptor repertoire to recognize molecules associated with pathogen invasion (Jones & Dangl, 2006).

Even though PTI and ETI are triggered by different mechanisms, their outcomes vary mostly in the intensity of the responses (van Schie & Takken, 2014), which are characterized by a variety of symptoms, the most extreme being cell death induction during the hypersensitive response (HR). Other symptoms include accumulation of reactive oxygen species (ROS), production of defence molecules such as salicylic acid (SA) and jasmonate (JA), cell wall strengthening, and activation of defence gene expression (Jones & Dangl, 2006; Muthamilarasan & Prasad, 2013).

A few *A. thaliana* mutants with improved pathogen resistance, like *edr1, cpr1*, *snc1, ssi4*, *rin4*, and *cpr5*, show deregulated, often constitutively activated immune responses, usually presenting disease symptoms even in the absence of any trigger (Bowling *et al.*, 1994; Adam *et al.*, 1999; Li *et al.*, 2001; Shirano *et al.*, 2002; Lorrain *et al.*, 2003; Belkhadir *et al.*, 2004; Landoni *et al.*, 2013). Another class of more

resistant mutants, which includes *pskr1*, shows enhanced induction of defence responses after PAMP treatment or pathogen challenge (Frye & Innes, 1998; Igarashi *et al.*, 2012; Mosher *et al.*, 2013).

Interestingly, in *A. thaliana*, components of the NUP107-160/NUP84 sub-complex, which comprise some of the HCSGs, have been shown to play roles in plant defence; mutations in NUP96, NUP160 and SEH1 impair innate immune responses and resistance-gene mediated defence (Zhang & Li, 2005; Wiermer *et al.*, 2012).

4.7 Role of compatibility factors in biotrophy

When a pathogen or, for that matter, any other microorganism, is able to overcome plant defence responses and undergo long-lasting interactions with their host, which in the case of biotrophic partners generally involve the formation of specialized feeding structures, a situation of compatibility takes place (reviewed in (van Schie & Takken, 2014)). Compatibility (also referred to as susceptibility) factor candidates have been described to have specific roles in the plant host, such as in cell wall composition, cell cycle progression, vesicle trafficking, cytoskeleton functionality, and detoxification. These factors are often manipulated by the pathogens to ultimately allow for a successful colonization, and in favour of its own growth (Vogel & Somerville, 2000; Vogel *et al.*, 2004; Haga *et al.*, 2007; Pareja-Jaime *et al.*, 2008). Based on the stage of the interaction the compatibility factors are involved in, three main mechanisms of action have been described: genes involved in facilitating host recognition and cell penetration, genes associated with the negative regulation of plant immunity, and genes required to maintain the interaction, allowing the pathogen to grow and proliferate (reviewed in (van Schie & Takken, 2014). The first described compatibility factor was the membrane-anchored protein Mildew Resistance *Locus* O (MLO), discovered in barley (Freisleben & Lein, 1942) and shown to be required for susceptibility to powdery mildew in, among others, *A. thaliana*, tomato, and wheat (Consonni *et al.*, 2006; Bai *et al.*, 2008; Varallyay *et al.*, 2012; Zheng *et al.*, 2013). MLO-dependent susceptibility requires vesicle trafficking as well as actin polarization (Consonni *et al.*, 2006; Miklis *et al.*, 2007). In barley, the protein HvRIC171 is recruited by the powdery mildew fungus *B. graminis* to the site of infection and supports fungal penetration (Schultheiss *et al.*, 2008). The powdery mildew fungus

Golovinomyces cichoracearum has been shown to modify plant cell wall components encoded by the genes *POWDERY MILDEW RESISTANT 4* (*PMR4*, a callose synthase), *PMR5* (unknown function), and *PMR6* (pectate lyase) to facilitate entrance into the cell without activating defence responses, and the corresponding mutants are therefore less susceptible to the infection (Vogel & Somerville, 2000; Vogel *et al.*, 2002; Vogel *et al.*, 2004). The mutant *pmr4* is less susceptible to *Hpa* as well (Vogel & Somerville, 2000). Several genes involved in amino acid metabolism have been reported to have a role in the *Hpa*-*A. thaliana* compatibility; *DOWNY MILDEW RESISTANT 1* (*DMR1*), for instance, encodes a homoserine kinase whose absence results in reduced susceptibility to *Hpa* (Van Damme *et al.*, 2005; van Damme *et al.*, 2009). A recent analysis of the *A. thaliana* susceptibility transcriptome was performed on laser-dissected *G. orontii* infection sites, revealing that the fungus induces genetic reprograming of the host cells to turn them into sources of nutrients (Chandran *et al.*, 2010). In this study, the expression of *MYB3R4*, encoding a transcription factor involved in cell cycle progression, was upregulated at the infection sites, with the cells undergoing endoreduplication events that result in an increased metabolic capacity.

In this work, we investigated the role of symbiosis-related genes in the interaction of hyphal microorganisms with *L. japonicus* and *A. thaliana*. In *L. japonicus*, the CSGs were assessed for involvement in the intracellular colonization by the root endophyte *P. indica*, while in *A. thaliana* the involvement of HCSGs in the fungal-mediated host growth promotion or their roles as possible compatibility factors in biotrophic interactions with *P. indica*, the oomycete *Hpa* and the powdery mildew fungus *E. cruciferarum* were investigated. To test for specificity to biotrophic associations that involve the formation of intracellular accommodation structures in *A. thaliana*, we included in our analysis the extracellular bacterial pathogen *Pseudomonas syringae* pv. tomato *DC3000*, which, contrary to *Hpa* and *E. cruciferarum*, grows in the apoplast without entering living plant cells.

5. Results

5.1 *A. thaliana* HCSG mutants reduce the reproductive success of the oomycete *Hpa*

We inspected the *A. thaliana* genome for the presence of HCSG and identified candidate orthologs of *POLLUX* and of the nucleoporins of the NUP107-160 subcomplex (Figures 2 + 3). A direct ortholog of *SYMRK*, a malectin-like domain leucine-rich repeat receptor-like kinase (MLD-LRR-RLK) (Antolin-Llovera *et al.*, 2014; Ried *et al.*, 2014) was deleted from the syntenic position in the *A. thaliana* genome (Kevei *et al.*, 2005). Nevertheless, the MLD-LRR-RLK protein subfamily is also present in *A. thaliana*. In fact, a specific expansion of this gene family took place in this plant (Shiu & Bleecker, 2003). We performed phylogenetic analyses with members of the *A. thaliana* MLD-LRR-RLK subfamily, *LjSYMRK*, and two additional MLD-LRR-RLKs from *L. japonicus* using the protein sequences of their kinase domain alone or only the extracytoplasmatic region. The clustering of the kinase domain sequences was similar to that of the whole (pruned) amino acid sequences previously published (Hok *et al.*, 2011). However, an analysis of the extracytoplasmatic region resulted in different tree topologies. For instance, while in the first tree the At5g48740 protein (which has an additional LRR domain) was identified as the closest related to LjSYMRK, in the second it clustered in a separate group. However, in both cases, the products of two *A. thaliana* genes, which we thus named *SYMRK-homologous Receptor-like Kinase* 1 (*ShRK1*) and *ShRK2,* were identified as the most closely related to *LjSYMRK* (this work and (Markmann *et al.*, 2008)).

All investigated *A. thaliana* HCSGs have identical exon-intron structure and domain organization to their CSG counterparts (Figure 2). By sequencing a PCR product amplified from *A. thaliana* Col-0 cDNA, we demonstrated that, contrary to the TAIR prediction, this was also the case for *NUP133*. The curated sequence has been submitted to the GenBank (accession number KM269292).

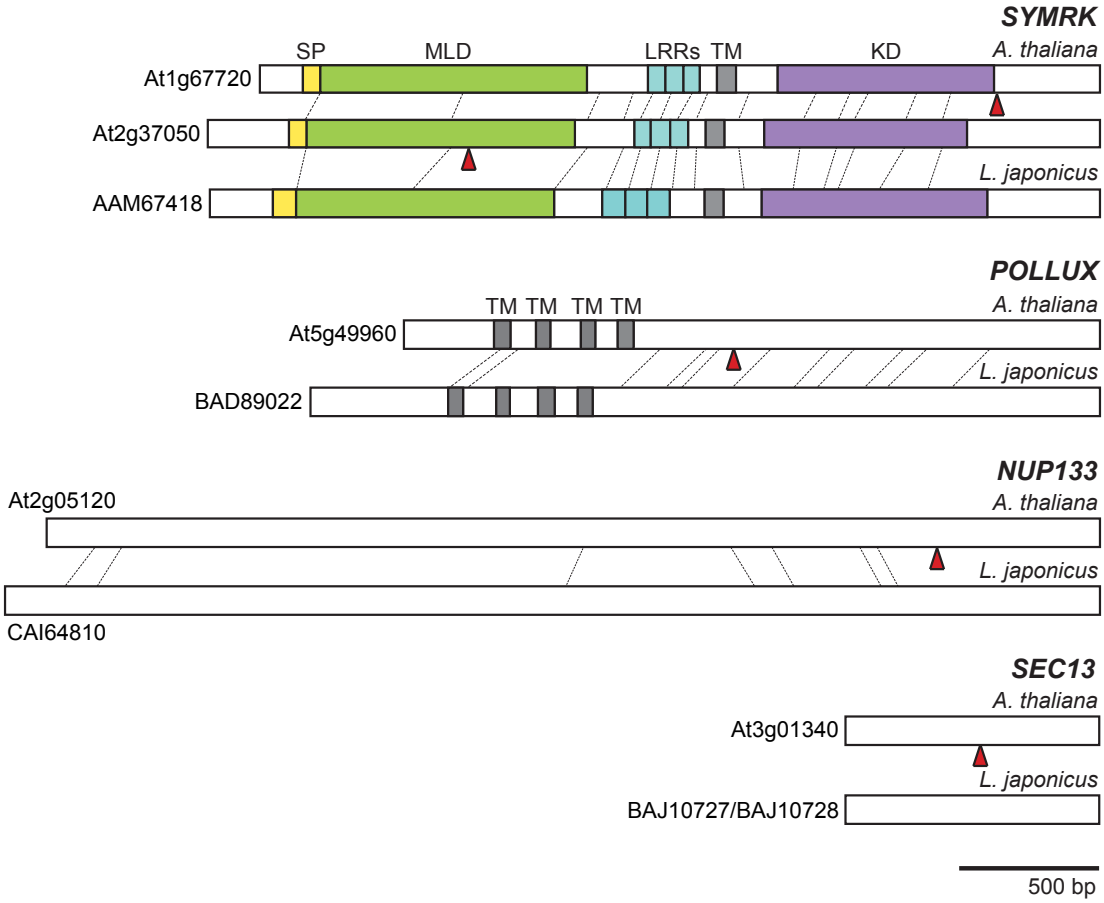

Figure 2: Comparison of gene structures and protein domains of *L. japonicus* common symbiosis genes (CSGs) and their closest homologs (HCSGs) in *A. thaliana*. Complete annotated genomic sequences were obtained from The Arabidopsis Information Resource (TAIR – www.arabidopsis.org) for *A. thaliana*, and from the KDRI website (Kazusa DNA Research Institute, Japan; http://www.kazusa.or.jp/lotus/) and the GenBank for *L. japonicus*. TAIR/GenBank protein identifiers are shown; dashed lines, positions of the introns in the original gene sequence; red triangles, positions of the T-DNA insertion in the respective mutants; SP, signal peptide; MLD, malectin-like domain; LRRs, leucine-rich repeats; TM, transmembrane domain; KD, kinase domain.

Gene	*L. japonicus* homolog (NCBI mRNA/protein IDs)	Gene ID	NASC ID	Protein Identity / Similarity To *L. japonicus*
POLLUX	AB162158 / BAD89022	At5g49960	N566135	71 / 82
SEC13	AB506697 / BAJ10727 - AB506698 / BAJ10728	At3g01340	N662322	80-82 / 92-91
NUP133	AJ890251 / CAI64810	At2g05120	N565761	55 / 71
SEH1	AB506696 / BAJ10726	At1g64350	N653094	64 / 78
NUP43	n.d.	At4g30840	N803490	n.d.
NUP85	AB284835 / BAF45348	At4g32910	N613274	61 / 75
NUP160	n.d.	At1g33410	N624418	n.d.
ShRK1	(*SYMRK*) AF492655 / AAM67418	At1g67720	N467036	33 (48KD) / 49 (68KD)
ShRK2		At2g37050	N643700	34 (48KD) / 50 (68KD)

Figure 3: ***A. thaliana* HCSGs with encoded proteins and their respective identities/similarities to their *L. japonicus* homologs.** Sequence identifiers and identities / similarities shared between the protein sequences of *A. thaliana* HCSGs and those of their respective *L. japonicus* counterparts. The numbers for AtSEC13 indicate the identity/similarity of its amino acid sequence to each of the two predicted LjSEC13 proteins. For ShRK1 and ShRK2, numbers in brackets refer to their kinase domain (KD) only. n.d., not detected. NASC ID: insertion mutant identifier.

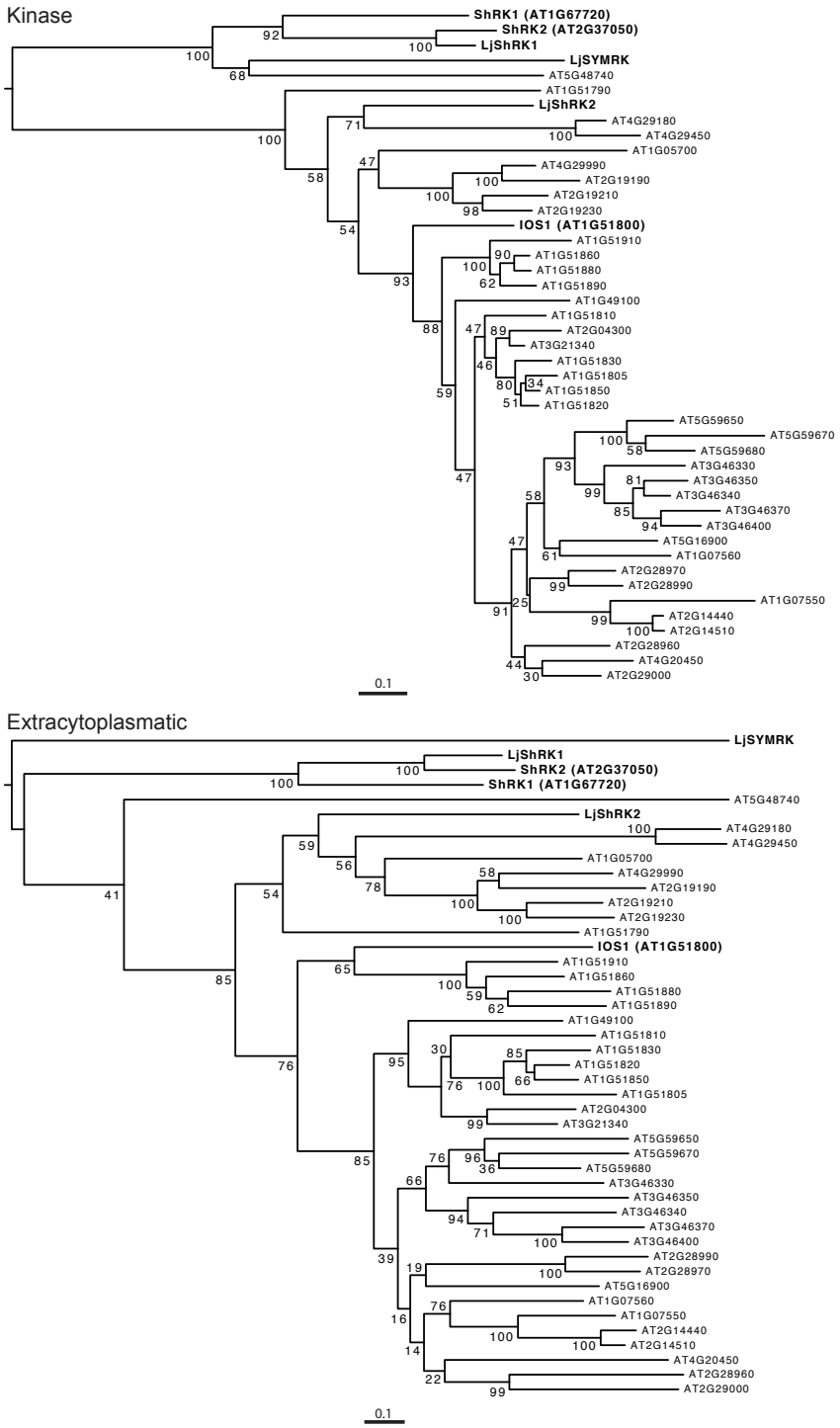

Figure 4: Maximum likelihood phylogenetic trees of MLD-LRR-RLKs from *A. thaliana* and *L. japonicus*. Maximum likelihood phylogenetic trees were constructed using the highly conserved kinase domains (upper tree) or the extracytoplasmatic regions minus signal peptide (lower tree) of MLD-LRR-RLK proteins from *A. thaliana*, where this family underwent a recent expansion (Shiu & Bleecker, 2003), and *L. japonicus*. Numbers on each node represent the respective bootstrap values. Scale bar = relative genetic distance (arbitrary unit).

Phylogenetic analyses were performed by Aline Banhara Pereira and Martina Katharina Ried. Aline Banhara Pereira prepared the figure.

Insertion mutant lines for *pollux*, *sec13*, *nup133*, *shrk1*, *shrk2*, and the double mutants *sec13* x *nup133* and *shrk1* x *shrk2* were analysed for their phenotype in the interaction with *Hpa*. In *pollux*, *shrk1*, *shrk2*, the double mutant *shrk1* x *shrk2,* and the disease resistant reference mutant *pskr1* (Mosher *et al.*, 2013), the reproductive success of *Hpa* isolate NoCo2, measured as sporangiophore number per cotyledon 4 days post infection (dpi), was significantly reduced and was restored in the available complementation lines (Figures 5 + 6).

To capture the potential structural or functional shifts of nucleoporins during evolution, we included a range of NUP107-160 subcomplex members in our analysis. The *sec13* and *nup133* single mutants and the double mutant *sec13* x *nup133* impaired *Hpa* reproductive success (Figures 5 + 6), while *seh1*, *nup43*, *nup85* and *nup160* did not (Figure 7). This pattern is not congruent with the observation in *L. japonicus*, in which *seh1*, *nup85* and *nup133* impaired symbiosis (Kanamori *et al.*, 2006; Alber *et al.*, 2007; Saito *et al.*, 2007), and may be explained by species-specific adaptations of the NUP107-160 subcomplex.

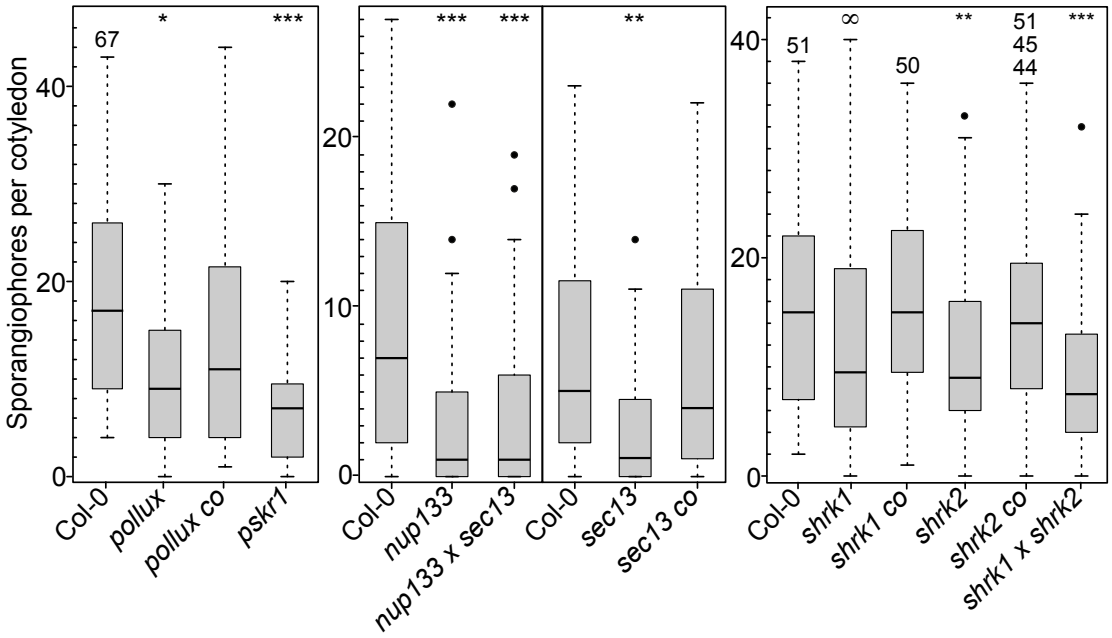

Figure 5: Mutation of *A. thaliana* HCSGs reduces the reproductive success of *Hpa*. Plots show the number of sporangiophores per infected cotyledon on ca. 50 cotyledons of *A. thaliana* wild-type (Col-0), the indicated mutants, and transgenic complementation lines (co) 4 dpi with *Hpa* isolate NoCo2. Dots: outliers. Numbers above upper whiskers indicate the values of individual outliers outside of the plotting area. Stars indicate significant differences to Col-0 (Wilcoxon–Mann–Whitney test with Bonferroni-Holm correction). ∞, $p = 0.067$, *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Constructs *pollux* co, *shrk1* co, and *shrk2* co were produced by Andreas Binder. Experiments were designed, performed, and analysed by Aline Banhara Pereira and Martina Katharina Ried. Martina Katharina Ried did the statistical analyses and prepared the figure.

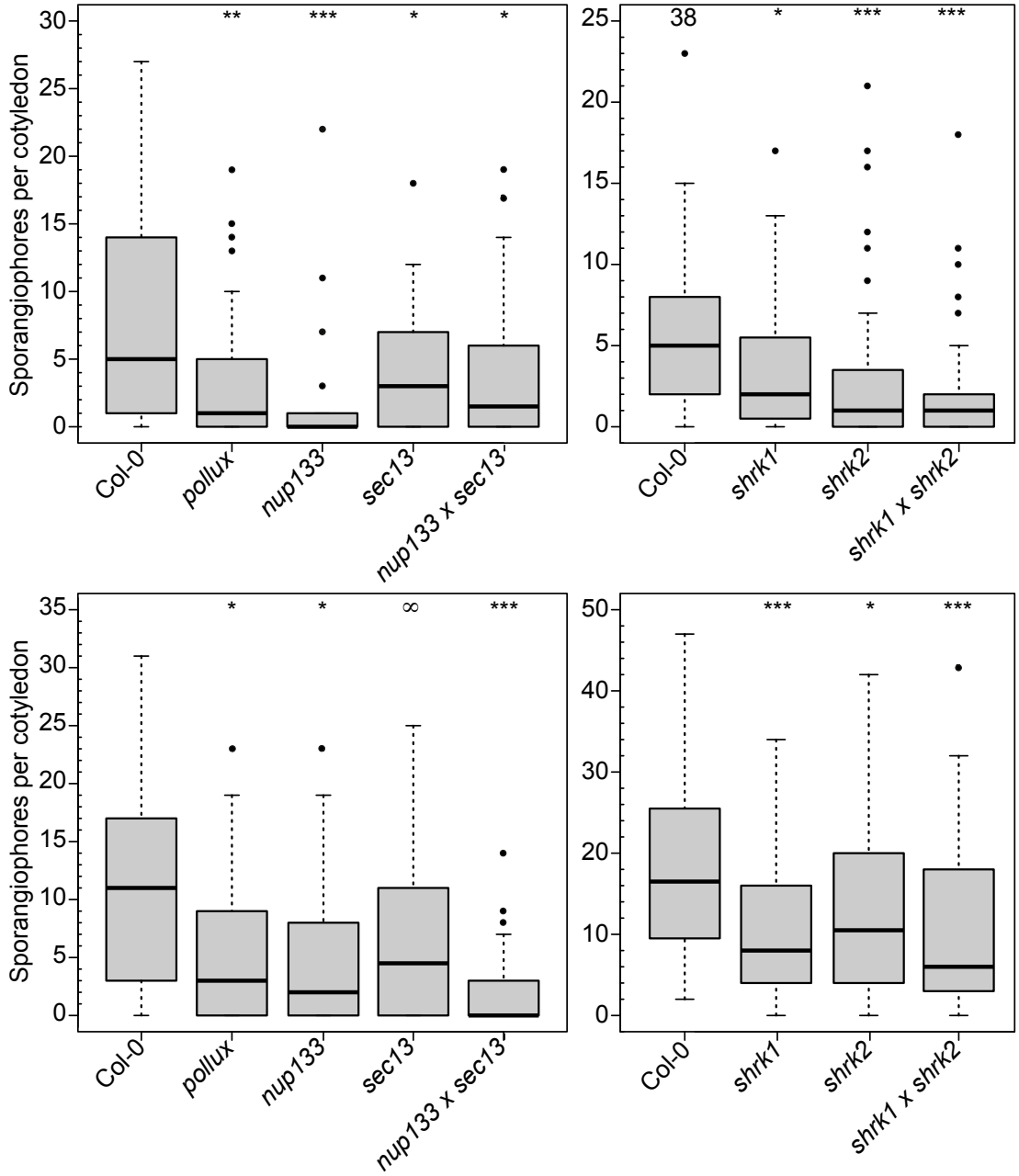

Figure 6: Reproductive success of *Hpa* is reduced in the HSCG mutants. Plots represent the number of sporangiophores per infected cotyledon on ca. 50 cotyledons of *A. thaliana* wild-type (Col-0) and the HSCGs mutants 4 dpi with *Hpa* isolate NoCo2. Two replicates are shown for each mutant set. Dots: outliers. Numbers above upper whiskers indicate the values of individual outliers outside of the plotting area. Significant differences to the wild-type (Col-0) were detected at the 5% significance level (Wilcoxon–Mann–Whitney with Bonferroni-Holm correction), except for *sec13* in the lower set, for which p was 0,065 (indicated with ∞). $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Experiments were designed, performed, and analysed by Aline Banhara Pereira and Martina Katharina Ried. Martina Katharina Ried did the statistical analyses and prepared the figure.

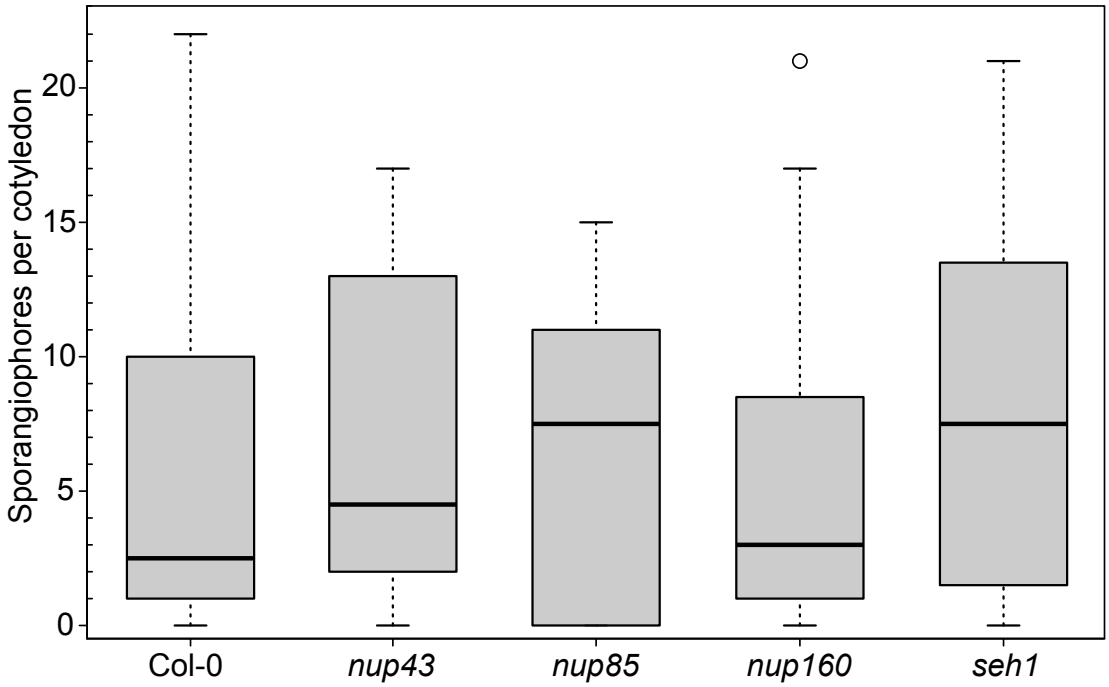

Figure 7: Reproductive success of *Hpa* is not affected in the *seh1*, *nup43*, *nup85*, and *nup160* mutants. Plots represent the number of sporangiophores per infected cotyledon on ca. 50 cotyledons of *A. thaliana* wild-type (Col-0) and the indicated mutants 4 dpi with *Hpa* isolate NoCo2. Open circle: outlier. No significant differences to the wild-type (Col-0) were detected at the 5% significance level (Wilcoxon–Mann–Whitney test with Bonferroni-Holm correction).

Experiment was designed and performed by Aline Banhara Pereira, and analysed by Aline Banhara Pereira and Martina Katharina Ried. Martina Katharina Ried did the statistical analyses and prepared the figure.

5.2. Haustorial development of *Hpa* is impaired in *A. thaliana* HCSG mutants

The reduced *Hpa* reproductive success could not be explained by a decreased frequency of haustoria formation. Other than a slight decrease in *pollux*, this frequency in the other mutants was indistinguishable from the wild-type (Figure 8). However, all *A. thaliana* HCSG mutants exhibited strikingly altered haustoria morphology. At 5 dpi the majority of haustoria in the wild-type had a globular, single-lobed appearance. Deviations from such morphology, which we generally called multilobed, were observed as well. The amount of multilobed haustoria in the HCSG mutants was significantly increased, a phenomenon that was alleviated in the available complementation lines (Figures 9 +10 + 11 + 12). In contrast to the HCSG mutants, the reference disease resistant mutant *pskr1*, which presents resistance to

disease due to constitutively activated defence responses (Mosher *et al.*, 2013), did not show any signs of altered haustorial development (Figure 9).

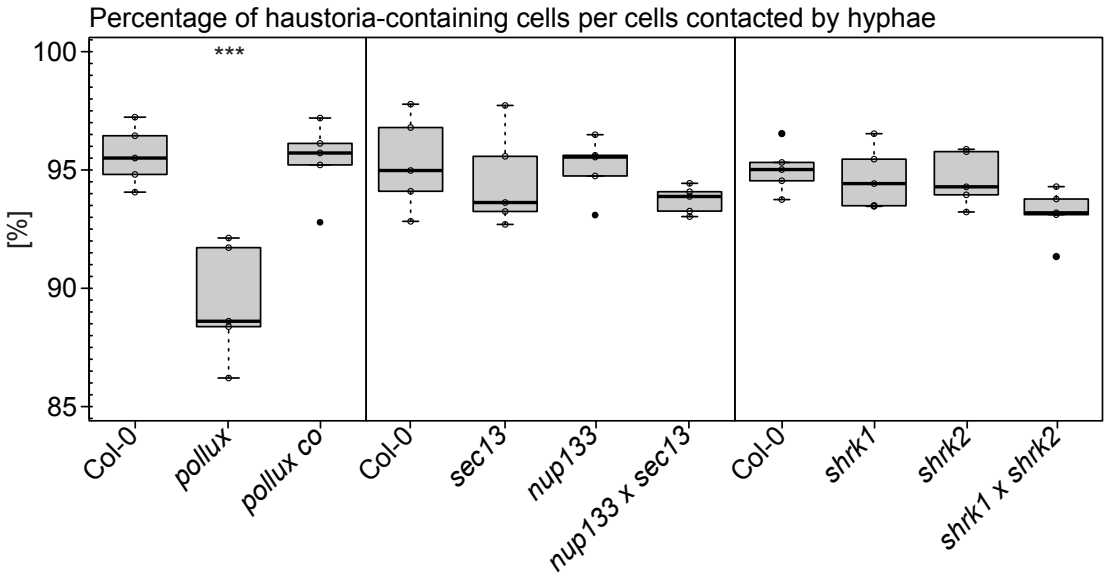

Figure 8: *Hpa* haustoria formation on *A. thaliana* HCSG mutants. Plots represent the mean percentage of haustoria-containing cells per cells contacted by hyphae on 5 leaves of *A. thaliana* wild-type (Col-0), the indicated mutants and transgenic complementation lines (co) at 5 dpi with *Hpa* isolate NoCo2. Ten independent hyphal strands were analysed on each leaf. Black circles: outliers. Stars indicate significant differences to Col-0 (Dunnett's Test with Bonferroni correction). *** $p < 0.001$.

Construct *pollux* co was produced by Andreas Binder. Experiment was designed, performed, and analysed by Aline Banhara Pereira and Martina Katharina Ried. Martina Katharina Ried did the statistical analyses and prepared the figure.

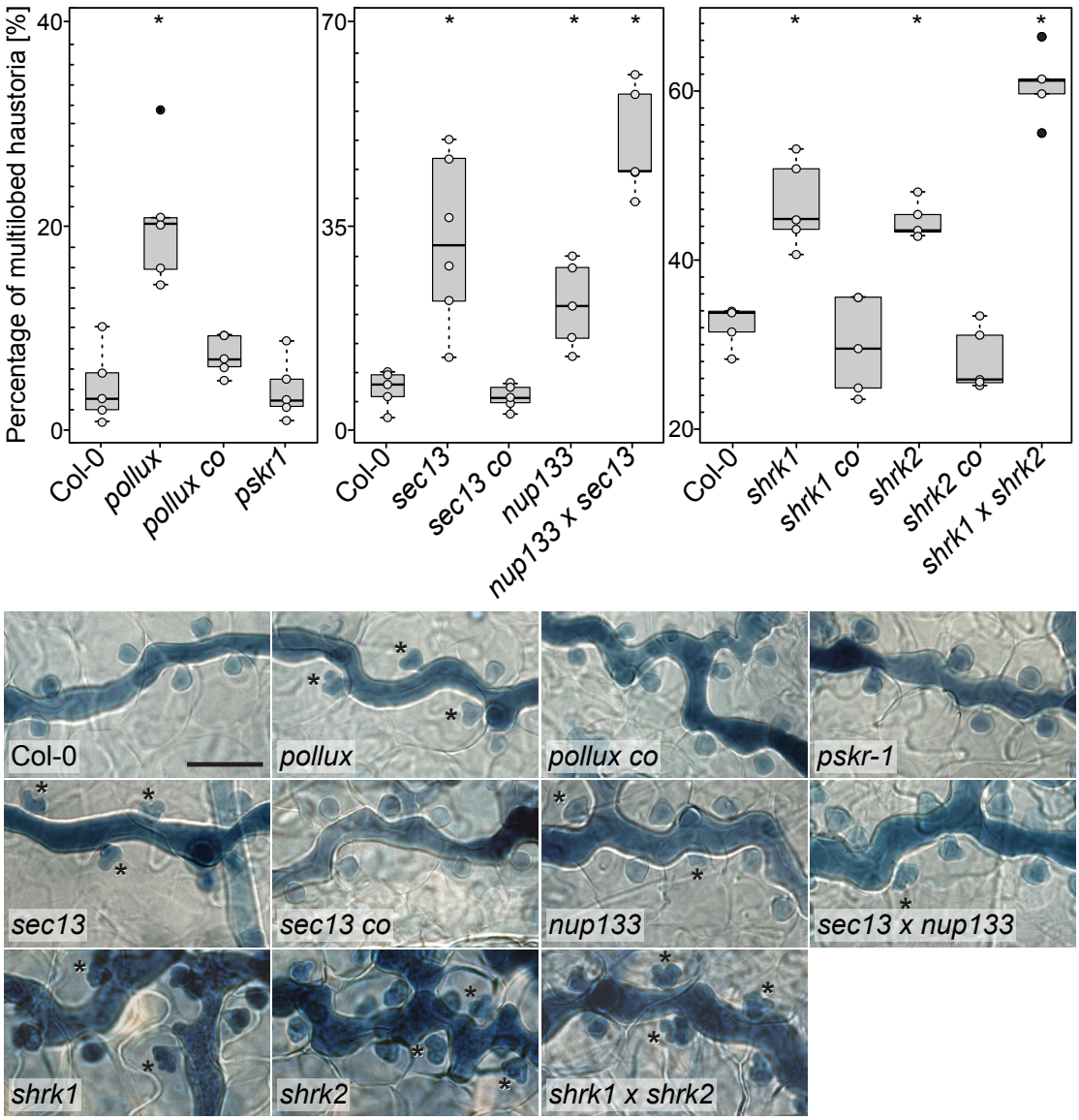

Figure 9: *Hpa* haustorium morphology is altered in *A. thaliana* HCSG mutants. *A. thaliana* wild-type (Col-0), the indicated HCSG mutants, and transgenic complementation lines (co) were analysed 5 dpi with *Hpa* isolate NoCo2. Upper panel: Plots show the percentage of multilobed haustoria among total haustoria. For each genotype, 5 leaves were analysed, and 10 hyphal strands with 15-30 haustoria each were counted on each leaf. Dots: outliers. Stars indicate significant differences to Col-0 (Wilcoxon–Mann–Whitney test with Bonferroni-Holm correction). *, $p < 0.05$. Lower panel: Representative pictures of hyphal strands that grow intercellularly in the mesophyll and intracellular haustoria. Stars indicate multilobed haustoria. Scale bars = 25 µm.

Constructs *pollux* co, *shrk1* co, and *shrk2* co were produced by Andreas Binder. Experiments were designed, performed, and analysed by Aline Banhara Pereira and Martina Katharina Ried. Martina Katharina Ried did the statistical analyses and prepared the figure.

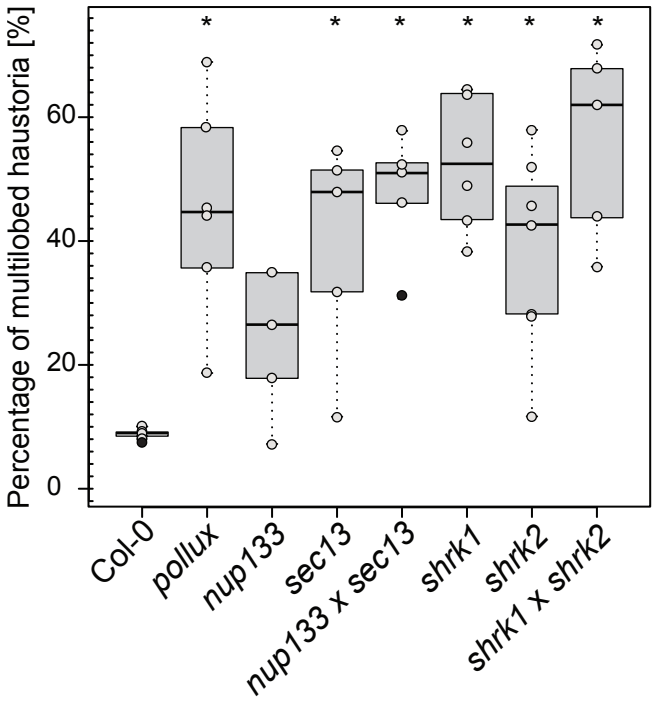

Figure 10: Frequency of multilobed haustoria on *A. thaliana* wild-type and on the HSCG mutants. *A. thaliana* wild-type (Col-0) and the HCSG mutants were analysed 5 dpi with *Hpa* isolate NoCo2. Plots show the percentage of multilobed haustoria among total haustoria. For each genotype, 5 leaves were analysed, and 10 hyphal strands with 15-30 haustoria each were counted on each leaf. Black dots: outliers. Stars indicate significant differences to Col-0 (Wilcoxon–Mann–Whitney test with Bonferroni-Holm correction). For *nup133*: p = 0,151. *, p < 0.05.

Experiment was designed, performed, and analysed by Aline Banhara Pereira and Martina Katharina Ried. Martina Katharina Ried did the statistical analyses and Aline Banhara Pereira prepared the figure.

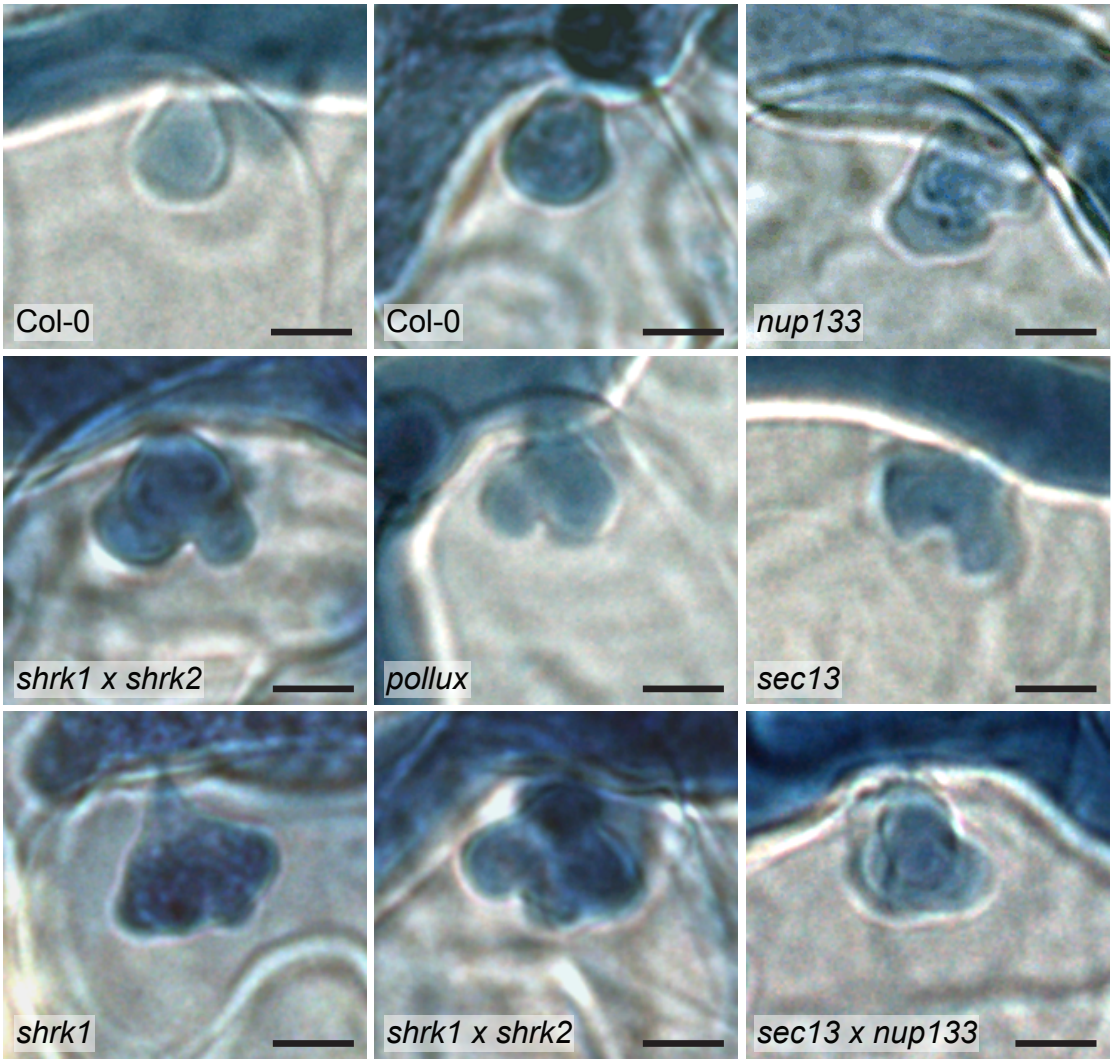

Figure 11: *Hpa* haustorium morphology is altered in *A. thaliana* HCSG mutants. Differential interference contrast microscopy of leaves of *A. thaliana* wild-type (Col-0) and HCSG mutants stained with trypan-blue lactophenol 5 dpi with *Hpa* isolate NoCo2. Scale bar = 25 μM.

Experiments was designed, performed, and analysed by Aline Banhara Pereira and Martina Katharina Ried. Aline Banhara Pereira prepared the figure.

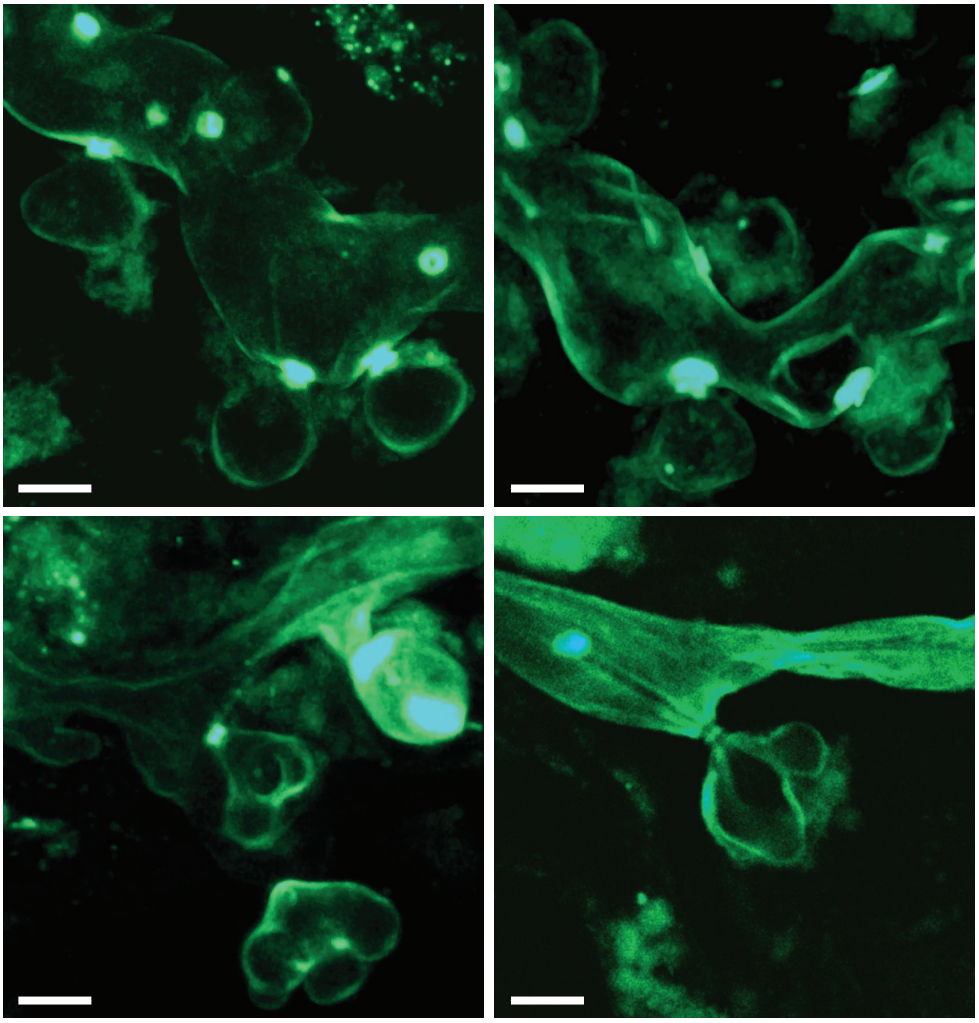

Figure 12*: Hpa* haustoria morphology. *Hpa* haustoria on wild-type *A. thaliana* (Col-0) leaves (upper row) 5 dpi, with the regular globular or pear-like morphology, and examples of multilobed haustoria observed in the *shrk1* x *shrk2* double mutant (lower row). Leaves were stained with anilin-blue and visualized with a CLSM. For every haustorium, the entry point can be identified by the formation of the usually bright callose neck. In the double mutant, multiple lobes are visualized forming in individual haustoria. Scale bar: 25 μM.

Experiment was designed, performed, and analysed by Aline Banhara Pereira and Martina Katharina Ried. Aline Banhara Pereira prepared the figure.

In both the wild-type and in *shrk1* x *shrk2*, the percentage of multilobed haustoria increased over time, but was significantly higher in *shrk1* x *shrk2* at each analysed time point (Figure 13), suggesting early haustoria aging in the double mutant. An altered morphology was also observed for *Hpa* haustoria in leaves of transgenic *A. thaliana* plants expressing the N-terminally YFP-tagged remorin AtREM1.2 (At3g61260) under the control of its native promoter (ProAt3g61260:YFP-At3g61260, (Jarsch *et al.*, 2014)). Similar to its homolog AtREM1.3 (Bozkurt *et al.*,

2014), AtREM1.2 localizes to the perihaustorial membrane. We observed that the loss of ProAt3g61260:YFP-At3g61260 fluorescence was associated with changes in the haustorium morphology, perhaps indicating an age-related alteration of the protein composition of the perihaustorial membrane (Figure 14). The impaired haustorial development in the HCGS mutants may decrease nutrient availability to the oomycete, with a consequent reduction in sporangiophore production. Collectively, the remarkably specific phenotype of altered haustorial development on all tested *A. thaliana* HCSG mutants pinpoints these genes as contributing to a program for the intracellular accommodation of this oomycetal pathogen. Interestingly, on the HCSG mutants we did not observe a consistent reduction in reproductive success of the powdery mildew fungus *Erysiphe cruciferarum* (Figure 15), which forms haustoria exclusively in epidermal cells. A morphological comparison of haustoria shape was not possible because of the highly variable haustorial morphology already in the wild-type interaction. It is therefore possible that the HCSGs do not play a role in this interaction, due to alternative pathways in the epidermis or distinct genetic requirements for the colonization between fungal and oomycetal intracellular pathogens. The *MLO* gene, for instance, is an epidermal compatibility factor required for powdery mildew fungus penetration (Consonni *et al.*, 2006), with no role in the *Hpa* infection reported to date.

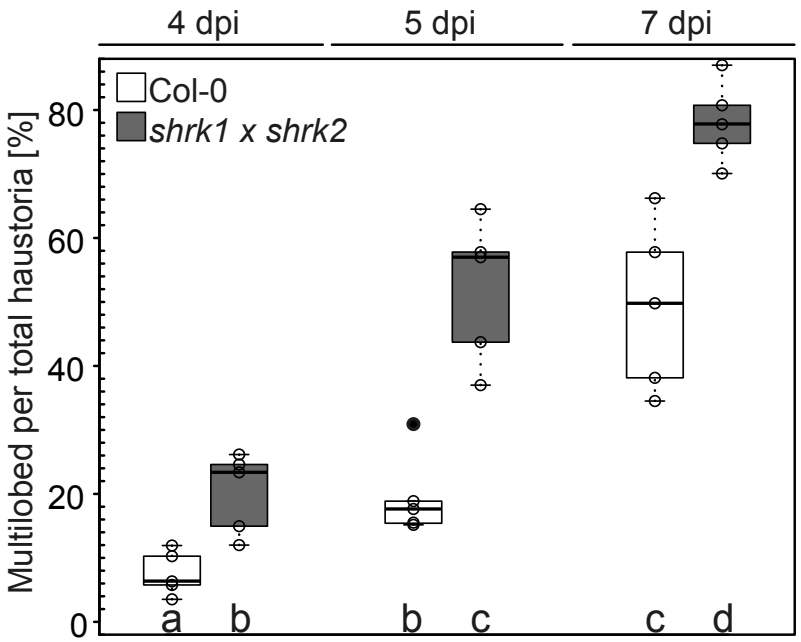

Figure 13*:* Time-course of haustoria development in *A. thaliana* wild-type and in *shrk1* x *shrk2.* Plots represent the mean percentage of multilobed haustoria per total haustoria on 5 leaves of *A. thaliana* wild-type (Col-0) and the *shrk1* x *shrk2* double mutant 4, 5 and 7 dpi with *Hpa* isolate NoCo2. On each leaf, 10 independent hyphal strands were analysed. Black circle: outlier. Stars represent significant differences (Wilcoxon–Mann–Whitney test with Bonferroni-Holm correction). **, $p < 0.01$.

Experiment was designed, performed, and analysed by Aline Banhara Pereira and Martina Katharina Ried. Martina Katharina Ried did the statistical analyses and prepared the figure.

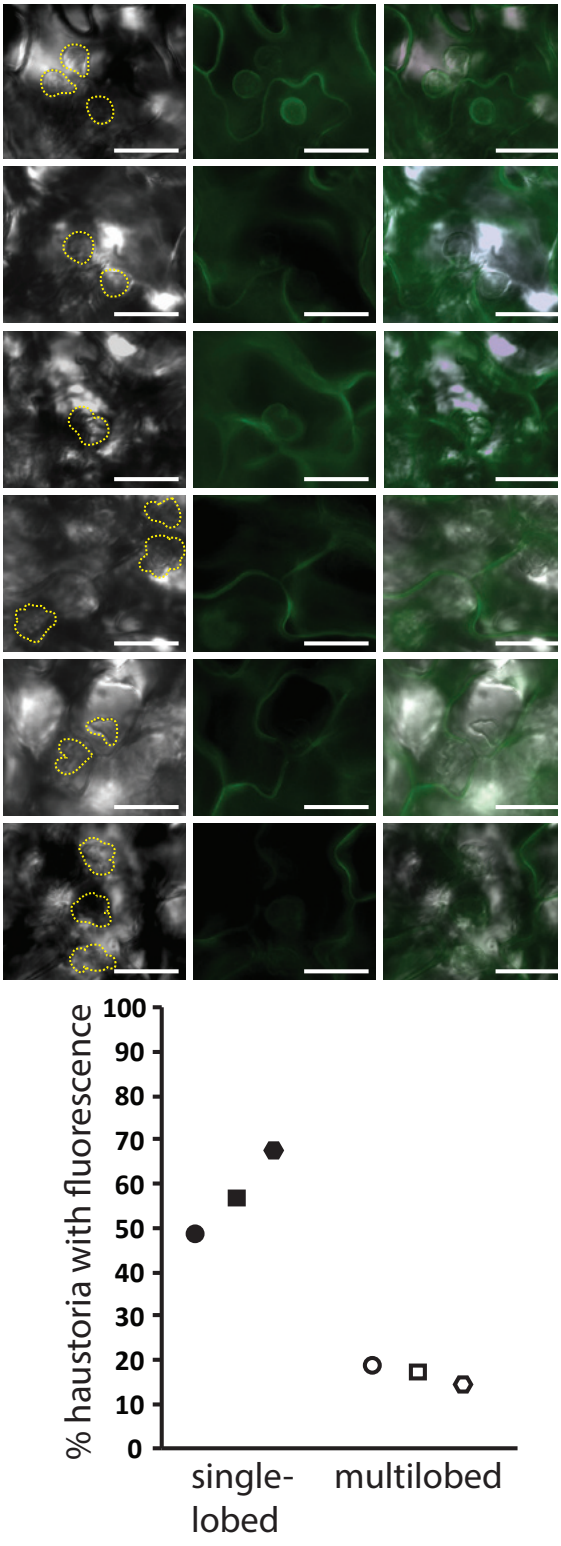

Figure 14: Quantification of haustoria with fluorescence on *ProAt3g61260:YFP-At3g61260* plants. *A. thaliana* plants expressing N-terminally YFP-tagged remorin AtREM1.2 under the control of its native promoter were infected with *Hpa* and inspected 8 dpi with an epifluorescence microscope. Quantification of haustoria with fluorescence on the perihaustorial membrane region was performed in triplicate. For each replicate consisting of 6 leaves, 100 single-lobed and 100 multilobed haustoria were counted and scored for detectable fluorescence. Exemplary images (differential interference contrast, fluorescent, merge) of labelled and non-labelled (completely dark) haustoria are shown. Yellow dashed lines delimitate the haustoria in the bright field images. Graph represents the percentage of haustoria with fluorescence as a function of morphology; distinct symbols refer to different replicates (1st replicate: 49% single-lobed/19% multilobed; 2nd replicate: 56% single-lobed/17% multilobed; 3rd replicate: 68% single-lobed/14% multilobed). Bar: 25 µM.

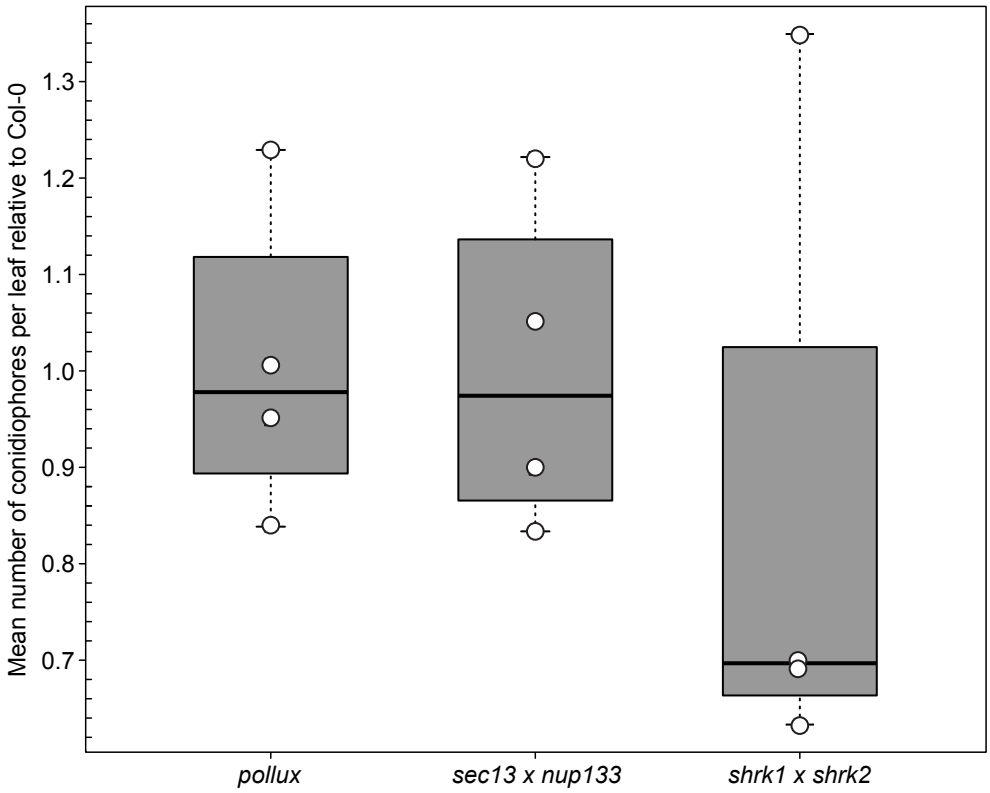

Figure 15: Mean number of conidiophores per leaf on *A. thaliana* HCSG mutants relative to the wild-type. Box-plots represent a compilation from four independent replicates and show mean number of conidiophore on HCSG mutant relative to the wild-type (Col-0) leaves 5 dai with 3-4 *E. cruciferarum* spores/mm^2. For each replicate, conidiophores/colony were counted on 10 colonies per leaf, on 5-10 leaves/genotype. No significant differences to the wild-type (Col-0) were detected at the 5% significance level (Dunnett's Test with Bonferroni-correction).

Experiment was designed and performed by Caroline Höfle, and analysed by Aline Banhara Pereira and Martina Katharina Ried. Martina Katharina Ried did the statistical analyses and prepared the figure.

5.3. *A. thaliana* HCSG mutants do not exhibit constitutive or enhanced defence responses

To investigate whether the reduced oomycete fitness on the HCSG mutants resulted from constitutively activated immune responses or from elevated defence responses upon pathogen perception, we first analysed the transcript levels of PAMP-induced marker genes under non-challenged conditions and 6 hours after flg22 treatment (Figures 16 + 17). The basal expression of *FLG22-INDUCED RECEPTOR-LIKE KINASE 1* (*FRK1*) (Asai *et al.*, 2002), *GLUTATHIONE S-TRANSFERASE 1* (*GST1)* (Grant *et al.*, 2000), *ETHYLENE RESPONSE FACTOR 1* (*ERF1*) (Solano *et al.*, 1998), and *PHOSPHATYDILINOSITOL-LIPID TRANSFER PROTEIN* (*PI-LTP*) (Denoux *et al.*, 2008) in the HCGS mutants did not differ from that detected in the wild-type or in the *FLAGELLIN SENSING 2* (*FLS2*; (Gomez-Gomez & Boller, 2000)) mutant (Figures 16 + 17). Moreover, 6 hours after flg22 treatment these genes were all upregulated in the HCSG mutants to values similar to the wild-type (Figures 16 + 17).

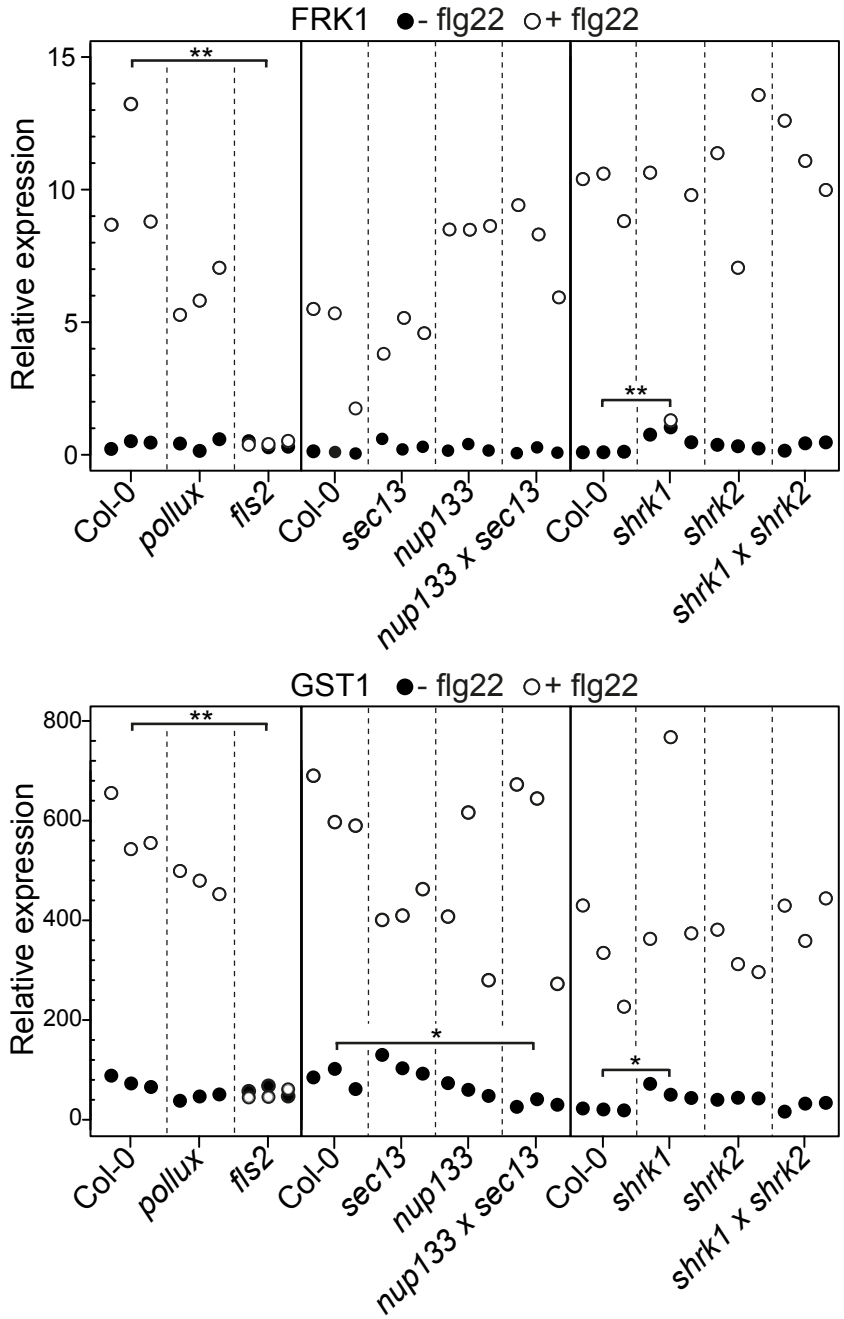

Figure 16: Basal expression levels and flg22-mediated induction of PAMP-responsive genes in *A. thaliana* HCSG mutants are not different from the wild-type. The relative expression of *FRK1* and *GST1* in mock-treated samples or in samples treated with 1 µM flg22 for 6h (+) was determined in three biological replicates for each genotype by qRT-PCR. The *fls2* mutant was the negative control. Transcript levels for every plant genotype and each treatment were determined with technical duplicates. Closed circle, mock-treated; open circle, flg22 treated. Stars label datasets significantly different from Col-0 (Dunnett's Test with Bonferroni correction). *, $p < 0.01$; **, $p < 0.001$.

Experiments were designed, performed and analysed by Aline Banhara Pereira and Martina Katharina Ried. Martina Katharina Ried did the statistical analyses and prepared the figure.

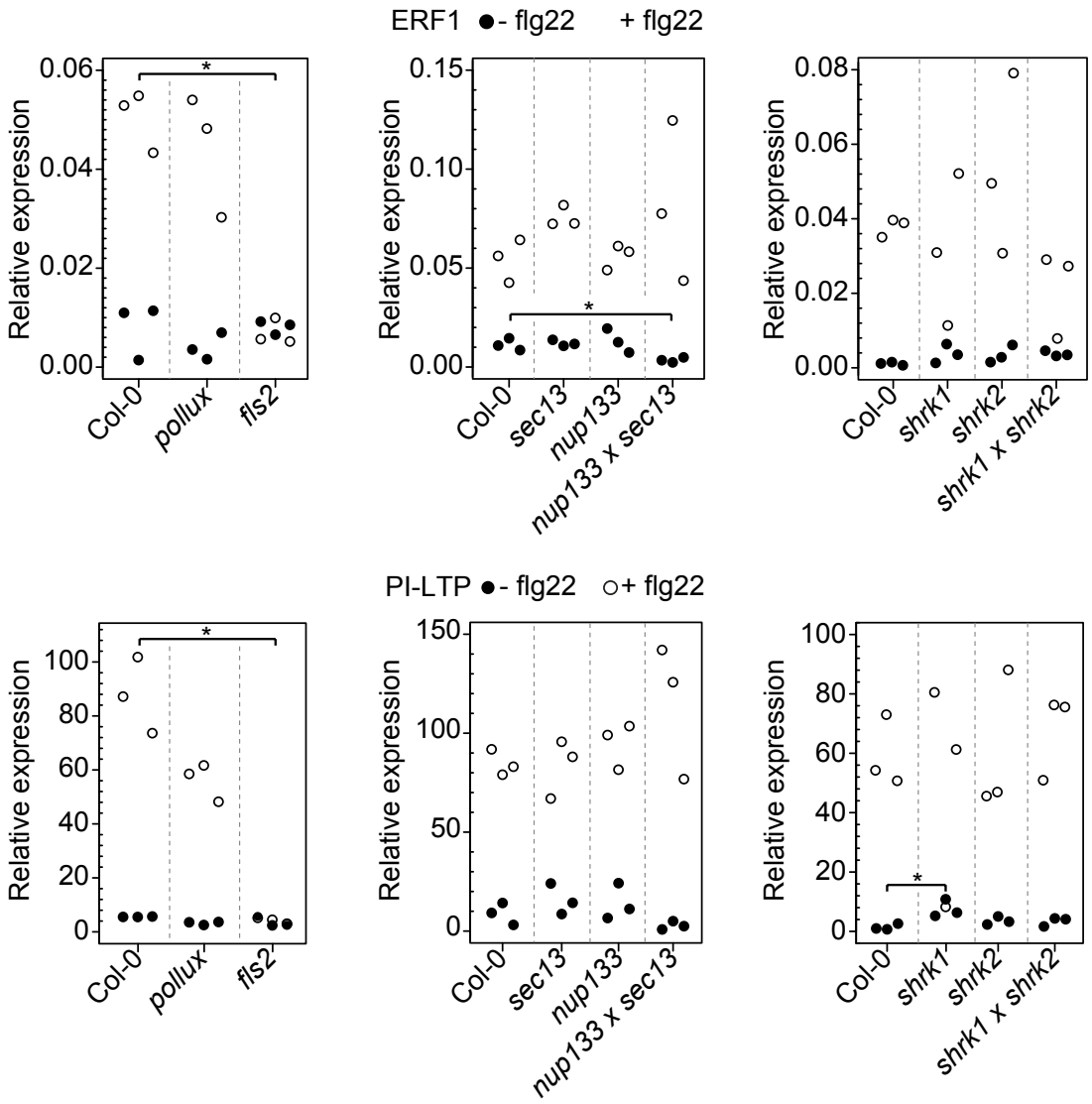

Figure 17: Basal expression levels and flg22-mediated induction of PAMP-responsive genes in *A. thaliana* HCSG mutants are not different from the wild-type. Relative expression of *ERF1* and *PI-LTP* in mock-treated samples (-) or in samples treated with 1 mM flg22 for 6h (+) were determined in three biological replicates for each genotype via qRT-PCR. The fls2 mutant was used as negative control. Transcript levels for every plant genotype and each treatment were determined through technical duplicates. Black circle, mock-treated; open circle, flg22 treated. Stars indicate significant differences to Col-0 (Dunnett's Test with Bonferroni correction). *, $p < 0.01$.

Experiments were designed, performed and analysed by Aline Banhara Pereira and Martina Katharina Ried. Martina Katharina Ried did the statistical analyses and prepared the figure.

Furthermore, the constitutive transcript levels of *PATHOGENESIS-RELATED GENE 1* (*PR1*), a marker gene for salicylic acid (SA)-mediated resistance (Ryals *et al.*, 1996), which is activated upon infection by biotrophic pathogens (Glazebrook, 2005), were not significantly increased in the HCSG mutants (Figure 18). In all cases, deviations observed for individual mutants were not consistent through the gene set and are thus unlikely responsible for the increased pathogen resistance.

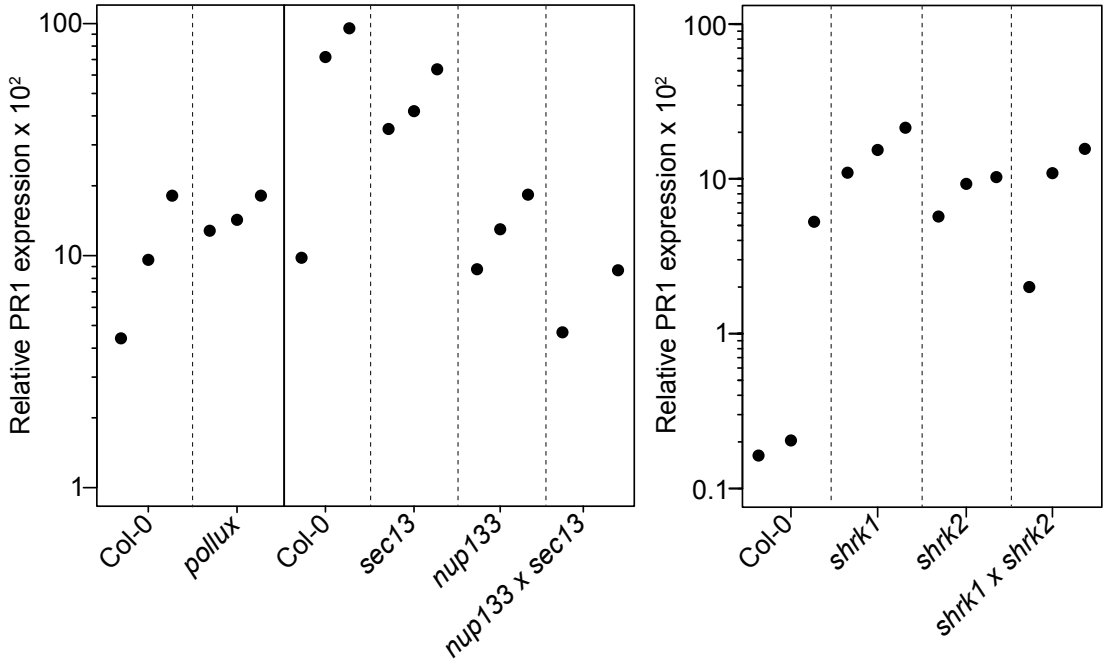

Figure 18: Transcript levels of defence marker gene *PR1* in *A. thaliana* HCSG mutants. Relative expression of *PR1* was determined in three mock-treated biological replicates for each genotype via qRT-PCR. Transcript levels for every plant genotype were determined through technical duplicates. No statistical differences to the wild-type (Col-0) were obtained at the 1% significance level (Dunnett's Test with Bonferroni correction).

Experiments were designed, performed and analysed by Aline Banhara Pereira and Martina Katharina Ried. Martina Katharina Ried did the statistical analyses and prepared the figure.

Symptoms typically associated with deregulated immune responses, such as constitutive or pathogen-induced hypersensitive response (HR) or defects in growth and development due to the hyper-activation of the SA-dependent defence pathway (Bowling *et al.*, 1997), were absent in the *A. thaliana* HCSG mutants (Figure 19 + 20). In addition, the ability of *Hpa* to suppress callose deposition around the haustorial neck region (Sohn *et al.*, 2007) was not disturbed (Figure 21). The NUP107-160 subcomplex has also been implicated in plant defence; mutations in *NUP96, NUP160* and *SEH1* impair basal and resistance-gene mediated immunity (Zhang & Li, 2005; Wiermer *et al.*, 2012). However, our mutants did not show such deregulation, possibly due to distinct roles of individual subcomplex components in compatibility and defence.

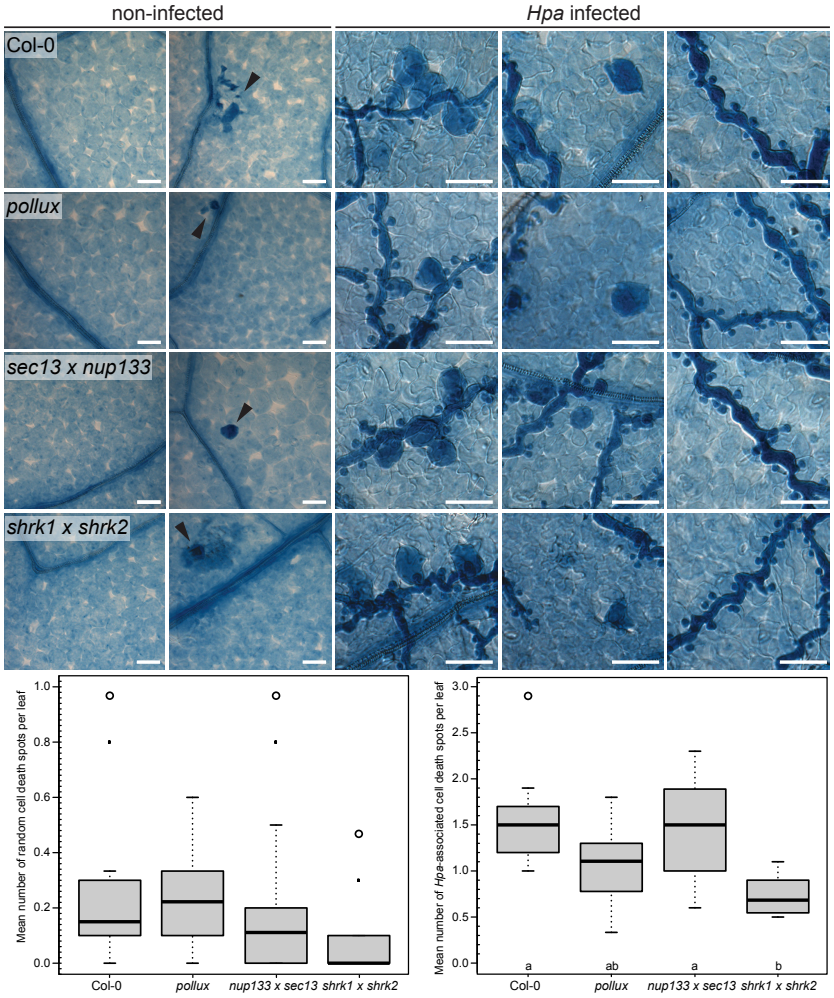

Figure 19: *A. thaliana* HCSG mutants do not show increased levels of spontaneous or pathogen-induced cell death. Upper panel: differential interference contrast microscopy of representative *A. thaliana* wild-type (Col-0) and HCSG leaves 4 dpi with *Hpa* isolate NoCo2. Most of the non-infected leaves display no sign of cell death (first column), but dark-blue stained dead cells are sporadically observed in non-infected leaves of both wild-type and HCSG mutants (arrows, second column). In infected leaves of the HCSG mutants, cell death is occasionally detected randomly on the leaf surface (third column) and in, or adjacent to, haustoria-containing cells in a frequency indistinguishable from the wild-type (fourth column). In all genotypes, infected leaves contain hyphal strands growing in the absence of any cell death (fifth column). Scale bar = 25 µm**.** Lower panel: plots show the mean number of random (left) or *Hpa*-associated (right) cell death spots per leaf on ca. 50 leaves per genotype 5 dpi with *Hpa* isolate NoCo2. Open circles: outliers. For statistical analysis, a one-way ANOVA followed by a Tukey's HSD was performed. Different letters indicate samples that are significantly different at the 5% significance level. Results were obtained from two independent experiments.
Experiments were designed, performed, and analysed by Aline Banhara Pereira and Martina Katharina Ried. Statistical analyses were done by Martina Katharina Ried. Aline Banhara Pereira prepared upper panel and Martina Katharina Ried prepared lower panel.

Figure 20*: A. thaliana* HCSG mutants do not show developmental or growth defects. 3-week-old *A. thaliana* wild-type (Col-0) plants grown alongside the indicated mutant lines under long day conditions (16h light). The dwarf phenotype of the mutant suppressor of *npr1-1, constitutive 1* (*snc1;* (Li *et al.*, 2001)) is included on the far right for comparison.

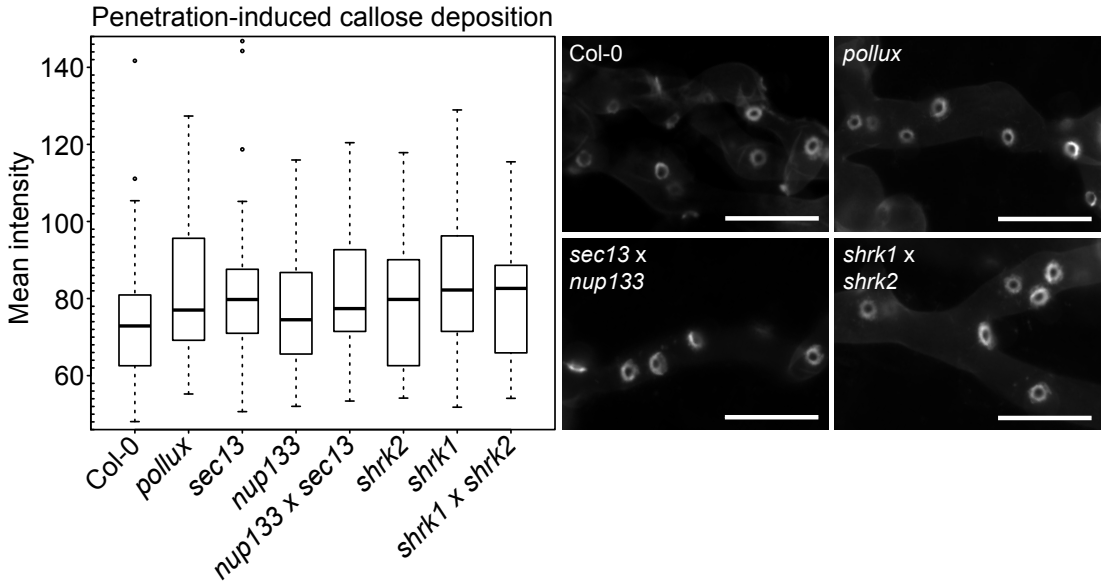

Figure 21: *Hpa* haustoria-associated callose deposition is not altered in the *A. thaliana* HCSG mutants. Plot shows the mean intensity of callose deposition on a total of ca. 50 oomycete penetration sites from 5 different leaves of *A. thaliana* wild-type (Col-0) and the HCSG mutants 4 dpi with *Hpa* isolate NoCo2. Black circles: outliers. For statistical analysis, a one-way ANOVA followed by a Tukey's HSD was performed. No significant differences were obtained at the 5% significance level. Representative pictures of haustoria-associated callose deposition on *Hpa* hyphae growing on the indicated mutants are shown on the right. Scale bar = 25 µm.

Experiment was designed and performed by Aline Banhara Pereira, and analysed by Aline Banhara Pereira and Martina Katharina Ried. Statistical analyses were done by Martina Katharina Ried. Aline Banhara Pereira prepared the right panel, and Martina Katharina prepared the left panel.

5.4. Growth kinetics of *Pseudomonas syringae* are not altered on the *A. thaliana* HCSG mutants

The bacterial pathogen *Pseudomonas syringe* DC3000 induces the activation of the SA- and the jasmonic acid (JA)-dependent defence signaling in the host (Farmer *et al.*, 2003; Thaler & Bostock, 2004), and overshooting activation of those pathways leads to increased *P. syringae* resistance. The growth of DC3000 wild-type or the avirulent *ΔAvrPto/PtoB* strain (Figure 22) was unaltered on the *A. thaliana* HCSG mutants, providing further evidence that they do not exhibit constitutive or enhanced activation of SA- and JA-dependent defences.

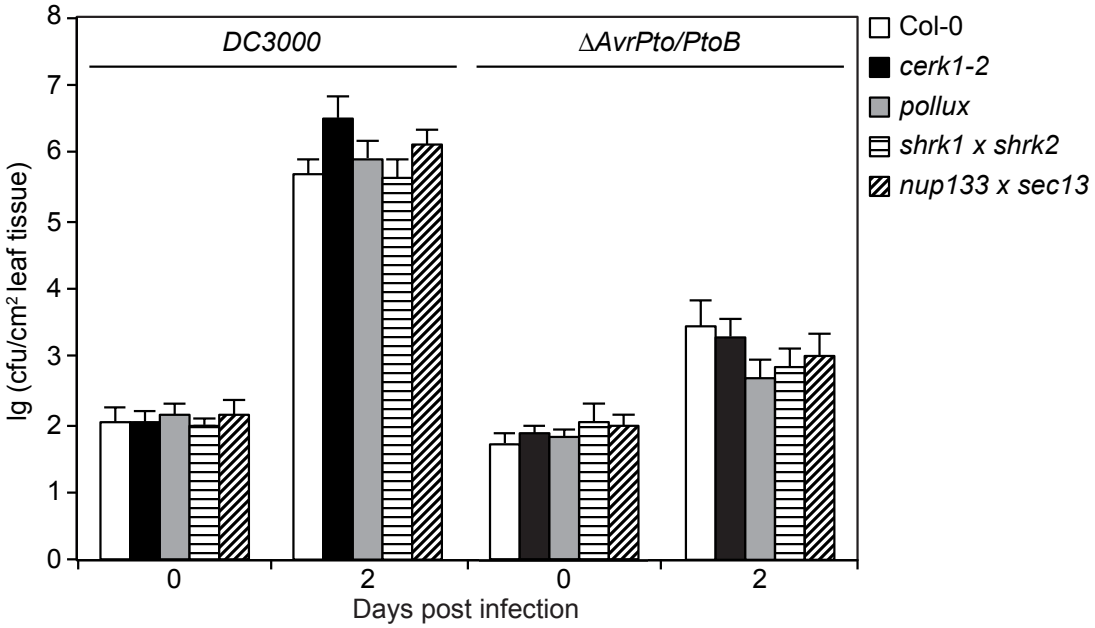

Figure 22: *A. thaliana* HCSG mutants support similar bacterial growth curves as the wild-type. Growth of *Pto* DC3000 (left) or *Pto* DC3000 Δ*AvrPto/AvrPtoB* (right) in *A. thaliana* wild-type (Col-0) or the HCSG mutants *pollux*, *shrk1 x shrk2* and *nup133 x sec13* was determined 2 or 4 days post infiltration of 10^4 colony forming units ml^{-1} (cfu/cm^2). Data represent means ± S.D. of six replicate measurements/genotype/data point. Results from one representative of at least four independent experiments are shown.

Experiments were designed, performed, and analysed by Andrea A. Gust and Thorsten Nürnberger. Figure was prepared by Andrea A. Gust.

5.5. *P. indica* colonization of *L. japonicus* roots occurs independently of CSGs

We investigated whether the classical CSGs are involved in the interaction between roots of the legume *L. japonicus* and *P. indica*. Gifu wild-type plants and common symbiosis mutants derived from this ecotype were analysed with regard to the ability of the fungus to penetrate root epidermal cells, undergo a biotrophic phase, and grow and sporulate inside these cells. For that, we inoculated *L. japonicus* roots with *P. indica* chlamydospores and performed microscopic analyses to assess colonization at 7 dpi and the presence of fungal spores at 14 dpi within cleared roots. Although fungal hyphae were clearly detected within the boundaries of root epidermal cells (Figure 23), it was not clear whether this was the result of biotrophic or necrotrophic growth. We therefore used the vital stain FM4-64 to label plant membranes as a proxy for the vitality status of the root cells. Using this technique we obtained clear evidence for *P. indica* hyphae within living root epidermal cells at 3 dpi. Intracellular biotrophic hyphae were detected in the wild-type and in the common symbiosis mutants (Figure 24). Moreover, intracellular sporulation was observed in roots of all genotypes (Figure 25), evidencing that the *P. indica* successfully completed its life cycle within the roots of the common symbiosis mutants. The fact that intracellular uptake and maintenance of *P. indica* hyphae occurred independent of the common symbiosis genes indicates that alternative pathways must exist for the biotrophic accommodation of *P. indica*.

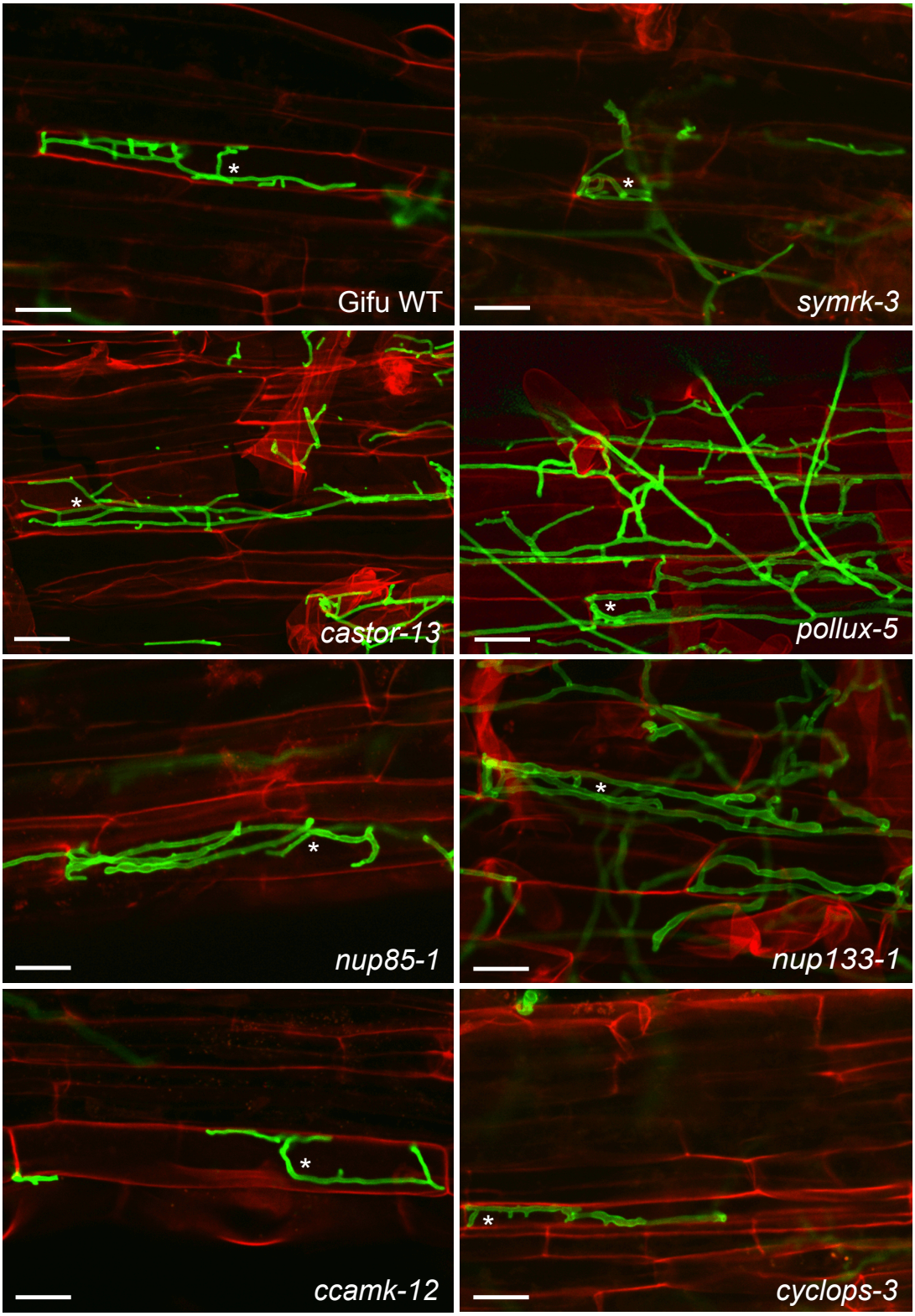

Figure 23: Intracellular growth of *P. indica* in root cells of *L. japonicus*. Wild-type and the indicated common symbiosis mutants were analysed 7 dpi with *P. indica* chlamydospores. Intracellular hyphae were present in all genotypes (examples are indicated by asterisks). Roots were cleared and double stained with propidium iodide (red), for cell wall visualization, and WGA-AF488 (green), for fungal structures. Scale bar: 25 µm.

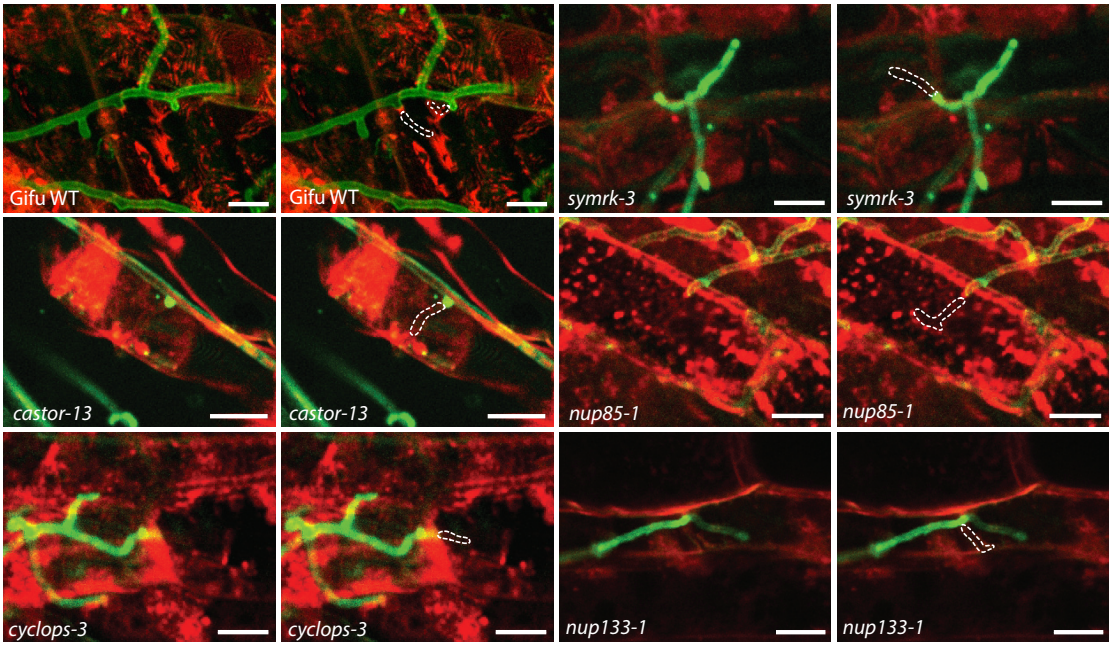

Figure 24*:* Biotrophic growth of *P. indica* in root cells of *L. japonicus*. Microscopy of wild-type and the indicated common symbiosis mutants was performed 3 dpi with *P. indica* chlamydospores. FM4-64-stained endomembranes (red) inside root cells indicates cell viability. Examples of biotrophic hyphae (delimited by dotted lines on the second and fourth columns) are shown within rhizodermal cells. In some cells, the FM4-64-stained membrane surrounding the invading hyphae is well detectable. While extracellular hyphae are stained with WGA-AF488 (green), biotrophic invasive hyphae are not or weakly fluorescent probably due to limited access of the reagent to the fungal cell wall in the fresh root cells. Confocal microscopic pictures were taken 3 dpi with *P. indica* chlamydospores with a Leica TCS-SP5 CLSM. Scale bar: 10 μm.

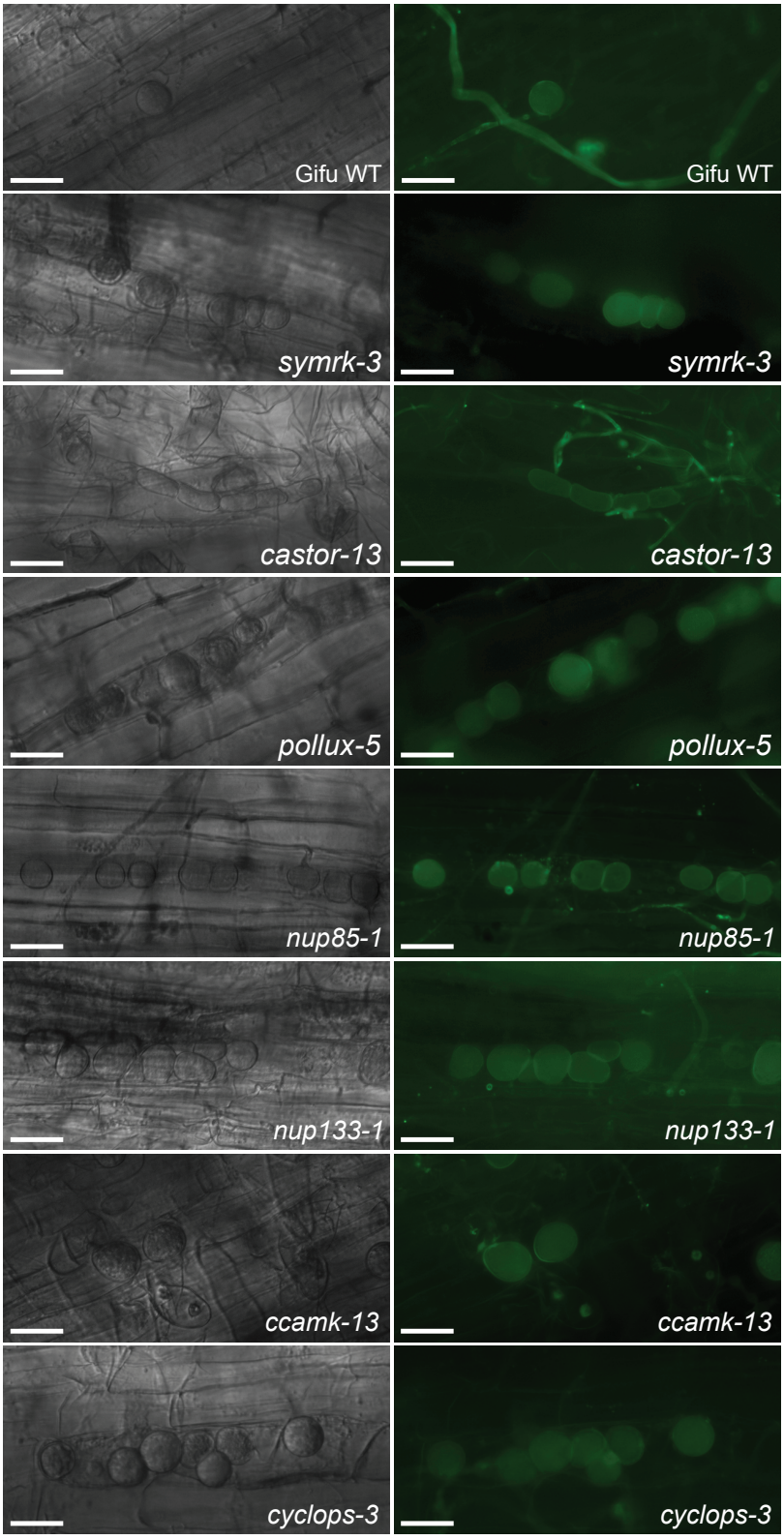

Figure 25: *P. indica* sporulation inside root cells of *L. japonicus*. Single and multiple spores are visible inside individual cleared root cells or root hairs of *L. japonicus* wild-type (Gifu) and in the indicated common symbiosis mutants at 14 dpi. Left column: differential interference contrast (DIC) microscopy; right column: WGA-AF488 (WGA) detected by fluorescence microscopy. Scale bar: 25 µm.

We analysed the relative content of fungal hyphae in roots by determining the ratio of fungal DNA to plant DNA. Interestingly, we detected a tendency for an increased relative amount of fungal DNA in most common symbiosis mutants, with a significant difference to the wild-type in *nup85-1*, *ccamk-12*, and *cyclops-3*, indicating that the fungal biomass in these mutant roots was higher than in the wild-type (Figure 26).

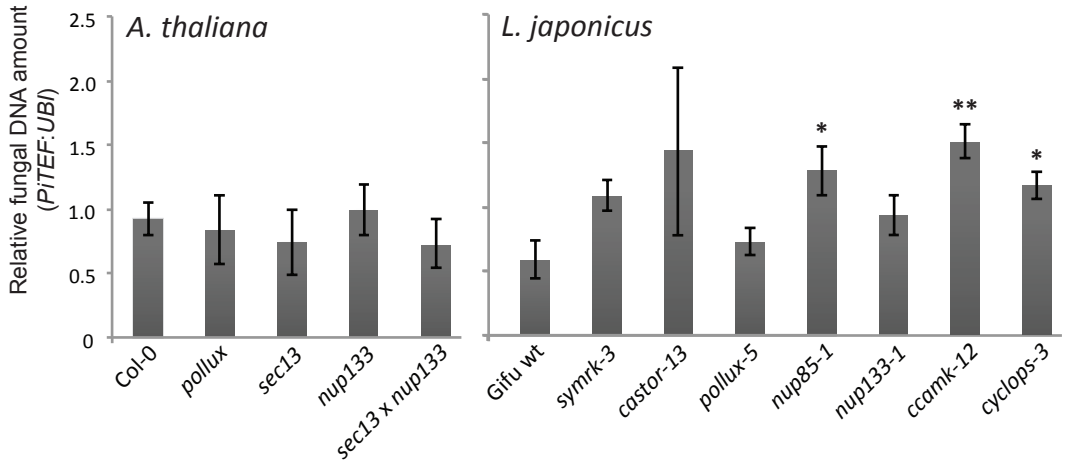

Figure 26: *P. indica* colonization in *A. thaliana* and *L. japonicus* wild-type and mutant roots. Real-time qPCR analysis were performed with DNA from surface-washed *P. indica* colonized roots using primers for the fungal gene *Transcription Elongation Factor* (*TEF*) and for *A. thaliana* and *L. japonicus Ubiquitin* (*UBI*) genes. Bars show ratios of fungal to plant DNA in 200 mg (ca. 10 plants) per sample of *L. japonicus* and *A. thaliana* roots colonized by *P. indica* at 14 dpi grown on modified HO medium. Error bars represent standard errors of the mean from three independent biological repetitions (except *pollux-5*, for which only one replicate was available). Data were analysed with a two-sided, unpaired t-test at the 5% significance level. *, $P < 0.05$; **, $P < 0.01$.

Experiments were designed by Alga Zuccaro and performed by Aline Banhara Pereira and Alga Zuccaro. Aline Banhara Pereira performed the statistical analyses and prepared the figure.

5.6. *P. indica* did not induce β-glucuronidase (GUS) activity in the *L. japonicus* symbiosis reporter line T90

The *L. japonicus* symbiosis-reporter line T90 was isolated in a screen for symbiosis-specific GUS expression from a promoter-tagging population (Webb *et al.*, 2000). The T90 reporter line is activated both in roots treated with nodulation factors and in roots inoculated with *Mesorhizobium loti*. It also shows GUS expression in the interaction with AM fungi (Radutoiu *et al.*, 2003; Kistner *et al.*, 2005). In order to check whether *P. indica* was able to induce the expression of GUS in the T90 line, we inoculated roots with either the *M. loti Ds*Red or with *P. indica* chlamydospores, or mock-inoculated with Tween water before histochemical staining with 5-bromo-4-chloro-3-indolyl glucuronide at 3, 7, and 14 dpi. No GUS activity (blue staining) was observed in *P. indica* or mock-inoculated roots. In roots inoculated with *M. loti Ds*Red, blue staining was detected in the root at all time points (Figure 27).

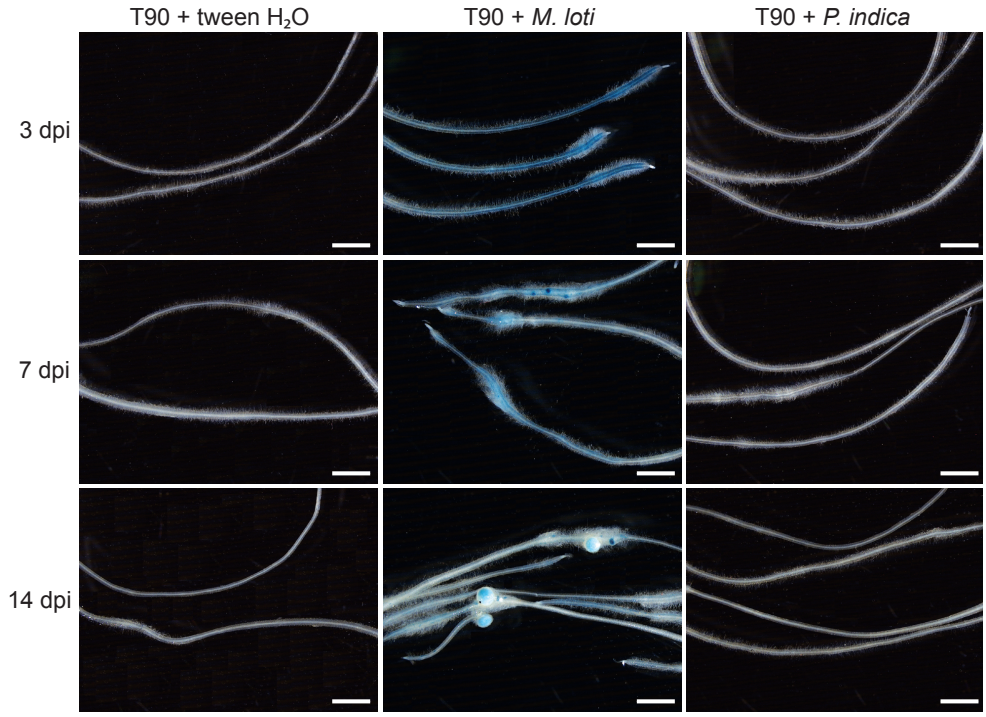

Figure 27: GUS expression in the *L. japonicus* symbiosis reporter line T90 inoculated with *P. indica*. At all evaluated time-points, GUS activity was not observed in T90 plants inoculated with *P. indica* chamydospores or mock-treated with Tween water. *Mesorhizobium loti Ds*Red was used as a positive control and activated GUS expression (blue) in the root or root nodules at all time-points analysed. Scale bar: 2.5 mm.

Experiment was designed and performed by Regina Kühner. Aline Banhara Pereira analysed the experiment and prepared the figure.

5.7. Intracellular colonization of *A. thaliana* roots by *P. indica* is independent of HCSGs

Our results from the mutant analysis in *L. japonicus* suggested that alternative plant genes support the infection by *P. indica*. Although the Brassicaceae, including *A. thaliana*, do not form AM and have lost several key CSGs during their evolutionary history, their genomes harbour homologs of CSGs (Delaux *et al.*, 2013; Delaux *et al.*, 2014; Banhara et al. unpublished). In order to determine the contribution of these genes to the interaction with *P. indica*, specially during the biotrophic phase, which presents more structural similarities to the root colonization by AM fungi, we carried out cytological analyses in roots of wild-type plants and of the HCSG mutants *pollux*, *sec13*, *nup133*, and the double mutants *sec13* x *nup133* and *shrk1* x *shrk2*. Hyphae growing within cells filled with FM4-64-stained endomembrane structures (Figure 28) were observed in all genotypes, suggesting that the penetrated cells were alive and consequently that *P. indica* is able to establish biotrophic growth in these HCSG mutants. Furthermore, the mutants did not differ in the relative colonization of their roots by *P. indica* at 14 dpi (Figure 26). In addition, we detected intracellular sporulation in rhizodermal cells of all genotypes (Fig. 29), indicating that the fungus could successfully complete its life cycle in the roots of the tested HCSG mutants.

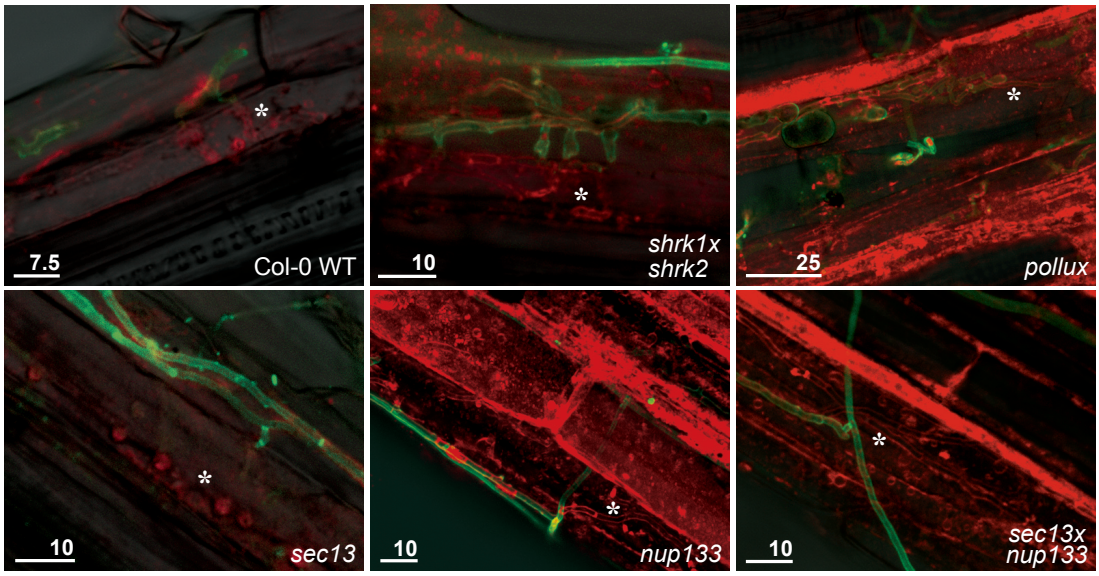

Figure 28: Biotrophic growth of *P. indica* within *A. thaliana* root cells. Intracellular hyphae (indicated by asterisks) are visible within rhizodermal cells of wild-type and the indicated mutants Extracellular hyphae are stained with WGA-AF488 (green). FM4-64-stained plant membranes (red) are visible inside the plant cells, and in some situations enveloping invading hyphae (arrowheads). Biotrophic intracellular hyphae are not or weakly fluorescent, probably due to limited access of WGA-AF488 to the fungal cell wall within root cells. Confocal microscopic pictures were taken with a Leica TCS-SP5 CLSM 14 dpi with *P. indica* chlamydospores. Lengths of scale bars (in μm) are shown on each image.

Experiments were designed, performed, and analysed by Aline Banhara Pereira, Yi Ding, and Alga Zuccaro. Aline Banhara Pereira prepared the figure.

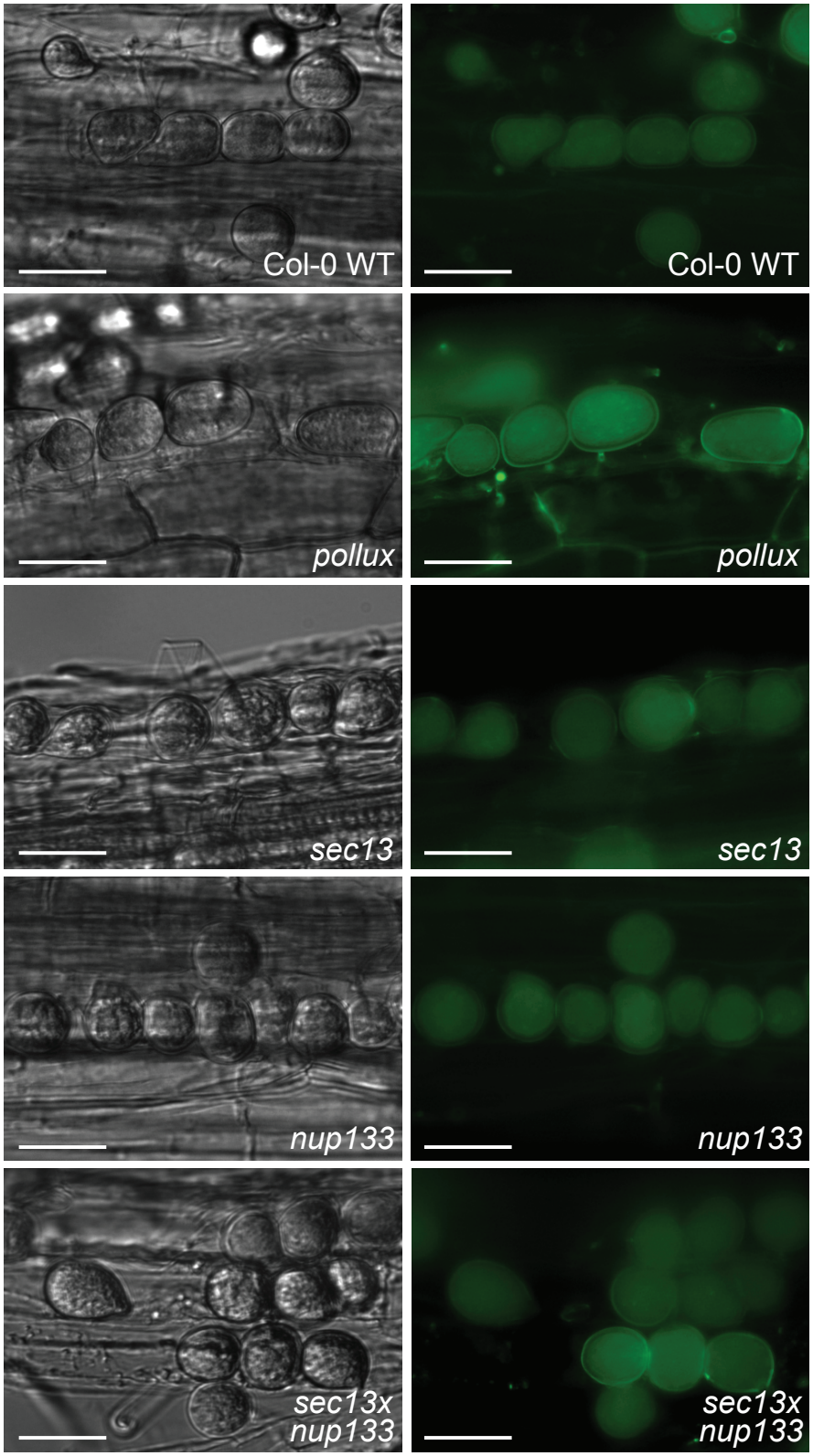

Figure 29: Intracellular sporulation of *P. indica* in *A. thaliana* roots. Multiple spores per root cell are present in the *A. thaliana* wild-type (Col-0) as well as in the indicated HCSG mutants at 14 dpi. Left column: differential interference contrast (DIC) microscopy. Right column: WGA-AF488 (WGA) detected by fluorescence microscopy. Scale bar: 25 µm.

5.8. Plant growth promotion by *P. indica* is influenced by nutrient availability

We previously reported growth promotion of *A. thaliana* plants upon co-culture with *P. indica* when grown on modified HO medium (Lahrmann *et al.*, 2013). To further explore the growth conditions supportive for *P. indica*-mediated growth promotion, we evaluated the effect of *P. indica* on *L. japonicus* and *A. thaliana* grown in soil with two different nutrient regimes. In both plant species, co-cultivation with *P. indica* in soil with high nutrient concentrations had little or no effect on the mean stem height. However, in soil with lower nutrient contents, *P. indica* inoculation roughly doubled the mean stem height of *A. thaliana* plants by 21 dpi, whereas there was only a small however significant effect on *L. japonicus* (Figure 30, Table 1). In general, the positive effect of co-cultivation with *P. indica* in low-nutrient soil was stronger on *A. thaliana* than in *L. japonicus*.

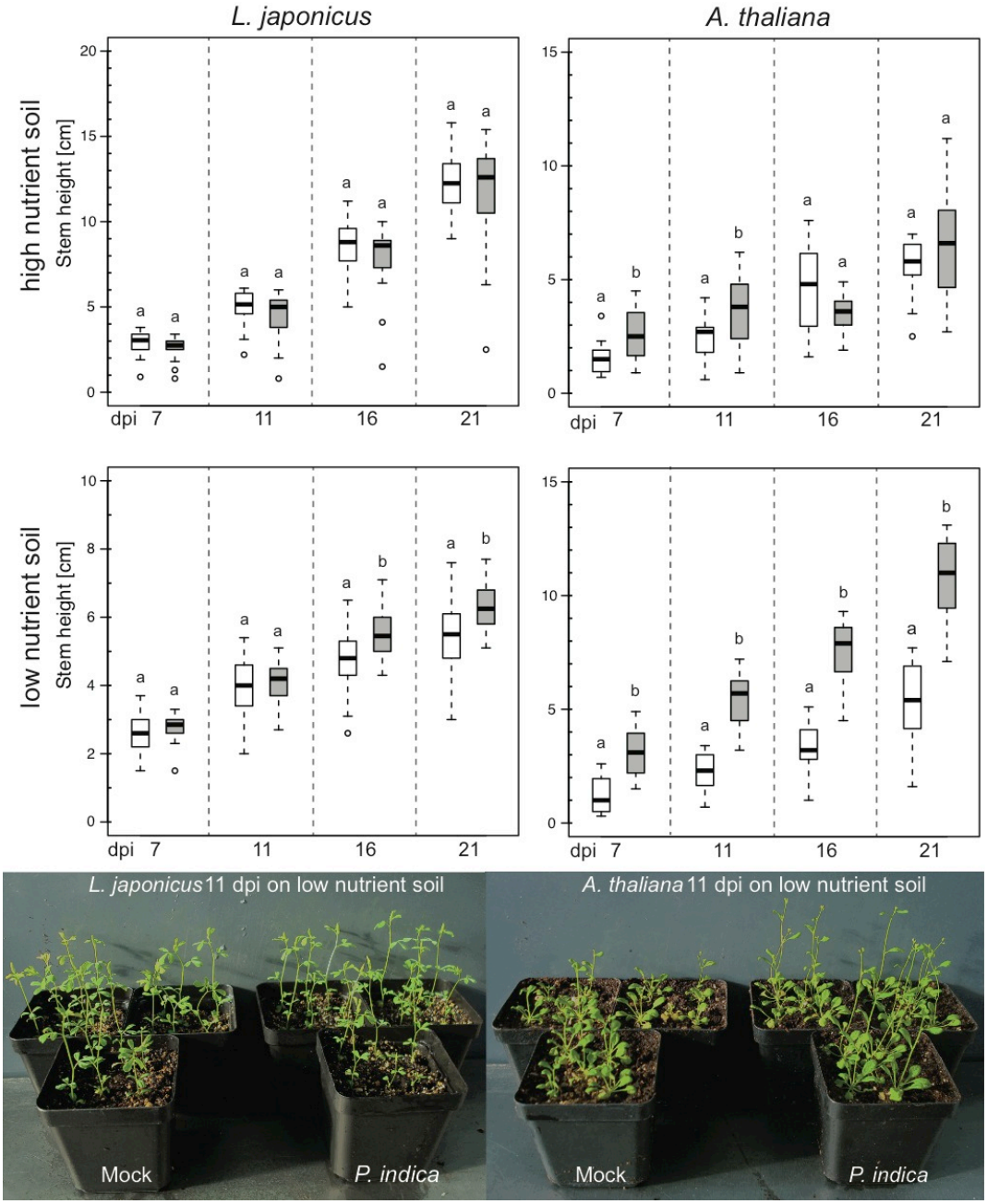

Figure 30: Effect of *P. indica* on the growth of *L. japonicus* and *A. thaliana* grown in soil with different nutrient availabilities. Box-plots represent stem height of plants (ca. 20/treatment) at the indicated dpi. On high-nutrient soil (500 mg l^{-1} N, 500 mg l^{-1} P, 500 mg l^{-1} K), inoculation with *P. indica* did not change plant stem height at 21 dpi ($p > 0.05$). On low-nutrient soil (50-100 mg l^{-1} N, 50-100 mg l^{-1} P, 100-150 mg l^{-1} K), *P. indica* inoculation led to an increase in the mean stem height at 21 dpi ($p < 0.05$). Statistical analyses were performed with a Kruskal-Wallis test followed by a Bonferroni-Holm correction using the mock-inoculated samples as control group. For each mock/*P. indica*-inoculated pair, box-plots that share the same letter do not significantly differ (at the 5% significance level). White boxes: mock; grey boxes: *P. indica*; open circles: outliers. Exemplary pictures of *P. indica*- and mock-inoculated plants grown in low nutrient soil. Experiments were performed three times with similar results.

When *A. thaliana* plants were grown on agar plates with ½ MS medium and no sugar, a growth promoting effect by inoculation with *P. indica* chlamydospores was observed. In contrast, when 0.05% sucrose was added to the medium, the plants were generally bigger and no increase was observed in the mean fresh weight of *P. indica*-inoculated plants compared to mock-treated plants (Figure 31, Table 1).

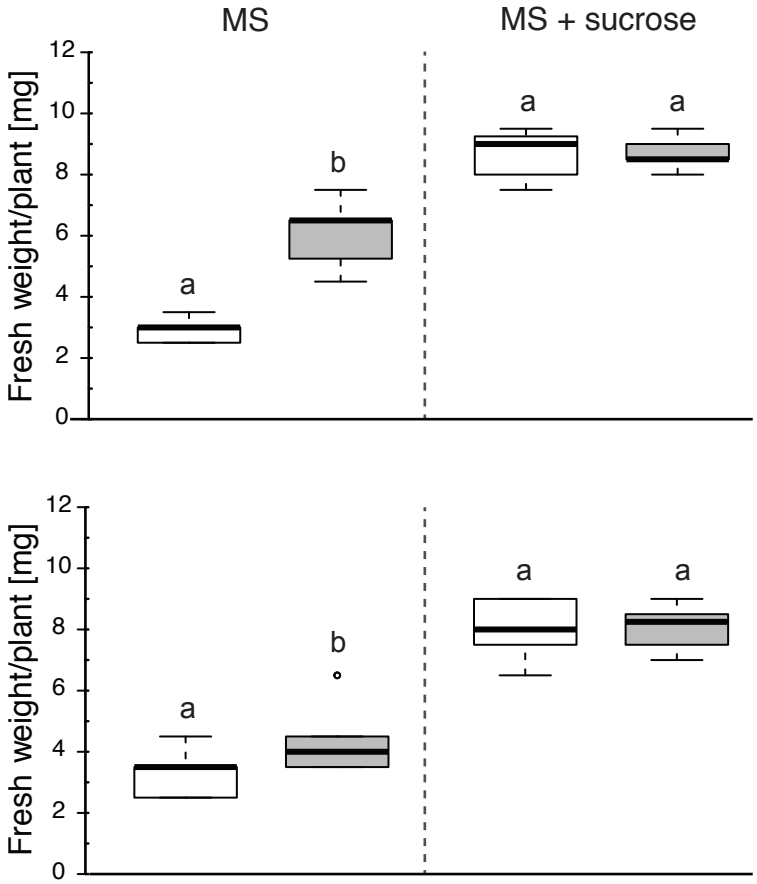

Figure 31: Effect of sucrose on the growth promotion of *A. thaliana* plants by *P. indica*. Seedlings (Col-0) (ca. 15/treatment) were grown on ½ MS with or without sucrose for 10 days before being inoculated with *P. indica* clamydospores (grey boxes) or mock-inoculated with Tween water (white boxes). Box plots represent the fresh weight of plants at 7 dpi. Without sucrose addition, the mean fresh weight of *P. indica*-inoculated plants was significantly higher than that of mock-treated plants ($p < 0.05$). In the presence of sucrose, the mean fresh weight of the plants grown in the presence of *P. indica* was not significantly different from that of mock-treated plants ($p > 0.05$). Statistical analyses were performed with a Kruskal-Wallis test followed by a Bonferroni-Holm correction using the mock-inoculated samples as control group. Plots sharing a letter do not significantly differ (significance level: 5%). Open circles: outliers. Results from two independent experiments are shown.

Since *A. thaliana* plants inoculated with *P. indica* exhibit increased uptake of nitrogen and phosphate (Shahollari *et al.*, 2007; Kumar *et al.*, 2011; Das *et al.*, 2014), we investigated whether the concentration of nitrate [supplied as $Ca(NO_3)_2$ and/or KNO_3], phosphate (KH_2PO_4), or ammonium (NH_4Cl) on modified HO medium influenced the growth promotion of *A. thaliana* plants by *P. indica*. Co-cultivation with the fungus had a positive effect on the mean fresh weight of the plants under all nutrient conditions tested, except on medium depleted of any nitrogen source, a condition that limited plant growth due to nitrogen starvation (Figure 32, Table 1).

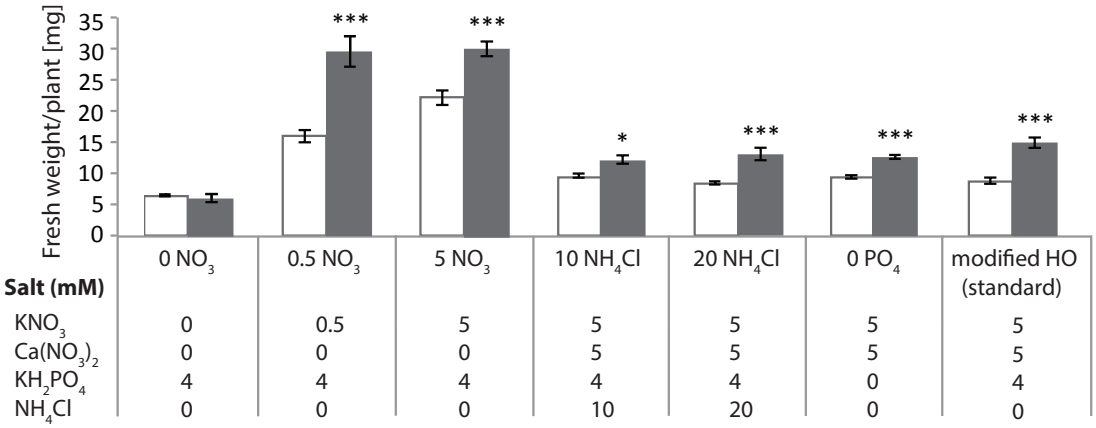

Figure 32: Influence of $Ca(NO_3)_2$, KNO_3, KH_2PO_4 or NH_4Cl concentrations on the growth promotion of *A. thaliana* by *P. indica*. Bars represent the fresh weight of *P. indica*-inoculated plants (ca. 10 plants/treatment) relative to the respective mock control at 7 dpi, and error bars refer to the standard deviation of the mean of the fresh weight values. Overall, co-cultivation with *P. indica* chlamydospores had a positive effect on the mean fresh weight of the plants, independent of nutrient concentration, except for medium depleted of any nitrogen source, on which the presence of the fungus did not significantly change plant biomass ($p > 0.05$). Plants were grown on the modified HO medium with the indicated salt concentrations. White box: mock; grey box: *P. indica*. Statistical analyses were performed with a Kruskal-Wallis test followed by a Bonferroni-Holm correction using the mock-inoculated samples as control group, and *P* was calculated on the absolute values. Each pair mock/*P. indica*-inoculated was compared individually. Stars indicate a significant difference (at the 5% significance level: 5%) relative to the non-inoculated control. *, $P < 0.05$; ***, $P < 0.001$.

Experiment was designed and performed by Aline Banhara Pereira. Statistical analyses were done by Aline Banhara Pereira and Alga Zuccaro, and Aline Banhara Pereira prepared the figure.

5.9. *A. thaliana* HCSGs are not required for *P. indica*-induced growth promotion

We investigated the influence of *A. thaliana* HCSGs on the host growth-promoting effect by *P. indica*. For that, we inoculated wild-type *A. thaliana* plants, the HCSG mutants *pollux*, *sec13*, *nup133*, and the double mutant *sec13* x *nup133* with chlamydospores of *P. indica.* As a specificity control, we included the closely related sebacinoid fungus *P. williamsii* (Basiewicz *et al.*, 2012; Lahrmann *et al.*, 2013). Plant biomass was evaluated 7 dpi. Plants inoculated with *P. indica* had a significant higher mean fresh weight than plants mock-inoculated with Tween water, whereas treatment with *P. williamsii* chlamydospores resulted in no or only a slight increase (for *sec13* x *nup133*) in plant biomass (Figure 33, Table 2).

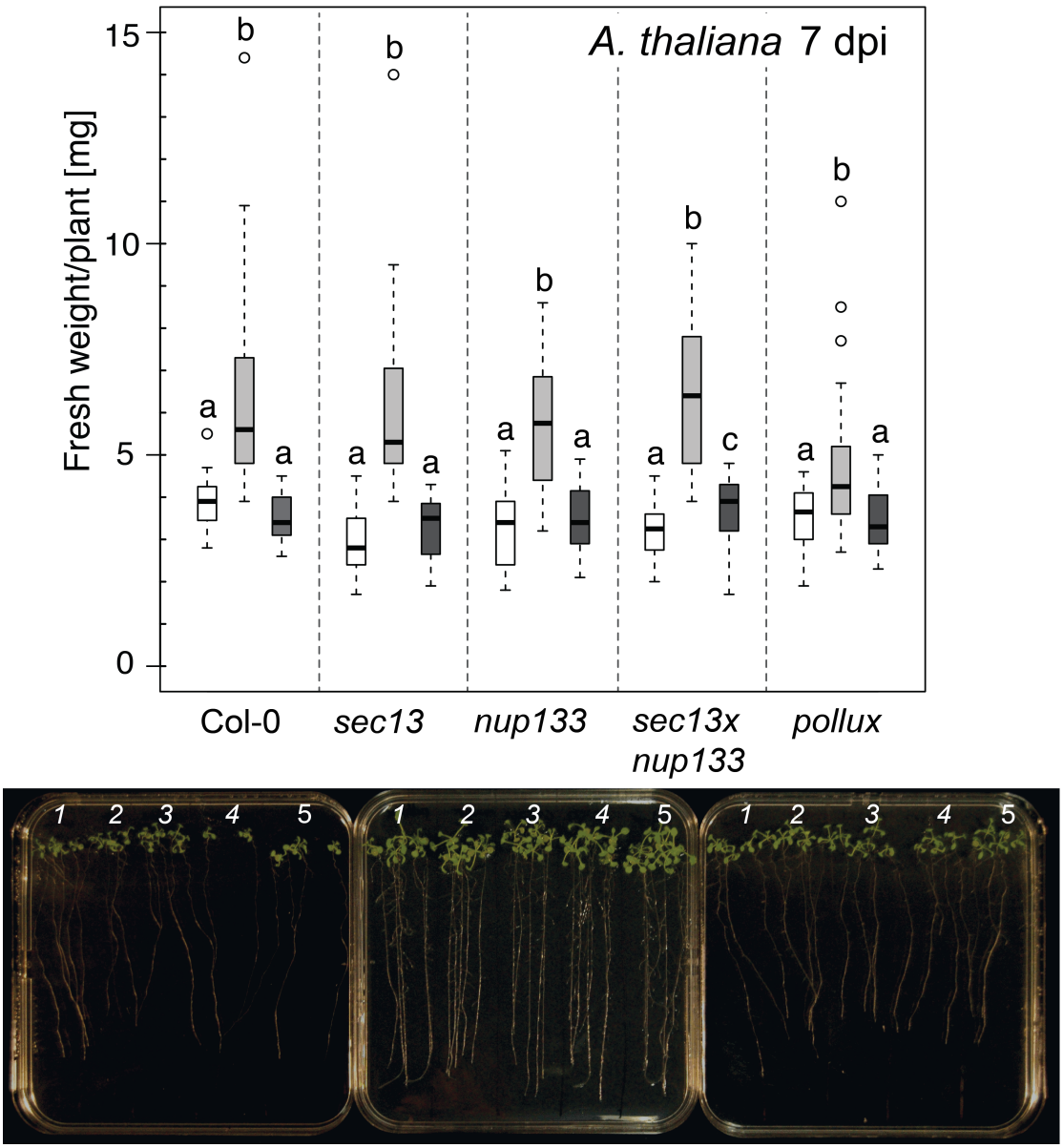

Figure 33: Biomass of *A. thaliana* wild-type or HCSG mutants upon inoculation with *P. indica* or *P. williamsii*. Upper panel: box-plots show the fresh weight of the indicated *A. thaliana* genotypes (ca. 15 plants/genotype) grown in the presence or absence of *P. indica* or *P. williamsii*. All plant genotypes accumulated more biomass upon co-cultivation with *P. indica* but not with *P. williamsii*. White box: mock; light grey box: *P. indica*; dark grey box: *P. williamsii*. Open circles: outliers. Statistical analyses were performed using a Kruskal-Wallis test followed by a Bonferroni-Holm correction using the mock-inoculated samples as control group. Boxes that do not share a letter are significantly different (at the 5% significance level). Comparisons among the three treatments were made for each genotype separately. Lower panel: representative plates showing sets of 14-day-old plants grown on modified HO medium are shown seven days after mock-treatment with Tween water (left) or inoculation with either *P. indica* (center) or *P. williamsii* (right) chlamydospores. 1: *pollux*; 2: *sec13* x *nup133*; 3: *nup133*; 4: *sec13*; 5: Col-0.

Table 1: Statistical values for pairwise comparisons performed with the Kruskal-Wallis test followed by a Bonferroni-Holm correction using the mock-inoculated samples as control group.

Growth in soil

A. thaliana

Condition	treatment	*P*
Low nutrient soil	mock x *P. indica*	0.01
	mock x *P. indica*	0.037
	mock x *P. indica*	0.175
	mock x *P. indica*	0.298
High nutrient soil	mock x *P. indica*	<0.001
	mock x *P. indica*	0.0
	mock x *P. indica*	0.0
	mock x *P. indica*	0.0

L. japonicus

Condition	treatment	dpi	*P*
High nutrient soil	*P. indica*	7	0.110
	P. indica	11	0.167
	P. indica	16	0.270
	P. indica	21	0.930
Low nutrient soil	*P. indica*	7	0.160
	P. indica	11	0.390
	P. indica	16	0.046
	P. indica	21	0.013

Growth on modified HO

Condition	treatment	*P*
0 mM KNO3	*P. indica*	0.770
0.5 mM KNO3	*P. indica*	<0.001
5 mM KNO3	*P. indica*	<0.001
10 mM NH4Cl	*P. indica*	0.025
20 mM NH4Cl	*P. indica*	<0.001
0 mM KH2PO4	*P. indica*	<0.001
Modified HO (standard)	*P. indica*	<0.001

Growth on MS		Fig. 31 upper	Fig. 31 lower
Condition	treatment	*P*	*P*
MS0	*P. indica*	0.0	0.0013
MS (+) sucrose	*P. indica*	0.3866	0.9211

P: probability at the 5% significance level
$P < 0.05$: significant biomass increase relative to mock

Table 2: Statistical values for multiple comparisons performed the data obtained with the inoculation of *A. thaliana* HSCS mutants with either *P. indica* or *P. williamsii* with the Kruskal-Wallis test followed by a Bonferroni-Holm correction using the mock-inoculated samples as control group.

Treatment	genotype	Effect	*P*
P. indica	Col(-)0	(+)	<0.001
P. williamsii	Col(-)0	none	0.110
P. indica	*pollux*	(+)	0.0086
P. williamsii	*pollux*	none	1.0
P. indica	*sec13*	(+)	<0.001
P. williamsii	*sec13*	none	0.35
P. indica	*nup133*	(+)	<0.001
P. williamsii	*nup133*	none	1.0
P. indica	*sec13* x *nup133*	(+)	<0.001
P. williamsii	*sec13* x *nup133*	(+)	0.042

(+): biomass increase

6. Discussion

6.1 *A. thaliana* mutants for HCSGs impair haustorial development and the reproductive success of a biotrophic oomycete

In this work, we demonstrated that the *A. thaliana* HCSGs *POLLUX*, *ShRK1*, *ShRK2*, *NUP133*, and *SEC13* are required for the full reproductive success of *Hpa* during colonization of *A. thaliana* leaves. This reduced susceptibility was related neither to reduced cell penetration frequency nor to constitutive or exacerbated activation of pathogen-associated molecular pattern (PAMP)-triggered plant immunity (Jones & Dangl, 2006) in the HCSG mutants. Instead, it was probably caused by an alteration in the haustorial development. The HCSG mutants presented a higher frequency of morphologically altered haustoria, and the change in morphology was associated with a loss of fluorescence generated by a perihaustorial marker protein, suggesting a modification in the protein composition of the perihaustorial membrane. We showed that, in the double mutant *shrk1* x *shrk2*, the haustoria seem to age earlier than in the wild-type, indicating a direct effect of the plant receptor kinases in the haustorial architecture of the oomycete. The haustorium was described to be the site of nutrient exchange with the plant host (Mendgen & Hahn, 2002; Voegele & Mendgen, 2003). Thus, our results suggest that the impaired haustorial development possibly results in a decreased nutrient availability to the oomycete, with a consequent reduction in the sporangiophore production.

Interestingly, the colonization by the extracellular bacterial pathogen *P. syringe* was normal in the *A. thaliana* HCSG mutants, indicating that the phenotype of altered haustorial development in all tested HCSG mutants is specific to biotrophic interactions that involve the formation of typical intracellular accommodation organs. To further test this hypothesis, we analysed the reproductive success of the powdery mildew fungus *E. cruciferarum* on *A. thaliana*, without revealing any consistent difference to the colonization pattern in wild-type leaves. Due to the highly variable morphology of the haustoria formed during colonization of wild-type leaves, we were unable to determine if the haustoria developed normally in the HCSG mutants. *E. cruciferarum* only penetrates epidermal cells (Micali *et al.*, 2008; Huesmann *et al.*,

2011; Weis *et al.*, 2014), while *Hpa* initially forms haustoria in two anticlinal epidermal cells but then proceeds to penetrate the mesophyll cells (Koch & Slusarenko, 1990; Slusarenko & Schlaich, 2003). Assuming that HCSGs do not play a role in this interaction, one could therefore propose that distinct pathways are used for colonization of the leaf epidermis and subjacent mesophyll cells. The *MLO* gene, for example, is a compatibility factor expressed in the epidermis and required for penetration of the powdery mildew fungus *G. orontii* into *A. thaliana* epidermal cells (Consonni *et al.*, 2006), with no reported role in the *Hpa* colonization. Alternatively, different genetic components may be required by intracellular oomycetal and fungal pathogens, with the pathway comprising the HCSGs being specifically recruited by *Hpa*. In this context, it would be interesting to investigate whether these genes are required for the interaction between *A. thaliana* and other biotrophic oomycetes, such as *Phytophthora parasitica*, member of a genus known to cause devastating diseases on economically important crops. *P. infestans*, for instance, is the causal agent of the late blight on potato and responsible for the Great Irish Famine in the 1840s (Fry, 2008; Haas *et al.*, 2009). Apart from penetrating *A. thaliana* leaves, *P. parasitica* is also able to enter root cells (Attard *et al.*, 2010; Wang *et al.*, 2011). This is particularly interesting, since the root is the organ where the CSG of legumes exert their role during AM symbiosis.

6.2 Symbiosis-related genes are not required for the colonization of *L. japonicus* and *A. thaliana* with the root endophytic fungus *P. indica*

The similarities between colonization of the outer cell layers of plant roots by AM fungi and *P. indica* led us to investigate whether symbiosis-related genes were required for *P. indica* colonization of *L. japonicus* and *A. thaliana* roots. In both wild-type plants and in the common symbiosis mutants, *P. indica* was able to successfully complete its life within root cells, where colonization went through an initial biotrophic phase, similar to what has been demonstrated for other species such as barley *and A. thaliana* (Jacobs *et al.*, 2011; Zuccaro *et al.*, 2011; Lahrmann & Zuccaro, 2012; Lahrmann *et al.*, 2013), and culminated with intracellular sporulation, indicating that the CSGs are not required for the intracellular colonization of *L. japonicus* roots by *P. indica*. This was further corroborated by the observation that the

inoculation of the T90 symbiosis reporter line with *P. indica* did not activate GUS expression, for which the common symbiosis genes are required (Kistner *et al.*, 2005; Gossmann *et al.*, 2012).

A core set of CSGs, including *CCaMK* and *CYCLOPS*, is absent from the *A. thaliana* genome (Delaux *et al.*, 2014). The observations that *P. indica* was able to establish a biotrophic colonization and sporulate in the roots of *A. thaliana* HCGS mutants, and the relative amount of fungal hyphae in these mutants was not altered, suggest that the HCSGs are not required for the intracellular colonization of *A. thaliana* by *P. indica*.

Together, our results imply the existence of compatibility factors in the *P. indica*-plant root that function independently of symbiosis-related genes.

6.3. Increased fungal biomass in *L. japonicus* common symbiosis mutants suggests cross-talk between symbiosis signalling and defence or developmental pathways

Interestingly, we detected a tendency for increased amount of intraradical hyphae in some of the common symbiosis mutants in comparison to the wild-type, with a significant difference for *nup85-1*, *ccamk-12*, and *cyclops-3*. This observation does not imply roles for the CSGs in the direct accommodation of *P. indica* within root cells as seen in the AM symbiosis. Instead, it suggests a negative role for these genes on fungal proliferation in the host roots, and might be related to possible overlaps between signalling pathways involved in symbiotic and pathogenic interactions, as already reported for a number of genes (reviewed in (Evangelisti *et al.*, 2014)). However, such effect was detected on *L. japonicus* but not on *A. thaliana* mutants, implying that the regulatory pathways affected in legumes may not be functional in *A. thaliana*.

In *A. thaliana* and in barley, the establishment or maintenance of the association between *P. indica* and plant roots requires that host defence responses are at some extent suppressed (Schäfer *et al.*, 2009; Jacobs *et al.*, 2011). Therefore, a situation in which the manipulation of the host immune system is not properly regulated could result in an increased susceptibility to the fungus, causing a more dense colonization. Hyper-infection by *P. indica* has been associated with deregulated defence responses at least in *A. thaliana* (Camehl *et al.*, 2010). In legumes, common symbiosis genes

have been connected to plant defence signaling as well. The Calcium and Calmodulin dependent protein Kinase gene *DMI3*, the *CCaMK* ortholog in *M. truncatula*, plays a role in the interaction with the hemi-biotrophic pathogenic fungus *Colletotrichum trifolii*; in the *dmi3* mutant, the switch from biotrophy to necrotrophy, which starts upon fungal penetration, occurred earlier (Genre *et al.*, 2009). In barley, a dense colonization by *P. indica* was shown in dead cells of the root differentiation and maturation zones (Deshmukh *et al.*, 2006). It will be therefore important to determine whether increased relative *P. indica* biomass in the roots of some of the *L. japonicus* common symbiosis mutants is associated with the necrotrophic colonization stage. This would explain the increased amount of intracellular hyphae that we detected for *ccamk-12* or perhaps through the same mechanism for the other common symbiosis mutants *nup85-1* and *cyclops-3*.

In addition, the bacterial PAMP (pathogen-associated molecular pattern) flg22 has been shown to play a negative role on rhizobial infection and on the *CCaMK*-induced nodule organogenesis during root nodule symbiosis between *L. japonicus* and *M. loti* (Lopez-Gomez *et al.*, 2011). In the same study, the nodule inception (NIN) transcription factor, which is a negative regulator of root cell competence to infection by rhizobia (Schauser *et al.*, 1999), was expressed after treatment with flg22. In *A. thaliana*, flg-22 mediated defence responses are suppressed by *P. indica* during the biotrophic phase (Jacobs *et al.*, 2011), implying a general negative effect of flg22 on infection by biotrophic microorganisms, and leaving us to speculate that the tendency for increased *P. indica* colonization in the roots of *L. japonicus* common symbiosis mutants results from alterations in the host immune system and the consequent inability to respond to invading microorganisms.

6.4. Growth promotion of *A. thaliana* by *P. indica* is independent of HCSGs but is affected by nutrient availability

In regard with *P. indica*-mediated biomass increase, considering that this trait is not robust and has not always been reproducible, we explored a range of distinct substrate and nutrient conditions and were able to confirm that the growth-promoting effect of *P. indica* (Peškan-Berghöfer *et al.*, 2004; Shahollari *et al.*, 2007; Sherameti *et al.*,

2008; Camehl *et al.*, 2010; Camehl *et al.*, 2011; Nongbri *et al.*, 2012; Lahrmann *et al.*, 2013; Venus & Oelmuller, 2013) on *A. thaliana* plants was present in all situations.

However, the mechanism by which the fungus promotes host growth is still highly debated. The importance of phythormones for the establishment of a proper association between *P. indica* and plant roots has been shown in a number of studies. On the one hand, jasmonic acid and gibberellins positively influence root colonization, while salicylic acid has inhibitory effects (Jacobs *et al.*, 2011). On the other hand, ethylene was described as being required for colonization by *P. indica* (Khatabi *et al.*, 2012), and genes involved in the synthesis of indole-3-acetaldoxime-derived compounds restricts and prevents excessive growth of *P. indica* inside *A. thaliana* roots (Nongbri *et al.*, 2012). Importantly, the fungus has been shown to produce the phytohormone auxin (Sirrenberg *et al.*, 2007; Lee *et al.*, 2011), which has been implicated in stimulation of plant growth (Davies, 2004). Nevertheless, it has been claimed this auxin does not directly affect host biomass increase in barley (Hilbert *et al.*, 2012) or *A. thaliana* (Vadassery *et al.*, 2008), and the mechanism by which *P. indica* promotes host growth remains therefore still to be elucidated.

Our results in soil indicate that a nutrient effect at least contributes to *P. indica*-induced growth promotion, since this trait was much more pronounced when the substrate contained lower amounts of nitrogen, phosphate and potassium, suggesting that high nutrient availability could have negative effects on the establishment or maintenance of the *P. indica* symbiosis. Nevertheless, in order to obtain a more clear role of nutrient concentration on the interaction between *P. indica* and plant roots, it will be crucial to investigate whether the elevated nutrient availability relates to a decreased root colonization by *P. indica,* similar to what has been described for as AM symbiosis (Olsson *et al.*, 2005; Breuillin *et al.*, 2010; Balzergue *et al.*, 2013; Bonneau *et al.*, 2013; Gosling *et al.*, 2013).

The influence of *P. indica* on increased uptake of nitrogen and phosphate by the plant host has already been reported (Kumar *et al.*, 2011; Das *et al.*, 2014). Therefore, we investigated whether the concentration of nitrogen and phosphate had any influence on the growth promotion effect of *P. indica* on plates. We observed that, regardless of salt concentration, the biomass of inoculated plants was always higher than that of non-inoculated individuals, except under nitrogen deficiency, which may have prevented the plants from properly growing even when *P. indica* was present. Though

nutrient concentration did not have an obvious influence on the *P. indica*-induced host biomass increase, the presence of one specific compound may have compensated for the absence of another, and do not exclude that an increased uptake of nitrate, ammonium and/or phosphate is involved in the incremented host growth, as indicated in (Sherameti *et al.*, 2005; Kumar *et al.*, 2011; Lahrmann *et al.*, 2013).

Das and colleagues reported that the total content of soluble sugar in rice plants inoculated with *P. indica* is higher than in non-inoculated plants, possibly due to an increased transport of carbohydrates to the fungus (Das *et al.*, 2014). In addition, it has been shown that, in *A. thaliana* and *Nicotiana tabacum* roots, *P. indica* inoculation stimulates the expression of starch degrading enzymes, resulting in higher availability of sugar to the plants and/or the fungus (Sherameti *et al.*, 2005). We observed that addition of sucrose to the culture medium abolished the growth-promoting effect by *P. indica* on *A. thaliana*, indicating that excess sugar may have caused the plants to grow to their maximal potential and masked any fungal effect on internal sucrose mobilization.

Even though our studies indicated no role of known symbioses-related genes in the association between plant roots and *P. indica* in regards with colonization, we explored their roles in the *P. indica*-induced host biomass increase, and showed that it was still present in the *A. thaliana* HCSG mutants *pollux*, *nup133*, and *sec13*. Thus, regardless of the mechanism involved in the growth promotion of *A. thaliana* by *P. indica*, it does not require the HCSG *POLLUX* and the nucleoporins genes *NUP133* and *SEC13*, confirming previous observations on *AtPOLLUX* (Shahollari *et al.*, 2007).

Altogether, our results support the idea that the interaction of plant roots with *P. indica* does not require the common symbiosis pathway in legumes or remnants of this signaling network in *A. thaliana*, and suggests that the fungus relies on alternative plant factors to establish its symbiosis with plant roots. However, it is still not clear whether a specific pre-determined genetic pathway is involved in the intracellular accommodation of *P. indica*, or if it is mainly a consequence of successful manipulation of plant host defence responses and phytohormone balance by the fungus (Evangelisti *et al.*, 2014).

While the presence of both *P. indica* and AM fungi results in increased host growth (Peškan-Berghöfer *et al.*, 2004; Javot *et al.*, 2007a; Shahollari *et al.*, 2007; Sherameti

et al., 2008; Smith & Read, 2008; Camehl *et al.*, 2010; Camehl *et al.*, 2011; Nongbri *et al.*, 2012; Lahrmann *et al.*, 2013; Venus & Oelmuller, 2013; Nouri *et al.*, 2014), only the first undergoes its entire life cycle as a biotroph, whereas in *P. indica* the biotrophic phase of root colonization is followed by a stage characterized by cell death and necrotrophy (Deshmukh *et al.*, 2006; Zuccaro *et al.*, 2011; Lahrmann & Zuccaro, 2012; Qiang *et al.*, 2012). There is evidence that excessive *P. indica* proliferation is associated with necrotrophy and/or with detrimental effects on the growth of the plant host (Deshmukh *et al.*, 2006; Camehl *et al.*, 2010; Nongbri *et al.*, 2012). Therefore, it will be interesting to explore whether the increased relative *P. indica* biomass in the roots of some *L. japonicus* common symbiosis mutants lead to host growth retardation, and whether this uncontrolled fungal proliferation occurs during the necrotrophic colonization stage. Such approach might help us to establish if the host growth-promoting effect by *P. indica* results from events that take place during the biotrophic phase of the interaction.

6.5. *A. thaliana* homologs of common symbiosis genes: compatibility factors for *Hpa* accommodation

Taken together, our results place the *A. thaliana* HCSGs in a pathway that is specifically recruited by the oomycete *Hpa* for its accommodation inside leaf epidermal and mesophyll cells. The phenotype of altered haustorial development was observed exclusively in the HCSG mutants infected with *Hpa*. The *A. thaliana pskr1* mutant, which presents disease resistance resulting from constitutive defence responses (Mosher *et al.*, 2013), showed normal haustoria formation. Considering that the HCSG mutants are less susceptible to the oomycete with no abnormal activation of the plant immune system, we propose that the HCSGs act as compatibility factors (opposed to disease resistance factors) in the association of *Hpa* with *A. thaliana*.

Even though many genes were described to play a role in disease resistance, only a few have been reported to contribute to pathogen colonization of the plant hosts. Compatibility factors are normally part of the plant machinery that is manipulated by the biotrophs for their own growth. In this context, the genes *DMR1* and *IOS1* are particularly interesting, since, like *ShRK1* and *ShRK2*, both encode kinase proteins that play a role in the colonization of *A. thaliana* by *Hpa* (Van Damme *et al.*, 2005;

van Damme *et al.*, 2009; Hok *et al.*, 2011). Moreover, *IOS1* is a member of the malectin-like domain leucine-rich repeat receptor-like kinase (MLD-LRR-RLK) family. *ShRK1* and *ShRK2*, which we reported to be the closest homologs of the CSG *SYMRK*, and *SYMRK* itself, belong to this family as well. The GDCP motif in the extracytoplasmatic portion of SYMRK, required for the establishment of the AM symbiosis in the root epidermis (Kosuta *et al.*, 2011; Antolin-Llovera *et al.*, 2014), is present in IOS1, ShRK1 and ShRK2, suggesting a possible role for this region in the *A. thaliana* association with *Hpa*. To confirm this hypothesis, it would be necessary to evaluate the oomycete colonization in mutants for genes encoding other members of the MLD-LRR-RLK family that lack the GDCP motif. One such candidate is At5g48740, whose kinase domain is the closest related to that of SYMRK, despite the fact that the identity between their extracellular domains is relatively lower.

Our finding that symbiosis-related genes may act as compatibility factors during the interaction of plants with both symbiotic and pathogenic microorganisms is reminiscent of the dual involvement of *RAM2* in AM symbiosis as well as in root colonization by an oomycete. In *M. truncatulra, RAM2*, which encodes a glycerol-3-phosphate acyl transferase required the production of cutin monomers, is required for the formation of hyphopodia, structures that attaches to the root surface at the fungal entry point preceding cell invasion during AM symbiosis (Gutjahr & Parniske, 2013), but also for the development of the highly specialized infection cells known as appressoria during root colonization by the oomycetal pathogen *Phytophthora palmivora* (Wang et al, 2012). In the *A. thaliana* HCSG mutants, however, the initial process of cell infection by *Hpa* appears unaffected. Rather, our work shows genetic commonalities in the formation of intracellular accommodation organs later in the development of symbiosis and disease.

6.6. "Common symbiosis pathway" in *A. thaliana*?

Based on the function of the legume CSGs, it is tempting to assume that the HCSGs are similarly involved in a signaling network that promotes oomycetal development. However, it is still not clear whether these genes are directly responsible for the intracellular accommodation of *Hpa* in *A. thaliana*. It is therefore important to investigate whether there are mechanisms commonly used by microorganisms during symbiotic and pathogenic interactions and which of them are controlled by the

HCSGs. In this context, even if the remnants of this ancient pathway play roles similar to those of their legume counterparts, the signaling pathway leading to the successful establishment of the pathogenic interaction would still have gaps, meaning that the branch that involves calcium signaling and decoding in the plant nucleus still needs to be identified. Even though we have not yet proven that nuclear calcium signatures are also required for *Hpa* infection, some evidences support this hypothesis. In legumes and most angiosperms, *POLLUX* seems to play a role exclusively in the generation of calcium spiking during the biotrophic association with AM fungi (Charpentier *et al.*, 2008; Venkateshwaran *et al.*, 2012), agreeing with our findings in *A. thaliana*, where a *pollux* mutant had a phenotype only during the biotrophic interaction with *Hpa*. Besides, the facts that *POLLUX* is conserved among angiosperms (Kistner & Parniske, 2002), and potentially functional in the root nodule symbiosis in *M. truncatula* (Venkateshwaran *et al.*, 2012), support the idea that *POLLUX* and the calcium spiking machinery is conserved in *A. thaliana* as well.

Assuming that nuclear calcium signatures are part of the plant program for the accommodation of *Hpa*, candidates with similar functions to *CCaMK* and *CYCLOPS* in signal decoding and transcriptional activation in the plant nucleus during AM symbiosis (Singh & Parniske, 2012; Singh *et al.*, 2014) must be identified in *A. thaliana*. This would allow us to fill in the gaps in the putative signaling pathway that leads to the establishment of the biotrophic association with an oomycetal pathogen and provide further evidence that, at least in part, an ancient pathway is conserved for the accommodation of biotrophic hyphal microorganisms in symbiosis and disease.

6.7. Evolutionary forces driving the retention of the HSCGs in *A. thaliana*

The finding that vestiges of an ancient pathway for the intracellular accommodation of beneficial microorganisms was retained in a plant that is not able to establish root symbiosis with AM fungi, and the symbiosis with *P. indica* was proven to be independent of HCSGs, suggests that pathogens may take advantage of symbiotic programs. The lack of obvious developmental phenotypes in any of the HCSG mutants indicates that these genes do not fulfil any vital housekeeping functions. This

raises the question why the HCSG genes were kept in the *A. thaliana* genome for no apparent reason other than supporting the lifestyle of an oomycetal pathogen.

Contrary to the fitness improvement for plants that are able to establish symbioses, there is no obvious selective advantage for plants that are infected by *Hpa* that would support the retention of the HCSGs in the *A. thaliana* genome. Considering that it is not likely that plants evolved genes solely to favour pathogen growth, it is important to investigate whether ecological conditions exist, under which plants colonized by the oomycete present selective advantages over non-infected plants.

Plants have evolved distinct signaling pathways to deal with different types of pathogenic agents. In general, they activate salicylic acid (SA)-mediated defence responses against biotrophic pathogens, and jasmonate (JA)-dependent defences in response to wounding associated with interaction with necrotrophic pathogens or with herbivore insects (Kessler & Baldwin, 2002; Glazebrook, 2005). Some necrotrophic microorganisms, such as the bacterial pathogen *P. syringae*, induce both the JA and the SA-mediated defence responses (Stout *et al.*, 1998; Preston *et al.*, 1999; Thaler & Bostock, 2004). In nature, plants are often simultaneously or subsequently faced with different sorts of attackers, and must therefore deal with such stresses by finely regulating and/or integrating the defence signaling pathways. Generally, the SA and the JA-mediated defence responses have antagonistic effects on each other, with the outcome being that, when one pathway is activated, the plants may be primed for an improved defence against organisms of the same type, but become more susceptible to pathogens that activate the other defence pathway (Spoel *et al.*, 2007; Koornneef & Pieterse, 2008).

Herbivore pathogens present themselves as some of the most damaging plagues to plant crops (Stout *et al.*, 2006). Therefore, the activation of one or another defence signaling pathway by pathogenic microorganisms and the effect they have on future attacks by herbivores must be taken into consideration. Reports have shown that phloem-sucking insect herbivores are associated with SA-mediated defence responses, whereas chewing Lepdopteran herbivores normally induce the JA-dependent responses (Moran & Thompson, 2001; Cooper *et al.*, 2004; Kaloshian & Walling, 2005; Musser *et al.*, 2005; Rodriguez-Saona *et al.*, 2005; Thompson & Goggin, 2006). As a result, infection by pathogens that induce either SA or JA-

mediated defences may render the plants more resistant to future attacks by aphids or chewing insects, respectively (Thaler *et al.*, 2010).

Besides the influence of the crosstalk between those defence signaling pathways and the potentially positive or detrimental effects it might have on the fitness of herbivores feeding on plants already infected by pathogens, insect performance can also be affected by certain compounds produced by plants as a general defence response, such as peroxidases (Anfoka & Buchenauer, 1997; Dowd & Lagrimini, 1997; Stout *et al.*, 1999), or directly by the presence of microorganisms colonizing the plant host. Despite reports that pathogen-infected plants may be preferred by certain herbivores as food sources (Cardoza *et al.*, 2003; Mondy & Corio-Costet, 2004), existing infections could also render the affected hosts more resistant to herbivores. This increased resistance is assumed to be caused by the presence of detrimental chemical compounds, alterations in the host metabolism or because pathogen structures themselves act as physical barriers (Stout *et al.*, 2006). Herbivores either avoid colonized leaves or show decreased fitness by feeding on infected, nutrient-depleted leaves. The avoidance behaviour was observed when adult *Phaedon cochleariae* beetles chose healthy chinese cabbage (*Brassica rapa*) leaves over leaves colonized by the fungus *Alternaria brassicae* for oviposition. In the same system, larvae that fed on colonized leaves transformed into pupa with reduced weight (Rostás & Hilker, 2002). When the two species of alpine beetles, *Oreina elongata* and *O. cacaliae*, were offered healthy leaves and leaves of the plant host *Adenostyles alliariae* infected with the rust fungus *Uromyces cacaliae*, they chose the first, and, when offered only infected material, larvae of both species presented lower growth rates, lower maximum weights and prolonged developmental times (Röder *et al.*, 2007). Similar effects were observed with the tree-way-interaction between the plant host *Plantago lanceolata*, the powdery mildew fungus *Podosphaera plantaginis*, and larvae of the lepdoptera *Melitaea cinxia* (Laine, 2004).

The symptoms caused by *Hpa* infection of *A. thaliana* are usually only mild (Slusarenko & Schlaich, 2003). In fact, even increased seed production solely due to infection by *Hpa* has been described in low fecund *A. thaliana* ecotypes (Salvaudon *et al.*, 2008). Despite the lack of severe symptoms, *Hpa* colonization still results in the induction of defence responses associated with biotrophic pathogens, such as SA-dependent defences (Glazebrook, 2005; Caillaud *et al.*, 2013; Asai *et al.*, 2014),

raising the possibility that such induction primes the infected plants to defend themselves more efficiently against more aggressive microbial pathogens. Even though both scenarios would justify the retention of genes facilitating oomycetal accommodation in the *A. thaliana* genome, our future studies will concentrate on a third aspect: the potential "protective" effects of *Hpa* colonization against herbivores. Though the oomycete does not typically activate defence pathways associated with herbivory, it is still fathomable that nutrient depletion on the infected leaves, or reduced physical accessibility deters herbivores from feeding on *Hpa*-colonized plants. In order to test this hypothesis, we are currently studying the responses of the leaf-chewing caterpillar of the Lepidoptera *Spodoptera littoralis* to both healthy wild-type *A. thaliana* leaves and leaves fully colonized by *Hpa* and superficially covered with oomycetal sporangiophores.

7. Conclusion

By investigating the role of the *A. thaliana* HCSGs in the establishment of biotrophic interactions with beneficial and pathogenic hyphal microorganisms, we showed that these genes are required for haustoria development and full reproductive success of the pathogenic oomycete *Hpa*. However, we could not detect an influence on the colonization of *A. thaliana* leaves by the powdery mildew fungus *E. cruciferarum* or the bacterial pathogen *P. syringe*. Likewise, the colonization of *A. thaliana* by *P. indica* or the associated growth promotion occurred independently of the HCSGs, even though the fungus is beneficial and grows inside roots, as do symbiotic AM fungi. Altogether, our results imply that the HCSGs have not been generally opted during evolution for the intracellular accommodation of hyphal pathogens and symbionts, but are required for the interaction with *Hpa* in particular. However, the cellular mechanisms involved in such interaction remain to be elucidated. Interestingly, though in *L. japonicus* the CSGs are not required for the biotrophic colonization of roots by *P. indica*, it appears that these genes have a negative role on fungal proliferation, suggesting an interconnection between symbiosis and defence signalling or plant developmental programs.

8. Materials and Methods

8.3. Fungal strains and growth conditions

P. indica ((Savita *et al.*, 1998), DSM11827, from Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany) and *P. williamsii* (Basiewicz *et al.*, 2012) were grown at 28°C, in the dark, on plates with solid (1.5% agar) complete medium (CM) (Pham *et al.*, 2004). For studies with plants grown in soil fungal mycelia were propagated in liquid CM medium, in the dark, at RT and 120 rpm shaking. Mycelium was washed three times with sterile distilled water directly prior to mixing with soil substrate.

For experiments on plates, fungal chlamydospores were obtained from four to six-week-old cultures, as follows: Tween water (0.002% Tween 20) was added to plates containing mycelium, which was rubbed off from the agar surface, and the resulting spore suspension was collected and centrifuged three times at 3.000 ***g*** for washing. Chlamydospore concentration was estimated using a Fuchs-Rosenthal counting chamber and adjusted to the desired $5x10^5$ ml^{-1} with Tween water.

8.4. Plant genotypes and growth conditions

For co-cultivation of *L. japonicus* with *P. indica,* seeds (Gifu ecotype wild-type and common symbiosis mutants; Table 3) were surface sterilized in a 2% sodium hypochlorite solution for 7 min, washed three times with sterile water for 5 min, and kept in water overnight until the seeds were swollen. Seeds were then put on plates with solid modified Hoagland's medium (5 mM KNO_3; 5 M $Ca(NO_3)_2$; 2 mM $MgSO_4$; 4 mM KH_2PO_4; 0.03 g l^{-1} Sprint 138 iron chelate; 0,1% micronutrients solution containing 2.86 g l^{-1} H_3BO_3; 1.81 g l^{-1} $MnCl_2.4H_2O$; 0.08 g l^{-1} $CuSO_4.5H_2O$; 0.02 g l^{-1} 85% $MoO_3.H_2O$; based on (Hoagland & Arnon, 1950)) and kept for 4 days at 24°C in the dark for germination.

Table 3: *L. japonicus* common symbiosis mutants screened for phenotypes associated with *P. indica* colonization.

Mutant	Reference
castor-13	(Imaizumi-Anraku *et al.*, 2005)
ccamk-12	(Perry *et al.*, 2009)
cyclops-3	(Schauser *et al.*, 1998; Szczyglowski *et al.*, 1998; Kistner *et al.*, 2005)
nup85-1	(Saito *et al.*, 2007)
nup133-1	(Schauser *et al.*, 1998; Kanamori *et al.*, 2006)
pollux-5	(Imaizumi-Anraku *et al.*, 2005)
symrk-3	(Stracke *et al.*, 2002; Demchenko *et al.*, 2004; Kistner *et al.*, 2005)

A. thaliana seeds (all ecotype Col-0) were obtained from "The Nottingham Arabidopsis Stock Centre" – NASC (55) or the GABI-DUPLO double mutant collection (Scholl *et al.*, 2000) (Table 4). For *in vitro* experiments, *A. thaliana* seeds were sterilized by incubation for 5 min in 70% ethanol/0.05% tween20, followed by 2 min in ethanol 100%. For *Hpa* infection, seeds were directly germinated in soil and grown for two weeks under long day conditions (16h light, 22°C µmol $m^{-2}s^{-1}$). For *Erysiphe cruciferarum* inoculation, *A. thaliana* plants were grown in a 2:1 soil/sand mixture. Seeds were stratified (4°C for 48h) prior to transfer into a growth chamber (10/14 light/dark cycle with 120 µmol m^{-2} s^{-1} light, 22°C day, 20°C night, 65% relative humidity). For elicitor (flg22) treatment, seeds were placed on half-strength MS plates (57) stratified for 48h at 4°C in the dark, and grown under long day conditions (16h light, 23°C, 85 µmol m^{-2} s^{-1}) for 8 days.

Table 4: *A. thaliana* HCSG mutants used in the screening for phenotypes associated with *P. indica* symbiosis.

Gene	Gene ID	NASC ID
POLLUX	At5g49960	N566135
SEC13	At3g01340	N662322
NUP133	At2g05120	N565761

For co-cultivation of *A. thaliana* with *P. indica* or *P. williamsii* and tests for plant growth promotion*, A. thaliana* seeds were sterilized as described above, left to dry and stratified for 48h at 4°C in the dark. Plants were then grown under long day conditions (16 h : 8 h, light : dark, at 23°C, 85 µmol $m^{-2}s^{-1}$), on ½ MS (Murashige & Skoog, 1962) with or without sucrose (0.05%) or on a modified Hoagland's (HO) medium solidified with gelrite 4 g l^{-1} , and four times more phosphate than in the original recipe. *L. japonicus* seeds (Table 3) were surface sterilized in a 2% sodium hypochlorite solution for 7 min, washed three times with sterile distilled water for 5 min, and kept in water overnight until the seeds were swollen. Seeds were then put on plates with solid modified HO and kept for 4 days at 24°C in the dark for germination.

Seven to ten-day-old *A. thaliana* or four-day-old *L. japonicus* plants were inoculated with 1 ml Tween water (0.002% Tween 20) or either *P. indica* or *P. williamsii* chlamydospores. Fungal chlamydospores were obtained from four to six-week-old cultures, as follows: Tween water was added to plates containing mycelium, which was rubbed off before the resulting spore suspension was collected and centrifuged three times at 3.000 ***g*** for washing. Chlamydospore concentration was estimated using a Fuchs-Rosenthal counting chamber and adjusted to the desired $5x10^5$ ml^{-1} with Tween water. Non-germinated seeds and unhealthy seedlings were removed from the plates before inoculation. Co-cultures or mock-inoculated plants were grown at 24°C under long day conditions (16 h : 8 h, light : dark, 200 µmol m^{-2} s^{-1}), and inspected for biomass seven days post inoculation (dpi).

For analysis of growth promotion on different nutrient conditions, mock- and *P. indica* inoculated plants were grown on the following media: modified HO with no KNO_3 or $Ca(NO_3)_2$, supplemented with 0.05 mM KNO_3, 0.5 mM KNO_3, or 5 mM KNO_3 (standard concentration), modified HO depleted of phosphate or with 4 mM KH_2PO_4 (standard concentration); and modified HO depleted of ammonium or with 10 mM NH_4Cl or 20 mM NH_4Cl. When necessary, potassium and calcium concentrations were normalized with KCl and $CaCl_2$, respectively.

For growth promotion experiments in soil, plants were grown on either low-nutrient soil (50-100 mg l^{-1} N, 50-100 mg l^{-1} P, 100-150 mg l^{-1} K) or high-nutrient soil (500 mg l^{-1} N, 500 mg l^{-1} P, 500 mg l^{-1} K) mixed with 1 g fresh or autoclaved mycelium per 100 g sterile substrate for inoculation and mock-inoculation, respectively. Plant height was determined 7, 11, 16, and 21 dpi.

8.5. *A. thaliana* stable transformation

Floral dipping was performed as described previously (Clough & Bent, 1998).

8.6. Pathogen assays and phenotypic analyses

Seven days after infection, *A. thaliana* leaves with sporulating *Hpa* isolate NoCo2 were harvested into 15 mL reaction tubes containing 10 mL dH_2O, and vortexed for 2s. The spore solution was then filtered through Miracloth filter and sprayed onto 12-day-old plants using a spraying gun. Subsequently, plants were placed into trays and covered with wet translucid lids. Trays were sealed to maintain high humidity, and plants were grown at 18°C under long day conditions (16h light, 85 µmol $m^{-2}s^{-1}$). Cotyledons (4 dpi) or leaves (4 or 5 dpi) were harvested and stained in 0.01 % trypan-blue-lactophenol (10 mL lactic acid, 10 mL glycerol, 10 g phenol, 10 mg trypan blue dissolved in 10 mL distilled water) for 3 min at 95°C and 5h at room temperature, followed by overnight clearing in saturated chloral hydrate (2.5g/mL) and mounting in glycerol for observation using differential interference contrast microscopy. For sporangiophore counting, a minimum of 50 cotyledons per genotype and replicate were analysed and the number of sporangiophores per infected cotyledon was plotted. For investigation of the haustoria shape and the penetration efficiency, a minimum of five leaves per genotype and replicate were analysed. On each leaf, the percentage of multilobed haustoria per total haustoria or the percentage of haustoria-containing cells per cells contacted by hyphae was calculated for 10 individual strands of hyphae. The mean for each leaf was built and plotted.

Erysiphe cruciferarum was grown on Col-0 to maintain aggressiveness and on susceptible *phytoalexin deficient 4* (*pad4*) mutants (Glazebrook *et al.*, 1996) for elevated conidia production. *A. thaliana* plants were placed under a polyamide net (0.2 mm^2) and inoculated at a density of 3–4 conidia mm^{-2}, by brushing conidia off of *pad4* plants through the net. Two leaves per plant were harvested, cleared and kept in acetic acid (25%) until analysis. Leaves were stained in acetic acid (25%) 1:9 + ink (Königsblau, Pelikan, 4001), washed in water, placed in water added of a few drops of Tween20, washed in water again, and analysed under a bright-flied microscope.

Bacterial strains *P. syringae* pv. *tomato* DC3000 or *Pto* DC3000 *ΔAvrPto/AvrPto* were grown and used for infection assays on leaves of 4-5-week-old *A. thaliana* plants as described previously (Lin & Martin, 2005; Kemmerling *et al.*, 2007).

8.7. Observation of fluorescently labelled haustoria

For fluorescent analysis of haustoria morphology, leaves of *A. thaliana* wild-type and the *shrk1* x *shrk2* double mutant were harvested at 5 dpi, cleared in 10M KOH for 5 min, stained with 0.05% anilin blue in 0.067M K_2HPO_4 for 20 min and observed with a CLSM (Leica SP5) using excitation at 360-380 nm and detection at 470-505 nm. Images were edited using imageJ with the "volume viewer" plugin (Schneider *et al.*, 2012).

For quantification of fluorescence associated with the perihaustorial membrane, wild-type *A. thaliana* (Col-0) plants transformed with the construct *ProAt3g61260:YFP-At3g61260* were infected with *Hpa* as described above, harvested at 8 dpi and observed with a microscope using differential interference contrast or epifluorescence with YFP filter settings (excitation at 500/20 nm and emission at 535/30 nm). The quantification was performed in triplicate. For each biological replicate consisting of 6 leaves, 100 single-lobed and 100 multilobed haustoria were counted and scored for detectable fluorescence. Haustoria without detectable fluorescence were visualized by differential interference contrast microscopy and scored as non-fluorescent. Leaves from *ProAt3g61260:YFP-At3g61260* plants were harvested at 8 dpi and observed with a CLSM (Leica SP5) using excitation at 510-520 nm and detection at 520-530 nm. 3D reconstructions of labelled haustoria were performed from a z-stack of 15 images (1,0-1,5 microns each) taken from fresh leaves at 5 or 6 dpi, using imageJ with the "3D viewer" plugin (Schneider *et al.*, 2012).

8.8. Analysis of oomycete-associated callose deposition

Oomycete-associated callose deposition was analysed on cotyledons of *A. thaliana* wild-type and HCSG mutants. Leaves were harvested at 4 dpi, cleared in 10M KOH for 5 min, stained with 0.05% anilin blue in 0.067M K_2HPO_4 for 20 min and mounted in glycerol for observation in an epifluorescence microscope (Leica DMI6000B) with CFP filter settings (excitation 436/10 nm and emission 465/30 nm). At least 50

pictures were taken per genotype. Regions of interest (ROIs) were selected in ImageJ (Schneider *et al.*, 2012), mean intensities were calculated from single ROIs and plotted.

8.9. Analyses of plant root colonization by *P. indica*

To observe *P. indica* intracellular sporulation in *A. thaliana* and *L. japonicus* roots, colonized roots at 14 dpi were incubated at 96°C for one (*A. thaliana*) or 10 min (*L. japonicus*) in 10% (w/v) KOH and double-stained in the dark at room temperature for 20 min with 10 µg ml^{-1} WGA-AF488 (Molecular Probes, Karlsruhe, Germany) to visualize fungal structures, and 10 µg ml^{-1} propidium iodide (PI) to visualize plant cell walls. Samples were analysed with a Leica DMI6000B microscope using differential interference contrast or epifluorescence (GFP filter set for WGA-AF488: excitation 450-490 nm, emission 500-550 nm; TX2 filter settings for PI: 540-580 nm excitation, 608-683 nm emission).

Root colonization and cell viability were analysed by confocal laser scanning microscopy (CLSM). Colonized roots at 7 dpi were stained for 10 min with 10 µg ml^{-1} WGA-AF488 (Molecular Probes, Karlsruhe, Germany) to visualize fungal structures. Membranes were stained with 3 µM FM4-64 (Molecular Probes, Karlsruhe, Germany) for 5 min. Root samples were imaged with TCS-SP5 confocal microscopes (Leica, Bensheim, Germany) with excitation at 488 nm for WGA-AF488 and detection at 500–540 nm. FM4-64 was excited at 633 nm and detected at 650–690 nm. Propidium iodide was excited at 540 nm and detected at 600-630 nm.

8.10. Elicitor treatment

For pre-incubation, eight-day-old seedlings were transferred to a 12-well plate (3 seedlings/well represent one biological replicate) with half-strength liquid MS medium (Murashige & Skoog, 1962) supplemented with 1% sucrose and incubated overnight under long-day conditions (16 h light, 22 °C, 100 µmol m^{-2} s^{-1}; 8 h dark, 18 °C) and 100 rpm shaking. On the following day the medium was exchanged, half the samples were supplemented with 1 µM flg22, and the other half kept in half-strength MS (Murashige & Skoog, 1962) as the mock controls. Plants were then incubated for

6h at 22°C and 100 rpm shaking. For every genotype, three biological replicates of treated and non-treated samples were harvested and immediately frozen in liquid N_2.

8.11. DNA/RNA extraction and qPCR analyses

For analyses of relative fungal/plant DNA content in roots of *A. thaliana* and *L. japonicus* colonized with *P. indica*, colonized roots (18 dpi) were thoroughly washed to remove fungal hyphae from the root surface. 200 mg of root material were then used for DNA extraction according to the protocol of Doyle and Doyle (Doyle & Doyle, 1987). Real-time qPCR analyses were performed from 10 ng DNA mixed with the appropriate primers in 10 µl SYBRgreen Supermix (BIORAD) using the following amplification protocol: 2'-95 °C; 40 x (30''-95°C; 30''-59°C; 30''-72 °C); melting curve 95°C – 60°C – 95°C. Relative DNA amount was calculated using the $2^{-\Delta\Delta Ct}$ method (Livak & Schmittgen, 2001).

For gene expression analyses before and after flg22 treatment, RNA extraction was performed using the Spectrum™ Plant Total RNA kit (Sigma-Aldrich), followed by DNaseI treatment (amplification grade DNaseI, Invitrogen™) for removal of genomic DNA. First strand cDNA synthesis was performed from 250 ng total RNA using the SuperScript® III First-Strand Synthesis SuperMix (Invitrogen™) with oligo(dT) primers. RT-qPCR was performed in 20 µL reactions containing 1x SYBR Green I (Invitrogen™) in a CFX96 Real-time PCR detection system (Bio-Rad). PCR program: 2'-95°C; 40 x (30''-95°C; 30''-60°C; 20''-72°C); melting curve 95°C – 60°C – 95°C. A primer list can be found in the supplemental material. Expression levels of target genes were normalized against the housekeeping genes *TIP41-like* and *PP2A* (Czechowski *et al.*, 2005) was used as a reference to calculate the relative expression of the target genes. The *fls2* mutant was used as internal control. For every genotype, three biological replicates and two technical duplicates were analysed.

Table 5: Oligonucleotides used in the analyses of relative fungal DNA content in *P. indica* colonized roots.

Expression analysis

Target	Sequence	Reference
P. indica TEF_fwd	TCGTCGCTGTCAACAAGATG	(Butehorn *et al.*, 2000)

P. indica TEF_rev	ACCGTCTTGGGGTTGTATC	
AtUBI_fwd	CCAAGCCGAAGAAGATCAAG	(Khatabi *et al.*, 2012)
AtUBI_rev	ACTCCTTCCTCAAACGCTGA	
LjUBI_fwd	ATGCAGATCTTCGTCAAGACCTTG	(Suzaki *et al.*, 2013)
LjUBI_rev	ACCTCCCCTCAGACGAAG	

Table 6: Oligonucleotides used to analyse gene expression patterns before and after flg22 treatment.

	Expression analysis	
Target	**Sequence**	**Reference**
PP2A	TAACGTGGCCAAAATGATGC GTTCTCCACAAC^CGCTTGGT	(Czechowski *et al.*, 2005)
TIP41-like	GTGAAAACTGTTGGAGAGAAGCAA TCAACTGGATACCCTTT^CGCA	(Czechowski *et al.*, 2005)
FRK1	ATCTTCGCTTGGAGCTTCTC TGCAGCGCAAGGACTAGAG	(He *et al.*, 2006)
GST1	TAATAAAAGTGGCGATGACC ACATTCAAATCAAACACTCG	(Grant *et al.*, 2000)
ERF1	TCGGCGATTCTCAATTTTTC ACAACCGGAGAACAACCATC-	(Denoux *et al.*, 2008)
PI-LTP	CTGGTTCATCCGGAAACTGT CCTTCTGTTGCAAACGTTGA	(Denoux *et al.*, 2008)
PR1	TTCTTCCCTCGAAAGCTCAA AAGGCCCACCAGAGTGTGTATG	(Schlicht & Kombrink, 2013)

8.12. β-glucuronidase (GUS) staining assays

GUS staining assays were performed with *L. japonicus* plants stably transformed with the symbiosis-reporter line T90 (Webb *et al.*, 2000). Colonized roots at 3 and 7 dpi with *P. indica* chlamydospores or Tween water were vacuum infiltrated with X-Gluc staining solution (100 mM NaxPO4 pH 7.0; 10 mM EDTA; 0.1 % Triton-X 100; 0.5 mg ml^{-1} X-Gluc; 1 mM $K_3[Fe(CN)_6]$; 1 mM $K_4[Fe(CN)_6].3\ H_2O$) three times for 10 min, followed by incubation at 37°C for 18 h in the dark. Root systems were analysed for GUS staining with a Leica MZFLIII stereomicroscope.

8.13. Gene structure and phylogenetic analyses

Analyses of gene structures and protein domain organization were performed using online databases TAIR (http://www.arabidopsis.org/) for *A. thaliana*, and the KDRI website (Kazusa DNA Research Institute, Japan; http://www.kazusa.or.jp/lotus/) and the GenBank for *L. japonicus*. BLAST searches were performed on TAIR (http://arabidopsis.org/Blast/index.jsp) with the *L. japonicus* genomic CSG sequences as query.

For phylogenetic studies, protein sequences of *A. thaliana* and *L. japonicus* MLD-LRR-RLKs (either only the highly conserved kinase domain or only the extracytoplasmic region without signal peptide) were aligned using MAFFT 6.822 (Katoh *et al.*, 2002) with the default settings (alignments are provided as supplemental files). The result of the alignments were used to create phylogenetic trees at the CIPRES web-portal with RAxML 7.2.7 (Stamatakis *et al.*, 2008) for fast maximum likelihood analyses using 100 bootstraps. For RAxML, the JTT PAM matrix for amino acid substitutions was chosen, and the GTRGAMMA model was used for both bootstrapping and tree inference.

8.14. Statistics and data visualisation

All statistical analyses and data plots have been performed and generated with R version 3.0.2 (2013-09-25) "Frisbee Sailing" (Team, 2013).

For analyses with *Hpa* and *E. cruciferarum*, the packages “Hmisc” (Harrell, 2014) “car” (Fox, 2011), “multcompView” (Graves *et al.*, 2012), and “multcomp” (Hothorn *et al.*, 2008) were used. For statistical analysis, data was either subjected to the nonparametric Wilcoxon–Mann–Whitney test with Bonferroni-Holm correction comparing mutant and complementation groups with Col-0, or was power transformed to improve normality and a one-way ANOVA followed by a Tukey’s HSD test or a Dunnett’s Test with Bonferroni correction was performed using Col-0 samples as control group.

For analyses involving the interaction *A. thaliana-P. indica*, we used the package ‘agricolae’ (de Mendiburu, 2010). Pairwise or multiple comparisons of the different subsets of data were performed with the Kruskal-Wallis test followed by a

Bonferroni-Holm correction using the mock-inoculated samples as control group. Two-sided, unpaired t-tests with equal variance were used to analyse relative amount of fungal DNA within plant roots. A 95% family-wise confidence level was set for all experiments.

8.15. Cloning

Constructs labelled with "GG" were generated via Golden Gate cloning. For details on assembly method, general modules and plasmids (G*xx*, BB*xx*), see (Binder *et al.*, 2014). Golden Gate constructs contain silent mutations to facilitate cloning. Constructs and oligonucleotides used for cloning are summarized on tables 7 and 8.

Table 7: Constructs used for cloning.

Entry clones / Golden Gate Level I & Level II plasmids (LI & LII)

Name	Description
pENTR-pSEC13:SEC13	Phusion PCR product consisting of SEC13 genomic construct amplified from *A. thaliana* gDNA with sec13co_FW and sec13co_RV, cloned into pENTR/D-TOPO (Invitrogen) via TOPO reaction
pENTR-pNUP133:NUP133 (GG)	NUP133 genomic construct with native promoter region (2158 bp). Introns 1 and 2 of *NUP133* were omitted for technical reasons. Final construct was assembled from 6 subcloned PCR fragments by BsaI cut ligation into pENTR-BsaI vector **Fragment: Primers – template** Fragment 1: N133-pro2_FW & N133-pro3_RV – *A. thaliana* gDNA Fragment 2: N133-pro3_FW & N133_Pro4_RV - *A. thaliana* gDNA Fragment 3: N133_ATG_FW & N133 mut_1b_RV - *A. thaliana* cDNA Fragment 4: N133_mut_1_FW & N133_mut_2_RV - *A. thaliana* gDNA Fragment 5: N133_mut2_FW & N133_e3_RV - *A. thaliana*

	gDNA Fragment 6: N133_e3_FW & N133_3'UTR2_RV- *A. thaliana* gDNA
LI A-C pPOLLUX (GG)	LI promoter element of POLLUX (1523 bp). Assembled from 2 PCR fragments amplified from *A. thaliana* gDNA by BpiI cut ligation into LI-BpiI vector Fragment 1: AtPol-Pro1+ & AtPol-Pro2- Fragment 2: AtPol-Pro3+ & AtPol-Pro4-
LI C-D POLLUX (GG)	LI element containing genomic *POLLUX*. Assembled from 2 PCR fragments amplified from *A. thaliana* gDNA by BpiI cut ligation into LI-BpiI vector. Fragment 1: AtPol1+ & AtPol2- Fragment 2: AtPol3+ & AtPol4-
LII F 1-2 pPOLLUX:POLLUX (GG)	Assembled by BsaI cut ligation from: LI A-C pPOLLUX + LI C-D POLLUX + LI dy D-E (BB8) + LI E-F 35S-T (G59) + LI F-G neo (G3) + LIIc F1-2 (BB30)
LII R 3-4 p35S-mCherry (GG)	Assembled by BsaI cut ligation from: LI A-B p35S (G5) + LI dy B-C (BB6) + LI C-D mCherry (G23) + LI dy D-E (BB8) + LI E-F nos-T (G6) + LI dy F-G (G9) + LIIc R 3-4 (BB33)
LII R 5-6 p35S-mCherry (GG)	Assembled by BsaI cut ligation from: LI A-B p35S (G5) + LI dy B-C (BB6) + LI C-D mCherry (G23) + LI dy D-E (BB8) + LI E-F HSP-T(G45) + LI dy F-G (G9) + LIIc R 5-6 (BB37)
LI A-C ShRK1 (GG)	Full length genomic DNA from ATG to codon prior to STOP (bases 1-4360); silent mutations introduced to remove type IIS restriction sites; obtained by gene synthesis (GenScript)
LI A-C ShRK2 (GG)	Full length genomic DNA from ATG to codon prior to STOP (bases 1-4185); silent mutations introduced to remove type IIS restriction sites; obtained by gene synthesis (GenScript)
LII F 1-2 pUBi: ShRK1-YFP (GG)	Assembled by BsaI cut ligation from: LI A-B pUBi (G7) + LI dy B-C (G4) + LI A-C ShRK1 + LI D-E YFP (G12) + LI E-F 35S-T (G59) + LI F-G hygro (G94) + LIIc F

	1-2 (BB30)
LII F 1-2 pUBi: ShRK2-YFP (GG)	Assembled by BsaI cut ligation from: LI A-B pUBi (G7) + LI dy B-C (G4) + LI A-C ShRK2 + LI D-E YFP (G12) + LI E-F 35S-T (G59) + LI F-G hygro (G94) + LIIc F 1-2 (BB30)
LII R 3-4 pUBi: ShRK1-CFP (GG)	Assembled by BsaI cut ligation from: LI A-B pUBi (G7) + LI dy B-C (G4) + LI A-C ShRK1 + LI D-E Cerulean (G14) + LI E-F HSP-T(G45) + LI dy F-G (G9) + LIIc R 3-4 (BB34)

Plasmids for stable transformation of *A. thaliana*

Name	**Description**
pSEC13:SEC13 + free mCherry	LR reaction of pENTR-pSEC13:SEC13 and PMDC99 (2); hygromycin resistance
pNUP133:NUP133	LR reaction of pENTR-pNUP133:NUP133 and pMDC99 (2); hygromycin resistance
pPOLLUX:POLLUX + free mCherry (GG)	Assembled by BpiI cut ligation from: LII F 1-2 pPOLLUX:POLLUX + LII 2-3 ins (BB43) + LII R 3-4 p35S-mCherry + LII dy 4-6 (BB41) + LIIIα fin (BB45); kanamycin resistance
pUBi:ShRK1-YFP + free mCherry (GG)	Assembled by BpiI cut ligation from: LII F 1-2 pUBi:ShRK1 + LII 2-3 ins (BB43) + LII R 3-4 p35S-mCherry + LII dy 4-6 (BB41) + LIIIα fin (BB45); hygromycin resistance
pUBi:ShRK2-YFP + free mCherry (GG)	Assembled by BpiI cut ligation from: LII F 1-2 pUBi:ShRK2 + LII 2-3 ins (BB43) + LII R 3-4 p35S-

	mCherry +
	LII dy 4-6 (BB41) + LIIIα fin (BB45); hygromycin resistance

Table 8: Oligonucleotides used for cloning

Plasmid construction

Name	Primer sequence (5' - 3')
sec13co_FW	CACCGGGAACACGGGAGAATAG
sec13co_RV	TTTTGCAATCTCTGTTGTCTGA
N133-pro2_FW	AGGGTCTCACACCGTTTTGAAAGACGGCATATTATGG
N133-pro3_RV	AGGGTCTCATACAAGGTCTTTTATTGCTTAAAACTCT
N133-pro3_FW	AGGGTCTCATGTACATTTATTTGTTTTCATTGATTG
N133_Pro4_RV	AGGGTCTCAACATTTTAAACCAGGAAGAGAGCGA
N133_ATG_FW	AGGGTCTCAATGTTCTCTCCATTGACGAAGA
N133_mut_1b_RW	AGGGTCTCATTTCTTTATCCATTCCACCGGA
N133_mut_1_FW	GGGGTCTCAGAAACCTGTCTTTCTTGGTTTATT
N133_mut_2_RV	GGGGTCTCAGCGACCGAGAAGCCCT
N133_mut_2_FW	GGGGTCTCATCGCGTAGTCCTGTTGGTGT
N133_e3_RV	AGGGTCTCACTCTCTGCAGTTGAGTTCCTAGTG
N133_e3_FW	AGGGTCTCAAGAGCCTGCGAACTCTCAAA
N133_3'UTR2_RV	AGGGTCTCACCTTGGTAGATTCGATACATCATAAAGAGG
AtPol-Pro1+	ATGAAGACTTTACGGGTCTCAGCGGAGCCCAATGACTTCCCACAC
AtPol-Pro2-	TAGAAGACAAATGACTACAGTTTCATGCCACCA
AtPol-Pro3+	ATGAAGACTTTCATCATTATGCTCATCTTGAATATGT
AtPol-Pro4-	TAGAAGACAACAGAGGTCTCAGGTGCGGGTTGAAGTAAGTAAATTGAGA
AtPol1+	ATGAAGACTTTACGGGTCTCACACCATGCCGATTCATACCCCTAGA
AtPol2-	TAGAAGACAACATCTTCTTTCTTCTGATTTGTTCGT
AtPol3+	ATGAAGACTTGATGTTCCTTTGAAGAAGAGACTAGC

AtPol4-	ATGAAGACTTCAGAGGTCTCACCTTCTGACTTGAGGCGATGACAAC

9. Declaration of contributions from other researchers

Aline Banhara Pereira, the author of this thesis, contributed to the experiments displayed in figures 1 to 33 as follows:

Figure 1: designed and prepared by Aline Banhara Pereira;

Figure 2 – 3, 23 – 25, 29 – 31, 33: Experiments were designed, performed and analysed by Aline Banhara Pereira, who prepared the corresponding figures;

Figure 4: Phylogenetic analyses were performed by Aline Banhara Pereira and Martina Katharina Ried. Aline Banhara Pereira prepared the figure;

Figure 5: Constructs *pollux* co, *shrk1* co, and *shrk2* co were produced by Andreas Binder. Experiments were designed, performed, and analysed by Aline Banhara Pereira and Martina Katharina Ried. Martina Katharina Ried did the statistical analyses and prepared the figure;

Figure 6: Experiments were designed, performed, and analysed by Aline Banhara Pereira and Martina Katharina Ried. Martina Katharina Ried did the statistical analyses and prepared the figure;

Figure 7: Experiment was designed and performed by Aline Banhara Pereira, and analysed by Aline Banhara Pereira and Martina Katharina Ried. Martina Katharina Ried did the statistical analyses and prepared the figure;

Figure 8: Construct *pollux* co was produced by Andreas Binder. Experiment was designed, performed, and analysed by Aline Banhara Pereira and Martina Katharina Ried. Martina Katharina Ried did the statistical analyses and prepared the figure;

Figure 9: Constructs *pollux* co, *shrk1* co, and *shrk2* co were produced by Andreas Binder. Experiments were designed, performed, and analysed by Aline Banhara Pereira and Martina Katharina Ried. Martina Katharina Ried did the statistical analyses and prepared the figure;

Figure 10: Experiment was designed, performed, and analysed by Aline Banhara Pereira and Martina Katharina Ried. Martina Katharina Ried did the statistical analyses and Aline Banhara Pereira prepared the figure;

Figure 11: Experiment was designed, performed, and analysed by Aline Banhara Pereira and Martina Katharina Ried. Aline Banhara Pereira prepared the figure;

Figure 12: Experiment was designed, performed, and analysed by Aline Banhara Pereira and Martina Katharina Ried. Aline Banhara Pereira prepared the figure;

Figure 13: Experiments was designed, performed, and analysed by Aline Banhara Pereira and Martina Katharina Ried. Martina Katharina Ried did the statistical analyses and prepared the figure;

Figure 14: Experiment was designed, performed, and analysed by Aline Banhara Pereira. Figure was prepared by Aline Banhara Pereira;

Figure 15: Experiment was designed and performed by Caroline Höfle, and analysed by Aline Banhara Pereira and Martina Katharina Ried. Martina Katharina Ried did the statistical analyses and prepared the figure;

Figure 16 – 18: Experiments were designed, performed and analysed by Aline Banhara Pereira and Martina Katharina Ried. Martina Katharina Ried did the statistical analyses and prepared the figure;

Figure 19 upper panel: Experiments were designed, performed, and analysed by Aline Banhara Pereira and Martina Katharina Ried. Statistical analyses were done by Martina Katharina Ried. Aline Banhara Pereira prepared upper panel and Martina Katharina Ried prepared lower panel;

Figure 20: Experiments were designed, performed and analysed by Aline Banhara Pereira. Aline Banhara Pereira prepared the figure;

Figure 21: Experiment was designed and performed by Aline Banhara Pereira, and analysed by Aline Banhara Pereira and Martina Katharina Ried. Statistical analyses were done by Martina Katharina Ried. Aline Banhara Pereira prepared the right panel, and Martina Katharina prepared the left panel;

Figure 22: Experiments were designed, performed, and analysed by Andrea A. Gust and Thorsten Nürnberger. Figure was prepared by Andrea A. Gust;

Figure 26: Experiments were designed by Alga Zuccaro and performed by Aline Banhara Pereira and Alga Zuccaro. Aline Banhara Pereira performed the statistical analyses and prepared the figure;

Figure 27: Experiment was designed and performed by Regina Kühner. Aline Banhara Pereira analysed the experiment and prepared the figure;

Figure 28: Experiments were designed, performed, and analysed by Aline Banhara Pereira, Yi Ding, and Alga Zuccaro. Aline Banhara Pereira prepared the figure;

Figure 32: Experiment was designed and performed by Aline Banhara Pereira. Statistical analyses were done by Aline Banhara Pereira and Alga Zuccaro, and Aline Banhara Pereira prepared the figure.

10. References

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11. List of figures

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Thank you Simon, for your love and support. And above all, thank you Theo, for being my little star.

14. Curriculum Vitae

Aline Banhara Pereira

Personal Data

Date of Birth	Oct. 04, 1982
Place of Birth	Rio de Janeiro, RJ, Brazil

Primary and Secondary Education

1987 - 1991	Colégio Santa Úrsula, Rio de Janeiro
1992 - 1998	Colégio Santo Antonio Maria Zaccaria, Rio de Janeiro
1999 - 2000	Colégio pH, Rio de Janeiro

Education

Nov 2014 (expected)	Dr.rer.nat. Genetics Ludwig-Maximilians-Universität München, Munich
Jun 2010 – May 2011	Maternity leave
Sep 2007 – May 2010	Genetics, predoctoral research Ludwig-Maximilians-Universität München, Munich
Mar 2005 – Feb 2007	Master of Science, Genetics Federal University of Rio de Janeiro, Rio de Janeiro
Mar 2001 – Feb 2005	Bachelor in Biological Sciences, Genetics Federal University of Rio de Janeiro, Rio de Janeiro

Research

2007 – 2014	Ludwig-Maximilians-Universität München, Genetics: doctoral research Thesis: Plant genes determining compatibility with hyphal pathogens and symbionts. Supervisor: Prof. Dr. Martin Parniske
2005 – 2007	Federal University of Rio de Janeiro, Genetics: Master of Science Dissertation: Anther development in *A. thaliana*: identification and characterization of a phosphatidylinositol kinase and a MYB-like transcription factor involved in microsporogenesis.

	Supervisor: Prof. Dr. Marcio Alves-Ferreira
2001 – 2005	Federal University of Rio de Janeiro, Genetics: Bachelor in Biological Sciences Dissertation: Identification of genes differentially expressed in nodulated cowpea (*Vigna unguiculata*) in response to heat-shock. Supervisor: Prof. Dr. Márcia M. A. N. Pinheiro Margis

Research Projects

2007 – present – The role of common symbiosis gene homologs in the interaction of *A. thaliana* with pathogenic and symbiotic microorganisms.

2005 – 2007 – Functional Genomics of plant reproduction: characterization of new genes for use in the Biotechnology.

2003 – 2004 – Identification and cloning of genes involved in heat shock resistance: a strategy for optimization of Biological Nitrogen Fixation in tropical legumes.

Publications

Banhara A.*, Ried, MK*, Binder A, Gust, AA, Höfler C, Hückelhoven R, Nürnberger T, Parniske M (2014). Symbiosis-related genes sustain the development of a downy mildew pathogen on *A. thaliana thaliana.* Under review.

* These authors contributed equally to the work

Lahrmann U, Ding Y, **Banhara A**, Rath M, Hajirezaei MR, Döhlemann S, von Wirén N, Parniske M, Zuccaro A (2013) Host-related metabolic cues affect colonization strategies of a root endophyte. Proc Natl Acad Sci USA. 110(34): 13965-70.

Alves-Ferreira M, Wellmer F, **Banhara A**, Kumar V, Riechmann JL, Meyerowitz EM (2007) Global expression profiling applied to the analysis of *A. thaliana* stamen development. Plant Physiol. 145: 747–762.

Teaching Experience

2001 – Colegio Ph, Rio de Janeiro, Brazil, Biology Tutor for undergraduate students.

2012 – LMU München, Munich, Germany, Tutor in the practical section of the Genetik II course by Prof. Martin Parniske.

Attended Meetings and Workshops

Talk

Banhara A, Alves-Ferreira M. Characterization of an A. thaliana Phosphatidylinositol 3- and 4- Kinase (PI3_PI4K) Knockout Mutant with Abnormal Microsporogenesis, XXXV Meeting of Brazilian Biochemistry and Molecular Biology Society, Águas de Lindóia SP, Brazil, 2006.

Posters

Keymer A, Gutjahr C, Banhara A, Hardel S, Parniske M. DISORGANIZED ARBUSCULE is required for arbuscule branching. SFB924 (Plant Biology of the Next Generation) Conference. Freising, Germany, 2013.

Banhara A, Binder A, Groth M, Parniske M. Nucleoporins in plant-microbe symbiosis and defence signaling, 10th European Nitrogen Fixation Meeting and 1st Molecular Mycorrhiza Meeting, Munich, Germany, 2012.

Banhara A, Alves-Ferreira M. Characterization of an A. thaliana Phosphatidylinositol 3- and 4- Kinase (PI3_PI4K) Knockout Mutant with Abnormal Microsporogenesis. 1st Brazilian Symposium of Molecular Plant Genetics, 2006, Natal, Brazil.

Alves-Ferreira M, Wellmer F, Banhara A, Kumar V, Riechmann JL, Meyerowitz EM. Identification and characterization of new genes critical to normal *A. thaliana thaliana* stamen development by microarray and functional analysis of flower mutants *apetala-3*, *sporocyteless* and *male-sterile-1*, XIX International Congress on Sexual Plant Reproduction, Budapeste, Hungary, 2006.

Banhara A, Alves-Ferreira M. Characterization of two novel genes encoding putative signal transduction components required to normal stamen development in *A. thaliana thaliana*, 51th Brazilian Congress on Genetics, 2005, Águas de Lindóia SP, Brazil, 2005.

Banhara A, de Araujo JLS. Estudo da tolerância da fixação biológica de nitrogênio a altas temperaturas através da análise do padrão de expressão gênica em nódulos de caupi (Vigna unguiculata) submetidos a estresse térmico, 51th Brazilian Congress on Genetics, 2005, Águas de Lindóia SP, Brazil, 2005.

Workshop

"Project Management (Natural Sciences and Medicine)." Graduate Center LMU – 24.25/10/2013.

Honors and Awards

2007 – Poster prize at the 1st Brazilian Symposium of Molecular Plant Genetics, Natal, Brazil.

2006 – "Grade A" Student Master Fellowship, Rio de Janeiro Research Council.

Fellowships

2007 – Fellowship for a 3 year PhD in Germany from the National Council for Scientific and Technological Development (CNPq) in collaboration with the Deustche Akademischer Austauschdienst (DAAD).

*Awarded but not utilized due to funding becoming available in the host research group.

2006 – 2007 – 'Grade A' Student Master Fellowship from Rio de Janeiro Research Council.

2005 – 2006 – Master Fellowship. National Council for Scientific and Technological Development, CNPq.

2003 – 2004 – Undergraduate student fellowship, National Council for Scientific and Technological Development, CNPq.

Supervision

Nadia Sinner (2012-2013) – *Hilfswissenschaftler*, HiWi.

Languages

Portuguese (native)
English (fluent)
German (advanced)

15. Appendix

Appendix 1: Pruned alignment of kinase domain of LjSYMRK (amino acid positions 593-870) and homologous stretches of related MLD-LRR-RLK sequences [LjSYMRK, LjShRK1, LjShRK2, ShRK1(AT1G67720), ShRK2(AT2G37050), AT5G48740, AT1G51790, IOS1(AT1g51800), AT1G51910, AT1G51890, AT1G51860, AT1G51880, AT1G07550, AT2G14440, AT2G14510, AT3G46350, AT3G46340, AT3G46370, AT3G46400, AT3G46330, AT5G59670, AT5G59680, AT5G59650, AT5G16900, AT1G07560, AT4G20450, AT2G28960, AT2G29000, AT2G28970, AT2G28990, AT1G491000, AT1G51810, AT1G51805, AT1G51830, AT1G51820, AT1G51850, AT2G04300, AT3G21340, AT1G05700, AT2G19210, AT2G19230, AT2G19190, AT4G29990, AT4G29180].

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LjSYMRK/1-278       1 ERYKTLIGEGGFGSVYRGTLND-GQEVAVKVRSATST-QGTREFDNELNLLSAIQHENLV
LjShRK1/1-278       1 NNFEKKIGSGGFGVVYYGKLKD-GKEIAVKVLTSNSY-QGKREFSNEVALLSRIHHRNLV
LjShRK2/1-272       1 RNLERIVGKGGFGIVYHGCVGD--IEVAVKMLS-PSA-QGYLQFQAEAKFLAKVHHKCLT
ShRK1/1-276         1 DNFSKKVGRGSFGSVYYGRMKD-GKEVAVKITADPSS-HLNRQFVTEVALLSRIHHRNLV
ShRK2/1-279         1 KKFEKRIGSGGFGIVYYGKTRE-GKEIAVKVLANNSY-QGKREFANEVTLLSRIHHRNLV
AT5G48740/1-277     1 RNFKEVIGRGSFGAVYRGKLPD-GKQVAVKVRFDRTQ-LGADSFINEVHLLSQIRHQNLV
AT1G51790/1-273     1 NGFDRDQGKVGFGRNYLGKLDG--KEVTVKLVSSLSS-QGYKQLRAEVKHLFRIHHKNLI
IOS1/1-274          1 NNFERVLGRGGFGVVYYGVLNN--EPVAVKMLTESTA-LGYKQFKAEVELLLRVHHKDLT
AT1G51910/1-272     1 NNFERVLGKGGYGRVYYGKLDD--TEVAVKMLFHSSAEQDYKHFKAEVELLLRVHHRHLV
AT1G51890/1-274     1 KNFERVLGKGGFGTVYHGNLDD--TQVAVKMLSHSSA-QGYKEFKAEVELLLRVHHRHLV
AT1G51860/1-274     1 NNFERVLGKGGFGTVYHGNLDG--AEVAVKMLSHSSA-QGYKEFKAEVELLLRVHHRHLV
AT1G51880/1-274     1 NNFERVLGKGGFGTVYHGNLED--TQVAVKMLSHSSA-QGYKEFKAEVELLLRVHHRNLV
AT1G07550/1-273     1 NNFQVVIGKGGFGVVYQGCLN--NEQAAIKVLSHSSA-QGYKEFKTEVELLLRVHHEKLV
AT2G14440/1-274     1 NNFEVVLGKGGFGVVYHGFLN--NEQVAVKVLSQSST-QGYKEFKTEVELLLRVHHVNLV
AT2G14510/1-274     1 NNFEVVLGKGGFGVVYHGFLN--NEQVAVKVLSQSST-QGYKEFKTEVELLLRVHHVNLV
AT3G46350/1-275     1 NNFQRALGEGGFGTVYHGDLDS-SQQVAVKLLSQSST-QGYKEFKAEVDLLLRVHHINLL
AT3G46340/1-276     1 KNLQRPLGEGGFGVVYHGDINGSSQQVAVKLLSQSST-QGYKEFKAEVELLLRVHHINLV
AT3G46370/1-275     1 KNFQKTLGEGGFGTVYYGNLNG-SEQVAVKVLSQSSS-QGYKHFKAEVELLLRVHHINLV
AT3G46400/1-275     1 KKFEKALGEGGFGIVYHGYLKN-VEQVAVKVLSQSSS-QGYKHFKAEVELLLRVHHINLV
AT3G46330/1-276     1 KNLQRPLGEGGFGVVYHGDLNG-SEQVAVKLLSQTSA-QGYKEFKAEVELLLRVHHINLV
AT5G59670/1-275     1 KNFQRVLGKGGFGMVYHGTVKG-SEQVAVKVLSQSST-QGSKEFKAEVDLLLRVHHTNLV
AT5G59680/1-275     1 NNFGRVVGEGGFGVVCHGTVNG-SEQVAVKLLSQSST-QGYKEFKAEVDLLLRVHHTNLV
AT5G59650/1-275     1 NNFQRVVGEGGFGVVCHGTING-SEQVAVKVLSQSSS-QGYKHFKAEVDLLLRVHHTNLV
AT5G16900/1-275     1 NNFERVIGEGGFGVVYHGYLND-SEQVAVKVLSPSSS-QGYKEFKAEVELLLRVHHINLV
AT1G07560/1-272     1 KKFERVLGKGGFGMVYHGYING-TEEVAVKLLSPSSA-QGYKEFKTEVELLLRVYHTNLV
AT4G20450/1-275     1 NNFERPLGEGGFGVVYHGNVND-NEQVAVKVLSESSA-QGYKQFKAEVDLLLRVHHINLV
AT2G28960/1-275     1 DNFERVLGEGGFGVVYHGILNG-TQPIAVKLLSQSSV-QGYKEFKAEVELLLRVHHVNLV
AT2G29000/1-275     1 NKFERVIGEGGFGIVYHGHLND-TEQVAVKLLSHSST-QGYKQFKAEVELLLRVHHTNLV
AT2G28970/1-275     1 NNFQRVLGEGGFGVVYHGCVNG-TQQVAVKLLSQSSS-QGYKHFKAEVELLMRVHHKNLV
AT2G28990/1-275     1 NNFDKALGEGGFGVVYHGFVNV-IEQVAVKLLSQSSS-QGYKHFKAEVELLMRVHHINLV
AT1G49100/1-275     1 NNFRSVLGKGGFGMVYHGYVNG-REQVAVKVLSHASK-HGHKQFKAEVELLLRVHHKNLV
AT1G51810/1-275     1 NNFQKILGKGGFGIVYYGSVNG-TEQVAVKMLSHSSA-QGYKQFKAEVELLLRVHHKNLV
AT1G51805/1-275     1 NNFQRILGKGGFGIVYHGFVNG-VEQVAVKILSHSSS-QGYKQFKAEVELLLRVHHKNLV
AT1G51830/1-275     1 NNFQRVLGKGGFGIVYHGLVNG-TEQVAIKILSHSSS-QGYKQFKAEVELLLRVHHKNLV
AT1G51820/1-275     1 NNFQRILGKGGFGMVYHGFVNG-TEQVAVKILSHSSS-QGYKQFKAEVELLLRVHHKNLV
AT1G51850/1-275     1 NNFQRILGKGGFGMVYHGFVNG-TEQVAVKILSHSSS-QGYKEFKAEVELLLRVHHKNLV
AT2G04300/1-275     1 NNFEKILGKGGFGMVYHGTVND-AEQVAVKMLSPSSS-QGYKEFKAEVELLLRVHHKNLV
AT3G21340/1-275     1 NNFERVLGKGGFGMVYHGTVNN-TEQVAVKMLSHSSS-QGYKEFKAEVELLLRVHHKNLV
AT1G05700/1-275     1 NNFGQVLGKGGFGTVYHGFYDN--LQVAVKLLSETSA-QGFKEFRSEVEVLVRVHHVNLT
AT2G19210/1-276     1 NNFERVLGQGGFGKVYHGVLND--DQVAVKILSESSA-QGYKEFRAEVELLLRVHHKNLT
AT2G19230/1-275     1 NNFERVLGQGGFGKVYYGVLRG--EQVAIKMLSKSSA-QGYKEFRAEVELLLRVHHKNLI
AT2G19190/1-273     1 NNFERVIGKGGFGKVYHGVING--EQVAVKVLSEESA-QGYKEFRAEVDLLMRVHHTNLT
AT4G29990/1-273     1 NNFERVLGKGGFGKVYHGFLNG--DQVAVKILSEEST-QGYKEFRAEVELLMRVHHTNLT
AT4G29180/1-277     1 NNFNKVIGKGGFGIVYLGSLED-GTEIAVKMISSSSSSQVSKEFQVEAELLLTVHHRNLA
AT4G29450/1-277     1 NNFNKVIGKGGFGIVYLGSLED-GTKIAVKMISSSSLSRASNQFQVEAELLLTVHHRNLA
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LjSYMRK/1-278      59 PLLGYCNESDQQILVYPFMSNGSLQDRLYGEPAKRKILDWPTRLSIALGAARGLAYLHTF
LjShRK1/1-278      59 QLLGYCREEGNSMLIYEFMHNGTLKEHLYGPLTHGRSINWIKRLEIAEDSAKGIEYLHTG
LjShRK2/1-272      57 ALIGYCDDGTNMALIYEYMANSDLAKHLSGKNE--NILGWNQRLQIAVDAAEGLEYLHHG
ShRK1/1-276        59 PLIGYCEEADRRILVYEYMHNGSLGDHLHGSSDY-KPLDWLTRLQIAQDAAKGLEYLHTG
ShRK2/1-279        59 QFLGYCQEEGKNMLVYEFMHNGTLKEHLYGVVPRDRRISWIKRLEIAEDAARGIEYLHTG
AT5G48740/1-277    59 SFEGFCYEPKRQILVYEYLSGGSLADHLYGPRSKRHSLNWVSRLKVAVDAAKGLDYLHNG
AT1G51790/1-273    58 TMLGYCNEGDKMAVIYEYMANGNLKQHIS-ENST-TVFSWEDRLGIAVDVAQGLEYLHTG
IOS1/1-274         58 CLVGYCEEGDKMSLIYEFMANGDLKEHLSGKRGP-SILTWEGRLRIAAESAQGLEYLHNG
AT1G51910/1-272    59 GLVGYCDDGDNFALIYEYMANGDLKENMSGNRSG-HVLSWENRMQIAMEAAQGLEYLHNG
AT1G51890/1-274    58 GLVGYCDDGDNLALIYEYMEKGDLRENMSGKHSV-NVLSWETRMQIAVEAAQGLEYLHNG
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AT1G51880/1-274    58 GLVGYCDDGDNLALIYEYMANGDLKENMSGKRGG-NVLTWENRMQIAVEAAQGLEYLHNG
AT1G07550/1-273    58 SLIGYCDDDNGLALIYELMGKGNLKEHLSGKPGC-SVLSWPIRLKIALESAIGIEYLHTG
AT2G14440/1-274    58 SLVGYCDKGNDLALIYEFMENGNLKEHLSGKRGG-PVLNWPGRLKIAIESALGIEYLHIG
AT2G14510/1-274    58 SLVGYCDEGIDLALIYEFMENGNLKEHLSGKRGG-SVLNWSSRLKIAIESALGIEYLHIG
AT3G46350/1-275    59 NLVGYCDERDHLALIYEYMSNGDLKHHLSGEHGG-SVLSWNIRLRIAVDAALGLEYLHIG
AT3G46340/1-276    60 SLVGYCDERDHLALIYEYMSNKDLKHHLSGKHGG-SVLKWNTRLQIAVDAALGLEYLHIG
AT3G46370/1-275    59 SLVGYCDERNHLALIYECMSNGDLKDHLSGKKGN-AVLKWSTRLRIAVDAALGLEYLHYG
AT3G46400/1-275    59 SLVGYCDEKDHLALIYEYMPNGDLKDHLSGKQGD-SVLEWTTRLQIAVDVALGLEYLHYG
AT3G46330/1-276    59 NLVGYCDEQDHFALIYEYMSNGDLHQHLSGKHGG-SVLNWGTRLQIAIEAALGLEYLHTG
AT5G59670/1-275    59 SLVGYCCEGDYLALVYEFLPNGDLKQHLSGKGGN-SIINWSIRLRIALEAALGLEYLHIG
AT5G59680/1-275    59 SLVGYCDEGDHLALIYEFVPNGDLRQHLSGKGGK-PIVNWGTRLRIAAEAALGLEYLHIG
AT5G59650/1-275    59 SLVGYCDERDHLALIYEFLPKGDLRQHLSGKSGG-SFINWGNRLRIALEAALGLEYLHSG
AT5G16900/1-275    59 SLVGYCDEQAHLALIYEYMANGDLKSHLSGKHGD-CVLKWENRLSIAVETALGLEYLHSG
AT1G07560/1-272    59 SLVGYCDEKDHLALIYQYMVNGDLKKHFSGS----SIISWVDRLNIAVDAASGLEYLHIG
AT4G20450/1-275    59 TLVGYCDEGQHLVLIYEYMSNGNLKQHLSGENSR-SPLSWENRLRIAAETAQGLEYLHIG
AT2G28960/1-275    59 SLVGYCDEESNLALLYEYAPNGDLKQHLSGERGG-SPLKWSSRLKIVVETAQGLEYLHTG
AT2G29000/1-275    59 NLVGYCNEEDHLALVYEYAANGDLKQHLSGESSS-AALNWASRLGIATETAQGLEYLHIG
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AT2G28990/1-275    59 SLVGYCDEGEHLALIYEYMPNGDLKQHLSGKHGG-FVLSWESRLKIVLDAALGLEYLHTG
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AT1G51810/1-275    59 GLVGYCEEGDKLALIYEYMANGDLDEHMSGKRGG-SILNWGTRLKIALEAAQGLEYLHNG
AT1G51805/1-275    59 GLVGYCDEGENMALIYEYMANGDLKEHMSGTRNR-FILNWETRLKIVIDSAQGLEYLHNG
AT1G51830/1-275    59 GLVGYCDEGENLALIYEYMANGDLKEHMSGTRNH-FILNWGTRLKIVVESAQGLEYLHNG
AT1G51820/1-275    59 GLVGYCDEGDNLALIYEYMANGDLKEHMSGTRNR-FILNWGTRLKIVIESAQGLEYLHNG
AT1G51850/1-275    59 GLVGYCDEGENMALIYEYMANGDLKEHMSGTRNR-FTLNWGTRLKIVVESAQGLEYLHNG
AT2G04300/1-275    59 GLVGYCDEGENLSLIYEYMAKGDLKEHMLGNQGV-SILDWKTRLKIVAESAQGLEYLHNG
AT3G21340/1-275    59 GLVGYCDEGENLALIYEYMANGDLREHMSGKRGG-SILNWETRLKIVVESAQGLEYLHNG
AT1G05700/1-275    58 ALIGYFHEGDQMGLIYEFMANGNMADHLAGKYQ--HTLSWRQRLQIALDAAQGLEYLHCG
AT2G19210/1-276    58 ALIGYCHEGKKMALIYEFMANGTLGDYLSGEKS--YVLSWEERLQISLDAAQGLEYLHNG
AT2G19230/1-275    58 ALIGYCHEGDQMALIYEYIGNGTLGDYLSGKNS--SILSWEERLQISLDAAQGLEYLHNG
AT2G19190/1-273    58 SLVGYCNEINHMVLIYEYMANENLGDYLAGKRS--FILSWEERLKISLDAAQGLEYLHNG
AT4G29990/1-273    58 SLIGYCNEDNHMALIYEYMANGNLGDYLSGKSS--LILSWEERLQISLDAAQGLEYLHYG
AT4G29180/1-277    60 SFVGYCDDGRSMALIYEYMANGNLQDYLSSENAE-D-LSWEKRLHIAIDSAQGLEYLHHG
AT4G29450/1-277    60 SFVGYCDDDRSMALIYEYMANGNLQAYLSSENAE-D-LSWEKRLHIAIDSAQGLEYLHDG
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LjSYMRK/1-278      119 PGRSVIHRDIKSSNILLDHSMCAKVADFGFSKYAPQEG-DSYVSLEVRGTAGYLDPEYYK
LjShRK1/1-278      119 CVPAVIHRDLKSSNILLDRQLRAKVSDFGLSK-LAVDG-VSHVSSIVRGTVGYLDPEYYI
LjShRK2/1-272      115 SNPPIVHRDVKSKNILLNEKFQAKLADFGLSKIFPNEG-DTHVYTVVAGTPGYLDPEYNR
ShRK1/1-276        118 CNPSIIHRDVKSSNILLDINMRAKVSDFGLSR-QTEED-LTHVSSVAKGTVGYLDPEYYA
ShRK2/1-279        119 CVPAIIHRDLKTSNILLDKHMRAKVSDFGLSK-FAVDG-TSHVSSIVRGTVGYLDPEYYI
AT5G48740/1-277    119 SEPRIIHRDVKSSNILLDKDMNAKVSDFGLSKQFTKAD-ASHITTVVKGTAGYLDPEYYS
AT1G51790/1-273    116 CKPPIIHRNVKCTNVFLDESFNAKLGGFGLSRAFDAAE-GSHLNTAIAGTPGYVDPEYYT
IOS1/1-274         117 CKPQIVHRDIKTTNILLNEKFQAKLADFGLSRSFPLGT-ETHVSTIVAGTPGYLDPEYYR
AT1G51910/1-272    118 SRPPMVHRDVKTTNILLNELYQAKLADFGLSRSSPVDG-ESYVSTIVAGTPGYLDPE---
AT1G51890/1-274    117 CRPPMVHRDVKPTNILLNERSQAKLADFGLSRSFPVDG-ESHVMTVVAGTPGYLDPEYYR
AT1G51860/1-274    117 CRPPMVHRDVKTTNILLNERCGAKLADFGLSRSFPIDG-ECHVSTVVAGTPGYLDPEYYR
AT1G51880/1-274    117 CTPPMVHRDVKTTNILLNERYGAKLADFGLSRSFPVDG-ESHVSTVVAGTPGYLDPEYYR
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AT2G14510/1-274    117 CQPPMVHRDVKSTNILLGLRFEAKLADFGLSRSFLVGS-QAHVSTNVAGTLGYLDPEYYL
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AT1G49100/1-275    118 CRPPIVHRDVKTANILLDEHFQAKLADFGLSRSFLNEG-ESHVSTVVAGTIGYLDPEYYR
AT1G51810/1-275    118 CKPLMVHRDVKTTNILLNEHFDTKLADFGLSRSFPIEG-ETHVSTVVAGTIGYLDPEYYR
AT1G51805/1-275    118 CKPLMVHRDVKTTNILLNEHFEAKLADFGLSRSFPIGG-ETHVSTVVAGTPGYLDPEYYK
AT1G51830/1-275    118 CKPLMVHRDIKTTNILLNEQFDAKLADFGLSRSFPIEG-ETHVSTAVAGTPGYLDPEYYR
AT1G51820/1-275    118 CKPPMVHRDVKTTNILLNEHFEAKLADFGLSRSFLIEG-ETHVSTVVAGTPGYLDPEYHR
AT1G51850/1-275    118 CKPPMVHRDVKTTNILLNEHFQAKLADFGLSRSFPIEG-ETHVSTVVAGTPGYLDPEYYK
AT2G04300/1-275    118 CKPPMVHRDVKTTNILLDEHFQAKLADFGLSRSFPLEG-ETRVDTVVAGTPGYLDPEYYR
AT3G21340/1-275    118 CKPPMVHRDVKTTNILLNEHLHAKLADFGLSRSFPIEG-ETHVSTVVAGTPGYLDPEYYR
AT1G05700/1-275    116 CKPPIVHRDVKTSNILLNEKNRAKLADFGLSRSFHTES-RSHVSTLVAGTPGYLDPLCFE
AT2G19210/1-276    116 CKPPIVQRDVKPANILINEKLQAKIADFGLSRSVALDG-NNQDTTAVAGTIGYLDPEYHL
AT2G19230/1-275    116 CKPPIVHRDVKPTNILINEKLQAKIADFGLSRSFTLEG-DSQVSTEVAGTIGYLDPEHYS
AT2G19190/1-273    116 CKPPIVHRDVKPTNILLNEKLQAKMADFGLSRSFSVEG-SGQISTVVAGSIGYLDPEYYS
AT4G29990/1-273    116 CKPPIVHRDVKPANILLNENLQAKIADFGLSRSFPVEG-SSQVSTVVAGTIGYLDPEYYA
AT4G29180/1-277    118 CRPPIVHRDVKTANILLNDNLEAKIADFGLSKVFPEDD-LSHVVTAVMGTPGYVDPEYYN
AT4G29450/1-277    118 CRPAIVHRDVKTANILINDNLEAKIADFGLSKVFPEDD-LSHVVTTVMGTPGYVDPEYYR
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LjSYMRK/1-278     178 TQQLSEKSDVFSFGVVLLEIVSGREPLNIKR-PRTEWSLVEWATPYI-RGSKVDEIVDPG
LjShRK1/1-278     177 SQQLTDKSDIYSFGVILLELISGQEAISNDSFGANCRNIVQWAKLHI-ESGDIQGIIDPA
LjShRK2/1-272     174 SSRLNEKSDVFSFGVVLLELITGQPAVTKTE---DKIHIIQWVSSLL-LQREVKDIVDPR
ShRK1/1-276       176 SQQLTEKSDVYSFGVVLFELLSGKKPVSAEDFGPEL-NIVHWARSLI-RKGDVCGIIDPC
ShRK2/1-279       177 SQQLTEKSDVYSFGVILLELMSGQEAISNESFGVNCRNIVQWAKMHI-DNGDIRGIIDPA
AT5G48740/1-277   178 TLQLTEKSDVYSFGVVLLELICGREPLSHSG-SPDSFNLVLWARPNL-QAGAFE-IVDDI
AT1G51790/1-273   175 SNMLTEKSDVYSFGVVLLEIVTAKPAIIKNE---ERMHISQWVESLL-SRENIVEILDPS
IOS1/1-274        176 TNWLTEKSDVFSFGVVLLELVTNQPVIDMKR---EKSHIAEWVGLML-SRGDINSIVDPK
AT1G51910/1-272   174 TNLLSEKTDVYSFGVVLLEIITNQPVIDTTR---EKAHITDWVGFKL-MEGDIRNIIDPK
AT1G51890/1-274   176 TNWLSEKSDVYSFGVVLLEIVTNQPVMNKNR---ERPHINEWVMFML-TNGDIKSIVDPK
AT1G51860/1-274   176 TNWLSEKSDVYSFGVVLLEIVTNQPVIDKTR---ERPHINDWVGFML-TKGDIKSIVDPK
AT1G51880/1-274   176 TNWLSEKSDVYSFGVVLLEIVTNQPVTDKTR---ERTHINEWVGSML-TKGDIKSILDPK
AT1G07550/1-273   175 TSLLSMKSDVYSFGVVLLEIISGQDVIDLSR---ENCNIVEWTSFIL-ENGDIESIVDPN
AT2G14440/1-274   176 KNWLTEKSDVYSFGIVLLEIITGQPVIEQSR---DKSYIVEWAKSML-ANGDIESIMDRN
AT2G14510/1-274   176 KNWLTEKSDVYSFGIVLLESITGQPVIEQSR---DKSYIVEWAKSML-ANGDIESIMDPN
AT3G46350/1-275   177 TSRLAEMSDVYSFGIVLLEIITNQRVIDKTR---EKPHITEWTAFML-NRGDITRIMDPN
AT3G46340/1-276   178 TGRLAEMSDVYSFGIVLLEIITNQRVIDPAR---EKSHITEWTAFML-NRGDITRIMDPN
AT3G46370/1-275   177 TCRLAEMSDVYSFGILLLEIITNQNVIDHAR---EKAHITEWVGLVL-KGGDVTRIVDPN
AT3G46400/1-275   177 TSRLAEMSDVYSFGIVLLEIITNQRVFDQAR---GKIHITEWVAFML-NRGDITRIVDPN
AT3G46330/1-276   178 TSELSEKSDVYSFGILLLEIITNQRVIDQTR---ENPNIAEWVTFVI-KKGDTSQIVDPK
AT5G59670/1-275   177 SGRLGEKSDVYSFGIVLLEMITNQPVINQTS---GDSHITQWVGFQM-NRGDILEIMDPN
AT5G59680/1-275   177 TSRLSEKSDVYSFGIVLLEMITNQAVIDRNR---RKSHITQWVGSEL-NGGDIAKIMDLK
AT5G59650/1-275   177 TTRLGEKSDVYSFGIVLLEIITNQPVIDQSR---SKSHISQWVGFEL-TRGDITKIMDPN
AT5G16900/1-275   177 TYRLTEKSDVYSFGIVLLEIITNQPVLEQAN---ENRHIAERVRTML-TRSDISTIVDPN
AT1G07560/1-272   174 TNRLSEKSDVYSFGVVLLEIITNKPVIDHNR---DMPHIAEWVKLML-TRGDISNIMDPK
AT4G20450/1-275   177 TNWLTEKSDVFSFGVVLLEIITSQPVIDQTR---EKSHIGEWVGFKL-TNGDIKNIVDPS
AT2G28960/1-275   177 TNRLNEKSDVYSFGIVLLEIITSRPVIQQTR---EKPHIAAWVGYML-TKGDIENVVDPR
AT2G29000/1-275   177 TNWLTEKSDVYSMGIVLLEIITNQPVIQQVR---EKPHIAEWVGLML-TKGDIKSIMDPK
AT2G28970/1-275   177 TNWLTEKSDVYSFGIVLLEIITNRPIIQQSR---EKPHLVEWVGFIV-RTGDIGNIVDPN
AT2G28990/1-275   177 TNWLTEKSDIYSFGIVLLEIISNRPIIQQSR---EKPHIVEWVSFMI-TKGDLRSIMDPN
AT1G49100/1-275   177 TNWLTEKSDVYSFGVVLLEIITNQRVIERTR---EKPHIAEWVNLMI-TKGDIRKIVDPN
AT1G51810/1-275   177 TNWLTEKSDVYSFGVVLLVMITNQPVIDQNR---EKRHIAEWVGGML-TKGDIKSITDPN
AT1G51805/1-275   177 TNRLTEKSDVYSFGIVLLEMITNRPVIDQSR---EKPYISEWVGIML-TKGDIISIMDPS
AT1G51830/1-275   177 TNWLTEKSDVYSFGVVLLEIITNQPVIDPRR---EKPHIAEWVGEVL-TKGDIKNIMDPS
AT1G51820/1-275   177 TNWLTEKSDVYSFGILLLEIITNRHVIDQSR---EKPHIGEWVGVML-TKGDIQSIMDPS
AT1G51850/1-275   177 TNWLTEKSDVYSFGIVLLELITNRPVIDKSR---EKPHIAEWVGVML-TKGDINSIMDPN
AT2G04300/1-275   177 TNWLNEKSDVYSFGIVLLEIITNQHVINQSR---EKPHIAEWVGVML-TKGDIKSIIDPK
AT3G21340/1-275   177 TNWLNEKSDVYSFGIVLLEIITNQLVINQSR---EKPHIAEWVGLML-TKGDIQNIMDPK
AT1G05700/1-275   175 TNGLNEKSDIYSFGVVLLEMITGKTVIKESQ--TKRVHVSDWVISILRSTNDVNNVIDSK
AT2G19210/1-276   175 TQKLSEKSDIYSFGVVLLEVVSGQPVIARSRTTAENIHITDRVDLML-STGDIRGIVDPK
AT2G19230/1-275   175 MQQFSEKSDVYSFGVVLLEVITGQPVISRSR-TEENRHISDRVSLML-SKGDIKSIVDPK
AT2G19190/1-273   175 TRQMNEKSDVYSLGVVLLEVITGQPAIASSK--TEKVHISDHVRSIL-ANGDIRGIVDQR
AT4G29990/1-273   175 TRQMNEKSDVYSFGVVLLEVITGKPAIWHSR--TESVHLSDQVGSML-ANGDIKGIVDQR
AT4G29180/1-277   177 TFKLNEKSDVYSFGIVLLELITGKRSIMKTD-DGEKMNVVHYVEPFL-KMGDIDGVVDPR
AT4G29450/1-277   177 TFVLNEKSDVYSFGVVLLELITGQRAIIKTE-EGDNISVIHYVWPFF-EARELDGVVDPL
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LjSYMRK/1-278      236 IK-GGYHAEAMWRVVEVALQCLEPFSTYRPSMVAIVRELEDALI
LjShRK1/1-278      236 LG-NDYDLQSMWKIAEKALMCVQPHGHMRPSISEVLKEIQDAIA
LjShRK2/1-272      230 LQ-GEFDIDSAKKALDTAMTCVAPTSINRPTMSHVVMELKLCLP
ShRK1/1-276        234 IA-SNVKIESVWRVAEVANQCVEQRGHNRPRMQEVIVAIQDAIR
ShRK2/1-279        236 LAEDDYSLQSMWKIAEKALLCVKPHGNMRPSMSEVQKDIQDAIR
AT5G48740/1-277    235 LK-ETFDPASMKKAASIAIRCVGRDASGRPSIAEVLTKLKEAYS
AT1G51790/1-273    231 LC-GDYDPNSAFKTVEIAVACVCRNSGDRPGMSQVVTALKESLA
IOS1/1-274         232 LQ-GDFDPNTIWKVVETAMTCLNPSSSRRPTMTQVVMDLKECLN
AT1G51910/1-272    230 LI-KEFDTNGVWKAVELALSCVNPTSNHRPTMPHVVMELKECLD
AT1G51890/1-274    232 LN-EDYDTNGVWKVVELALACVNPSSSRRPTMPHVVMELNECLA
AT1G51860/1-274    232 LM-GDYDTNGAWKIVELALACVNPSSNRRPTMAHVVMELNDCVA
AT1G51880/1-274    232 LM-GDYDTNGAWKIVELALACVNPSSNRRPTMAHVVTELNECVA
AT1G07550/1-273    231 LH-QDYDTSSAWKVVELAMSCVNRTSKERPNMSQVVHVLNECLE
AT2G14440/1-274    232 LH-QDYDTSSSWKALELAMLCINPSSTLRPNMTRVAHELNECLE
AT2G14510/1-274    232 LH-QDYDSSSSWKALELAMLCINPSSTQRPNMTRVAHELNECLE
AT3G46350/1-275    233 LN-GDYNSHSVWRALELAMSCANPSSENRPSMSQVVAELKECLI
AT3G46340/1-276    234 LQ-GDYNSRSVWRALELAMMCANPSSEKRPSMSQVVIELKECIR
AT3G46370/1-275    233 LD-GEYNSRSVWRALELAMSCANPSSEHRPIMSQVVIDLKECLN
AT3G46400/1-275    233 LH-GEYNSRSVWRAVELAMSCANPSSEYRPNMSQVVIELKECLT
AT3G46330/1-276    234 LH-GNYDTHSVWRALEVAMSCANPSSVKRPNMSQVIINLKECLA
AT5G59670/1-275    233 LR-KDYNINSAWRALELAMSCAYPSSSKRPSMSQVIHELKECIA
AT5G59680/1-275    233 LN-GDYDSRSAWRALELAMSCADPTSARRPTMSHVVIELKECLV
AT5G59650/1-275    233 LN-GDYESRSVWRVLELAMSCANPSSVNRPNMSQVANELKECLV
AT5G16900/1-275    233 LI-GEYDSGSVRKALKLAMSCVDPSPVARPDMSHVVQELKQCIK
AT1G07560/1-272    230 LQ-GVYDSGSAWKALELAMTCVNPSSLKRPNMSHVVHELKECLV
AT4G20450/1-275    233 MN-GDYDSSSLWKALELAMSCVSPSSSGRPNMSQVANELQECLL
AT2G28960/1-275    233 LN-RDYEPTSVWKALEIAMSCVNPSSEKRPTMSQVTNELKQCLT
AT2G29000/1-275    233 LN-GEYDSSSVWKALELAMSCVNPSSGGRPTMSQVISELKECLI
AT2G28970/1-275    233 LH-GAYDVGSVWKAIELAMSCVNISSARRPSMSQVVSDLKECVI
AT2G28990/1-275    233 LH-QDYDIGSVWKAIELAMSCVSLSSARRPNMSRVVNELKECLI
AT1G49100/1-275    233 LK-GDYHSDSVWKFVELAMTCVNDSSATRPTMTQVVTELTECVT
AT1G51810/1-275    233 LL-GDYNSGSVWKAVELAMSCMNPSSMTRPTMSQVVFELKECLA
AT1G51805/1-275    233 LN-GDYDSGSVWKAVELAMSCLNPSSTRRPTMSQVLIALNECLV
AT1G51830/1-275    233 LN-GDYDSTSVWKAVELAMCCLNPSSARRPNMSQVVIELNECLT
AT1G51820/1-275    233 LN-EDYDSGSVWKAVELAMSCLNHSSARRPTMSQVVIELNECLA
AT1G51850/1-275    233 LN-EDYDSGSVWKAVELAMSCLNPSSARRPTMSQVVIELNECIA
AT2G04300/1-275    233 FS-GDYDAGSVWRAVELAMSCVNPSSTGRPTMSQVVIELNECLA
AT3G21340/1-275    233 LY-GDYDSGSVWRAVELAMSCLNPSSARRPTMSQVVIELNECLS
AT1G05700/1-275    233 MA-KDFDVNSVWKVVELALSSVSQNVSDRPNMPHIVRGLNECLQ
AT2G19210/1-276    234 LG-ERFDAGSAWKITEVAMACASSSSKNRPTMSHVVAELKESVS
AT2G19230/1-275    233 LG-ERFNAGLAWKITEVALACASESTKTRLTMSQVVAELKESLC
AT2G19190/1-273    232 LR-ERYDVGSAWKMSEIALACTEHTSAQRPTMSQVVMELKQIV-
AT4G29990/1-273    232 LG-DRFEVGSAWKITELALACASESSEQRPTMSQVVMELKQSI-
AT4G29180/1-277    235 LH-GDFSSNSAWKFVEVAMSCVRDRGTNRPNTNQIVSDLKQCLA
AT4G29450/1-277    235 LR-GDFSQDSAWKFVDVAMSCVRDKGSNRPTMNQIVAELKQCLA
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Appendix 2: Pruned alignment of extracytoplasmatic region of LjSYMRK (amino acid positions 30-517) and homologous stretches of related MLD-LRR-RLKs (sequences as above; conserved GDPC motifs are at alignment positions 464-467).

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LjSYMRK/1-509       1 -MMELPATRILSQAVTCFLCLYIFIGSASATEGFESIACCAD----LNYTDPLTTLNYTT
LjShRK1/1-522       1 PFFLSLTLLLLLRL--------S--SA-QMK-GFVSLDCGGK----ENFTD-EIGLQWTP
LjShRK2/1-506       1 --MAGL-LLLLVFQLSWTLPII--VHA-QDQSGFISIDCGLEDE--PSYTDETTSIHYTS
ShRK1_(AT1/1-533    1 ------MGLCLAQLAVTCLFLVPFVLS-QVT-EFVSIDCGCS----SNYTDPRTGLGWVS
ShRK2_(AT2/1-529    1 --MVRISLLLLCLLVSTCLFTSS--SA-QAP-GFVSLDCGGA----EPFTD-ELGLKWSP
AT5G48740/1-492     1 ---------MLFWVLLSSFCVFCF----SSPDGFLSLSCGG-----SSYT-AAYNISWVS
AT1G51790/1-518     1 --MMTSKAKALTF--ICCVALLNLAIA-QDQSGFISIDCGLQPEN-SSYTETSTDIKYVS
IOS1(AT1G5/1-513    1 --MAFSSCFLLVLLQIFSALLLCL--A-QDQSGFISLDCGSPRE--TSFREKTTNITYIS
AT1G51910/1-508     1 --MKTMNGFLLLS--TIAFAVFHLVQA-QSQSGFISLDCGLIPKD-TTYTEQITNITYIS
AT1G51890/1-499     1 --MR----FLSFL--IFVFAVLGLVQA-QDQSGFISLDCGLVPTE-ITYVEKSTNITYRS
AT1G51860/1-511     1 --MKSLHWFLHLL--IIAFTVLRSVEA-QNQAGFISLDCGLVPKE-TTYTEKSTNITYKS
AT1G51880/1-511     1 --MKSIHGFLLFL--ITAYVILESVQA-QDQLGFISLDCGLVPKN-ATYTEKTTNITYKS
AT1G07550/1-504     1 --MDTCTRLLFA-A-CATLSILHLVQS-QNQQGFISLDCGLASNE-SPYNEANSNLTYIS
AT2G14440/1-502     1 --METRSKLMLL-A-CATFSIISLVKS-QNQQGFISLYCGLPSNE-SPYIEPLTNLTYIS
AT2G14510/1-508     1 --METRNKFMLL-A-CATFSIMSLVKS-QNQQGFISLDCGLPSKE-S-YIEPSSNLTFIS
AT3G46350/1-491     1 --MNSSHELLLTAL-IATFAIFHLVQA-QEQEGFISLDCGLAPTEPSPYTEPVTTLQYSS
AT3G46340/1-513     1 --MEFPHSVLLVVLIIATFAISNLVQAEEDQEGFISLDCGLPPNEVSPYIEPFTGLRFSS
AT3G46370/1-427     1 ------------------------------------------------------------
AT3G46400/1-508     1 --MESSHRFLLVALTVA----SSIIHLVQAQAGFISLDCGLSPNEQSPYVELETGLQFLS
AT3G46330/1-516     1 --MKNLCWVFLSLFWFGVFLIIRFAEG-QNQEGFISLDCGLPLNEP-PYIESETGIQFSS
AT5G59670/1-499     1 --MESSFGLLLAL--L-TLTIIHIVQA-QDPQGFISLDCGLPANETSPYTETQTGLLFSS
AT5G59680/1-508     1 --MERSLELLLLL--IRTLAIIHISQA-QSQQGFISLDCGLPANEPSPYTEPRTGLQFSS
AT5G59650/1-509     1 --MDSPCWLLLLL--LGAFAIIGCVQA-QDQQEFISLDCGLPMTEPSSYTESVTGLRFSS
AT5G16900/1-505     1 --MEDRHRYLFFI-----FAIIHYVQA---QQGFISLDCGLPSNE-PPYIEPVTGLVFSS
AT1G07560/1-512     1 --MKNLRGLLLAFL-VLSLGISDFLRA-QDQQGFISLDCGLQADE-SPYTEPLTKLTFTS
AT4G20450/1-530     1 --MEGIHKLIFLAL-IWIFLITNIVDA-QDQQGFISLDCGMPRNE-SSYTDESTGLNFSS
AT2G28960/1-507     1 --MEGRRQRLLVFI-FGALAITHLVQA-QPPDGFISLDCGLPVNE-SPYTDPRTGLTFSS
AT2G29000/1-506     1 --MEGHRGLLLALI-VNIFSIVHLVHA-QNPEGFISLDCGLPAKE-SPYTESTTSLVFTS
AT2G28970/1-409     1 ------MMSHLLLAIIGTFAVI--VGA-QKQEGFISLDCGFPIEE-SPYSDPSTGLTFTS
AT2G28990/1-506     1 ------MKIHLLLAMIGTFVVI--IGA-QDQEGFISLDCGLPSDE-SPYDDSFNGLTFTS
AT1G49100/1-518     1 MEKYFHGVLCVFIITVAF---IHVVQA-QDPNGFITLDCGLLPDG-SPYTNPSTGLTFTS
AT1G51810/1-384     1 ------------------------------------------------------------
AT1G51805/1-504     1 MESH-------RVFVATFMLILHLVQA-QDQPGFINVDCGLLPRD-SPYNALGTGLVYTS
AT1G51830/1-315     1 ------------------------------MTVFFINDC--------------------
AT1G51820/1-504     1 MERH-------FVFIATYLLIFHLVQA-QNQTGFISVDCGLSLLE-SPYDAPQTGLTYTS
AT1G51850/1-486     1 MERH-------CVLVATFLLMLHIVHA-QDQIGFISVDCGLAPRE-SPYNEAKTGLTYTS
AT2G04300/1-480     1 MKTHPQAILLCVLFFITF-GLLHVVEA-GNQEGFISLDCGLSPNE-PPYVDAATDLTYTT
AT3G21340/1-520     1 MEYHPQAIRLCALIFISFYALLHLVEA-QDQKGFISLDCGSLPNE-PPYNDPSTGLTYST
AT1G05700/1-507     1 --MEEFRFLYLIYSAAFALCLVVSVLA-QDQSGFISIDCGIPSG--SSYKDDTTGINYVS
AT2G19210/1-516     1 --MVHYNFLSLIIFACFFAVFVLLVRA-QDQSGFVSIDCGIPED--SSYNDETTDIKYVS
AT2G19230/1-516     1 --MGNFNFLPLVSFASFVVVLV-LVCA-QDQSGFVSIDCGIPED--SSYYDEKTDIKYIS
AT2G19190/1-516     1 --MAMLKSLSSILFTSFALLFF-LVHA-QDQSGFISIDCGIPDD--SSYNDETTGIKYVS
AT4G29990/1-511     1 --MTRLRLLSWISITS----CVCLVFA-QDQSGFISIDCGIPDD--SSYTDEKTNMKYVS
AT4G29180/1-509     1 -----MGAHSVFLILFSVIAIAIVVHG-QGQAGFISIDCGSPPN--INYVDTDTGISYTW
AT4G29450/1-513     1 -----MRANLVFGI-FCALVTTILVHG-QDQSGYISIDCGIPPY--DTPEDTMTNINYVS
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LjSYMRK/1-509      56 DYTWFSDKRSC-----RKIPETELRNRSNENVRLFDIDEGKRCYNLPTIKNG--VYLIRG
LjShRK1/1-522      44 DDKMS-YGEISTI------SVTNETRKQYMTLRHFPADSRKYCYTLDVVSRT--RYLLRT
LjShRK2/1-506      53 DVNFTDTGVSHSI---SPKYE-ASLERQFWNVRSFP-GGRRNCYTLVVPQGRSKKYLVRA
ShRK1_(AT1/1-533   49 DSEIIKQGKPVTL------ANTNWNSMQYRRRRDFPTDNKKYCYRLSTKERR--RYIVRT
ShRK2_(AT2/1-529   50 DNHLI-YGETANI------SSVNETRTQYTTLRHFPADSRKYCYTLNVTSRN--RYLIRA
AT5G48740/1-492    42 DNDYIETGNTTTVTYAEGNSTSSV------PIRLFPDPQGRQCYKLPVRKDLS-SVLIRA
AT1G51790/1-518    55 DSSYTDTGTSYFV---APENRQNM-KQSMWSVRSFP-EGIRNCYTIAVNSST--KYLIRA
IOS1(AT1G5/1-513   54 DANFINTGVGGSI---KQGYR-TQFQQQTWNLRSFP-QGIRNCYTLNLTIGD--EYLIRA
AT1G51910/1-508    55 DADYIDSGLTERI---SDSYKSQL-QQQTWTLRSFP-EGQRNCYNFNLKANL--KYLIRG
AT1G51890/1-499    51 DATYIDSGVPGKI---NEVYRTQF-QQQIWALRSFP-EGQRNCYNFSLTAKR--KYLIRG
AT1G51860/1-511    55 DVDYIDSGLVGKI---NDAYKTQF-QQQVWAVRSFP-VGQRNCYNVNLTANN--KYLIRG
AT1G51880/1-511    55 DANYIDSGLVGRI---SAEYKAQL-QQQTWTVRSFP-EGERNCYNFNLTAKS--RYLIRA
AT1G07550/1-504    55 DADFIQGGKTGNV---QKDLLMKL-RKPYTVLRYFP-DGIRNCYSLNVKQDT--NYLIRV
AT2G14440/1-502    55 DVNFVRGGKTGNI---KNNSDIDFTSRPYKVLRYFP-EGIRNCYSLSVKQGT--KYLIRT
AT2G14510/1-508    54 DVNFIRGGKTGNI---QNNSRTNFIFKPFKVLRYFP-DGIRNCYSLSVKQGT--KYLIRT
AT3G46350/1-491    57 DSNFIQSGKLGRI---DTSLQTFF-LKQQTTLRYFP-DGIRNCYNLTVKQGT--NYLIRA
AT3G46340/1-513    59 DSSFIQSGKIGKV---DKSFEATT-LKSYMTLRYFP-DGKRNCYNLIVKQGK--TYMIRA
AT3G46370/1-427     1 ------------------------------------MRNCYNLSVHKET--KYLIRV
AT3G46400/1-508    55 DSSFIQSGKIGRI---DASLESKY-PRSQTTLRYFP-DGIRNCYNVNVYKGT--NYLIRA
AT3G46330/1-516    57 DENFIQSGKTGRI---PKNLESEN-LKQYATLRYFP-DGIRNCYDLRVEEGR--NYLIRA
AT5G59670/1-499    55 DATFIQSGKTGRV---QANQESKF-LKPYRTLRYFP-EGVRNCYNLSVFKER--KYLIAA
AT5G59680/1-508    56 DAAFIQSGKIGRI---QANLEADF-LKPSTTMRYFP-DGKRNCYNLNVEKGR--NHLIRA
AT5G59650/1-509    56 DAEFIQTGESGKI---QASMENDY-LKPYTRLRYFP-EERRNCYSLSVDKNR--KYLIRA
AT5G16900/1-505    50 DADHIPSGISGRI---QKNLEAVH-IKPYLFLRYFP-DGLRNCYTLDVLQNR--RYMIKA
AT1G07560/1-512    56 DADFIKSGKSGKI---QNVPGMEY-IKPYTVLRYFP-DGVRNCYTLIVIQGT--NYLIVA
AT4G20450/1-530    56 DADFISSGKSGTIKTEDSDSGVKY-IKPYKQLRYFP-EGARNCYNLTVMQGT--HYLIRA
AT2G28960/1-507    56 DADFILSGLRGE-----AGDDNTYIYRQYKDLRYFP-DGIRNCYNLKVEQGI--NYLIRA
AT2G29000/1-506    56 DANFISSGISTKL---PKHDD----YKPYNFLRYFP-DGTRHCYDLSVKQGT--NYLIRA
AT2G28970/1-409    51 DSTFIQTGESGRV---DKELNKIF-RKPYLTLRYFP-EGKRNC----------------
AT2G28990/1-506    51 DSTFIQTGKIDSV---DKDLNINL-SKQYLTLRYFP-EGKRNCYSLDVKRGT--TYLIVV
AT1G49100/1-518    56 DSSFIESGKNGRV---SKDSERNF-EKAFVTLRYFP-DGERNCYNLNVTQGT--NYLIRA
AT1G51810/1-384     1 ------------------------------------------------------------
AT1G51805/1-504    52 DVGLVSSGKTGKI---AKEFEENN-STPNLTLRYFP-DGARNCYNLNVSRDT--NYMIKA
AT1G51830/1-315    10 ------------------------------------------------------------
AT1G51820/1-504    52 DADLVASGKTGRL---AKEFEPLV-DKPTLTLRYFP-EGVRNCYNLNVTSDT--NYLIKA
AT1G51850/1-486    52 DDGLVNVGKPGRI---AKEFEPLA-DKPTLTLRYFP-EGVRNCYNLNVTSDT--NYLIKA
AT2G04300/1-480    58 DNDFVQSGKTGTI---DKELESTY-NKPILQLRYFP-EGVRNCYTLNVTLGT--NYLIRA
AT3G21340/1-520    59 DDGFVQSGKTGRI---QKAFESIF-SKPSLKLRYFP-DGFRNCYTLNVTQDT--NYLIKA
AT1G05700/1-507    56 DSSFVETGVSKSI---PFTAQ-----RQLQNLRSFP-EGSRNCYTLIPIQGKGKKYLIRA
AT2G19210/1-516    56 DAAFVESGTIHSI---DPEFQTSSLEKQFQNVRSFP-EGNRNCYDVKPPQGKGFKYLIRT
AT2G19230/1-516    55 DAAFVESGTIHSI---DSKFQKKNLEKQFQKVRSFP-EGKKNCYDVQPPQGKGFKYLIRT
AT2G19190/1-516    55 DSAFVDSGTTKRI---AAQFQSSGFDRHLLNVRSFP-QSKRSCYDVPTPRGKGFKYLIRT
AT4G29990/1-511    52 DLGFVESGTSHSI---VSDLQTTSLERQFQNVRSFP-EGKRNCYDIRPQQGKGFKYLIRT
AT4G29180/1-509    53 DAPFINAGVNLNVSEEYGYPKNPVLPFPLADVRSFP-QGNRNCYTLTPSDGKGNLYLIRA
AT4G29450/1-513    52 DEAFITTGVNFKVSEEYGYPKNPVLLSTLAEVRAFP-QGNRNCYTLKLSQGKDHLYLIRA
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LjSYMRK/1-509      109 TFPFDSLN------SSFNASIGVTQLGAVR------------SSRLQDLEIVFRATKDYI
LjShRK1/1-522       95 TFLYGNFD-SNNVYPKFDISVGATHWSTIV-----IS----DANTIEVRELIFWASSPTV
LjShRK2/1-506      108 RFVYGNYD-GNGSLPEFDIYLGDKWWESLV-----FE----DASSVITKEIIYAASSDYV
ShRK1_(AT1/1-533   101 TFLYGGLG-SEEAYPKFQLYLDATKWATVT-----IQ----EVSRVYVEELIVRATSSYV
ShRK2_(AT2/1-529   101 TFLYGNFDNSNNVYPKFDISLGATHWATIV-----IS----ETYIIETAELVFLASSPTV
AT5G48740/1-492     95 TFVYRNYD-SQNSPPAFHVSLGRRITSTVD-----LR-----TNDPWIEELVWPVNNDSL
AT1G51790/1-518    108 DFMYGNYD-SRNEIPGFDLHLGPNKWDTVELVSPLQT---------VSKEIIYYVLTDTI
IOS1(AT1G5/1-513   107 NFLHGGYD-DKPST-QFELYLGPNLWSTVT-----TT----NETEASIFEMIHILTTDRL
AT1G51910/1-508    108 TFVYGNYD-GLNQMPKFDLHIGPNKWTSVI-------LEGVANA--TIFEIIHVLTQDRL
AT1G51890/1-499    104 TFIYGNYD-GLNQLPSFDLYIGPNKWTSVS-------IPGVRNG--SVSEMIHVLRQDHL
AT1G51860/1-511    108 TFVYGNYD-GLNQFPSFDLHIGPNKWSSVK-------ILGVTNT--SMHEIIHVVPQDSL
AT1G51880/1-511    108 TFTYGNYD-GLRQVPKFDIHIGPSKWTSVK-------LDGVGNG--AVLEMIHVLTQDRL
AT1G07550/1-504    108 MFRYGNYD-GLNNSPRFDLYLGPNIWTTID-----MGKS--GDG--VLEEIIHITRSNIL
AT2G14440/1-502    109 LFFYGNYD-GLNTSPRFDLFLGPNIWTSVD-----VQKVDGGDG--VIEEIIHVTRCNIL
AT2G14510/1-508    108 LFYYGNYD-GLNTSPRFDLFLGPNIWTSVD-----VLIADVGDG--VVEEIVHVTRSNIL
AT3G46350/1-491    110 RFTYGNYD-GRNMSPTFDLYLGPNLWKRID-----MTKL--QNKVSTLEEITYIPLSNSL
AT3G46340/1-513    112 TALYGNYD-GLNISPKFDLYIGANFWTTLD-----AGEY--LSG--VVEEVNYIPRSNSL
AT3G46370/1-427     20 TSNYGNYD-GRNEPPRFDLYLGPNFWVTID-----LGKH--VNG-DTWKEIIHIPKSNSL
AT3G46400/1-508    108 TINYGNYD-GLNISPRFDLYIGPNFWVTID-----LEKH--VGG-DTWEEIIHIPKSNSL
AT3G46330/1-516    110 TFFYGNFD-GLNVSPEFDMHIGPNKWTTID-----LQIV--PDG--TVKEIIHIPRSNSL
AT5G59670/1-499    108 SFLYGNYD-GHNIAPVFDLYLGPNLWAKID-----L-QD--VNG--TGEEILHIPTSNSL
AT5G59680/1-508    109 RFVYGNYD-GRDTGPKFDLYLGPNPWATID-----LAKQ--VNG--TRPEIMHIPTSNKL
AT5G59650/1-509    109 RFIYGNYD-GRNSNPIFELHLGPNLWATID-----LQKF--VNG--TMEEILHTPTSNSL
AT5G16900/1-505    103 VFVYGNYD-GYNDYPSFDLYLGPNKWVRVD-----LEGK--VNG--SVEEIIHIPSSNSL
AT1G07560/1-512    109 MFTYGNYD-NLNTHPKFDLYLGPNIWTTVD-----LQRN--VNG--TRAEIIHIPRSTSL
AT4G20450/1-530    112 VFVYGNYD--LKQRPKFDLYLGPNFWTTIN-----LQRIWLQDG--TVEEVIHMPKSNNL
AT2G28960/1-507    108 GFGYGNYD-GLNVYPKFDLHVGPNMWIAV---DLEFGKD---------REIIYMTTSNLL
AT2G29000/1-506    106 SFVYGNYD-GRNIMPRFDLYIGPNIWAVVSELDL-YSPE---------EEIIHMTKSTSL
AT2G28970/1-409     89 ------------------------------------------------------------
AT2G28990/1-506    104 SFVYGNYD-GLNRDPNFDIHLGPNKWKRID-----LDGE--KEG-TREEIIHKARSNSL
AT1G49100/1-518    109 AFLYGNYD-GLNTVPNFDLFIGPNKVTTVN-----FNAT--GGG--VFVEIIHMSRSTPL
AT1G51810/1-384      1 ------------------------MWITVN-----------TDN--TIKEILHVSKSNTL
AT1G51805/1-504    105 TFVYGNYD-GHKDEPNFDLYLGPNLWATVS-----------RSE--TVEEIIHVTKSDSL
AT1G51830/1-315     10 ------------------------------------------------------------
AT1G51820/1-504    105 TFVYGNYD-GLNVGPNFNLYLGPNLWTTVS-----------SND--TIEEIILVTRSNSL
AT1G51850/1-486    105 TFVYGNYD-GLNVGPNFDLYFGPNLWTT-------------------------------
AT2G04300/1-480    111 SFVYGNYD-GLNKELEFDLYLGPNLWANVNTAVYLMNGV--TT-----EEIIHSTKSKVL
AT3G21340/1-520    112 VFVYGNYD-GLNNPPSFDLYLGPNLWVTVD-----MNGR--TNG--TIQEIIHKTISKSL
AT1G05700/1-507    107 SFMYGNYD-GENGSPEFDLFLGGNIWDTVL-----LS----NGSSIVSKEVVYLSQSENI
AT2G19210/1-516    112 RFMYGNYD-NLGKAPDFDLYLGFNIWDSVT-----ID----NATTIVTKEIIHTLRSDHV
AT2G19230/1-516    111 RFMYGNYD-NLGKAPDFDLYLGVNLWDSVT-----LE----NSTTIVTKEIIYTLRSDKV
AT2G19190/1-516    111 RFMYGNYD-DLGRVPEFDLYLGVNFWDSVK-----LD----DATTILNKEIITIPLLDNV
AT4G29990/1-511    108 RFMYGNYD-GFSKTPEFDLYIGANLWESVV-----LI----NETAIMTKEIIYTPPSDHI
AT4G29180/1-509    112 SFMYGNYD-GKNALPEFDLYVNVNFWTSVK-----LR----NASENVIKEILSFAESDTI
AT4G29450/1-513    111 SFMYGNYD-GKKALPEFDLYVNVNFWSTVK-----FK----NASDQVTKEILSFAESDTI
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LjSYMRK/1-509      151 DFCLLK--GEVYPFISQLELRPSPEE-YLQDFPTS--VLKLISRNNL-GDT----KDDIR
LjShRK1/1-522      145 SVCLSN-ATTGQPFISTLELRQFNGSVYYTDY-EEHFYLSVSARINFGAES----DAPIR
LjShRK2/1-506      158 HVCLFN-TGKGTPFISVLELRVLNSDAYLV---N---SLELLARFDVGLRD----GEIIR
ShRK1_(AT1/1-533   151 DVCVCC-AITGSPFMSTLELRPLNLSMYATDY-EDNFFLKVAARVNFGAPN----MDALR
ShRK2_(AT2/1-529   152 SVCLSN-ATTGQPFISTLELRQLSGSMYGSMLSEDRFYLSVAARINFGAES----EASVR
AT5G48740/1-492    144 LLCLLAVKGRGIPVISSLEVRPLPLGSYKYSLEG---SPDIILRRSYRINSGYT-NGTIR
AT1G51790/1-518    158 QVCLVN-TGNGTPFISVLELRQLPNSSYAAQS-E---SLQLFQRLDFGSTT----NLTVR
IOS1(AT1G5/1-513   156 QICLVK-TGNATPFISALELRKLMNTTYLTRQ-G---SLQTFIRADVGATV----NQGYR
AT1G51910/1-508    158 QVCLVK-TGQTTPFISSLELRPLNNDTYVTQG-G---SLMSFARIYF-PKT----AYFLR
AT1G51890/1-499    154 QICLVK-TGETTPFISSLELRPLNNNTYVTKS-G---SLIVVARLYF-SPT----PPFLR
AT1G51860/1-511    158 EVCLVK-TGPTTPFISSLEVRPLNNESYLTQS-G---SLMLFARVYFPSSS----SSFIR
AT1G51880/1-511    158 QICLVK-TGKGIPFISSLELRPLNNNTYLTQS-G---SLIGFARVFF-SAT----PTFIR
AT1G07550/1-504    158 DICLVK-TGTSTPMISSIELRPLLYDTYIAQT-G---SLRNYNRFYF-TDS----NNYIR
AT2G14440/1-502    161 DICLVK-TGTTTPMISAIELRPLRYDTYTART-G---SLKKILHFYF-TNS----GKEVR
AT2G14510/1-508    160 DICLVK-TGTSTPMISAIELRPLRYDTYTART-G---SLKSMAHFYF-TNS----DEAIR
AT3G46350/1-491    162 DVCLVK-TNTTIPFISALELRPLPSNSYITTA-G---SLRTFVRFCF-SNS----VEDIR
AT3G46340/1-513    162 DVCLVK-TDTSTPFLSLLELRPLDNDSYLTGS-G---SLKTFRRYYL-SNS----ESVIA
AT3G46370/1-427     71 DVCLIK-TGTTTPIISTLELRSLPKYSYNAIS-G---SLKSTLRAFL-SES----TEVIR
AT3G46400/1-508    159 DVCLIK-TGTSTPIISVLELRSLPNNTYITES-G---SLKSILRSYL-SVS----TKVIR
AT3G46330/1-516    160 QICLVK-TGATIPMISALELRPLANDTYIAKS-G---SLKYYFRMYL-SNA----TVLLR
AT5G59670/1-499    157 QICLVQ-TGETTPLISSLELRPMRTGSYTTVS-G---SLKTYRRLYF-KKS----GSRLR
AT5G59680/1-508    159 QVCLVK-TGETTPLISVLEVRPMGSGTYLTKS-G---SLKLYYREYF-SKS----DSSLR
AT5G59650/1-509    159 NVCLVK-TGTTTPLISALELRPLGNNSYLT-D-G---SLNLFVRIYL-NKT----DGFLR
AT5G16900/1-505    153 QICLVK-TGNSLPFISALELRLLRNDTYVVQD-V---SLKHLFRRYY-RQS----DRLIR
AT1G07560/1-512    159 QICLVK-TGTTTPLISALELRPLRNNTYIPQS-G---SLKTLFRVHL-TDS----KETVR
AT4G20450/1-530    163 DICLVK-TGTTTPFISSLELRPLRDDTYTTTT-G---SLKLISRWYF-RKPFPTLESIIR
AT2G28960/1-507    155 QICLVK-TGSTIPMISTLELRPLRNDSYLTQF-G---PLDLIYRRAYSSNS----TGFIR
AT2G29000/1-506    155 QICLVK-TGPTTPFISTLELRPLRNDNYITQS-G---SLKLMQRMCM-TET----VSTLR
AT2G28970/1-409     89 ----------------------------------SLRNSFRVHC-STS----DSEIR
AT2G28990/1-506    154 DICLVK-TGETLPIISAIEIRPLRNNTYVTQS-G---SLMMSFRVYL-SNS----DASIR
AT1G49100/1-518    159 DICLVK-TGTTTPMISTLELRPLRSDTYISAI-GS--SLLLYFRGYL-NDS----GVVLR
AT1G51810/1-384     24 QVCLVK-TGTSIPYINTLELRPLADDIYTNES-G---SLNYLFRVYY-SNL----KGYIE
AT1G51805/1-504    151 QVCLAK-TGDFIPFINILELRPLKKNVYVTES-G---SLKLLFRKYF-SDS----GQTIR
AT1G51830/1-315     10 ------------------------------------------------------------VR
AT1G51820/1-504    151 QVCLVK-TGISIPFINMLELRPMKKNMYVTQS-G---SLKYLFRGYI-SNS----STRIR
AT1G51850/1-486    132 -VCLIK-TGISIPFINVLELRPMKKNMYVTQG-E---SLNYLFRVYI-SNS----STRIR
AT2G04300/1-480    163 QVCLIK-TGESIPIINSLELRPLINDTYNTQS-G---SLKYLFRNYF-STS----RRIIR
AT3G21340/1-520    162 QVCLVK-TGTSSPMINTLELRPLKNNTYNTQS-G---SLKYFFRYYF-SGS----GQNIR
AT1G05700/1-507    157 FVCLGN-KGKGTPFISTLELRFLGNDTYDSPN-G---ALFFSRRWDLRSLM----GSPVR
AT2G19210/1-516    162 HVCLVD-KNRGTPFLSALEIRLLKSNTYETPY-D---SLILFKRWDLGGLG----ALPVR
AT2G19230/1-516    161 HVCLVD-KERGTPFLSVLELRLLKNNIYETAS-D---SLMLYRRWDLGATG----DLPAR
AT2G19190/1-516    161 QVCVVD-KNAGTPFLSVLEIRLLLNTTYETPY-D---ALTLLRRLDYSKTG----KLPSR
AT4G29990/1-511    158 HVCLVD-KNRGTPFLSVLEIRFLKNDTYDTPY-E---ALMLGRRWDFGTAT----NLQIR
AT4G29180/1-509    162 YVCLVN-KGKGTPFISALELRPMNSSIYGTEF-GRNVSLVLYQRWDT-GYL----NGTGR
AT4G29450/1-513    161 YVCLVN-KGKGTPFISGLELRPVNSSIYGTEF-GRNVSLVLYRRWDI-GYL----NGTGR
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LjSYMRK/1-509      201 FPVDQSDRIWKA-----------SSISSSAVPLSSNVSNVDLNANVTPPLTVLQTALT--
LjShRK1/1-522      199 YPDDPFDRIWESDSVKKANYLVDVAPGTTKISTKEPIDV---NRDEMPPGRVMQTAVVGT
LjShRK2/1-506      207 YPDDTFDRMWTP--------YNSIE--WKLMNTSLTIDQPSFNFLPLPPSIVSSTAAIPA
ShRK1_(AT1/1-533   205 YPDDPYDRIWESDINKRPNYLVGVAPGTTRINTSKTINT---LTREYPPMKVMQTAVVGT
ShRK2_(AT2/1-529   207 YPDDPYDRIWESDLQKKPNYLVDVAAGTVRVSTTLPIES---RVDDRPPQKVMQTAVVGT
AT5G48740/1-492    200 YPSDPFDRIWDPDQSYSPFHASWSFNGLTKLN--------SFNITENPPASVLKTARILA
AT1G51790/1-518    209 YPNDVFDRIWFP-------ATPNGTKPLSDPSTSLTSNS-TGN--FRLPQVVMRTGIVPD
IOS1(AT1G5/1-513   207 YGIDVFDRVWTP--------YNFGN--WSQISTNQSV---NINNDYQPPEIAMVTASVPT
AT1G51910/1-508    208 YSDDLYDRVWVP-------FSQ-NE--TVSLSTNLPVDT-SSN-SYNVPQNVANSAIIPA
AT1G51890/1-499    204 YDEDVHDRIWIP-------FLD-NK--NSLLSTELSVDT--SN-FYNVPQTVAKTAAVPL
AT1G51860/1-511    209 YDEDIHDRVWNS-------FTD-DE--TVWISTDLPIDT--SN-SYDMPQSVMKTAAVPK
AT1G51880/1-511    208 YDEDIHDRVWVR-------QFG-NG--LKSISTDLLVDT--SN-PYDVPQAVAKTACVPS
AT1G07550/1-504    208 YPQDVHDRIWVP-------LIL-PE--WTHINTSHHVID-SID-GYDPPQDVLRTGAMPA
AT2G14440/1-502    211 YPEDVYDRVWIP-------HSQ-PE--WTQINTTRNVSG-FSD-GYNPPQDVIKTASIPT
AT2G14510/1-508    210 YPEDVYDRVWMP-------YSQ-PE--WTQINTTRNVSG-FSD-GYNPPQGVIQTASIPT
AT3G46350/1-491    212 FPMDVHDRMWES-------YFD-DD--WTQISTSLTVNT-S-D-SFRLPQAALITAATPA
AT3G46340/1-513    212 YPEDVKDRIWEP-------TFD-SE--WKQIWTTLKPNN-S-N-GYLVPKNVLMTAAIPA
AT3G46370/1-427    121 YPNDFYDRMWVP-------HFE-TE--WKQISTNLKVNS-S-N-GYLLPQDVLMTAAIPV
AT3G46400/1-508    209 YPDDFYDRKWVP-------YFE-SE--WRQISTILKVNN-TIN-GFLAPQEVLMTAAVPS
AT3G46330/1-516    210 YPKDVYDRSWVP-------YIQ-PE--WNQISTTSNVSN-K-N-HYDPPQVALKMAATPT
AT5G59670/1-499    207 YSKDVYDRSWFP-------RFM-DE--WTQISTALGVIN-T-N-IYQPPEDALKNAATPT
AT5G59680/1-508    209 YPDDIYDRQWTS-------FFD-TE--WTQINTTSDVGN-S-N-DYKPPKVALTTAAIPT
AT5G59650/1-509    208 YPDDIYDRRWHN-------YFMVDD--WTQIFTTLEVTN-D-N-NYEPPKKALAAAATPS
AT5G16900/1-505    203 YPDDVYDRVWSP-------FFL-PE--WTQITTSLDVNN-S-N-NYEPPKAALTSAATPG
AT1G07560/1-512    209 YPEDVHDRLWSP-------FFM-PE--WRLLRTSLTVNT-SDDNGYDIPEDVVVTAATPA
AT4G20450/1-530    217 HPDDVHDRLWDV-------YHADEE--WTDINTTTPVNT-TVN-AFDLPQAIISKASIPQ
AT2G28960/1-507    206 YPDDIFDRKWDR-------YNE-FE--TD-VNTTLNVRS-S-S-PFQVPEAVSRMGITPE
AT2G29000/1-506    205 YPDDVYDRLWYT-------DGI-YE--TKAVKTALSVNS-T-N-PFELPQVIIRSAATPV
AT2G28970/1-409    107 YDDDSYDRVWYP-------FFS-SS--FSYITTSLNINN-S-D-TFEIPKAALKSAATPK
AT2G28990/1-506    204 YADDVHDRIWSP-------FNG-SS--HTHITTDLNINN-S-N-AYEIPKNILQTAAIPR
AT1G49100/1-518    210 YPDDVNDRRWFP--------FSYKE--WKIVTTTLNVNT-S-N-GFDLPQGAMASAATRV
AT1G51810/1-384     74 YPDDVHDRIWKQ-------ILPYQD--WQILTTNLQINV-S-N-DYDLPQRVMKTAVTPI
AT1G51805/1-504    201 YPDDIYDRVWHA-------SFLENN--WAQVSTTLGVNV-T-D-NYDLSQDVMATGATPL
AT1G51830/1-315     12 FPDDVYDRKWYP-------IF-QNS--WTQVTTNLNVNI-S-T-IYELPQSVMSTAATPL
AT1G51820/1-504    201 FPDDVYDRKWYP-------LF-DDS--WTQVTTNLKVNT-S-I-TYELPQSVMAKAATPI
AT1G51850/1-486    181 FPDDVYDRKWYP-------YF-DNS--WTQVTTTLDVNT-S-L-TYELPQSVMAKAATPI
AT2G04300/1-480    213 YPNDVNDRHWYP-------FFDEDA--WTELTTNLNVNS-S-N-GYDPPKFVMASASTPI
AT3G21340/1-520    212 YPDDVNDRKWYP-------FFDAKE--WTELTTNLNINS-S-N-GYAPPEVVMASASTPI
AT1G05700/1-507    208 YDDDVYDRIWIP--------RNFGY--CREINTSLPV-T-SDNNSYSLSSLVMSTAMTPI
AT2G19210/1-516    213 YKDDVFDRIWIP--------LRFPK--YTIFNASLTIDS-NNNEGFQPARFVMNTATSPE
AT2G19230/1-516    212 YKDDIFDRFWMP--------LMFPN--FLILNTSLMIDP-TSSNGFLPPSVVMSTAVAPM
AT2G19190/1-516    212 YKDDIYDRIWTP-------RIVSSE--YKILNTSLTVDQ-FLNNGYQPASTVMSTAETAR
AT4G29990/1-511    209 YKDDFYDRIWMP--------YKSPY--QKTLNTSLTIDE-TNHNGFRPASIVMRSAIAPG
AT4G29180/1-509    215 YQKDTYDRIWSP-------YSP-VS--WNTTMTTGYIDI-FQS-GYRPPDEVIKTAASPK
AT4G29450/1-513    214 YQDDRFDRIWSP-------YSSNIS--WNSIITSGYIDV-FQN-GYCPPDEVIKTAAAPE
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LjSYMRK/1-509      248 ----DPERLEFIHTDLETEDYGYRVFLYFLELDR-TLQAGQ-RVFDIY------------
LjShRK1/1-522      256 NGS------LTYRMNLDGFPGIGWAVCYFAEIE--DLPQNESRKFRLVLPGQPDISKAVV
LjShRK2/1-506      257 NVN-DN---IEFYYHPKYNASTYYMYMYFDEIK--KLQANQIREFDIF------------
ShRK1_(AT1/1-533   262 QGL------ISYRLNLEDFPANARAYAYFAEIE--ELGANETRKFKLVQPYFPDYSNAVV
ShRK2_(AT2/1-529   264 NGS------LTYRMNLDGFPGFGWAFTYFAEIE--DLAEDESRKFRLVLPEQPEYSKSVV
AT5G48740/1-492    252 RKE-S----LSYTLSLHTPGD-YYIILYFAGIL--SL----SPSFSVT------------
AT1G51790/1-518    259 NPR-GF---VDFGWIPDDPSLEFFFYLYFTELQQPNSGTVETREFVIL------------
IOS1(AT1G5/1-513   254 DPD-AA---MNISLVGVERTVQFYVFMHFAEIQ--ELKSNDTREFNIM------------
AT1G51910/1-508    256 EAT-HP---LNIWWDLQNINAPSYVYMHFAEIQ--NLKANDIREFNIT------------
AT1G51890/1-499    251 NAT-QP---LKINWSLDDITSQSYIYMHFAEIE--NLEANETREFNIT------------
AT1G51860/1-511    256 NAS-EP---WLLWWTLDENTAQSYVYMHFAEVQ--NLTANETREFNIT------------
AT1G51880/1-511    255 NAS-QP---LIFDWTLDNITSQSYVYMHFAEIQ--TLKDNDIREFNIT------------
AT1G07550/1-504    256 NAS-DP---MTITWNLKTATDQVYGYIYIAEIM--EVQANETREFEVV------------
AT2G14440/1-502    259 NVS-EP---LTFTWMSESSDDETYAYLYFAEIQ--QLKANETRQFKIL------------
AT2G14510/1-508    258 NGS-EP---LTFTWNLESSDDETYAYLFFAEIQ--QLKVNETREFKIL------------
AT3G46350/1-491    259 KDG-PSYIGITFSTSSE---ERFFIYLHFSEVQ--ALRANETREFNIS------------
AT3G46340/1-513    259 NDS-AP---FRFTEELDSPTDELYVYLHFSEVQ--SLQANESREFDIL------------
AT3G46370/1-427    168 NTS-AR---LSFTENLEFPHDELYLYFHFSEVQ--VLQANQSREFSIL------------
AT3G46400/1-508    257 NAS-VP---LSFTKDLEFPKDKLYFYFHFSEIQ--PLQANQSREFSIL------------
AT3G46330/1-516    257 NLD-AA---LTMVWRLENPDDQIYLYMHFSEIQ--VLKANDTREFDII------------
AT5G59670/1-499    254 DAS-AP---LTFKWNSEKLDVQYYFYAHYAEIQ--DLQANDTREFNIL------------
AT5G59680/1-508    256 NAS-AP---LTNEWSSVNPDEQYYVYAHFSEIQ--ELQANETREFNML------------
AT5G59650/1-509    256 NAS-AP---LTISWPPDNPGDQYYLYSHFSEIQ--DLQTNDTREFDIL------------
AT5G16900/1-505    250 DNG-TR---LTIIWTLDNPDEQIHLYVHFAELE--PVGENTTRTFYFV------------
AT1G07560/1-512    258 NVS-SP---LTISWNLETPDDLVYAYLHVAEIQ--SLRENDTREFNIS------------
AT4G20450/1-530    266 VAS-DT---WSTTWSIQNPDDDVHVYLHFAEIQ--ALKPSDTREFSIL------------
AT2G28960/1-507    252 NAS-LP---LRFYVSLDDDSDKVNVYFHFAEIQ--ALRGNETREFDIE------------
AT2G29000/1-506    252 NSS-EP---ITVEYGGYSSGDQVYLYLHFAEIQ--TLKASDNREFDIV------------
AT2G28970/1-409    154 NAS-AP---LIITWKPRPSNAEVYFYLHFAEIQ--TLAANETREFDIV------------
AT2G28990/1-506    251 NAS-AP---LIITWDPLPINAEVYLYMHFAEIQ--TLEANETRQFDVI------------
AT1G49100/1-518    257 NDN-GT---WEFPWSLEDSTTRFHIYLHFAELQ--TLLANETREFNVL------------
AT1G51810/1-384    122 KASTTT---MEFPWNLEPPTSQFYLFLHFAELQ--SLQANETREFNVV------------
AT1G51805/1-504    249 NDS-ET---LNITWNVEPPTTKVYSYMHFAELE--TLRANDTREFNVM------------
AT1G51830/1-315     59 NAN-AT---LNITWTIEPPTTPFYSYIHFAELQ--SLRANDTREFNVT------------
AT1G51820/1-504    248 KAN-DT---LNITWTVEPPTTQFYSYVHIAEIQ--ALRANETREFNVT------------
AT1G51850/1-486    228 KAN-DT---LNITWTVEPPTTKFYSYMHFAELQ--TLRANDAREFNVT------------
AT2G04300/1-480    261 SKN-AP---FNFTWSLIPSTAKFYSYMHFADIQ--TLQANETREFDMM------------
AT3G21340/1-520    260 STF-GT---WNFSWLLPSSTTQFYVYMHFAEIQ--TLRSLDTREFKVT------------
AT1G05700/1-507    256 NTT-RP---ITMTLENSDPNVRYFVYMHFAEVEDLSLKPNQTREFDIS------------
AT2G19210/1-516    262 DLS-QD---IIFSWEPKDPTWKYFVYMHFAEVV--ELPSNETREFKVL------------
AT2G19230/1-516    261 NSSIEQ---IMVYWEPRDPNWKFYIYIHFAEVE--KLPSNETREFSVF------------
AT2G19190/1-516    262 NES-LY---LTLSFRPPDPNAKFYVYMHFAEIE--VLKSNQTREFSIW------------
AT4G29990/1-511    258 NES-NP---LKFNWAPDDPRSKFYIYMHFAEVR--ELQRNETREFDIY------------
AT4G29180/1-509    263 SDD-EP---LELSWTSSDPDTRFYAYLYFAELE--NLKRNESREIKIF------------
AT4G29450/1-513    263 NVD-DP---LELFWTSDDPNVRFYAYLYFAELE--TLEKNETRKIKIL------------
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LjSYMRK/1-509      290 -VNSEIK---------KESFDVLAGGSNYRYDVLDISASGSLNV-TLVKASKS-EFGPLL
LjShRK1/1-522      308 NIEENALGKYRLYEPGYTNLSLPFVLSF-----------------RFGKTSDS-TRGPLL
LjShRK2/1-506      299 -VN-GKLFNNDPVNPVYL--KSLYYISAI--------AKPHLEL-WINRTSRS-TLPPLI
ShRK1_(AT1/1-533   314 NIAENANGSYTLYEPSYMNVTLDFVLTF-----------------SFGKTKDS-TQGPLL
ShRK2_(AT2/1-529   316 NIKENTQRPYRVYAPGYPNITLPFVLNF-----------------RFAKTADS-SRGPIL
AT5G48740/1-492    288 -INDEVK--QSDYTVTSSEAGTLYFTQKG--------------ISKLNITLRKIKFNPQV
AT1G51790/1-518    303 -LNGKSF--GEPLSLNYFRTLALFTSNPL--------KAESFQF-SLRQTQSS-SLPPLI
IOS1(AT1G5/1-513   296 -YN--NKHIYGPFRPLNFTTSSVFTPTEV-----VADANGQYIF-SLQRTGNS-TLPPLL
AT1G51910/1-508    298 -YNGGQVW-ESSIRPHNLSITTISSPTAL-----NS-SDGFFNF-TFTMTTTS-TLPPLI
AT1G51890/1-499    293 -YNGGENW-FSYFRPPKFRITTVYNPAAV-----SS-LDGNFNF-TFSMTGNS-THPPLI
AT1G51860/1-511    298 -YNGGLRW-FSYLRPPNLSISTIFNPRAV-----SS-SNGIFNF-TFAMTGNS-TLPPLL
AT1G51880/1-511    297 -YNGGQNV-YSYLRPEKFEISTLFDSKPL-----SS-PDGSFSL-SFTKTGNS-TLPPLI
AT1G07550/1-504    298 -VNNKVH--FDPFRPTRFEAQVMFNNVPL-----TC-EGGFCRL-QLIKTPKS-TLPPLM
AT2G14440/1-502    301 -VNGVYY--ID-YIPRKFEAETLITPAAL-----KC-GGGVCRV-QLSKTPKS-TLPPQM
AT2G14510/1-508    300 -ANGVDY--ID-YTPWKFEARTLSNPAPL-----KC-EGGVCRV-QLSKTPKS-TLPPLM
AT3G46350/1-491    301 -INGESV--ADLYRP--------------------------------LSRTQSS-THPPMI
AT3G46340/1-513    301 -WSGEVA--YEAFIPEYLNITTIQTNTPV-----TC-PGGKCNL-ELKRTKNS-THPPLI
AT3G46370/1-427    210 -WNGMVI--YPDFIPDYLGAATVYNPSPS-----LC-EVGKCLL-ELERTQKS-TLPPLL
AT3G46400/1-508    299 -WNGEII--IPTLSPKYLKASTLYSVSPF-----VC-EVGKCLL-ELKRTQNS-TLPPLL
AT3G46330/1-516    299 -LNGETIN-TRGVTPKYLEIMTWLTTNPR-----QC-NGGICRM-QLTKTQKS-TLPPLL
AT5G59670/1-499    296 -LNGQNLSVTGPEVPDKLSIKTFQSSSPI-----SC-NGWACNF-QLIRTKRS-TLPPLL
AT5G59680/1-508    298 -LNGKLF--FGPVVPPKLAISTILSVSPN-----TC-EGGECNL-QLIRTNRS-TLPPLL
AT5G59650/1-509    298 -WDGAVV--EEGFIPPKLGVTTIHNLSPV-----TC-KGENCIY-QLIKTSRS-TLPSLL
AT5G16900/1-505    292 -VNGKISY-DESITPLDLAVSTVETVVN------KC-DGGNCSL-QLVRSEASPVRVPLV
AT1G07560/1-512    300 -AGQDVN--YGPVSPDEFLVGTLFNTSPV-----KC-EGGTCHL-QLIKTPKS-TLPPLL
AT4G20450/1-530    308 -WNKNTII-RDYYSPLEFMADTVPIRTSS-----KCGDDGFCSL-DLTRTKSS-TLPPYC
AT2G28960/1-507    294 -LEEDII--QSAYSPTMLQSDTKYNLSPH-----KC-SSGLCYL-KLVRTPRS-TLPPLI
AT2G29000/1-506    294 -WANNIK--KLAYKPKVSQIDTLLNTSPN-----KC-DNTFCKA-FLVRTQRS-TLPPLL
AT2G28970/1-409    196 -FKGNFN--YSAFSPTKLELLTFFTSGPV-----QC-DSDGCNL-QLVRTPNS-TLPPLI
AT2G28990/1-506    293 -LRGNFN--HSGFSPTKLKVFTLYTEEPM-----KC-GSEGCYL-QLVKTPNS-TLPPLI
AT1G49100/1-518    299 -LNGKVY--YGPYSPKMLSIDTM---SPQPDSTLTC-KGGSCLL-QLVKTTKS-TLPPLI
AT1G51810/1-384    165 -LNGNVT--FKSYSPKFLEMQTVYSTAPK-----QC-DGGKCLL-QLVKTSRS-TLPPLI
AT1G51805/1-504    291 -LNGNDL--FGPYSPIPLKTETETNLKPE-----EC-EDGACIL-QLVKTSKS-TLPPLL
AT1G51830/1-315    101 -LNGEYT--IGPYSPKPLKTETIQDLSPE-----QC-NGGACIL-QLVETLKS-TLPPLL
AT1G51820/1-504    290 -LNGEYT--FGPFSPIPLKTASIVDLSPG-----QC-DGGRCIL-QVVKTLKS-TLPPLL
AT1G51850/1-486    270 -MNGIYT--YGPYSPKPLKTETIYDKIPE-----QC-DGGACLL-QVVKTLKS-TLPPLL
AT2G04300/1-480    303 -LNGNLA--LER-------------------------------------------------
AT3G21340/1-520    302 -LNGKLA--YERYSPKTLATETIFYSTPQ-----QC-EDGTCLL-ELTKTPKS-TLPPLM
AT1G05700/1-507    300 -IN--GVTVAAGFSPKYLQTNTFFL-NPE--------SQSKIAF-SLVRTPKS-TLPPIV
AT2G19210/1-516    304 -LNEKEINMSS-FSPRYLYTDTLFVQNPV--------SGPKLEF-RLQQTPRS-TLPPII
AT2G19230/1-516    304 -LNKEQIDTTSVFRPSYLYTDTLYVQNPV--------SGPFLEF-VLRQGVKS-TRPPIM
AT2G19190/1-516    304 -LN--EDVISPSFKLRYLLTDTFVTPDPV--------SGITINFSLLQPPGEF-VLPPII
AT4G29990/1-511    300 -IN--DVILAENFRPFYLFTDTRSTVDPV--------GRKMNEI-VLQRTGVS-TLPPII
AT4G29180/1-509    305 -WNGSPVS--GAFNPSPEYSMTVSNSRAF--------TGKDHWI-SVQKTAES-TRPPIL
AT4G29450/1-513    305 -WNGSPVS-ETSFEPSSKYSTTFSNPRAF--------TGKDHWI-SIQKTVDS-TLPPIL
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LjSYMRK/1-509      338 NAYEILQVRPWIEE-TNQTDVGVIQKMREELLLQNSESWS---GDPCI--LLPWKGIACD
LjShRK1/1-522      350 NAMEINKYLE---KNGGSPDGEAISSVLSHY---SSADWAQEGGDPCLPVPWSW--IRCS
LjShRK2/1-506      345 NAIEIYMTKDFLQSQTYQTDADAIINVKSIYGI-KR-NWQ---GDPCIPLAYLWDGLNCS
ShRK1_(AT1/1-533   356 NAIEISKYLPISVKTDRS-DVSVLDAIRSMS---PDSDWASEGGDPCIPVLWSW--VNCS
ShRK2_(AT2/1-529   358 NAMEISKYLR---KSDGSVDATVMANVASLY---SSTEWAQEGGDPCSPSPWSW--VQCN
AT5G48740/1-492    331 SALEVYEILQIPPE-ASSTTVSALKVIEQFTG--QDLGWQ---DDPCTP--LPWNHIECE
AT1G51790/1-518    350 NAMETYFVNKLPQSSTDPNDLSAMRNIKSAYKV-KR-NWE---GDVCVPQAYTWEGLNCS
IOS1(AT1G5/1-513   346 NAMEIYSVNLLPQQETDRKEVDAMMNIKSAYGV-NKIDWE---GDPCVPLDYKWSGVNCT
AT1G51910/1-508    348 NALEVYTLVENLLLETYQDEVSAMMNIKKTYGLSKKISWQ---GDPCSPQIYRWEGLNCL
AT1G51890/1-499    343 NGLEIYQVLELPQLDTYQDEVSAMMNIKTIYGLSKRSSWQ---GDPCAPELYRWEGLNCS
AT1G51860/1-511    348 NALEIYTVVDILQLETNKDEVSAMMNIKETYGLSKKISWQ---GDPCAPQLYRWEGLNCS
AT1G51880/1-511    347 NGLEIYKVLDLLELETDQDEVSAMINIKATYDLSKKVSWQ---GDPCAPKSYQWEGLNCS
AT1G07550/1-504    347 NAFEIFTGIEFPQSETNQNDVIAVKNIQASYGL-NRISWQ---GDPCVPKQFLWTGLSCN
AT2G14440/1-502    349 NAIEIFSVIQFPQSDTNTDEVIAIKNIQSTYKV-SRISWQ---GDPCVPIQFSWMGVSCN
AT2G14510/1-508    348 NAIEIFSVIQFPQSDTNTDEVIAIKKIQSTYQL-SRISWQ---GDPCVPKQFSWMGVSCN
AT3G46350/1-491    326 NAIEIFLVSELLQSETYENDVIAIKKIKDTYGL-QLISWQ---GDPCVPRLYKWDGLDCT
AT3G46340/1-513    350 NAIEFYTVVNFPQLETNETDVVAIKDIKATYEL-NRITWQ---GDPCVPQKFIWEGLDCN
AT3G46370/1-427    259 NAIEVFTVMNFPQSETNDDDVIAITKIKDTHRL-NRTSWQ---GDPCVPQLFSWAGLSCI
AT3G46400/1-508    348 TAIEVFTVIDFPQSKTNEDDVSAIKNIKDTHGL-SRVSWQ---GDPCVPRQFLWEGLSCN
AT3G46330/1-516    349 NAFEVYSVLQLPQSQTNEIEVVAIKNIRTTYGL-SRISWQ---GDPCVPKQFLWDGLNCN
AT5G59670/1-499    347 NALEVYTVIQFPRSETDESDVVAMKNISASYGL-SRINWQ---GDPCFPQQLRWDALDCT
AT5G59680/1-508    347 NAYEVYKVIQFPQLETNETDVSAVKNIQATYEL-SRINWQ---SDPCVPQQFMWDGLNCS
AT5G59650/1-509    347 NALEIYTVIQFPRNQLSSTSVVAVKNIEAAYKL-SRIRWQ---GDPCVPQKYAWDGLNCS
AT5G16900/1-505    342 NAMEAFTAIKFPHSETNPDDVISIKVIQATYEL-SRVDWQ---GDPCLPQQFLWTGLNCS
AT1G07560/1-512    349 NAIEAFITVEFPQSETNANDVLAIKSIETSYGL-SRISWQ---GDPCVPQQLLWDGLTCE
AT4G20450/1-530    359 NAMEVFGLLQLLQTETDENDVTTLKNIQATYRI-QKTNWQ---GDPCVPIQFIWTGLNCS
AT2G28960/1-507    343 SAIEAFKVVDFPYAETNPNDVAAMKDIEAFYGL-KMISWQ---GDPCVPELLKWEDLKCS
AT2G29000/1-506    343 NAYEVYILVEFPYSETHPDDVVAIKKIKAAYGL-KIISWQ---GDPCLPREYKWEYIECS
AT2G28970/1-409    245 NALEAYTIIEFPQLETSLSDVNAIKNIKATYRL-SKTSWQ---GDPCLPQELSWENLRCS
AT2G28990/1-506    342 NAIEAYSVIEFSQLETSLSDVDAIKNIKNTYKL-NKITWQ---GDPCLPQDLSWESIRCT
AT1G49100/1-518    350 NAIELFTVVEFPQSETNQDEVIAIKKIQLTYGL-SRINWQ---GDPCVPEQFLWAGLKCS
AT1G51810/1-384    214 NAMEAYTVLDFPQIETNVDEVIAIKNIQSTYGL-SKTTWQ---GDPCVPKKFLWDGLNCN
AT1G51805/1-504    340 NAIEAFTVIDFLQVETDEDDAAAIKNVQNAYGLINRSSWQ---GDPCVPKQYSWDGLKCS
AT1G51830/1-315    150 NAIEAFTVIDFPQMETNEDDVTGINDVQNTYGL-NRISWQ---GDPCVPKQYSWDGLNCN
AT1G51820/1-504    339 NAIEAFTVIDFPQMETNENDVAGIKNVQGTYGL-SRISWQ---GDPCVPKQLLWDGLNCK
AT1G51850/1-486    319 NAIEAFTVIDFPQMETNGDDVDAIKNVQDTYGI-SRISWQ---GDPCVPKLFLWDGLNCN
AT2G04300/1-480    312 -ALEVFTVIDFPELETNQDDVIAIKNIQNTYGV-SKTSWQ---GDPCVPKRFMWDGLNCN
AT3G21340/1-520    351 NALEVFTVIDFPQMETNPDDVAAIKSIQSTYGL-SKISWQ---GDPCVPKQFLWEGLNCN
AT1G05700/1-507    346 NALEIYVANSFSQSLTNQEDGDAVTSLKTSYKV-KK-NWH---GDPCLPNDYIWEGLNCS
AT2G19210/1-516    352 NAIETYRVNEFLQSPTDQQDVDAIMRIKSKYGV-KK-SWL---GDPCAPVKYPWKDINCS
AT2G19230/1-516    353 NAIETYRTNEFLDLPTDQNDVDAIMKIKTKYKV-KK-NWL---GDPCAPFGYPWQGINCS
AT2G19190/1-516    352 NALEVYQVNEFLQIPTHPQDVDAMRKIKATYRV-KK-NWQ---GDPCVPVDYSWEGIDCI
AT4G29990/1-511    347 NAIEIYQINEFLQLPTDQQDVDAMTKIKFKYRV-KK-NWQ---GDPCVPVDNSWEGLECL
AT4G29180/1-509    352 NAIEIFSAQSLDEFYTRIDDVQAIESIKSTYKV-NKI-WT---GDPCSPRLFPWEGIGCS
AT4G29450/1-513    353 NAIEIFTAQSLDEFSTTIEDIHAIESIKATYKV-NKV-WS---GDPCSPRLFPWEGVGCS
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LjSYMRK/1-509      392 --GSNGSSVITKLDLSSSNLKGLIPSSIAEMTNLETLNISHNSFDG-SVPSFLSSL--LI
LjShRK1/1-522      402 ---SDIQPRIVSILLSSKNLTGNIPLDITKLTGLVELWLDGNMLTG-PIPDF-TGCMDLK
LjShRK2/1-506      400 YAESD-SPRIIYLNLSSSGLIGNIAPSISNMKSIEYLDLSNNNLTG-ALPDFLSQLRFLR
ShRK1_(AT1/1-533   410 ---STSPPRVTKIALSRKNLRGEIPPGINYMEALTELWLDDNELTG-TLPDM-SKLVNLK
ShRK2_(AT2/1-529   410 ---SDPQPRVVAIKLSSMNLTGNIPSDLVKLTGLVELWLDGNSFTG-PIPDF-SRCPNLE
AT5G48740/1-492    383 ------GNRVTSLDLHNTSLTGAIQSELEDLVNLEVLDLQNNSLQG-SVPETLGKLKKLR
AT1G51790/1-518    405 -FNGTNMPRVIALNLSSAGLTGEITSDISRLSQLQILDLSNNNLSGPAVPAFLAQLQFLR
IOS1(AT1G5/1-513   402 YVDNE-TPKIISLDLSTSGLTGEILEFISDLTSLEVLDLSNNSLTG-SVPEFLANMETLK
AT1G51910/1-508    405 YLD-SDQPLITSLNLRTSGLTGIITHDISNLIQLRELDLSDNDLSG-EIPDFLADMKMLT
AT1G51890/1-499    400 YPN-FAPPQIISLNLSGSNLSGTITSDISKLTHLRELDLSNNDLSG-DIPFVFSDMKNLT
AT1G51860/1-511    405 YPD-SEGSRIISLNLNGSELTGSITSDISKLTLLTVLDLSNNDLSG-DIPTFFAEMKSLK
AT1G51880/1-511    404 YPN-SDQPRIISLNLAENKLTGTITPEISKLTQLIELDLSKNDLSG-EIPEFFADMKLLK
AT1G07550/1-504    403 VIDVSTPPRIVKLDLSSSGLNGVIPPSIQNLTQLQELDLSQNNLTG-KVPEFLAKMKYLL
AT2G14440/1-502    405 VIDISTPPRIISLDLSSSGLTGVITPSIQNLTMLRELDLSNNNLTG-EVPEFLATIKPLL
AT2G14510/1-508    404 VIDISTPPRIISLDLSLSGLTGVISPSIQNLTMLRELDLSNNNLTG-EVPEFLATIKPLL
AT3G46350/1-491    382 DTDTYIAPRITSLKLSSKGLTGTIAADIQYLTSLEKLDLSDNKLVG-VVPEFLANMKSLM
AT3G46340/1-513    406 SKDALTLPRITSLNLSSTGLTGNIAAGIQNLTHLDKLDLSNNNLTG-GVPEFLASMKSLS
AT3G46370/1-427    315 DTNVSTPPRIISLNLSSSGLTGNIATGIQNLTKLQKLDLSNNNLTG-VVPEFLANMKSLL
AT3G46400/1-508    404 DKNVSASPRITSLNLSSSGLVGTIPSGIQNFTLLEKLDLSNNNLTG-LVPEFLAKMETLL
AT3G46330/1-516    405 ITDISAPPRIISLNLSSSGLSGTIVSNFQNLAHLESLDLSNNSLSG-IVPEFLATMKSLL
AT5G59670/1-499    403 NRNISQPPRITSLNLSSSRLNGTIAAAIQSITQLETLDLSYNNLTG-EVPEFLGKMKSLS
AT5G59680/1-508    403 ITDITTPPRITTLNLSSSGLTGTITAAIQNLTTLEKLDLSNNNLTG-EVPEFLSNMKSLL
AT5G59650/1-509    403 NTDVSKPPRVLSLNLSSSGLTGIIAAAIQNLTHLEKLDLSNNTLTG-VVPEFLAQMKSLV
AT5G16900/1-505    398 YMNMSTSPRIISLDLSSHKLTGKIVPDIQNLTQLQKLDLSNNKLTG-GVPEFLANMKSLL
AT1G07560/1-512    405 YTNMSTPPRIHSLDLSSSELTGIIVPEIQNLTELKKLDFSNNNLTG-GVPEFLAKMKSLL
AT4G20450/1-530    415 NMFPSIPPRITSIDFSNFGLNGTITSDIQYLNQLQKLDLSNNNLTG-KVPEFLAKMKLLT
AT2G28960/1-507    399 YTNKSTPPRIISLDLSSRGLKGVIAPAFQNLTELRKLDLSNNSFTG-GVPEFLASMKSLS
AT2G29000/1-506    399 YTNNSIPPRIISLDLSNRGLKGIIEPVLQNLTQLEKLDLSINRLSG-EVPEFLANMKSLS
AT2G28970/1-409    301 YTNSSTPPKIISLNLSASGLTGSLPSVFQNLTQIQELDLSNNSLTG-LVPSFLANIKSLS
AT2G28990/1-506    398 YVDGSTSPTIISLDLSKSGLNGSIPQILQNFTQLQELDLSNNSLTG-PVPIFLANMKTLS
AT1G49100/1-518    406 NINSSTPPTITFLNLSSSGLTGIISPSIQNLTHLQELDLSNNDLTG-DVPEFLADIKSLL
AT1G51810/1-384    270 NSDDSTPPIITSLNLSSSGLTGIIVLTIQNLANLQELDLSNNNLSG-GVPEFLADMKSLL
AT1G51805/1-504    397 YSD-STPPIINFLDLSASGLTGIIAPAIQNLTHLEILALSNNNLTG-EVPEFLADLKSIM
AT1G51830/1-315    206 NSDISIPPIIISLDLSSSGLNGVITQGIQNLTHLQYLDLSDNNLTG-DIPKFLADIQSLL
AT1G51820/1-504    395 NSDISTPPIITSLDLSSSGLTGIITQAIKNLTHLQILDLSDNNLTG-EVPEFLADIKSLL
AT1G51850/1-486    375 NSDNSTSPIITSLDLSSSGLTGSITQAIQNLTNLQELDLSDNNLTG-EIPDFLGDIKSLL
AT2G04300/1-480    367 NSYISTPPTITFLNLSSSHLTGIIASAIQNLTHLQNLDLSNNNLTG-GVPEFLAGLKSLL
AT3G21340/1-520    407 NLDNSTPPIVTSLNLSSSHLTGIIAQGIQNLTHLQELDLSNNNLTG-GIPEFLADIKSLL
AT1G05700/1-507    401 Y-DSLTPPRITSLNLSSSGLTGHISSSFSNLTMIQELDLSNNGLTG-DIPEFLSKLKFLR
AT2G19210/1-516    407 YVDNE-SPRIISVNLSSSGLTGEIDAAFSNLTLLHILDLSNNSLTG-KIPDFLGNLHNLT
AT2G19230/1-516    408 YTANN-PPRIISVNLSFSGLTGQIDPVFITLTPLQKLDLSNNRLTG-TVPDFLANLPDLT
AT2G19190/1-516    407 QSDNTTNPRVVSLNISFSELRGQIDPAFSNLTSIRKLDLSGNTLTG-EIPAFLANLPNLT
AT4G29990/1-511    402 HSDNNTSPKSIALNLSSSGLTGQIDPAFANLTSINKLDLSNNSLTG-KVPDFLASLPNLT
AT4G29180/1-509    407 --YNTSSYQIKSLNLSSSGLHGPIAFAFRNLSLLESLDLSNNNLKG-IVPEFLADLKYLK
AT4G29450/1-513    408 --DNNNNHQIKSLNLSSSGLLGPIVLAFRNLSLLESLDLSNNDLQQ-NVPEFLADLKHLK
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LjSYMRK/1-509      447 SVDLSYN-DLM-GKLPESIVKL---PHLKSLYFGCNEHMSPEDPANMNSSLINTDYGRCK
LjShRK1/1-522      457 IIHLENN-QFS-GALPTSLVNL---PKLRELWVQNNMLSGTVPSDLLSKDLVLNYSGNVK
LjShRK2/1-506      458 VLNLEGN-QLS-GTIPMPLTVRSKNDLL-ESNFGGN------------PDLCSPGSCN--
ShRK1_(AT1/1-533   465 IMHLENN-QLS-GSLPPYLAHL---PNLQELSIENNSFKGKIPSALLKGKVLFKYNNNPE
ShRK2_(AT2/1-529   465 IIHLENN-RLT-GKIPSSLTKL---PNLKELYLQNNVLTGTIPSD-LAKDVISNFSGNLN
AT5G48740/1-492    436 LLNLENN-NLV-GPLPQSL----NITGL-EVRITGN------------PCLSFSSSCNNV
AT1G51790/1-518    464 VLHLANN-QLS-GPIPSSLIER-------LDSFSGN------------PSICSANACEEV
IOS1(AT1G5/1-513   460 LINLSGN-ELN-GSIPATLLDKERRGSI-TLSIEGN------------TGLCSSTSCA--
AT1G51910/1-508    463 LVNLKGNPKLN-LTVPDSIKHRINNKSL-KLIIDEN----------------Q----SSE
AT1G51890/1-499    458 LINLSGNKNLN-RSVPETLQKRIDNKSL-TLIRDE----------------------TGK
AT1G51860/1-511    463 LINLSGNPNLNLTAIPDSLQQRVNSKSL-TLILGEN----------------L--TLTPK
AT1G51880/1-511    462 LINLSGNLGLN-STIPDSIQQRLDSKSL-ILILSKT----------------VTKTVTLK
AT1G07550/1-504    462 VINLSGN-KLS-GLVPQALLDRKK-EGL-KLLV--D------------ENMICVSCGTRF
AT2G14440/1-502    464 VIHLRGN-NLR-GSVPQALQDRENNDGL-KLL----------------------RGKHQ
AT2G14510/1-508    463 VIHLRGN-NLR-GSVPQALQDREKNDGL-KLFV--D------------PNI--TRRGKHQ
AT3G46350/1-491    441 FINLTKN-DLH-GSIPQALRDREK-KGL-KILFDGDK-----------NDPCLSTSCN--
AT3G46340/1-513    465 FINLSKN-NLN-GSIPQALLKREK-DGL-KLSVDE-------------QIRCFPGSCV--
AT3G46370/1-427    374 FIDLRKN-KLN-GSIPKTLLDRKK-KGL-QLFVDGDDDK-------GDDNKCLSGSCV--
AT3G46400/1-508    463 FIDLRKN-KLN-GSIPNTLRDREK-KGL-QIFVDG-------------DNTCL--SCV--
AT3G46330/1-516    464 VINLSGN-KLS-GAIPQALRDRER-EGL-KLNVLGN------------KELCLSSTCIDK
AT5G59670/1-499    462 VINLSGN-NLN-GSIPQALR---K-KRL-KLYLEGN------------PRL-IKPPKK--
AT5G59680/1-508    462 VINLSGN-DLN-GTIPQSLQ---R-KGL-ELLYQGN------------PRL-ISPGSTET
AT5G59650/1-509    462 IINLSGN-NLS-GPLPQGLR---R-EGL-ELLVQGN------------PRLCLSGSCTEK
AT5G16900/1-505    457 FINLSNN-NLV-GSIPQALLDR---KNL-KLEFEGN------------PKLCATGPCNSS
AT1G07560/1-512    464 VINLSGN-NLS-GSVPQALLNKVK-NGL-KLNIQGN------------PNLCFSSSCNKK
AT4G20450/1-530    474 FINLSGN-NLS-GSIPQSLLNMEK-NGLITLLYNGN-------------NLCLDPSCESE
AT2G28960/1-507    458 IINLNWN-DLT-GPLPKLLLDREK-NGL-KLTIQGN------------PKLCNDASCKNN
AT2G29000/1-506    458 NINLSWN-NLK-GLIPPALEEKRK-NGL-KLNTQGN------------QNLCPGDECKRS
AT2G28970/1-409    360 LLDLSGN-NFT-GSVPQTLLDREK-EGL-VLKLEGN------------PELCKFSSCNPK
AT2G28990/1-506    457 LINLSGN-NLS-GSVPQALLDKEK-EGL-VLKLEGN------------PDLCKSSFCNTE
AT1G49100/1-518    465 IINLSGN-NFS-GQLPQKLIDK---KRL-KLNVEGNP-----------KLLCTKGPCGNK
AT1G51810/1-384    329 VINLSGN-NLS-GVVPQKLIEK---KML-KLNIEGNP-----------KLNCTVESCVNK
AT1G51805/1-504    455 VIDLRGN-NLS-GPVPASLLQK---KGL-MLHLDDNP-----------HILCTTGSCMHK
AT1G51830/1-315    265 VINLSGN-NLT-GSVPLSLLQK---KGL-KLNVEGNP-----------HLLCTDGLCVNK
AT1G51820/1-504    454 VINLSGN-NLS-GSVPPSLLQK---KGM-KLNVEGNP-----------HILCTTGSCVKK
AT1G51850/1-486    434 VINLSGN-NLS-GSVPPSLLQK---KGM-KLNVEGNP-----------HLLCTADSCVKK
AT2G04300/1-480    426 VINLSGN-NLS-GSVPQTLLQK---KGL-KLNLEGNI-----------YLNCPDGSCVSK
AT3G21340/1-520    466 VINLSGN-NFN-GSIPQILLQK---KGL-KLILEGNA-----------NLICPDGLCVNK
AT1G05700/1-507    459 VLNLENN-TLT-GSVPSELLERSNTGSF-SLRLGEN------------PGLCTEISCR--
AT2G19210/1-516    465 ELNLEGN-KLS-GAIPVKLLERSNKKLI-LLRIDGN------------PDLCVSASCQIS
AT2G19230/1-516    466 ELNLEEN-KLT-GILPEKLLERSKDGSL-SLRVGGN------------PDLCVSDSCR--
AT2G19190/1-516    466 ELNVEGN-KLT-GIVPQRLHERSKNGSL-SLRFGRN------------PDLCLSDSCS--
AT4G29990/1-511    461 ELNLEGN-KLT-GSIPAKLLEKSKDGSL-SLRFGGN------------PDLCQSPSCQ--
AT4G29180/1-509    464 SLNLKGN-NLT-GFIPRSLRKRATANGL-ALSVDE-------------QNICHSRSCRDG
AT4G29450/1-513    465 VLNLKGN-NFT-GFIPKSLMKKLKAGLL-TLSADE-------------QNLCN--SCQEK
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LjSYMRK/1-509     502 GKESRFGQ--------
LjShRK1/1-522     512 LHKGSRRKSHM-----
LjShRK2/1-506     501 QKNGNK----------
ShRK1_(AT1/1-533  520 LQNEAQRKHFWQIL—
ShRK2_(AT2/1-529  519 LEKSGDKGKKL-----
AT5G48740/1-492   477 SSTIDTPQVTIPINKK
AT1G51790/1-518   503 SQNRSKKNKLPSFVIP
IOS1(AT1G5/1-513  503 --TTKKKKKNTVI---
AT1G51910/1-508   501 KHGIKFPL--------
AT1G51890/1-499   494 NSTNVV----------
AT1G51860/1-511   504 KESKKVPM--------
AT1G51880/1-511   504 GKSKKVPM--------
AT1G07550/1-504   504 P---------------
AT2G14440/1-502   498 PKSWL-----------
AT2G14510/1-508   504 PKSWL-----------
AT3G46350/1-491   484 -PKKKFSVM-------
AT3G46340/1-513   506 ITKKKFPV--------
AT3G46370/1-427   421 -PKMKFPL--------
AT3G46400/1-508   502 -PKNKFPM--------
AT3G46330/1-516   508 PKKKVAVKV-------
AT5G59670/1-499       ---------------
AT5G59680/1-508   502 KSGKSFP---------
AT5G59650/1-509   503 NSKKKFP---------
AT5G16900/1-505   499 SGNKETT---------
AT1G07560/1-512   508 KNSIM-----------
AT4G20450/1-530   518 TGPGNNKKKLLVP---
AT2G28960/1-507   502 NNQTYI----------
AT2G29000/1-506   502 IPKFP-----------
AT2G28970/1-409   404 KKKGLL----------
AT2G28990/1-506   501 KKNKFL----------
AT1G49100/1-518   508 PGEGGHPKKSI-----
AT1G51810/1-384   372 DEEGGRQIKSMTI---
AT1G51805/1-504   498 G-EGEKKS--------
AT1G51830/1-315   308 G-DGHKKKS-------
AT1G51820/1-504   497 KEDGHKKK--------
AT1G51850/1-486   477 GEDGHKKKSV------
AT2G04300/1-480   469 DGNGGAKKKNVV----
AT3G21340/1-520   509 AGNGGAKKMNVV----
AT1G05700/1-507   502 KSNSKK----------
AT2G19210/1-516   510 DEKTKKN---------
AT2G19230/1-516   509 NKKTERKE--------
AT2G19190/1-516   509 --NTKKKNKN------
AT4G29990/1-511   504 -TTTKKKIG-------
AT4G29180/1-509   508 NR--------------
AT4G29450/1-513   507 KKKKSMV---------
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Appendix 3: Host-related metabolic cues affect colonization strategies of a root endophyte

Lahrmann U, Ding Y, **Banhara A**, Rath M, Hajirezaei MR, Dohlemann S, Von Wiren N, Parniske M & Zuccaro A (2013) Host-related metabolic cues affect colonization strategies of a root endophyte. *Proc Natl Acad Sci U S A* **110**:13965-70. doi: 10.1073/pnas.1301653110.

Contributions by Aline Banhara**:**

- Designed, performed, and analysed all experiments concerning growth promotion of *A. thaliana* by *Piriformospora indica* on plants grown on a modified Hoagland's medium;
- Analysed results of *P. indica* on biomass of plants grown on 1/10 PNM medium;
- Prepared the corresponding Figure S4.

Host-related metabolic cues affect colonization strategies of a root endophyte

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The mechanisms underpinning broad compatibility in root symbiosis are largely unexplored. The generalist root endophyte *Piriformospora indica* establishes long-lasting interactions with morphologically and biochemically different hosts, stimulating their growth, alleviating salt stress, and inducing local and systemic resistance to pathogens. Cytological studies and global investigations of fungal transcriptional responses to colonization of barley and *Arabidopsis* at different symbiotic stages identified host-dependent colonization strategies and host-specifically induced effector candidates. Here, we show that in *Arabidopsis*, *P. indica* establishes and maintains biotrophic nutrition within living epidermal cells, whereas in barley the symbiont undergoes a nutritional switch to saprotrophy that is associated with the production of secondary thinner hyphae in dead cortex cells. Consistent with a diversified trophic behavior and with the occurrence of nitrogen deficiency at the onset of saprotrophy in barley, fungal genes encoding hydrolytic enzymes and nutrient transporters were highly induced in this host but not in *Arabidopsis*. Silencing of the high-affinity ammonium transporter *PiAMT1* gene, whose transcripts are accumulating during nitrogen starvation and in barley, resulted in enhanced colonization of this host, whereas it had no effect on the colonization of *Arabidopsis*. Increased levels of free amino acids and reduced enzymatic activity for the cell-death marker VPE (vacuolar-processing enzyme) in colonized barley roots coincided with an extended biotrophic lifestyle of *P. indica* upon silencing of *PiAMT1*. This suggests that PiAmt1 functions as a nitrogen sensor mediating the signal that triggers the *in planta* activation of the saprotrophic program. Thus, host-related metabolic cues affect the expression of *P. indica*'s alternative lifestyles.

root cortical cell death | RCD | broad-host range | biotrophy | mutualism

Upon plant colonization, fungi adopt different strategies to gain access to host nutrients. Whereas necrotrophs kill plant cells with subsequent saprotrophic nutrition, other fungi maintain biotrophic relationships with their hosts either transiently (hemibiotrophs) or as lifelong interactions. The degree of specialization to a particular host and the host's metabolic status may greatly influence plant colonization (1–4). Broad-host range root endophytes undergo long-term interactions with a large variety of plants, thereby playing a significant role in natural and managed ecosystems and in the evolution of land plants. To establish and maintain a compatible interaction with different hosts, these endophytes must respond and adapt to host-specific signals. Alternative lifestyles and colonization strategies in different host species thus may be a consequence of this adaptation to highly variable host environments. In this study, we addressed the question of whether endophytes adopt different strategies during colonization of distinct hosts or whether their success resides in a general colonization strategy. An interesting system to explore this issue is the mutualistic root endophyte *Piriformospora indica* (Basidiomycota, Sebacinales), with its large number of plant hosts. Among others, this generalist can establish a mutualistic interaction with roots of the agriculturally important monocot barley (*Hordeum vulgare*) and the dicot model plant *Arabidopsis thaliana* (5, 6), two biochemically and morphologically distinct plants. Cytological studies in both hosts have shown that *P. indica* has a biphasic colonization strategy (7–10). Initial root cell invasions are biotrophic where colonized host cells maintain membrane integrity and invasive hyphae of *P. indica* remain enveloped by the host plasma membrane and thus are not accessible to cell wall stains such as wheat germ agglutinin–Alexa Fluor 488 (WGA-AF488) conjugate (7, 8, 11). Later, *P. indica* is found more often in dead or dying host cells (9, 10), especially in the root cortex of barley. Colonization at later stages was shown to be reduced by overexpression of the negative cell death regulator BAX inhibitor 1 in barley and to be mediated by an endoplasmic reticulum stress-triggered caspase-dependent cell death in *Arabidopsis* (9, 10). Questions arise as to what extent hemibiotrophy in a root endophyte reflects a general colonization strategy of the symbiont to benefit from different plants and how the symbiotic lifestyle is influenced by the hosts. Our study reveals that broad compatibility in a root endophyte is associated with phenotypic plasticity of the symbiont and with the expression of alternative lifestyle strategies in a host-dependent way.

Results

Expression Patterns of *P. indica* Genes, Including Those Encoding Small Secreted Proteins, Support a Diversified Colonization Strategy for Barley and *Arabidopsis*. We hypothesized that successful root colonization of different hosts would require host-related colonization strategies, and we studied these differences by a global characterization of fungal transcriptional responses to barley and *Arabidopsis* at different developmental stages. A customized Agilent microarray was designed to monitor *P. indica*'s gene expression during root colonization of plants grown on sugar-free minimal medium (PNM) at 3 (early biotrophic phase) and 14 (late saprotrophic phase) days post inoculation (dpi). Fungal material grown on PNM was used as a control. From the 11,463 *P. indica* genes represented on the microarray chip, about 2,400 genes were differentially regulated at the early biotrophic phase in barley compared with the control. At this time point, 3,162 fungal genes were differentially regulated in colonized *Arabidopsis* compared with the control. At the late colonization stage, far more genes (4,482 genes) were differentially regulated in

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The authors declare no conflict of interest.

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Data deposition: The data reported in this paper has been deposited in the Gene Expression Omnibus (GEO) database, www.ncbi.nlm.nih.gov/geo (accession no. GSE47775).

[1]U.L and Y.D. contributed equally to this work.

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MICROBIOLOGY

colonized barley than in colonized *Arabidopsis* roots (1,948 genes; Dataset S1). In total, about 70% (2,023) of the *in planta*-induced genes (2,861 genes) encoding intracellular proteins and 60% (277 of 463 induced genes) of those encoding putative secreted proteins had host-specific expression profiles (*SI Appendix*, Fig. S1). In particular, 123 of the 216 induced genes encoding small secreted proteins (SSPs; <300 amino acids), also known as putative effectors (12), were either *Arabidopsis* or barley responsive (Fig. 1*A*), suggesting that colonization of different hosts may require exploitation of distinct effectors that can interact with elements characteristic to each host. SSPs recently were shown to facilitate colonization by manipulating host defense and reprogramming plant metabolism during symbiosis (13, 14). Many of these proteins are cysteine rich (15) or possess distinctive features, such as a regular pattern of histidine and alanine residues found in all members of the *P. indica*-specific DELD family (7, 8). Eighteen of the 29 genes encoding *P. indica* DELD proteins were plant responsive and largely induced in barley but to a lesser extent in *Arabidopsis* (Fig. 1*B* and Dataset S2). Additionally, a small set of 21 SSPs were identified that are expressed in both hosts at comparable symbiotic stages (Fig. 1*A*). These SSPs may represent general determinants that target conserved recognition and signaling pathways in roots. Congruent with the broad definition of effectors (12), at the early symbiotic stage, 16 and 14 of *P. indica*'s top 20 up-regulated SSPs in *Arabidopsis* and in barley, respectively, encoded proteins with no known functional domains. A different situation was found at 14 dpi, when 50% of *P. indica*'s SSPs induced during colonization of barley, but not of *Arabidopsis*, encoded putative hydrolases (mainly glycoside hydrolase families GH10, GH11, and GH61; Dataset S3). Taken together, these data are consistent with a diversified colonization strategy for barley and for *Arabidopsis*, especially at 14 dpi, and prompted us to further characterize the two interactions at this stage.

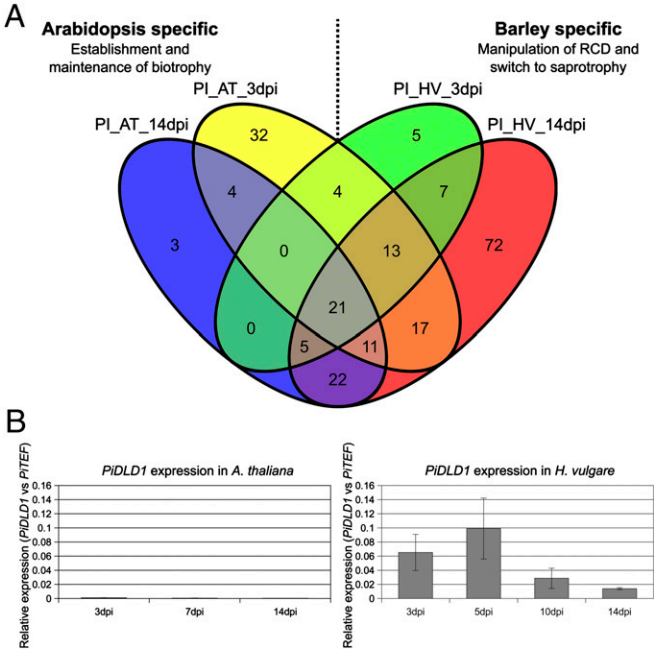

Fig. 1. Host-dependent expression profiles of *in planta*-induced *P. indica* genes encoding SSPs (<300 amino acids). (*A*) Number of SSPs significantly induced at 3 and 14 dpi during *Arabidopsis* (PI_AT) and barley (PI_HV) colonization, calculated vs. PNM control. (*B*) RT-qPCR analysis of *PiDLD1* (PIIN_05872), a member of the *P. indica*-specific putative effector family DELD, during colonization of *Arabidopsis* (*Left*) and barley (*Right*) at different time points from three independent experiments.

***P. indica* Undergoes Major Trophic, Phenotypic, and Transcriptional Rearrangements During Colonization of Barley, Whereas It Maintains a Predominant Biotrophic Nutrition in *Arabidopsis*.** Transcriptional data, together with cytological analyses, show that in barley, in response to a progressively increasing natural root cortical cell death (RCD) in older/basal root zones (16), *P. indica* undergoes a distinct nutritional shift from biotrophic to saprotrophic nutrition associated with the production of secondary thinner hyphae and with the secretion of hydrolytic enzymes (Fig. 2 *A–D* and *SI Appendix*, Fig. S2). Thus, after biotrophic colonization of the outermost cell layer (3–5 dpi), on its route toward the endodermis, the fungus progresses inter- and intracellularly by digestion of barley cortical cell walls, which became most evident at 30 dpi (Fig. 2*C*), but without visible root necrosis at the macroscopic level (10, 17). Host cell wall appositions, named papillae, often are visible beneath the site of fungal penetration attempts of living host barley cells (8), and at later colonization stages, hyphal contact with the endodermis, which is not penetrated (10), results in a strong autofluorescence of the host cell wall, indicating activation of plant defense upon attempted penetration of the root vasculature (*SI Appendix*, Fig. S3). Despite a predominant saprotrophic lifestyle, the presence of *P. indica* in barley leads to beneficial effects (6, 17).

Colonization of *Arabidopsis* by *P. indica* results in growth promotion (5) (*SI Appendix*, Fig. S4) and is characterized by a long-term feeding relationship with living host cells via the production of thicker bulbous invasive hyphae in epidermal cells and no sign for papillae formation upon penetration (Fig. 2 *F* and *G* and *SI Appendix*, Fig. S5). We show here that these intracellular, non–WGA-stainable multilobed invasive hyphae are present throughout the *Arabidopsis* colonization process, leading to a nondestructive progression within the epidermis and cortex layers, as demonstrated by colonized living root cells capable of endocytosis also at later time points (Fig. 2*F*). Consistent with this observation is the reduced expression of *P. indica* genes involved in host cell wall and lipid degradation at 3 and 14 dpi in this host compared with the situation in barley (Fig. 2*D* and *SI Appendix*, Fig. S2). The occurrence of a long-term biotrophic nutrition in *Arabidopsis* and of a switch to a saprotrophic nutrition in barley is supported further by comparative expression analyses of fungal genes involved in primary metabolism and nutrient transport. Whereas in colonized *Arabidopsis* fungal transcripts for amino acid biosynthetic processes and glycolysis are abundant, they show lower expression values in colonized barley at 14 dpi (*SI Appendix*, Fig. S6 and Dataset S4*A*). Conversely, at this time point, transcripts encoding fungal carbohydrate and nitrogen transporters are strongly induced during colonization of barley (Fig. 2*E* and *SI Appendix*, Fig. S7 and Dataset S4*B*). In particular, the high-affinity ammonium transporter PiAmt1 (PIIN_02036), whose transcripts are accumulating upon nitrogen starvation in axenic culture (*SI Appendix*, Fig. S8*A*), is induced during the late saprotrophic phase in colonized barley but to a much lower extent in colonized *Arabidopsis* (*SI Appendix*, Fig. S9 *A* and *B*), strongly indicating that in barley, but not in *Arabidopsis*, a status of nitrogen depletion is reached.

Transcripts for *P. indica* ABC transporters and other transporters most probably implicated in detoxification processes are well represented in both hosts, but to a greater extent in colonized *Arabidopsis* at 14 dpi (*SI Appendix*, Figs. S2 and S10). ABC transporters are efflux pumps that have been implicated in resistance to antifungal compounds in various host–fungal interactions, but little is known about their substrate specificities (18–20). Their host-dependent expression profiles therefore might represent a fungal stress response matched to distinct phytoalexins and other antifungal compounds produced by different hosts (21).

The Switch to Saprotrophic Nutrition in Barley Is Affected by Nitrogen Availability in a PiAMT1-Dependent Manner. To address whether nitrogen availability is one of the key switches to saprotrophic nutrition *in planta*, *P. indica* RNAi strains carrying a silencing

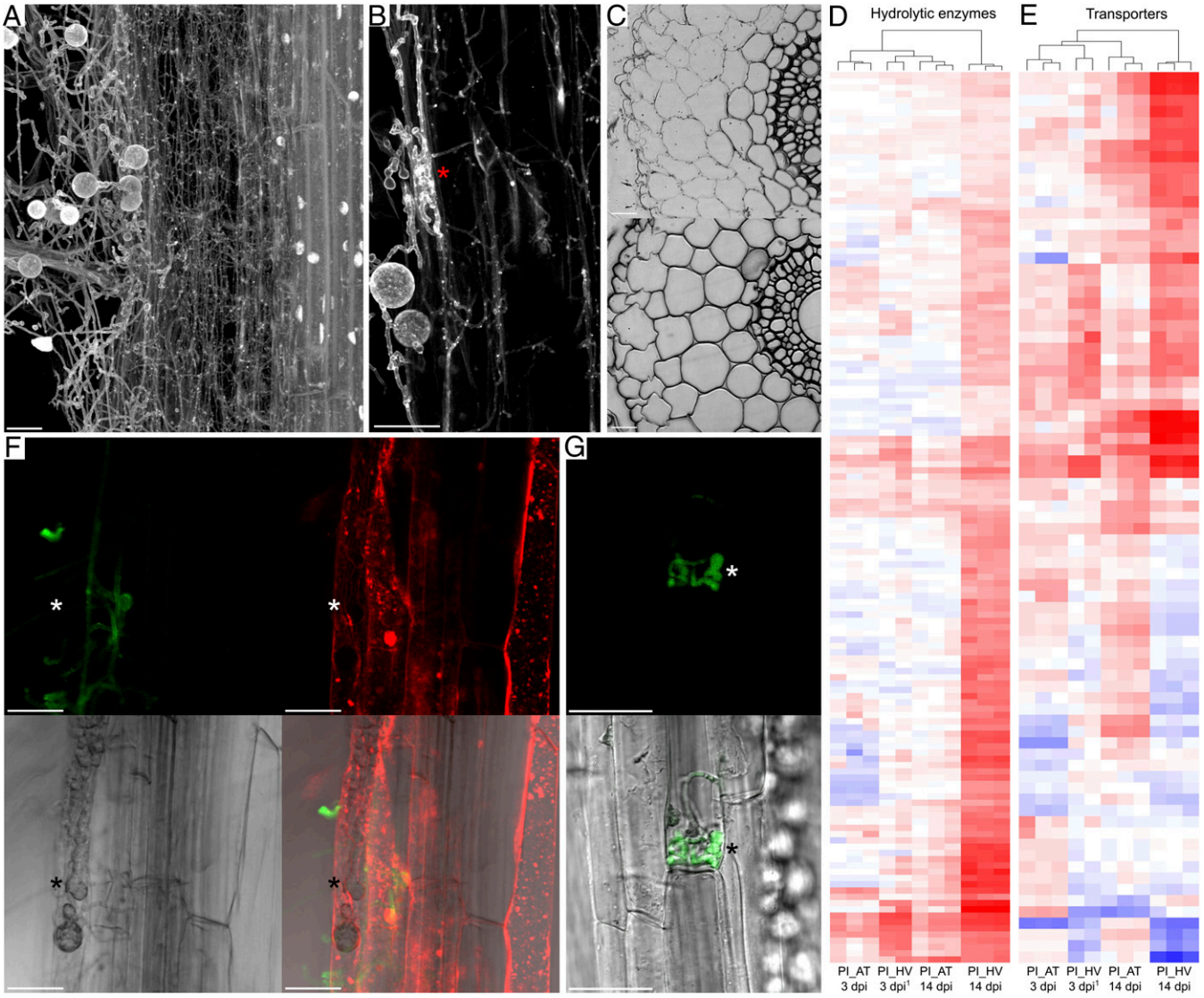

Fig. 2. Colonization patterns and *P. indica* gene expression polymorphisms correlate with extended biotrophy in *Arabidopsis* (AT) and with a switch from biotrophic to saprotrophic nutrition during colonization of barley (HV). (*A*) Maximum projection of a barley root colonized by *P. indica* at 30 dpi. Broad extraradical hyphae are visible at the boundary of the epidermis, whereas thin secondary hyphae are filling the cortical cells. Host nuclei are absent in the cortex cells while the cylinder is undamaged and preserves intact nuclei. The root was stained with acid fuchsine. (*B*) Closer view of biotrophic broad invasive hyphae within a barley epidermal cell and secondary hyphae in cortical cells (*). (*C*) Transverse 4-μm sections of barley roots inoculated with *P. indica* (30 dpi), stained with toluidine blue. (*Upper*) Heavily colonized cortex cells. (*Lower*) A noncolonized part of the root. (*D* and *E*) Heat map showing $\log_2$-fold expression changes of *P. indica* genes encoding (*D*) hydrolytic enzymes and (*E*) transporters. Significant (*t* test, $P < 0.05$) $\log_2$-fold expression changes were calculated vs. PNM control. A consistent divergence is observed at 14 dpi, where a strong induction is visible for most of these genes during colonization of barley only (for a detailed overview, see Dataset S1). [1]Raw expression data for barley 3 dpi were retrieved from ref. 8. Color coding indicates up-regulation (red) and down-regulation (blue) of genes. (*F*) Biotrophic broad invasive hyphae (white *) in *Arabidopsis* epidermal cell at 14 dpi. In contrast to extracellular hyphae, invasive hyphae are not stainable with WGA-AF488 (green) because of the presence of a plant-derived membrane. Endomembrane structures stained by FM4-64 (red) are visible inside the plant cells, indicating cell viability. (*G*) *Arabidopsis* epidermal cell with biotrophic invasive hyphae (white *) of *P. indica* GFP strain (14 dpi). The scale bars represent 25 μm.

construct targeting the high-affinity ammonium transporter *PiAMT1* were generated. The success of transformation for selected *P. indica* RNAi strains was confirmed by Southern blot, and the efficiency of silencing was verified by quantitative PCR (qPCR) experiments (Fig. 3*A* and *SI Appendix*, Fig. S11*A*). PiAmt1 displays strong homology (75% amino acid residues identity) to the high-affinity ammonium transporters AMT1 and AMT2 from *Hebeloma cylindrosporum* and to the high-affinity ammonium permease MEP2 from *Saccharomyces cerevisiae* (*SI Appendix*, Fig. S8*B*). These transporters were proposed to function as ammonium sensors, generating downstream signals in response to nitrogen starvation (22, 23). In yeast, MEP2 is required for pseudohyphal growth, which is the outgrowth of nuclear-free hyphae to better forage the medium at low N availability (22, 24, 25). The ammonium import function of PiAmt1 was verified by yeast complementation (*SI Appendix*, Fig. S8*C*), and the predicted topological structure of the *P. indica* Amt1 polypeptide demonstrated the presence of a long cytoplasmic tail at the C-terminus, which might be involved in downstream signaling (*SI Appendix*, Fig. S8*D*). To verify that ammonium uptake by the *P. indica* RNAi strains is reduced relative to wild-type (WT) and empty-vector (EV) controls, these strains were analyzed for growth on minimal medium with a low concentration of ammonium as the sole nitrogen source. Their growth on complex

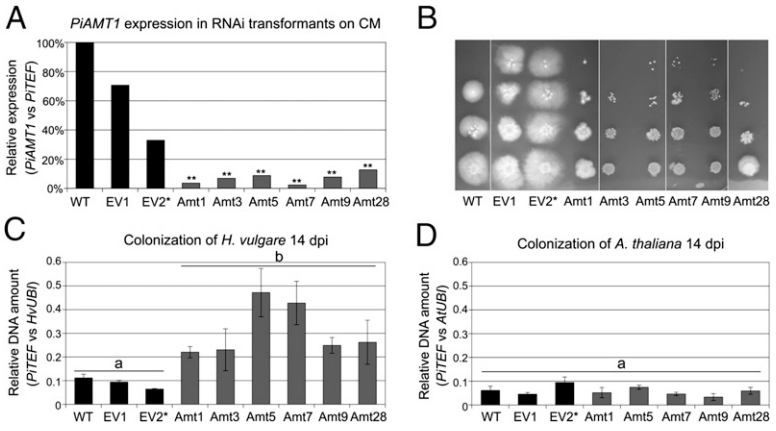

Fig. 3. Silencing of *PiAMT1* affects *P. indica* colonization of barley but not of *Arabidopsis*. (*A*) Relative expression of *PiAMT* in *P. indica* WT and EV controls and RNAi strains grown in liquid complete medium (CM) for 7 d. **ANOVA, $P < 0.01$. (*B*) Colony phenotype of *P. indica* WT and transformants on yeast nitrogen base medium supplemented with 2 mM NH_4Cl as the sole nitrogen source, 14 dpi. (*C* and *D*) Relative amount of fungal DNA in (*C*) barley and (*D*) in *Arabidopsis* roots colonized by *P. indica* WT or transformants at 14 dpi. Plants were grown on 1/10 PNM. Error bars represent SE of the mean from three independent biological repetitions. Grouping was done by ANOVA. *Southern blot analyses showed that all transformed strains had one to two integrations of the plasmids, with the exception of EV2, which had multiple integrations (*SI Appendix*, Fig. S11).

media and media supplied with large amounts of ammonium was not affected (*SI Appendix*, Fig. S11*B*), whereas hyphal growth at low N was suppressed (Fig. 3*B*). The RNAi strains were investigated further for altered root colonization. Despite a substantial reduction in growth under a low supply of ammonium as the sole nitrogen source and the absence of any evidence for a compensatory up-regulation of the second ammonium transporter *PiAMT2* (*SI Appendix*, Fig. S9*C*), the RNAi strains displayed a significantly increased colonization of barley roots at 14 dpi compared with the WT and EV controls, calculated as the ratio of fungal DNA to plant DNA (elogation factor *PiTEF*/ubiquitin *HvUBI*) (Fig. 3*C*) or as the ratio of *PiTEF* to *HvUBI* transcripts (*SI*

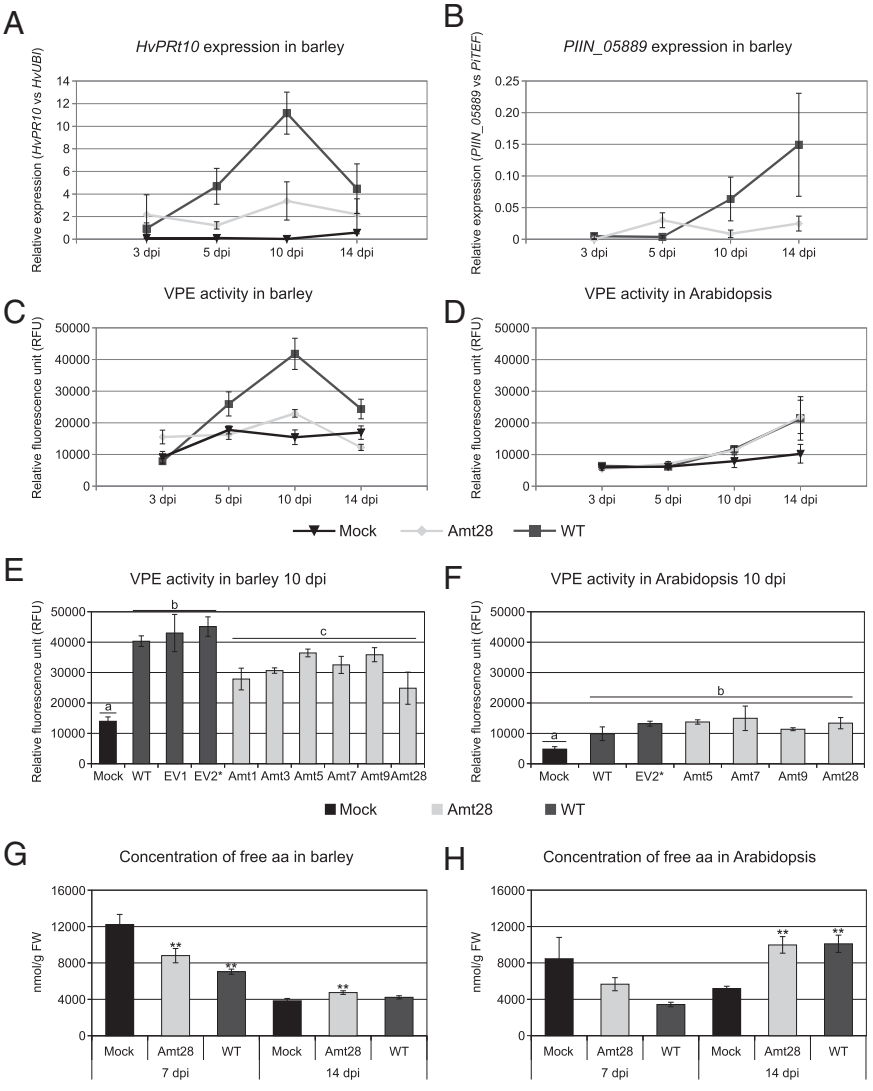

Fig. 4. Gene expression profiles, VPE-like enzymatic activity, and amino acid levels in barley roots suggest extended biotrophy in *P. indica* AMT1-RNAi strains compared with WT. (*A*) Relative expression of the plant defense-related gene *PR10* and (*B*) of the *P. indica* gene PIIN_05889 encoding a putative xylanase, during colonization of barley by the *P. indica* WT and RNAi strain Amt28 at different time points. Expression data are standardized relative to *HvUBI* or to *PiTEF*. SEs are calculated from three independent biological repetitions. (*C*) VPE-like enzymatic activities during biotrophic (≤5 dpi) and cell death-associated colonization (≥10 dpi) of barley roots colonized by *P. indica* WT and RNAi strain Amt28 or mock treated. For the assay, the fluorescent VPE-specific substrate Ac-ESEN-MCA was added to the root extracts for spectrophotometric determination of enzymatic activities (for details, see *SI Appendix*). (*D*) VPE-like enzymatic activities during early (3 and 5 dpi) and late (10 and 14 dpi) colonization of *Arabidopsis* roots. (*E*) VPE-like enzymatic activities at the onset of saprotrophy (10 dpi) in barley roots and (*F*) in *Arabidopsis* roots. (*G*) Concentrations of free amino acids in the roots of barley noncolonized or colonized by *P. indica* WT and RNAi strain Amt28 at 7 and 14 dpi. (*H*) Concentrations of free amino acids in the roots of *Arabidopsis* at 7 and 14 dpi. Error bars represent SEM from three to four independent biological repetitions. **ANOVA, $P < 0.01$. Columns not sharing a letter are significantly different (ANOVA, $P < 0.01$).

Appendix, Fig. S9*D*). The temporal expression pattern for transcripts encoding the barley pathogenesis-related gene PR10 was examined to determine the plant's response to colonization by the *P. indica* WT and the RNAi strain Amt28. A decreased transcript accumulation for the PR gene in roots colonized by the RNAi strain compared with the WT strain at the onset of saprotrophy was observed (Fig. 4*A*). Consistent with this, expression of the *P. indica* saprotrophic marker gene, PIIN_05889 (encoding a putative xylanase), was reduced in the RNAi strain compared with *P. indica* WT (Fig. 4*B*). This indicates an extended biotrophic phase of the RNAi strain Amt28, which likely contributes to the enhanced colonization of barley roots at 14 dpi. To verify this hypothesis, we measured the activity of the vacuolar processing enzyme (VPE). This is a cysteine protease with caspase-like activity responsible for the maturation of various vacuolar proteins in higher plants and reported to be induced in dying cells (9, 26). VPE-like enzymatic activity increased significantly in barley roots colonized by *P. indica* WT compared with barley mock treated and barley roots colonized by the RNAi strain Amt28, especially at the onset of saprotrophy (Fig. 4*C*). Analyses of the VPE-like enzymatic activity performed at 10 dpi with the other RNAi strains and EV controls confirmed this finding (Fig. 4*E*). Remarkably, in the RNAi strains, elicitation of growth promotion was not affected compared with the WT situation (*SI Appendix*, Fig. S12). This indicates that an extended biotrophic phase does not seem to influence *P. indica*'s beneficial effects on barley.

Consistent with low expression of the *PiAMT1* gene in *Arabidopsis* at 14 dpi, analyses of the *P. indica* RNAi strains impaired in ammonium uptake and possibly ammonium sensing displayed no difference in their colonization patterns compared with the WT and EV controls (Fig. 3*D* and *SI Appendix*, Fig. S9*E*). Similarly, no differences in the VPE-like enzymatic activity were observed in *Arabidopsis* roots colonized by *P. indica* WT or RNAi strain Amt28, in which both displayed moderate increased activity compared with mock control at later stages (Fig. 4 *D* and *F*). We therefore concluded that PiAmt1 is not required for biotrophic growth but is involved in the lifestyle switch of *P. indica* to saprotrophic growth as observed in barley.

Quantification of free amino acid levels in barley roots by ultra-pressure reversed-phase chromatography (see *SI Appendix* for details on this method) showed that amino acid concentrations decreased significantly in colonized roots by *P. indica* WT compared with roots colonized by the RNAi strain Amt28 and control roots at the onset of the saprotrophic stage/late biotrophic stage (Fig. 4*G* and *SI Appendix*, Fig. S13). At 14 dpi, the concentrations of free amino acids were remarkably lower than at 7 dpi in the older/basal root zone of barley, irrespective of colonization (Fig. 4*G* and *SI Appendix*, Fig. S13). In particular, asparagine and glutamine decreased by approximately five- and sevenfold, respectively, in control roots (*SI Appendix*, Fig. S13). This might be the result of reallocation of nitrogen to younger root zones in response to developmental RCD (16) and is in agreement with the microarray data, which indicate limited nitrogen availability to *P. indica* in the basal root zone of barley at 14 dpi. In *Arabidopsis*, colonization by *P. indica* WT decreased amino acid concentrations 7 dpi (Fig. 4*H* and *SI Appendix*, Fig. S13), whereas the level of free amino acids increased significantly 14 dpi (Fig. 4*H* and *SI Appendix*, Fig. S13). The altered organic nitrogen allocation upon *P. indica* colonization at 14 dpi was mainly the result of changes in asparagine and glutamine (*SI Appendix*, Fig. S13) and suggests that nitrogen supply to the fungus is not limited in this phase. Biotrophic and hemibiotrophic fungi have been shown to induce plant nitrogen mobilization and accumulation at the site of infection. In particular, nitrogen-rich amino acids such as glutamine and asparagine have been identified as the major forms of nitrogen reallocation during infection of different plant hosts (27–29). Changes in free amino acid pools during biotrophy have been speculated to reflect the demand for organic nitrogen by the fungus or to be required to launch defense responses by the host (27, 30). Asparagine and glutamine are the preferred nitrogen source of *P. indica* in axenic culture, and when grown on these amino acids as the sole nitrogen source, *P. indica* produces enlarged hyphae that resemble the multilobed biotrophic hyphae *in planta* (*SI Appendix*, Fig. S14). On nitrate-containing medium, known to be a poor nitrogen source for *P. indica* (8), or on medium without nitrogen, *P. indica* hyphae are thin and less branched, similar to the secondary hyphae found in barley at later colonization stages (*SI Appendix*, Fig. S14). Asparagine and glutamine therefore may represent a ready source of organic nitrogen during biotrophy.

Taken together, these results strongly suggest that host-related nutritional cues affect *P. indica*'s lifestyle and that the switch from biotrophic to saprotrophic nutrition during colonization of barley is affected by PiAmt1 and by nitrogen availability.

Discussion

Establishment of biotrophy during colonization of *Arabidopsis* and barley by *P. indica* is an important feature of the symbioses with both plant hosts, and it implies a strong interdependence between host metabolism and fungal nutrient uptake in this endophyte. Transcriptional profiling revealed that far fewer genes need to be induced to maintain biotrophy compared with those necessary for coordinating the switch to saprotrophy and the manipulation of RCD. Evolution of obligate biotrophy was shown to correlate, among others, with the loss of genes involved in nitrate metabolism, and it is speculated that host plants provide a ready source of organic nitrogen in the form of amino acids (15). In the draft genome of *P. indica*, no nitrate transporters or reductases were found (8), indicating that nitrate is not essential for this symbiont. *P. indica* can grow on ammonium and on glutamine and asparagine, which are its favorite nitrogen sources. The low expression of the *P. indica* high-affinity ammonium transporter in *Arabidopsis* at later colonization stages suggests that

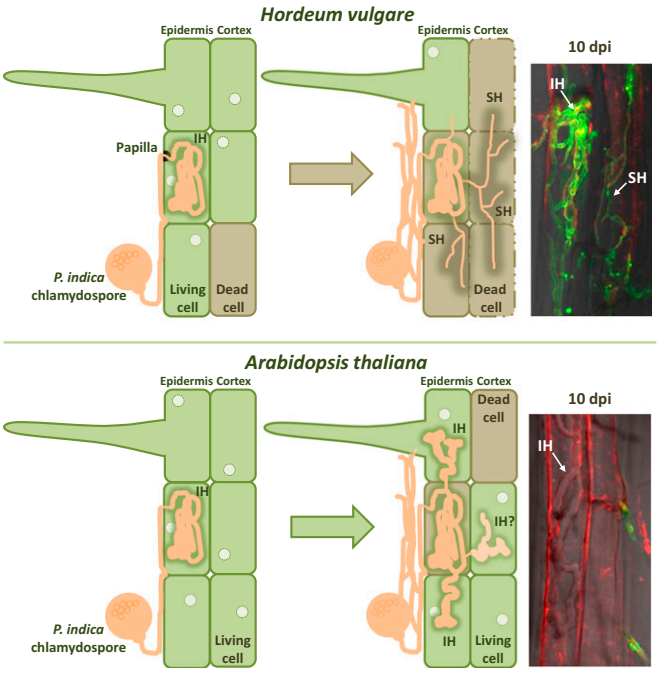

Fig. 5. Schematic representation of *P. indica* colonization strategies at different symbiotic stages in barley and in *Arabidopsis*. (*Upper*) Invasive hyphae (IH) and secondary thin hyphae (SH) of *P. indica* in barley dead cells (10 dpi). (*Lower*) *P. indica* non–WGA-stainable biotrophic broad invasive hyphae in an *Arabidopsis* epidermal cell (10 dpi). Fungal structures were stained with WGA-AF488 (green); membranes were stained with FM4-64 (red). Staining of fungal structures by WGA-AF488 is enhanced in dead host cells. In *H. vulgare* roots, cell death is initiated a few days after germination by developmental RCD (16) and is increased by *P. indica* colonization.

an adequate source of nitrogen is provided by this host during colonization. Increased concentrations of free amino acids, principally of glutamine and asparagine upon *P. indica* colonization of this host at 14 dpi, strongly support this conclusion. The induction of *PiAMT1* and other fungal nitrogen transporters in barley leads to the assumption that this plant cannot provide *P. indica* with sufficient organic nitrogen during the onset of the RCD program and that nitrogen depletion therefore may function as a trigger for the *in planta* expression of fungal genes encoding hydrolytic enzymes and for the activation of the saprotrophic program. Silencing of the high-affinity ammonium transporter *PiAMT1* by RNAi resulted in reduced expression of fungal xylanase, barley defense response, and VPE activity in colonized roots, supporting the hypothesis that PiAmt1 is involved in sensing the N status and in downstream signaling upon nitrogen depletion in *P. indica*. Because biotrophic growth of *P. indica* in *Arabidopsis* was independent of PiAmt1, we conclude that expression and a signaling function of PiAmt1 are needed for the switch of *P. indica*'s lifestyle to saprotrophy. Loss or suppression of the expression of *PiAMT1* would represent a step toward a progressively more intimate biotrophic association with its hosts. The maintenance of this gene at the expense of biotrophy might benefit *P. indica* during prolonged saprotrophic growth on decaying plant material, making this fungus also able to survive in the absence of living hosts or on dying host cells.

Plant-associated fungi are either specialists, which are adapted to one or a few distinct hosts, or generalists that can thrive in highly variable host environments. Specialists and their hosts are in an evolutionary arms race that leads to the development of fungal tools and colonization strategies that are efficiently tailored to the respective host. Conversely, broad-host range species must evolve adaptations to cope with a plethora of different host-associated signals and host-specific defense mechanisms. The evolutionary force, in this case, possibly drives the expansion and diversification of the fungal toolkit and the host-adapted gene expression to better suit different plants. Recently it was shown that the obligate biotrophic ascomycete powdery mildew pathogen, *Blumeria graminis* f. sp. *hordei*, which can grow and reproduce only on living cells of its natural host, barley, displays a conserved transcriptional program during early pathogenesis on barley and on immunocompromised *Arabidopsis* (31). Although we cannot exclude that at early time points (<2 dpi, prepenetration stage) a certain conservation of the transcriptional program may be present, our data show that the broad-host range fungal root symbiont *P. indica* responds differently to divergent hosts, especially at later time points during establishment and maintenance of the intracellular biotrophic interaction.

In conclusion, the transcriptional and phenotypic plasticity of *P. indica* during symbioses (summarized in Fig. 5) establishes a highly adaptive capacity in a root endophyte with broad compatibility that can reconfigure itself and its lifestyle in response to different environmental and host signals.

Material and Methods

Microarray Analyses. Microarray experiments were performed with total RNA extracted from *P. indica*-inoculated barley and *Arabidopsis* roots. As a control, total RNA from *P. indica* grown on 1/10 PNM–agar was used. Root 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. For details, see *SI Appendix*.

RNAi Vector Construction and *P. indica* Transformation. A 570-bp fragment of the *PiAMT1* gene was amplified by PCR (Dataset S5) from cDNA and inserted in the EcoRV site of the convergent dual-promoter vector pPiRNAi (17). *P. indica* was transformed with vector pPiRNAi-AMT1 and the EV control as described in ref. 17. For details, see *SI Appendix*.

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Supplementary Materials:

Material and Methods

Supplementary Figures 1 to 15

Supplementary Datasets 1 to 5

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.

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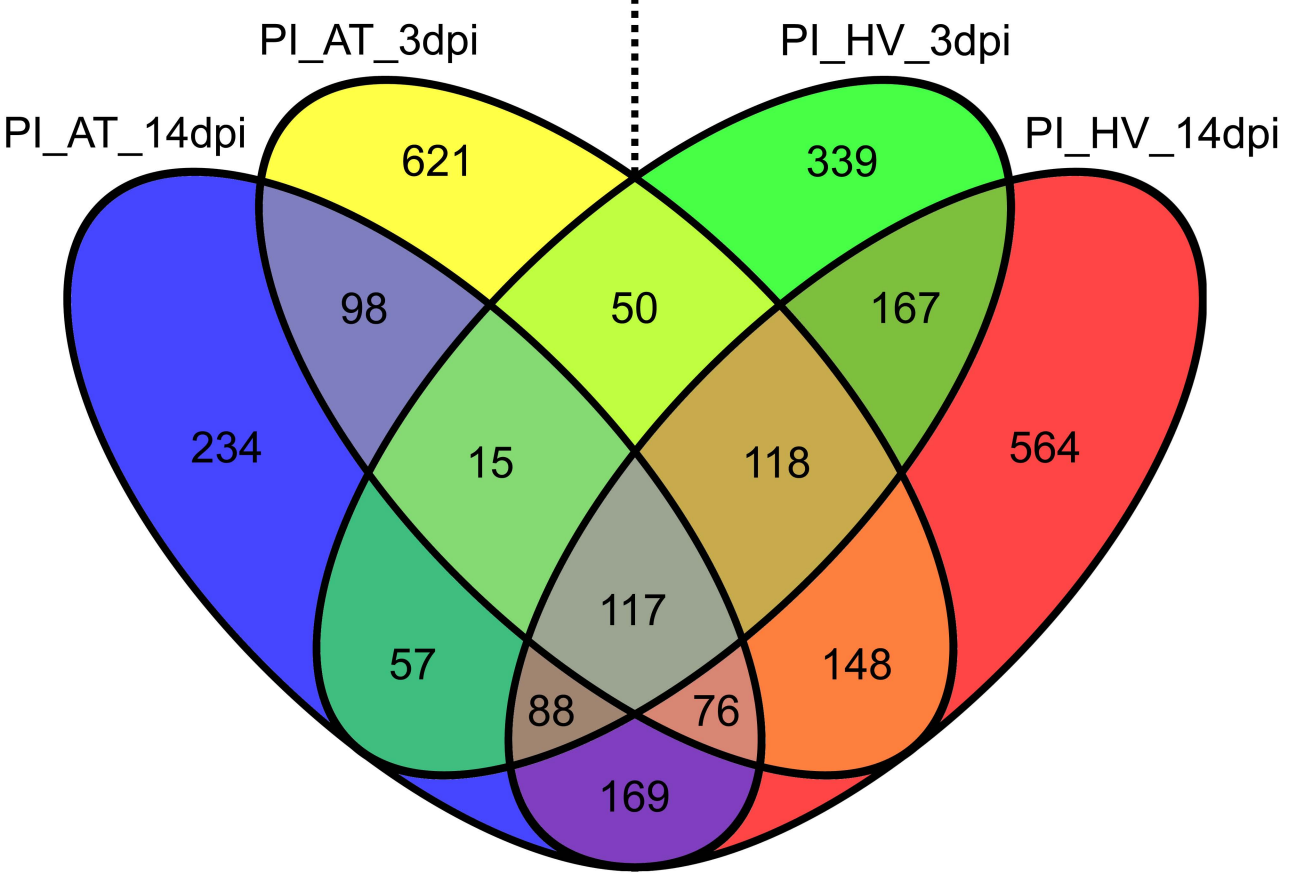
A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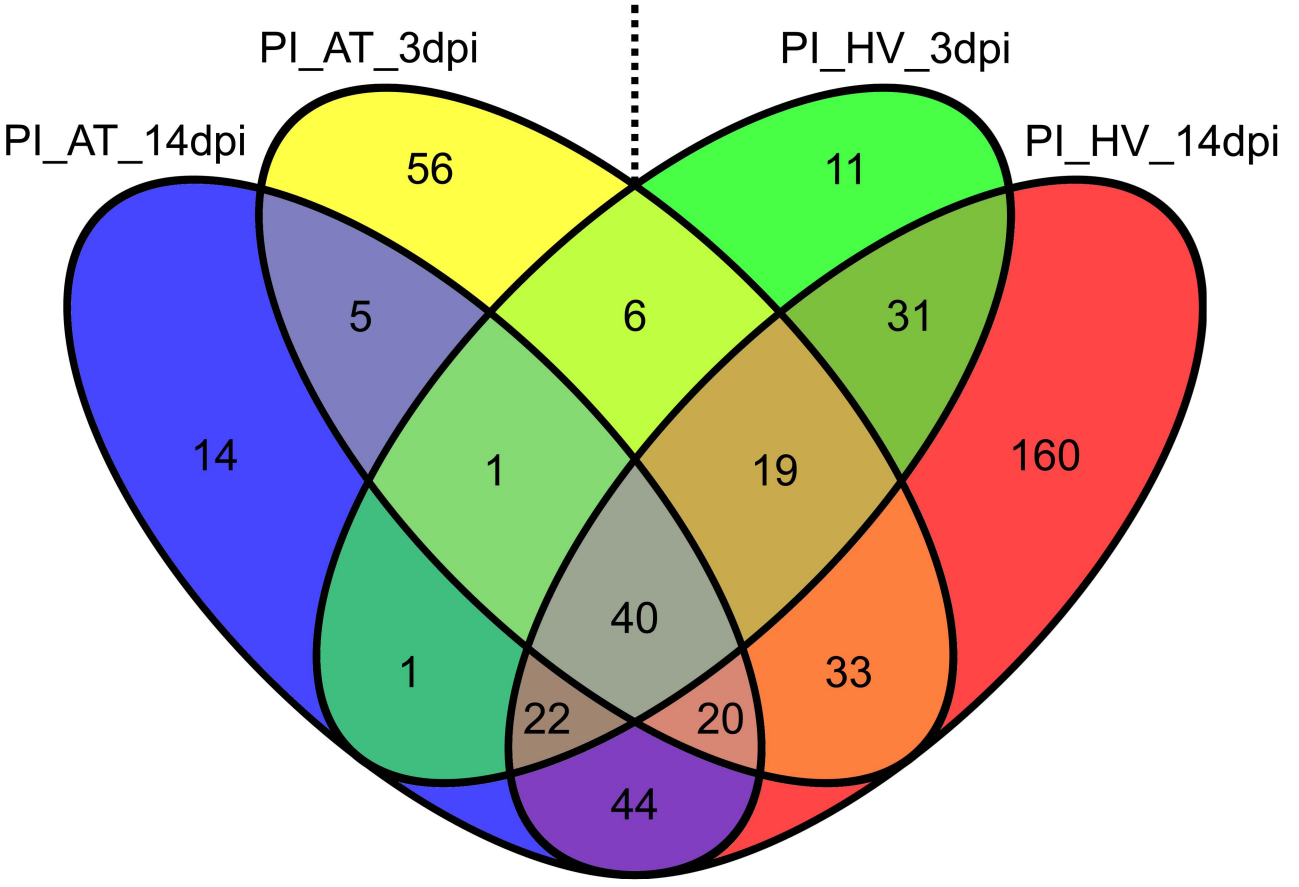
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160
40
1
33
22
20
44

Hydrolytic enzymes

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PIIN_03660 – GH_43
PIIN_02711 – GH_3
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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
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PIIN_04140 – Pep_C14
PIIN_06296 – GH_45
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PIIN_01084 – GH_35
PIIN_05614 – GH_47
PIIN_06730 – Pep_C2
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PIIN_10057 – GH_10
PIIN_04931 – Pep_M28
PIIN_00112 – Pep_M14
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PIIN_06648 – Pep_M43
PIIN_10522 – GH_11
PIIN_05605 – Pep_M20
PIIN_04202 – GH_28
PIIN_04249 – Pep_M16_C
PIIN_10741 – GH_28
PIIN_04139 – Pep_C14
PIIN_06172 – Lip_GDSL
PIIN_04136 – Pep_C14
PIIN_01870 – GH_88
PIIN_10086 – Pep_C14
PIIN_00863 – Pep_S66
PIIN_00599 – GH_61
PIIN_07359 – GH_26
PIIN_05519 – Lip_GDSL
PIIN_07549 – GH_43
PIIN_06881 – GH_43
PIIN_07331 – Lip_GDSL
PIIN_06360 – GH_10
PIIN_00424 – Pep_M36
PIIN_02991 – Pep_M36
PIIN_07574 – Pep_M1
PIIN_03543 – GH_18
PIIN_07553 – Lip_GDSL
PIIN_04364 – GH_10
PIIN_01083 – GH_35
PIIN_00917 – GH_2
PIIN_03039 – GH_3
PIIN_03991 – GH_10
PIIN_05547 – GH_43
PIIN_00392 – GH_3
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
PIIN_05699 – GH_61
PIIN_06155 – GH_61
PIIN_07320 – Lip_GDSL
PIIN_02110 – GH_61
PIIN_03993 – GH_10
PIIN_06162 – Lip_GDSL
PIIN_06127 – GH_61
PIIN_01486 – GH_61
PIIN_01487 – GH_61
PIIN_04707 – GH_62
PIIN_04357 – GH_61
PIIN_02795 – GH_47
PIIN_03996 – GH_10
PIIN_03975 – GH_61
PIIN_06164 – Lip_GDSL
PIIN_04374 – Pep_S8
PIIN_07245 – Lip_GDSL
PIIN_05707 – GH_28
PIIN_04706 – GH_62
PIIN_07922 – GH_10
PIIN_06118 – GH_61
PIIN_06260 – Pep_S9
PIIN_00353 – GH_6
PIIN_02752 – GH_6
PIIN_07749 – Lip_3
PIIN_06258 – Pep_M35
PIIN_10572 – GH_11
PIIN_04356 – GH_61
PIIN_06405 – Lip_GDSL
PIIN_00723 – Lip_GDSL
PIIN_03577 – GH_1
PIIN_05887 – GH_11
PIIN_04708 – GH_62
PIIN_06594 – GH_7
PIIN_08199 – GH_61
PIIN_06862 – Lip_GDSL
PIIN_05886 – GH_11
PIIN_06854 – GH_11
PIIN_08401 – GH_61
PIIN_05889 – GH_11
PIIN_03994 – GH_10
PIIN_05862 – GH_11
PIIN_06890 – GH_11
PIIN_07098 – GH_61
PIIN_09690 – GH_11
PIIN_05863 – GH_11
PIIN_01483 – GH_61
PIIN_07545 – GH_43
PIIN_10315 – GH_61
PIIN_03992 – GH_10
PIIN_05861 – GH_11
PIIN_05057 – GH_10
PIIN_10660 – GH_61
PIIN_11387 – GH_10
PIIN_10892 – GH_11
PIIN_03997 – GH_10
PIIN_09050 – Pep_M36
PIIN_01932 – Pep_M43
PIIN_06646 – Pep_M43
PIIN_10078 – GH_31
PIIN_06156 – GH_61
PIIN_06647 – Pep_M43
PIIN_08869 – GH_11
PIIN_08706 – Lip_3
PIIN_09579 – Pep_S10
PIIN_06081 – Pep_S24
PIIN_10098 – GH_31
PIIN_10097 – GH_31
PIIN_10677 – GH_15
PIIN_04795 – Pep_M16
PIIN_06650 – Pep_M43
PIIN_06117 – GH_61
PIIN_06889 – GH_11
PIIN_01936 – Pep_M43
PIIN_08949 – GH_43
PIIN_10588 – Pep_M43

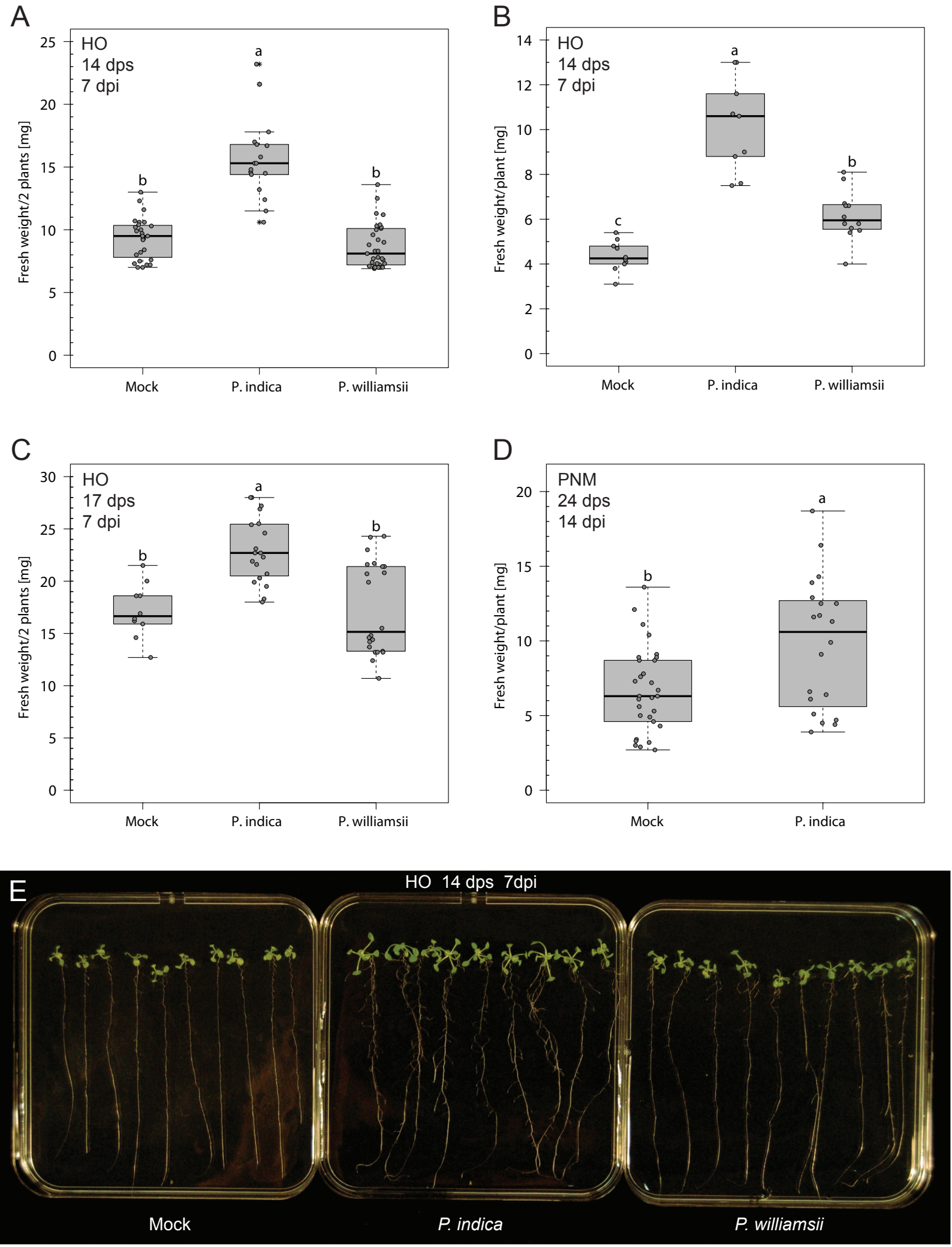
PI_HV_14d1
PI_HV_14d3
PI_HV_14d2
PI_HV_3d1
PI_HV_3d2
PI_AT_14d3
PI_AT_14d2
PI_AT_14d1
PI_PNM_3d3
PI_PNM_3d1
PI_PNM_3d2
PI_AT_3d2
PI_AT_3d3
PI_AT_3d1

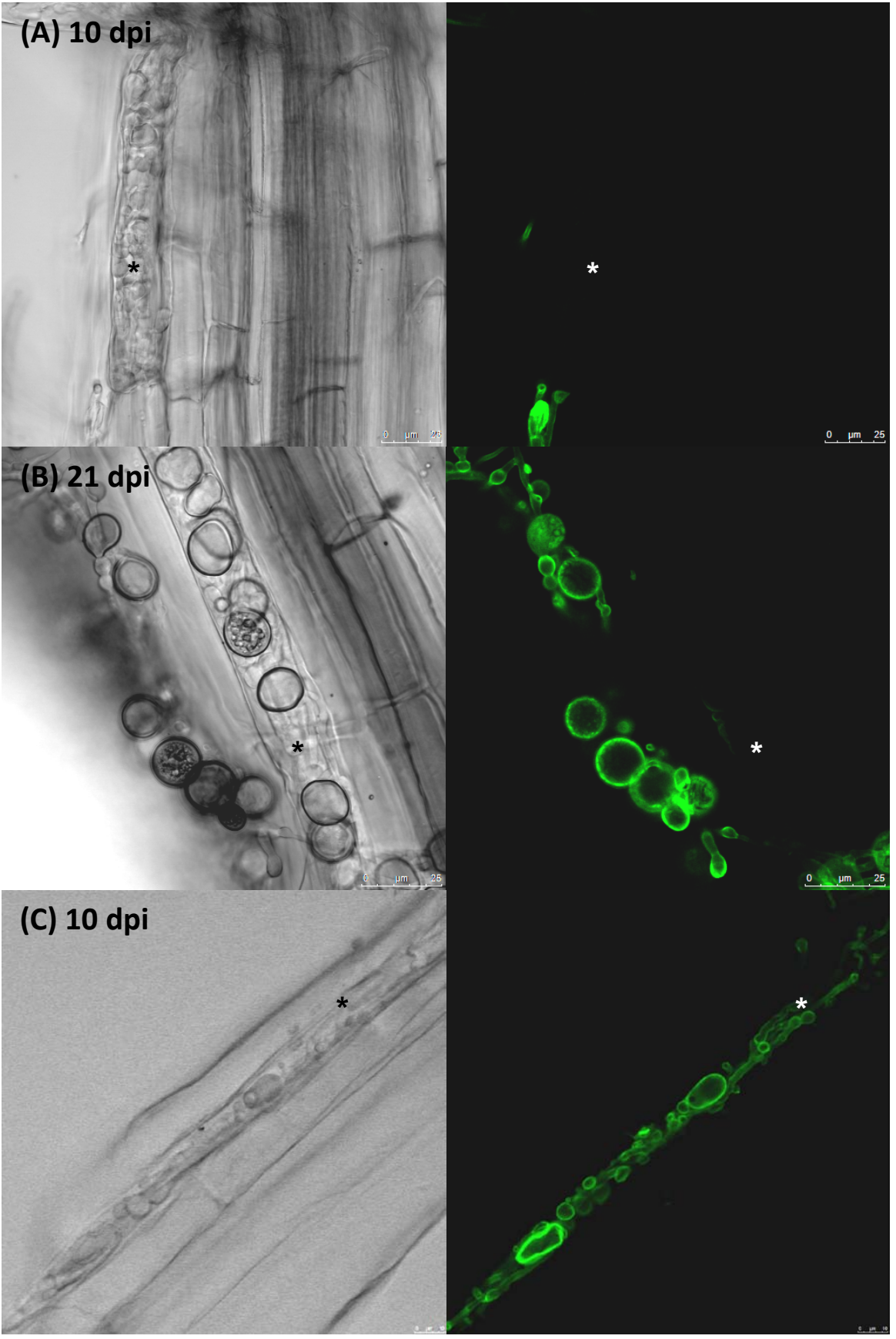
Transporters

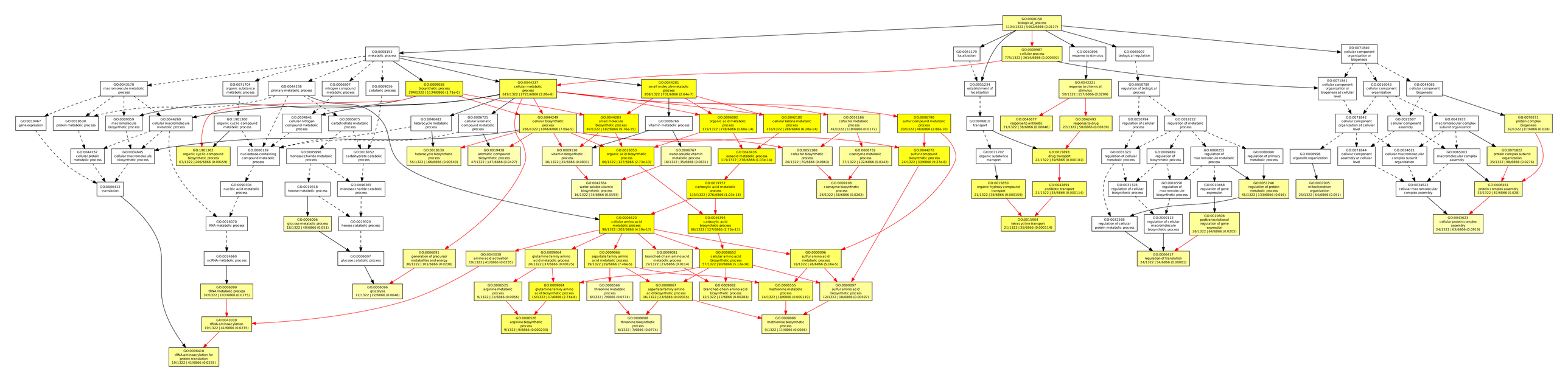
PIIN_05314 – carbohydrate
PIIN_04449 – metal, mitochondrion
PIIN_05146 – metal, mitochondrion
PIIN_06807 – toxin efflux, ABC
PIIN_02728 – unclassified
PIIN_03445 – toxin efflux, ABC
PIIN_03569 – unclassified
PIIN_04975 – phosphate
PIIN_05763 – metal, mitochondrion
PIIN_08131 – phosphate
PIIN_00903 – 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
PIIN_06080 – toxin efflux, ABC
PIIN_02349 – sulfate
PIIN_00491 – toxin efflux, ABC
PIIN_02059 – carbohydrate
PIIN_06815 – unclassified
PIIN_08729 – metal, mitochondrion
PIIN_03420 – metal, mitochondrion
PIIN_00579 – toxin efflux, ABC, etc
PIIN_01532 – metal, mitochondrion
PIIN_03843 – toxin efflux, ABC
PIIN_02738 – carbohydrate
PIIN_01740 – unclassified
PIIN_04113 – unclassified
PIIN_01050 – metal, mitochondrion
PIIN_08798 – metal, mitochondrion
PIIN_08122 – phosphate
PIIN_03707 – carbohydrate
PIIN_09045 – toxin efflux, ABC
PIIN_00819 – carbohydrate
PIIN_07467 – toxin efflux, ABC
PIIN_01500 – phosphate
PIIN_08285 – toxin efflux, ABC
PIIN_03404 – unclassified
PIIN_00119 – metal, mitochondrion
PIIN_07932 – metal, mitochondrion
PIIN_00838 – unclassified
PIIN_06706 – unclassified
PIIN_05124 – toxin efflux, ABC
PIIN_01480 – metal, mitochondrion
PIIN_08632 – unclassified
PIIN_06392 – metal, mitochondrion
PIIN_01841 – metal, mitochondrion
PIIN_09353 – toxin efflux, ABC
PIIN_06378 – nitrogen
PIIN_11264 – toxin efflux, ABC
PIIN_01726 – nitrogen
PIIN_04034 – nitrogen
PIIN_07488 – nitrogen
PIIN_03174 – nitrogen
PIIN_07528 – toxin efflux, ABC
PIIN_03607 – nitrogen
PIIN_05937 – toxin efflux, ABC
PIIN_06574 – unclassified
PIIN_03125 – nitrogen
PIIN_05189 – nitrogen
PIIN_05732 – unclassified
PIIN_07257 – carbohydrate
PIIN_00748 – toxin efflux, ABC
PIIN_02380 – toxin efflux, ABC
PIIN_09386 – metal, mitochondrion
PIIN_02107 – metal, mitochondrion
PIIN_07440 – carbohydrate
PIIN_06025 – toxin efflux, ABC
PIIN_03282 – metal, mitochondrion
PIIN_04428 – toxin efflux, ABC, etc
PIIN_07747 – metal, mitochondrion
PIIN_07616 – unclassified
PIIN_07652 – toxin efflux, ABC
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
PIIN_04016 – unclassified
PIIN_06542 – metal, mitochondrion
PIIN_01021 – metal, mitochondrion
PIIN_01857 – toxin efflux, ABC
PIIN_04696 – unclassified
PIIN_06643 – nitrogen
PIIN_04496 – metal, mitochondrion
PIIN_03275 – nitrogen
PIIN_06028 – toxin efflux, ABC
PIIN_00335 – carbohydrate
PIIN_04851 – toxin efflux, ABC
PIIN_05671 – toxin efflux, ABC
PIIN_06219 – metal, mitochondrion
PIIN_05792 – metal, mitochondrion
PIIN_02487 – toxin efflux, ABC
PIIN_07950 – carbohydrate
PIIN_09860 – toxin efflux, ABC
PIIN_02271 – nitrogen
PIIN_01789 – metal, mitochondrion
PIIN_02479 – metal, mitochondrion
PIIN_03410 – nitrogen
PIIN_10125 – toxin efflux, ABC
PIIN_04334 – toxin efflux, ABC
PIIN_08951 – metal, mitochondrion
PIIN_09208 – toxin efflux, ABC
PIIN_08071 – nitrogen
PIIN_05934 – toxin efflux, ABC
PIIN_11202 – toxin efflux, ABC
PIIN_02871 – metal, mitochondrion
PIIN_07604 – carbohydrate
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
PIIN_06879 – nitrogen
PIIN_10480 – nitrogen
PIIN_02854 – carbohydrate
PIIN_02855 – carbohydrate
PIIN_06341 – phosphate
PIIN_01718 – carbohydrate
PIIN_06466 – metal, mitochondrion
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
PIIN_07655 – toxin efflux, ABC
PIIN_11606 – carbohydrate
PIIN_11197 – toxin efflux, ABC
PIIN_10387 – toxin efflux, ABC
PIIN_00337 – carbohydrate
PIIN_01526 – toxin efflux, ABC
PIIN_03408 – nitrogen
PIIN_09315 – toxin efflux, ABC
PIIN_03368 – carbohydrate
PIIN_04623 – toxin efflux, ABC
PIIN_10126 – carbohydrate
PIIN_06024 – toxin efflux, ABC
PIIN_03608 – nitrogen
PIIN_05175 – unclassified
PIIN_07018 – toxin efflux, ABC
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
PIIN_09765 – toxin efflux, ABC
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

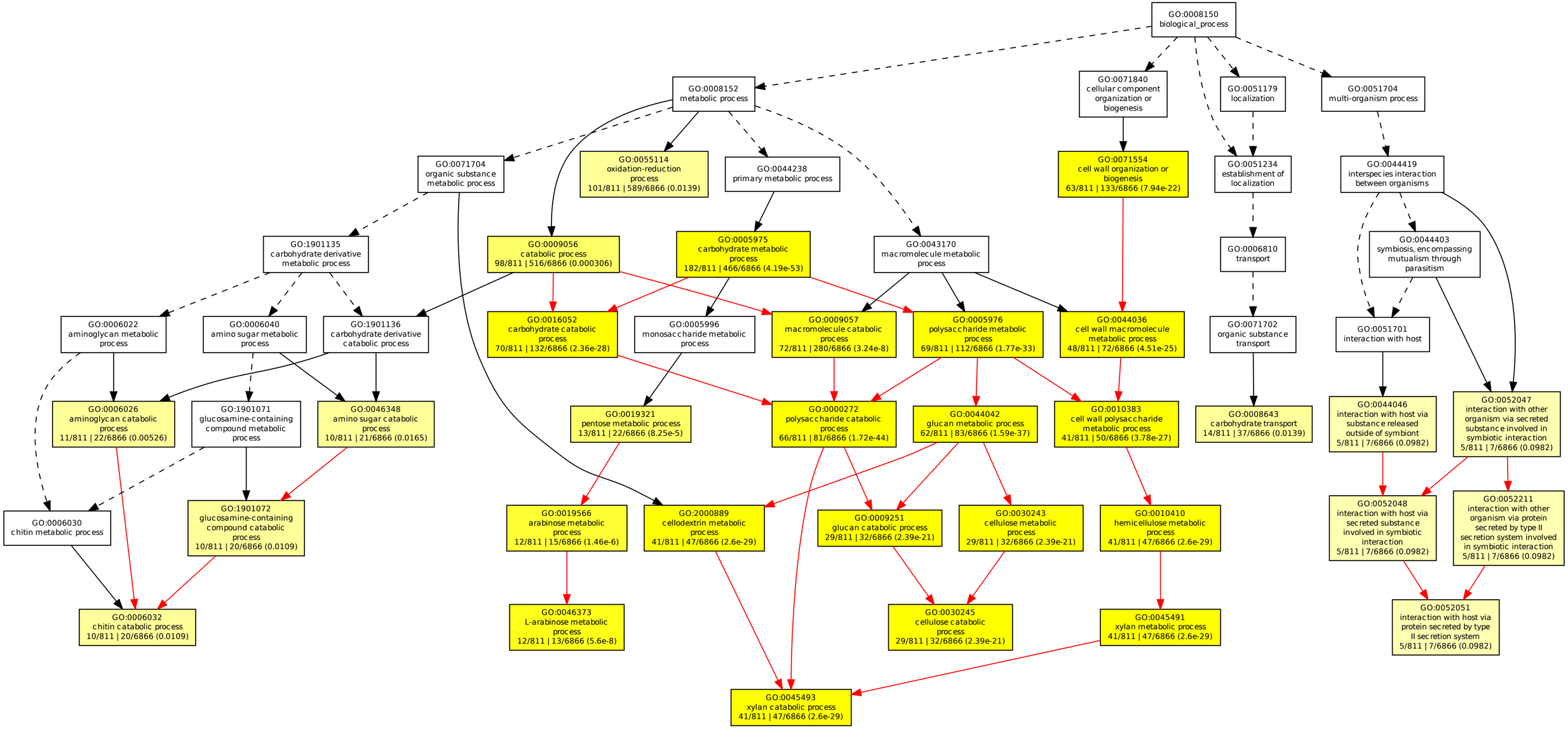
PI_HV_14d3
PI_HV_14d1
PI_HV_14d2
PI_AT_14d3
PI_AT_14d2
PI_AT_14d1
PI_HV_3d1
PI_HV_3d2
PI_PNM_3d3
PI_PNM_3d1
PI_PNM_3d2
PI_AT_3d3
PI_AT_3d1
PI_AT_3d2


0
µm
25

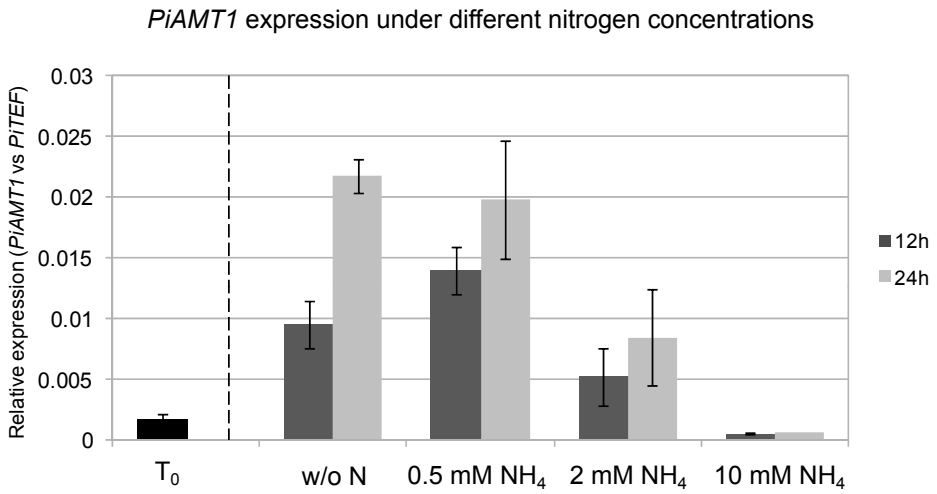

A
HO
14 dps
7 dpi
Fresh weight/2 plants [mg]
0
5
10
15
20
25
a
b
b
Mock
P. indica
P. williamsii
B
HO
14 dps
7 dpi
Fresh weight/plant [mg]
0
2
4
6
8
10
12
14
c
a
b
Mock
P. indica
P. williamsii
C
HO
17 dps
7 dpi
Fresh weight/2 plants [mg]
0
5
10
15
20
25
30
b
a
b
Mock
P. indica
P. williamsii
D
PNM
24 dps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

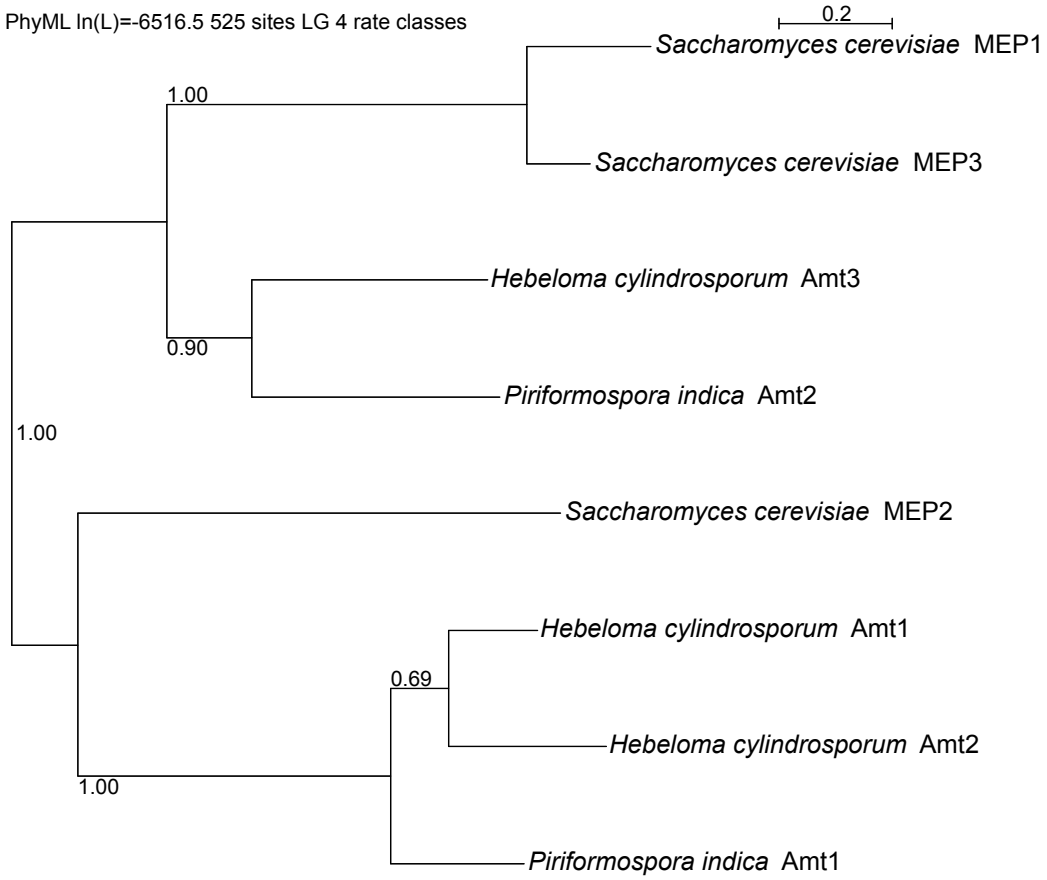

GO:0008150
biological_process
1104/1322 | 5462/6866 (0.0117)
GO:0051179
localization
GO:0009987
cellular process
775/1322 | 3614/6866 (0.000392)
GO:0050896
response to stimulus
GO:0065007
biological regulation
GO:0008152
metabolic process
GO:0071840
cellular component organization or biogenesis
GO:0051234
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: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: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:0032502
developmental process
GO:0043933
macromolecular complex subunit organization
GO:0070271
protein complex biogenesis
32/1322 | 87/6866 (0.028)
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:0097975
carbohydrate metabolic process
GO:0046483
heterocycle metabolic process
GO:0006725
cellular aromatic compound metabolic process
GO:0044249
cellular biosynthetic process
266/1322 | 1046/6866 (7.09e-5)
GO:0044283
small molecule biosynthetic process
87/1322 | 182/6866 (9.76e-15)
GO:0006766
vitamin metabolic process
GO:0006682
organic acid metabolic process
115/1322 | 278/6866 (1.68e-14)
GO:0042180
cellular ketone metabolic process
116/1322 | 286/6866 (6.28e-14)
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:0046907
intracellular transport
GO:0032259
methylation
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 | 127/6866 (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 | 33/6866 (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:0080090
regulation of primary metabolic process
GO:0007005
mitochondrion organization
25/1322 | 64/6866 (0.051)
GO:0006996
organelle organization
GO:0071844
cellular component assembly at cellular level
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:0090304
nucleic acid metabolic process
GO:0019318
hexose metabolic process
GO:0043365
monosaccharide catabolic process
GO:0015850
organic hydroxy compound transport
21/1322 | 36/6866 (0.000159)
GO:0042891
antibiotic transport
21/1322 | 35/6866 (0.000114)
GO:0033036
macromolecule localization
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:0006006
glucose metabolic process
18/1322 | 40/6866 (0.051)
GO:0019320
hexose catabolic process
GO:0015804
tetracycline transport
21/1322 | 35/6866 (0.000114)
GO:0032268
regulation of cellular protein metabolic process
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:0006520
cellular amino acid metabolic process
98/1322 | 202/6866 (4.19e-17)
GO:0046394
carboxylic acid biosynthetic process
66/1322 | 127/6866 (2.73e-13)
GO:0006417
regulation of translation
24/1322 | 54/6866 (0.00801)
GO:0034660
ncRNA metabolic process
GO:0006399
tRNA metabolic process
37/1322 | 103/6866 (0.0175)
GO:0006091
generation of precursor metabolites and energy
36/1322 | 101/6866 (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:0006525
arginine metabolic process
9/1322 | 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 (0.00015)
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:0043039
tRNA aminoacylation
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:0006418
tRNA aminoacylation for protein translation
19/1322 | 41/6866 (0.0235)


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)

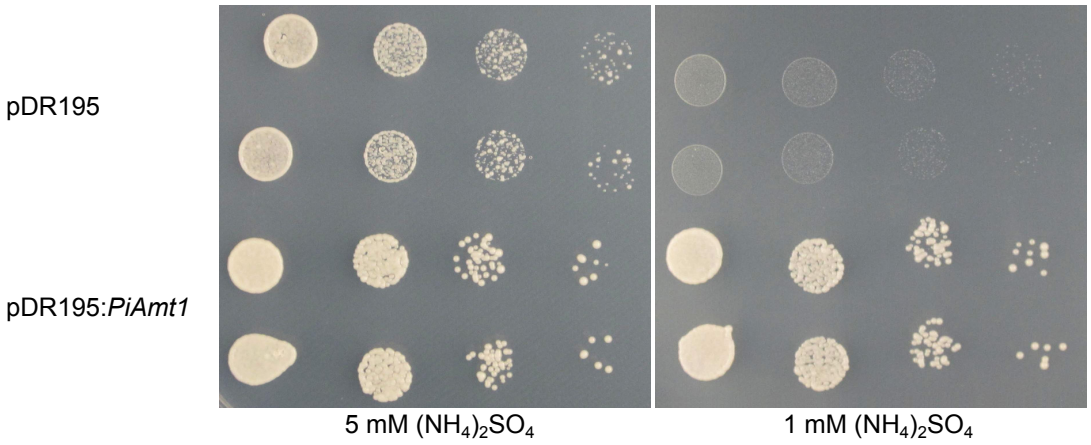
A


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

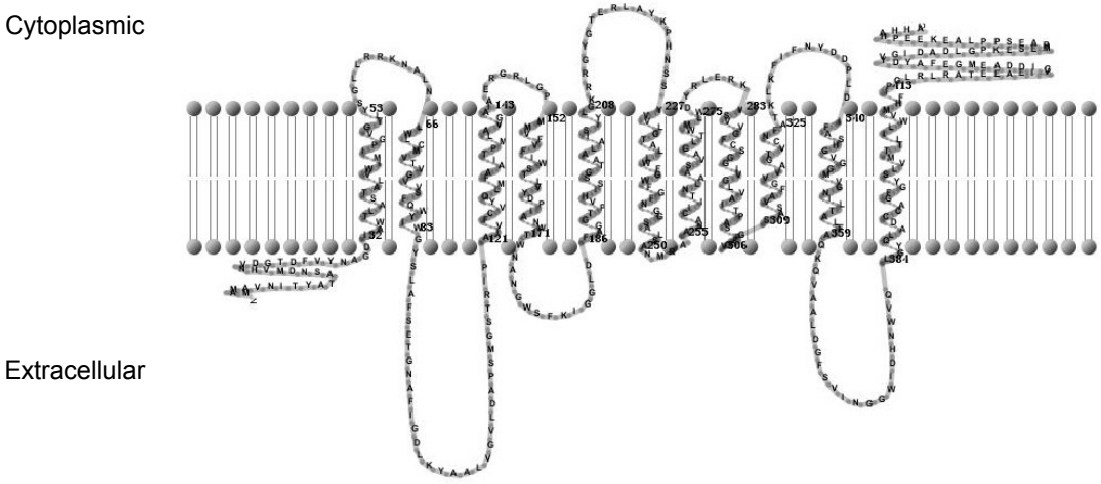
B


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

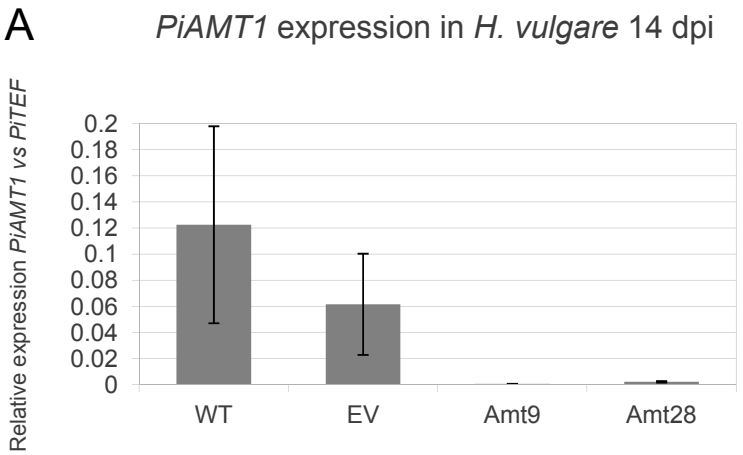
C


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

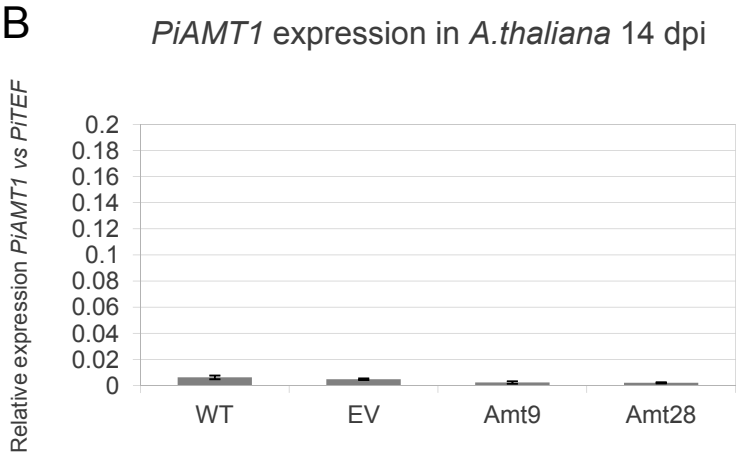
D


Cytoplasmic
Extracellular

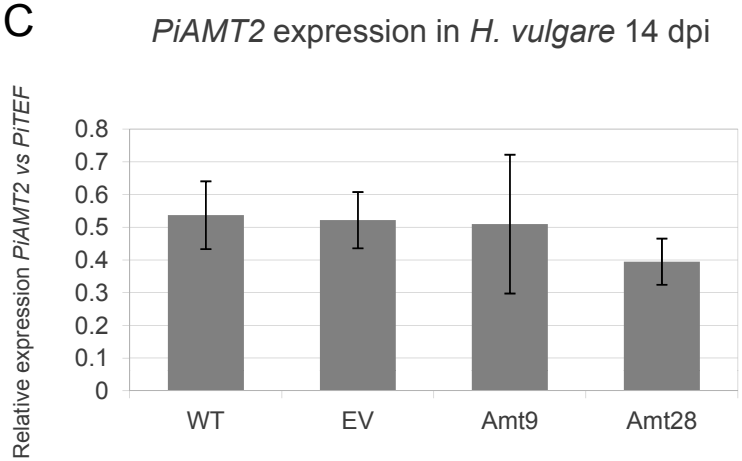
A


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

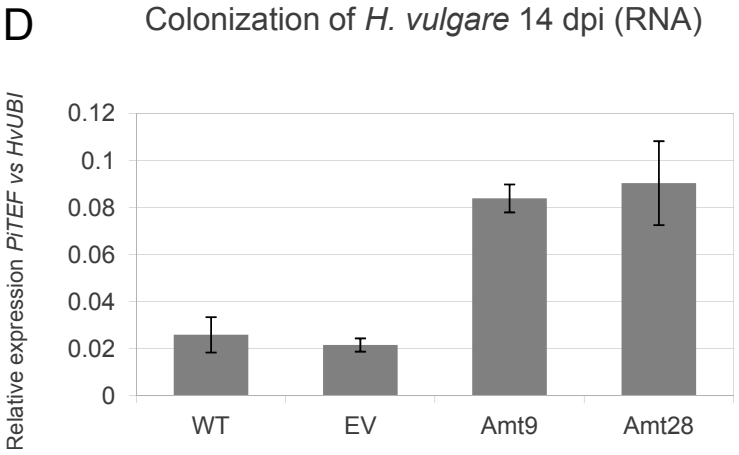
B


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

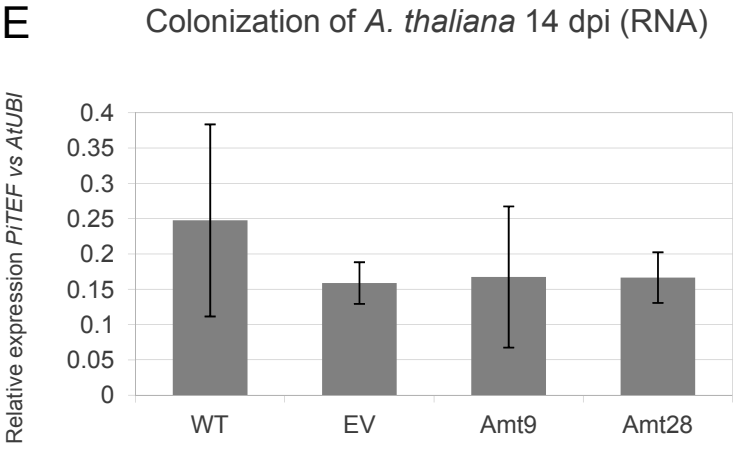
C

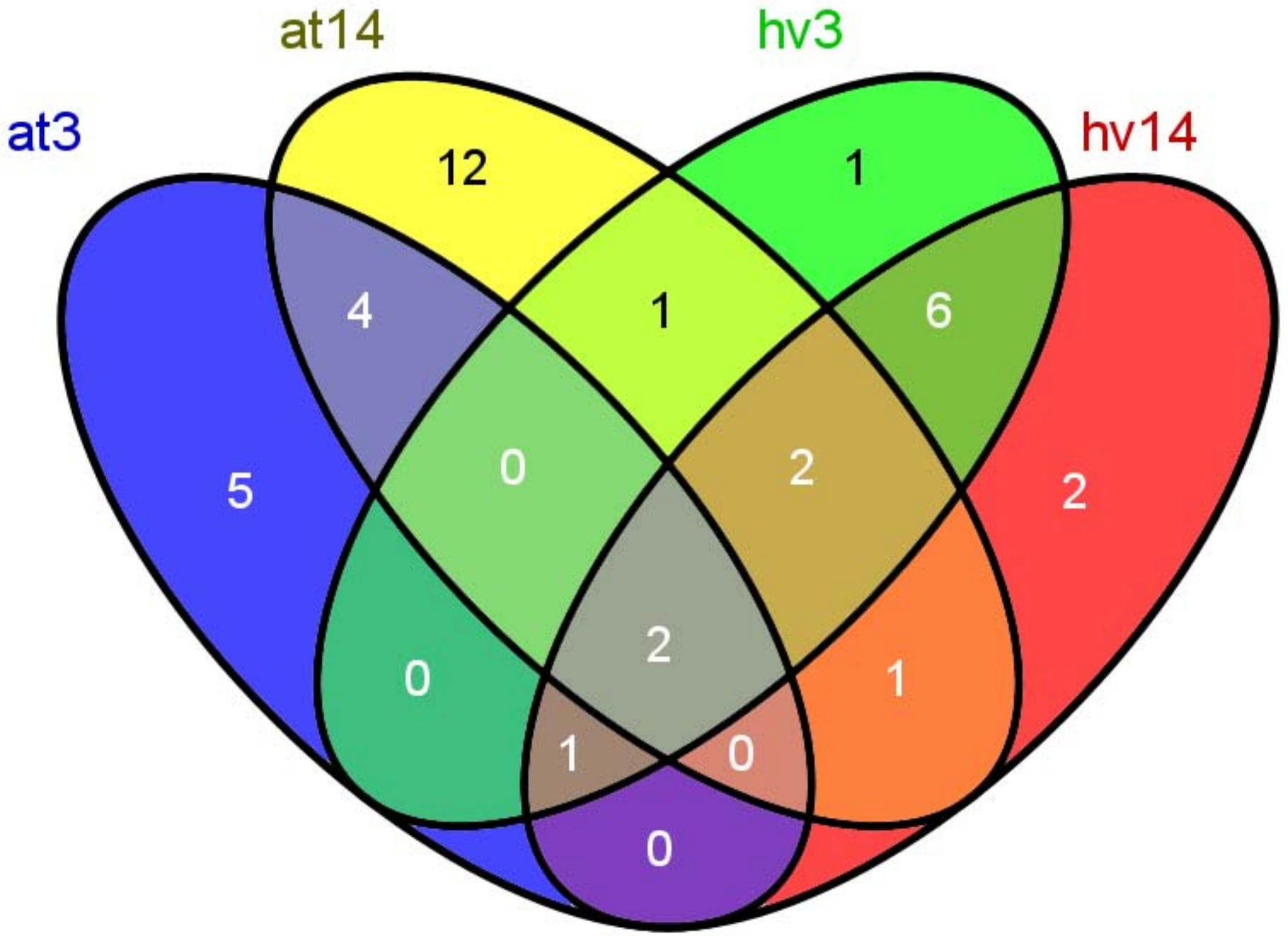

PiAMT2 expression in *H. vulgare* 14 dpi
Relative expression *PiAMT2 vs PiTEF*
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
WT
EV
Amt9
Amt28

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

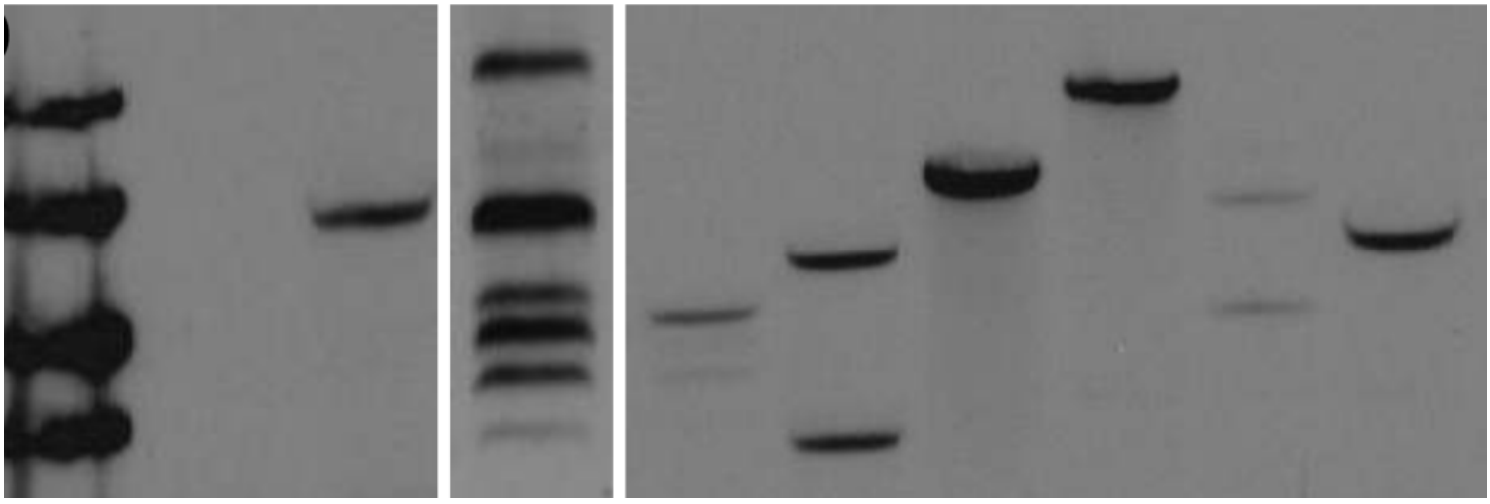
E


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

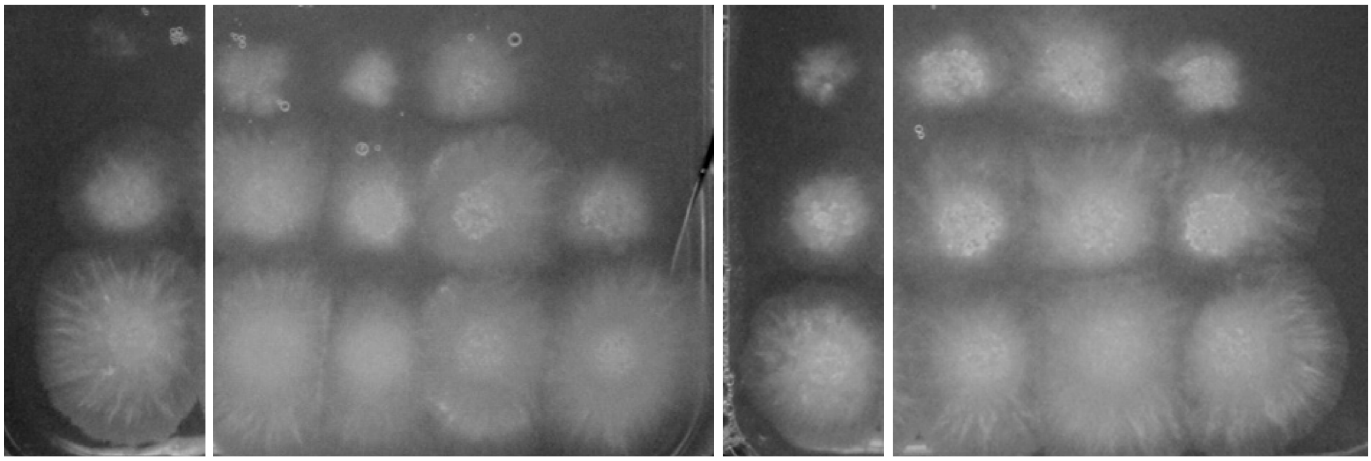
A

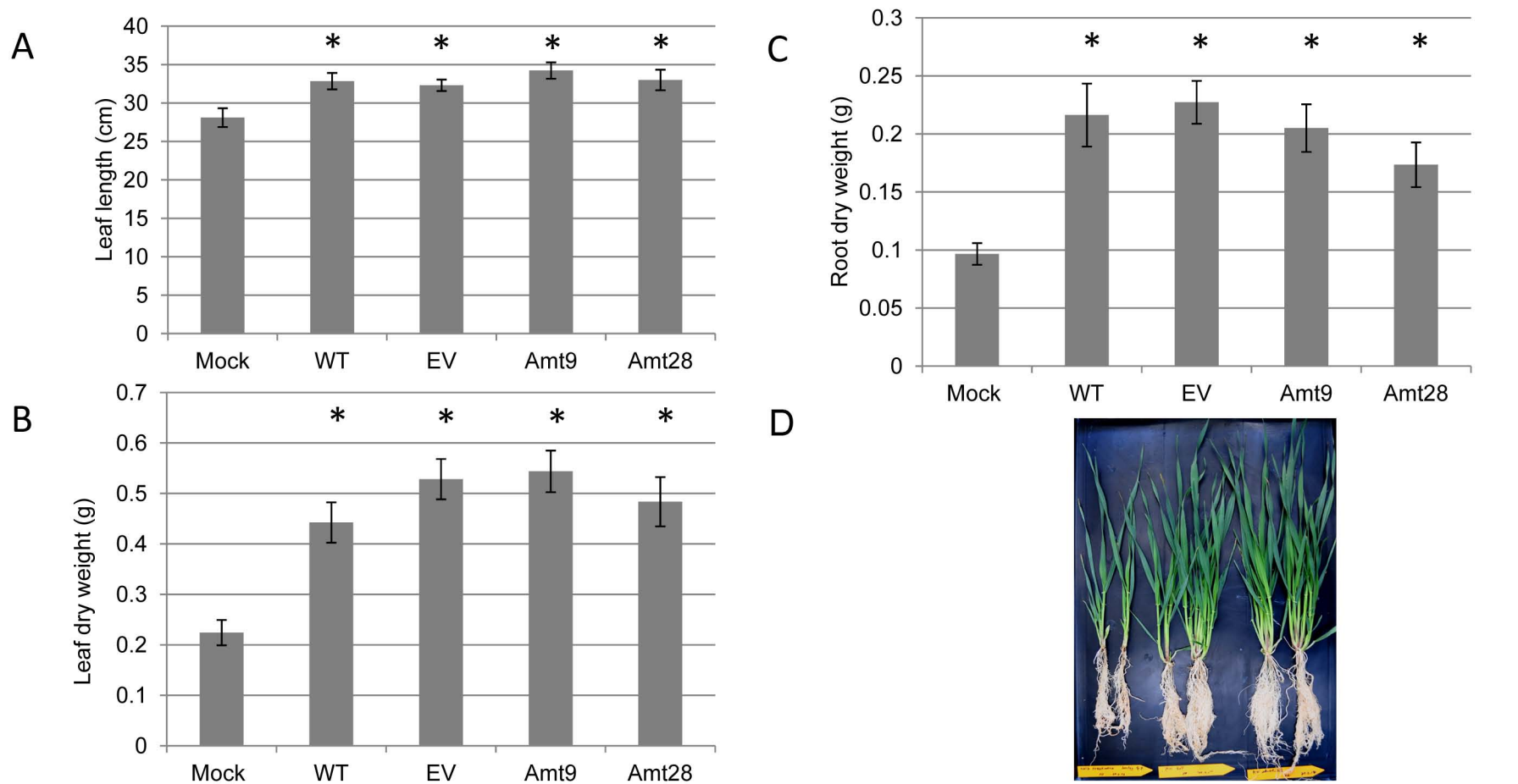
Southern blot

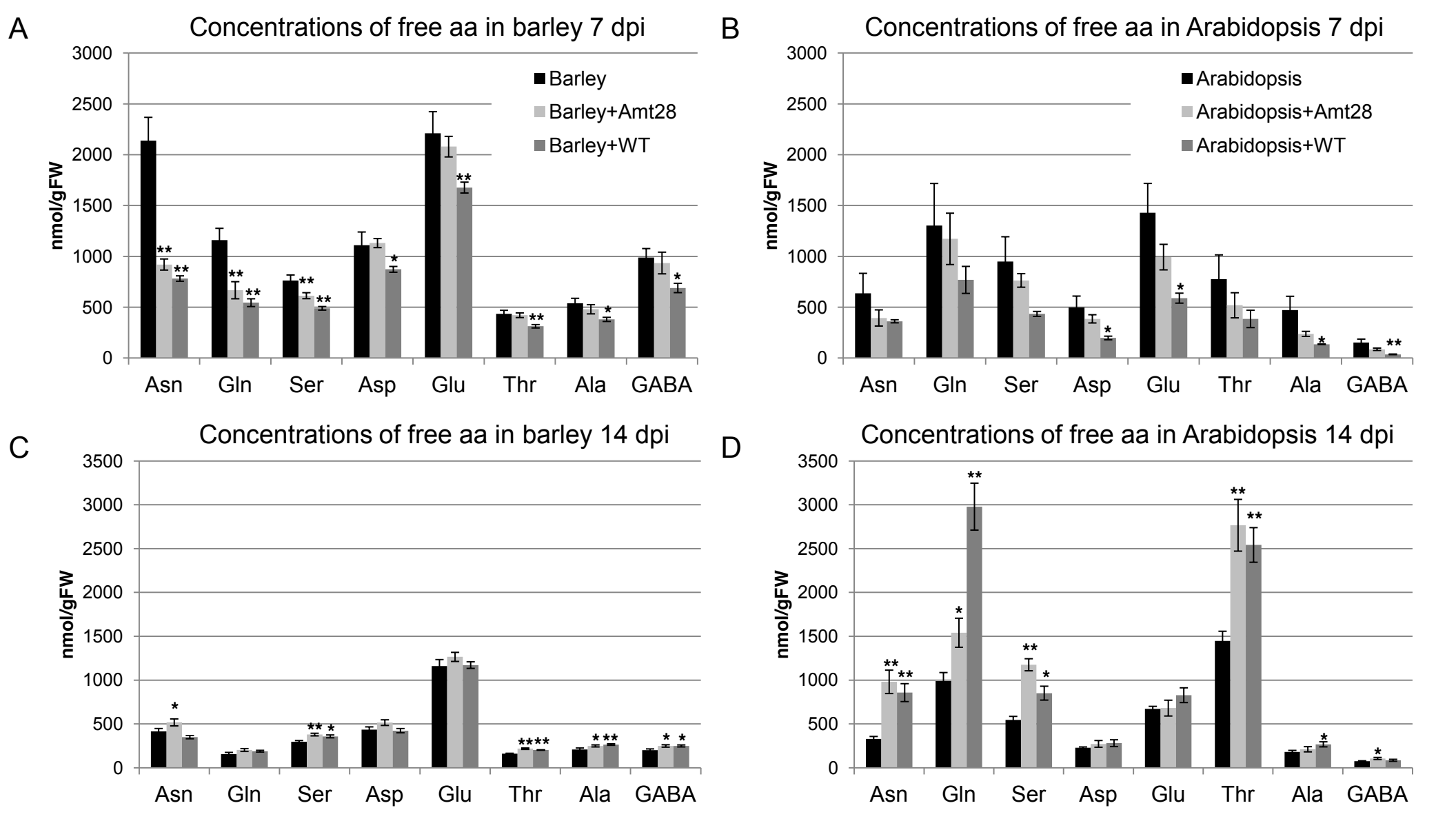


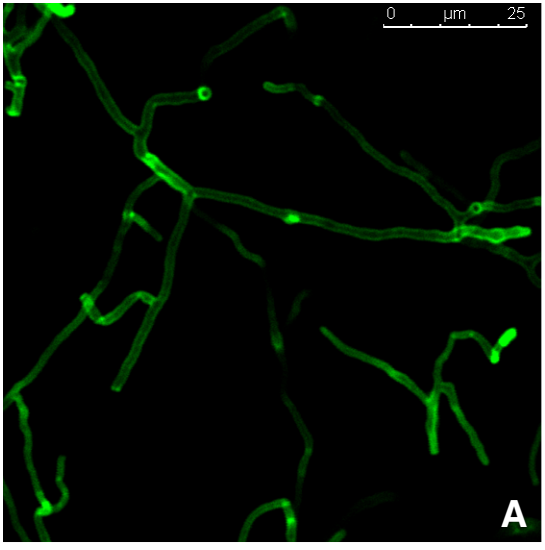
B

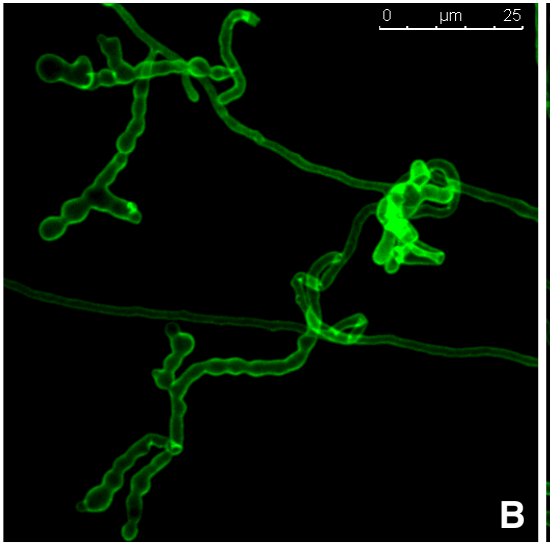
P. indica strains on YNB + 10 mM ammonium

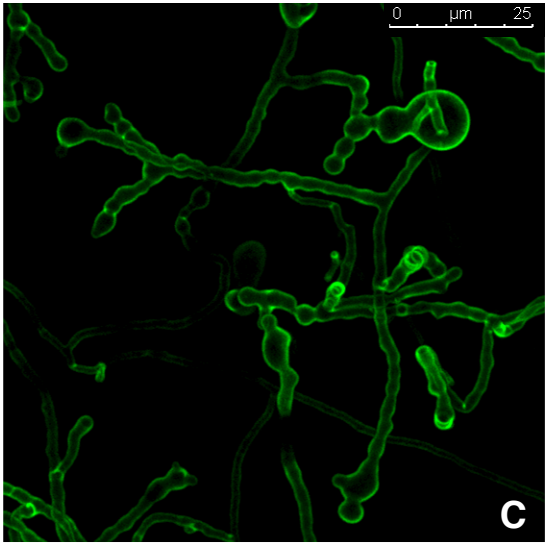


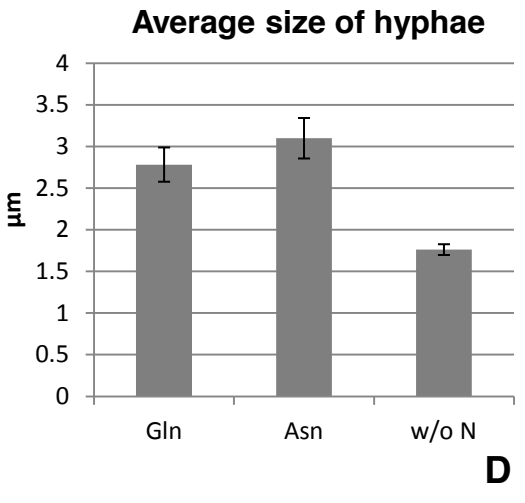

A
Leaf length (cm)
40
35
30
25
20
15
10
5
0
*
*
*
*
Mock
WT
EV
Amt9
Amt28
B
Leaf dry weight (g)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
*
*
*
*
Mock
WT
EV
Amt9
Amt28
C
Root dry weight (g)
0.3
0.25
0.2
0.15
0.1
0.05
0
*
*
*
*
Mock
WT
EV
Amt9
Amt28
D

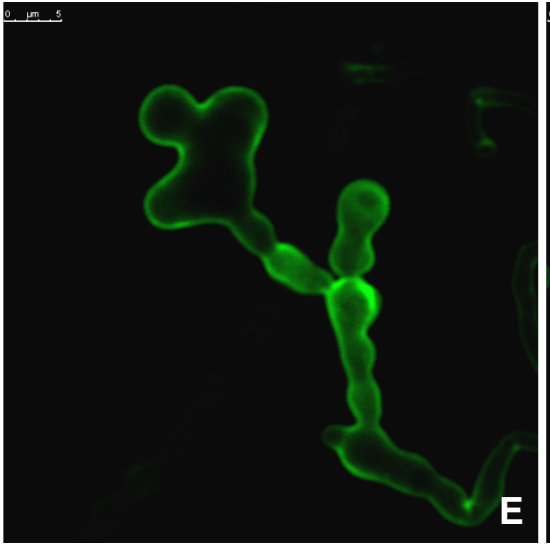

A
Concentrations of free aa in barley 7 dpi
nmol/gFW
Barley
Barley+Amt28
Barley+WT
Asn
Gln
Ser
Asp
Glu
Thr
Ala
GABA
B
Concentrations of free aa in Arabidopsis 7 dpi
Arabidopsis
Arabidopsis+Amt28
Arabidopsis+WT
C
Concentrations of free aa in barley 14 dpi
D
Concentrations of free aa in Arabidopsis 14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

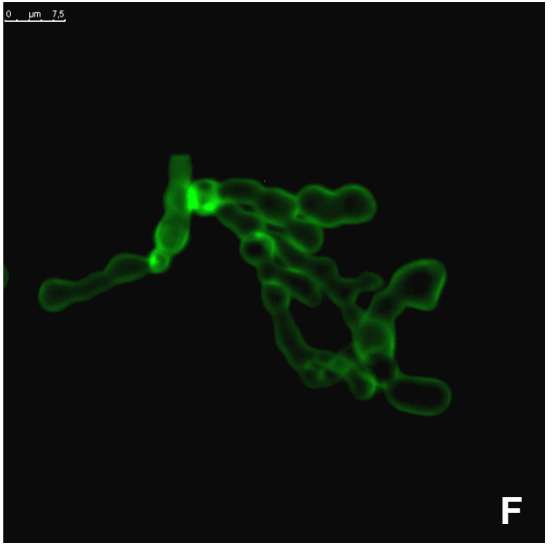

0 µm 25
A

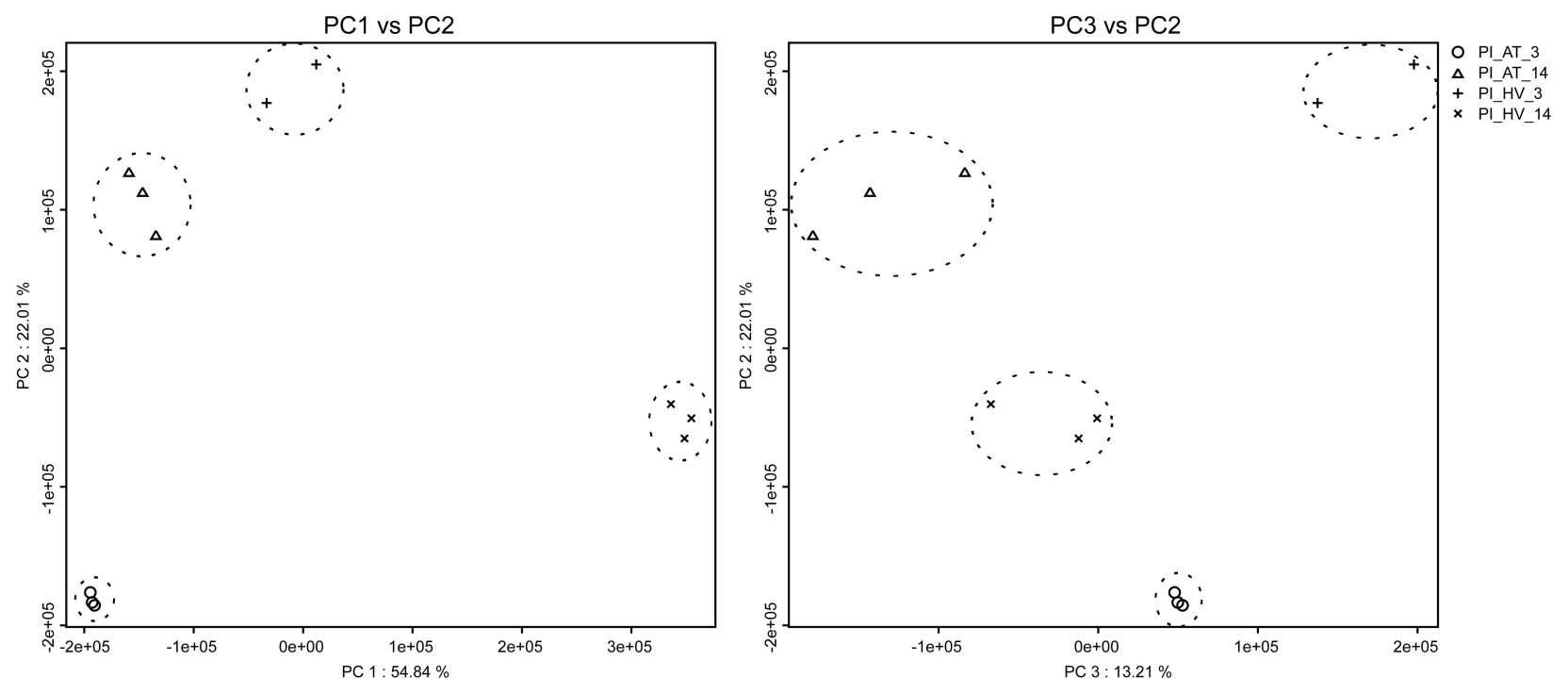

0 µm 25
B


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