Symbiosis Receptor-like Kinase and its *Arabidopsis* homologs in the interaction with beneficial and pathogenic microbes

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Eidesstattliche Versicherung

Hiermit versichere ich an Eides statt, dass die vorliegende Dissertation von mir selbstständig und ohne unerlaubte Hilfe angefertigt ist.

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Erklärung

Hiermit erkläre ich, dass die Dissertation nicht ganz oder in wesentlichen Teilen einer anderen Prüfungskommission vorgelegt worden ist. Ich habe nicht versucht, anderweitig eine Dissertation einzureichen oder mich einer Doktorprüfung zu unterziehen.

München, den 19.11.2014

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Martina Katharina Ried

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I. ABBREVIATION INDEX

° C	degree(s) Celsius
Δ	delta
µ	micro
A / ala	alanine
aa	amino acid
AM	arbuscular mycorrhiza
ANOVA	analysis of variance
BAK1	BRI1-associated Receptor Kinase 1
BF	bright field
bp	base pair(s)
Bcp1	Blue Copper-binding Protein 1
BLAST	basic local alignment search tool
BRI1	Brassinosteroid Insensitive 1
C / cys	cysteine
CCaMK	Calcium Calmodulin dependent Kinase
cDNA	complementary DNA
CEBiP	Chitin oligosaccharide Elicitor-Binding Protein
CERK1	Chitin Elicitor Receptor Kinase 1
CFP	Cyan Fluorescent Protein
CLSM	confocal laser microscopy
CO	chitin oligomer
co	complementation line
Col-0	Columbia-0
CrRLK1L	*Catharanthus roseus* RLK1-like
CSGs	common symbiosis genes
D / asp	aspartic acid
Da	Dalton
DAMP	damage-associated molecular pattern
DMR1	Downy Mildew Resistant 1
dpi	days post infection/inoculation
dpt	days post transformation
EFR	EF-Tu Receptor
ENOD11	Early Nodulin 11
ER	endoplasmatic reticulum
ERF1	Ethylene Response Factor 1
ETI	effector-triggered immunity

EV	empty vector
FLS2	Flagellin-Sensing 2
FP	Fahraeus medium
g	gravitational acceleration / gram(s)
G / gly	glycine
GFP	Green Fluorescent Protein
GG	golden gate
GPAT	Glycerol-Phosphate Acyl-Transferase
GUS	β-glucuronidase
h	hour(s)
HCSGs	homologs of common symbiosis genes
HMGR1	3-hydroxy-3-methylglutaryl CoA-reductase1
HO	Hoagland's
Hpa	*Hyaloperonospora arabidopsidis*
HR	hypersensitive response
HSD	honestly significant difference
IE	immuno enrichment
IOS1	Impaired Oomycete Susceptibility 1
IPD3	Interacting Protein of DMI3
IQR	inter quartile range
JA	jasmonic acid
k	kilo
KD	kinase domain
KDRI	Kazusa DNA Research Institute
L	litre(s)
LCO	lipo-chitooligosaccharide
LRR	leucine-rich repeat
LYP	LysM domain protein
LysM	lysin motif
M	molar
m	meter(s) / mili
min	minute(s)
MLD	malectin-like domain
MLO	Mildew resistance Locus O
mOr	mOrange
MPK	Map Kinase
mRNA	messenger RNA
MS	Murashige and Skoog

n	nano
N / asn	asparagine
NASC	The Nottingham Arabidopsis Stock Centre
NF	nodulation factor
NFP	Nod Factor Perception
NFR1	Nod Factor Receptor 1
NFR5	Nod Factor Receptor 5
NF-Y	Nuclear Factor-Y subunit
NORK	Nodulation Receptor Kinase
NUP	nucleoporin
OD	optical density
P / pro	proline
p	p-value
PAD4	Phytoalexin Deficient 4
PAMP	pathogen-associated molecular pattern
PCR	polymerase chain reaction
PGN	peptidoglycan
PI-LTP	Phosphatidylinositol-lipid Transfer Protein
PMR4	Powdery Mildew Resistant 4
PPA	prepenetration apparatus
PR1	Pathogenesis-Related Gene 1
PRR	pattern recognition receptor
PSKR1	Phytosulfokine Receptor 1
PTI	PAMP-triggered immunity
PUB1	E3 ubiquitin ligases Plant U-box protein 1
Q / gln	glutamine
qRT-PCR	quantitative real-time PCR
R genes	resistance genes
RAM2	Required for Arbuscular Mycorrhiza 2
RFP	Red Fluorescent Protein
RLK	receptor-like kinase
RNS	root nodule symbiosis
ROI	region of interest
ROP6	Rho-like small GTPase 6
ROS	reactive oxygen species
rpm	rounds per minute
RT	room temperature
s	second(s)

S / ser	serine
S genes	susceptibility genes
SA	salicylic acid
ShRK	SYMRK-homologous Receptor Kinase
S.D.	standard deviation
SEH	SEC13 homolog
SIE3	SYMRK-interacting E3 ubiquitin ligase
SINA4	SEVEN IN ABSENTIA 4
SIP1	SYMRK-interacting protein 1
SIP2	SYMRK-interacting protein 2
SNC1	Suppressor of *npr1-1*, constitutive 1
SP	signal peptide
SYMREM1	Symbiotic Remorin 1
SYMRK	Symbiosis Receptor-like Kinase
T / thr	threonine
TAIR	The Arabidopsis Information Resource
TM	transmembrane domain
Tukey's HSD	Tukey's Honestly Significant Difference
UB / Ubi	Ubiquitin
WB	western blot
X-Gluc	5-bromo-4-chloro-3-indolyl glucuronide
Y / tyr	tyrosine
YFP	Yellow Fluorescent Protein

II. LIST OF PUBLICATIONS

Research papers and manuscripts

- 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 *Arabidopsis thaliana.* Under review.

  * These authors contributed equally to the work

- **Ried MK**, Antolín-Llovera M & Parniske M. 2014. Spontaneous symbiotic reprogramming of plant roots triggered by receptor-like kinases. *Elife*. doi: 10.7554/eLife.03891

- Antolín-Llovera M, **Ried MK** & Parniske M. 2014. Cleavage of the SYMBIOSIS RECEPTOR-LIKE KINASE ectodomain promotes complex formation with Nod Factor Receptor 5. *Curr Biol* **24**:422-7. doi: 10.1016/j.cub.2013.12.053.

- Den Herder G, Yoshida S, Antolín-Llovera M, **Ried MK** & Parniske M. 2012. *Lotus japonicus* E3 ligase SEVEN IN ABSENTIA4 destabilizes the symbiosis receptor-like kinase SYMRK and negatively regulates rhizobial infection. *Plant Cell* **24**:1691-707. doi: 10.1105/tpc.110.082248.

Reviews

- Antolín-Llovera M, Petutschnig EK, **Ried MK**, Lipka V, Nürnberger T, Robatzek S & Parniske M. 2014a. Knowing your friends and foes - plant receptor-like kinases as initiators of symbiosis or defence. *New Phytol* **204**:791-802. doi: 10.1111/nph.13117.

- Antolín-Llovera M, **Ried MK**, Binder A & Parniske M. 2012. Receptor kinase signaling pathways in plant-microbe interactions. *Annu Rev Phytopathol* **50**:451-73. doi: 10.1146/annurev-phyto-081211-173002.

III. DECLARATION OF CONTRIBUTION AS CO-AUTHOR
III. ERKLÄRUNG ÜBER DIE ERBRACHTE LEISTUNG ALS KOAUTOR

Martina Katharina Ried, the author of this thesis, contributed to the following manuscripts as listed:

Martina Katharina Ried, die Autorin der vorliegenden Dissertation, hat die folgenden Leistungen zu den aufgeführten Manuskripten beigetragen:

Manuscript I: Cleavage of the SYMBIOSIS RECEPTOR-LIKE KINASE Ectodomain Promotes Complex Formation with Nod Factor Receptor 5. (Results - Paper I)

Reference: Antolín-Llovera M, **Ried MK** & Parniske M. 2014b. Cleavage of the SYMBIOSIS RECEPTOR-LIKE KINASE ectodomain promotes complex formation with Nod Factor Receptor 5. *Curr. Biol.* **24**:422-7. doi: 10.1016/j.cub.2013.12.053.

Martina Katharina Ried:

- designed, performed and analysed interaction studies in *Nicotiana benthamiana* and prepared the corresponding figure.
- *entwarf, führte durch und analysierte Interaktionsstudien in Nicotiana benthamiana und erstellte die zugehörige Abbildung.*

Manuscript II: Spontaneous symbiotic reprogramming of plant roots triggered by receptor-like kinases. (Results – Paper II)

Reference: **Ried MK**, Antolín-Llovera M & Parniske M. 2014. Spontaneous symbiotic reprogramming of plant roots triggered by receptor-like kinases. *Elife***.** doi: 10.7554/eLife.03891

Martina Katharina Ried:

- designed all experiments except for those presented in Figure 5, in the upper panel of Figure 11, and in Figures 12 + 13.
- performed all experiments except for those presented in Figure 5, Figure 6, in the upper panel of Figure 11, and in Figures 12 + 13.
- analysed all experiments.
- performed all statistical analyses except for the data presented in Figure 6.
- created all figures.

- wrote the manuscript, which was edited by Martin Parniske.

- *entwarf sämtliche Experimente, mit Ausnahme von den in Abbildung 5, im oberen Panel von Abbildung 11, und den in Abbildungen 12 + 13 dargestellten.*
- *führte sämtliche Experimente durch, mit Ausnahme der in Abbildung 5, in Abbildung 6, im oberen Panel von Abbildung 11, und den in Abbildungen 12 +13 dargestellten.*
- *analysierte sämtliche Experimente.*
- *führte sämtliche statistischen Auswertungen durch, mit Ausnahme der in Abbildung 6 dargestellten.*
- *erstellte sämtliche Abbildungen.*
- *schrieb das Manuskript, welches von Martin Parniske editiert wurde.*

Manuscript III: Symbiosis-related genes sustain the development of a downy mildew pathogen on *Arabidopsis thaliana.* (Results - Paper III)

Reference: 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 *Arabidopsis thaliana.* Under review.

* These authors contributed equally to the work

Martina Katharina Ried:

- designed all experiments that deal with receptor-like kinases except for those presented in Figure 29 + 30, and in Figure 37.
- performed all experiments that deal with receptor-like kinases except for those presented in Figure 30, in Figure 35, and in Figure 37.
- analysed all experiments that deal with receptor-like kinases.
- performed all statistical analyses expect for the data presented in Figure 38.
- created all figures except for Figure 17 - 19, Figure 25 - 27, Figure 29, and Figure 35.
- wrote the first draft of the manuscript together with Aline Banhara Pereira, and contributed to the writing of all following versions of the manuscript, which was edited by Martin Parniske.

- *entwarf sämtliche Experimente, in denen Rezeptor Kinasen untersucht wurden, mit Ausnahme von den in Abbildung 29 + 30, und in Abbildung 37 dargestellten.*
- *führte sämtliche Experimente durch, in denen Rezeptor Kinasen untersucht wurden, mit Ausnahme von den in Abbildung 30, in Abbildung 35, und in Abbildung 37 dargestellten.*
- *analysierte sämtliche Experimente, in denen Rezeptor Kinasen untersucht wurden.*

- *führte sämtliche statistischen Auswertungen durch, mit Ausnahme der in Abbildung 37 dargestellten.*
- *erstellte sämtliche Abbildungen, mit Ausnahme von Abbildung 17 - 19, Abbildung 25 - 27, Abbildung 14, und Abbildung 35.*
- *schrieb den ersten Entwurf des Manuskripts zusammen mit Aline Banhara Pereira und half beim Schreiben aller weiteren Versionen des Manuskriptes, das von Martin Parniske editiert wurde.*

Signature of co-first author

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Aline Banhara Pereira

Signature of Supervisor

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Prof. Dr. Martin Parniske

IV. SUMMARY

Plants engage in two major types of plant root endosymbioses with beneficial microbes to evade nutrient deficiencies. Arbuscular mycorrhiza (AM), whose origin dates back approximately 450 million years, is a symbiotic relationship between phosphate-acquiring fungi of the phylum *Glomeromycota* and 70 – 90 % of the recent land plants. In contrast, root nodule symbiosis (RNS) with nitrogen-fixing bacteria is restricted to plants of four orders within the Eurosid I clade (*Fabales*, *Fagales*, *Rosales*, *Cucurbitales*). Almost all Angiosperms possess an ancient genetic programme essential for the intracellular accommodation of AM fungi, and it is believed that this programme has been co-opted by the evolutionary much younger RNS. As a consequence, this set of genes has been named common symbiosis genes (CSG).

In legume RNS, gram-negative rhizobia exude lipo-chitooligosaccharides (LCOs) into the rhizosphere, so-called nodulation factors (NF), which are recognized by the lysin motif (LysM)-type receptor-like kinases (RLK) Nod Factor Receptor 1 (NFR1) and NFR5 in *L. japonicus.* NFR1 and NFR5 represent the entry point for NF-mediated signalling and are indispensable for the successful establishment of this plant-microbe mutualism. Symbiosis Receptor-like kinase (SYMRK) contains a malectin-like domain (MLD) followed by three leucine-rich repeats (LRRs) in its extracytoplasmic region. In contrast to NFR1 and NFR5, SYMRK is crucial for the development of AM as well as RNS, but the mechanisms that discriminate between the two distinct symbiotic developmental outcomes and the connection between SYMRK and the NFRs remained enigmatic. We were able to demonstrate complex formation of full-length SYMRK and NFR1 or NFR5 upon overexpression in *Nicotiana benthamiana*. Dependent on the presence of the conserved GDPC motif that connects the LRRs with the MLD, SYMRK undergoes constitutive cleavage *in planta,* giving rise to the highly unstable SYMRK-ΔMLD, a SYMRK version that lacks the MLD but retains the three LRRs. NFR5 interacted specifically and strongly with SYMRK-ΔMLD that outcompeted full-length SYMRK in co-immunoenrichment experiments in *N. benthamiana*.

Interestingly, expression of any of the three *RLK* genes from the strong *LjUbiquitin* promoter in *L. japonicus* roots resulted in the spontaneous formation of root nodules and the activation of RNS-related promoters and genes in the absence of any external symbiotic stimulation, demonstrating an active role of SYMRK in symbiosis signalling. This phenomenon was accompanied by the association of overexpressed NFR1 or NFR5 with endogenous SYMRK in *L. japonicus* roots regardless of the presence of rhizobia. We thus hypothesize that overexpression of one of the *RLKs* results in spontaneous complex formation, which subsequently leads to auto-activation of RNS-signalling. In addition, we

could show that the dominant active *SYMRK* allele is sufficient to activate RNS-signalling upstream of CSGs required for the generation or decoding of calcium-spiking in both symbioses and independently of either *NFR*.

The observation that only overexpression of *SYMRK* - but not of *NFR1* or *NFR5* - activated the AM-related *SbtM1* promoter and the expression of AM-related genes suggests that signalling specificity towards the two different symbiotic programs is achieved at the level of the receptors.

Arabidopsis thaliana belongs to one out of five plant lineages that have lost the ability to form AM, which is accompanied by the specific loss of CSGs in their genomes. *Arabidopsis* can, however, be colonized by the biotrophic oomycete *Hyaloperonospora arabidopsidis,* and the feeding organs of this microbe exhibit structural similarities to AM fungal arbuscules and are accommodated inside plant leaf cells. Besides the loss of certain CSGs, *Arabidopsis* retained homologs of *SYMRK (ShRKs)* in its genome. We report that mutations in these genes caused a reduced amount of oomycetal sporangiophores and alterations in the shape of the haustoria, while they did not result in constitutive resistance or exacerbated activation of defence responses. Therefore, we postulate genetic commonalities between the genetic programmes for the development of intracellular accommodation structures in symbiotic and pathogenic interactions and put forward a model in which pathogens might exploit symbiotic programmes.

V. ZUSAMMENFASSUNG

Es gibt zwei Hauptarten von Endosymbiosen, bei denen Pflanzenwurzeln Verbindungen mit nützlichen Mikroben eingehen, um Nährstoffknappheiten zu überwinden. Arbuskuläre Mykorrhiza (AM) bezeichnet dabei die symbiontische Beziehung zwischen 70 – 90 % der Landpflanzen und Pilzen des Phylums *Glomeromycota*, die die Pflanze mit Phosphat versorgen. Ihr Ursprung reicht ca. 450 Millionen Jahre zurück. Im Gegensatz dazu ist die Fähigkeit zur Wurzelknöllchensymbiose (root nodule symbiosis; RNS) mit Stickstoff-fixierenden Bakterien auf Pflanzen aus vier Ordnungen innerhalb der Eurosiden I Klasse (*Fabales*, *Fagales*, *Cucurbitales*, *Rosales*) beschränkt. Nahezu alle Angiospermen besitzen ein konserviertes genetisches Programm bestehend aus einem Set an Genen, das für die intrazelluläre Beherbergung von AM Pilzen unabdingbar ist und im Zuge der Entstehung der wesentlich jüngeren RNS rekrutiert wurde. Diese Gene wurden konsequenterweise „common symbiosis genes" (CSG) genannt.

In der Leguminosen-RNS geben gram-negative Rhizobien Lipo-chitooligosaccharide (LCOs) in die Erde um die Pflanzenwurzel ab. Diese LCOs werden Nodulations Faktoren (NF) genannt und in *L. japonicus* von den Rezeptor-artigen Kinasen (receptor-like kinases; RLKs) Nod Factor Receptor 1 (NFR1) und NFR5, die zum lysin motif (LysM)-Typ der RLKs gehören, erkannt. NFR1 und NFR5 stellen den Eintrittspunkt der NF-vermittelten Signaltransduktion dar und sind für die erfolgreiche Ausbildung dieses Pflanzen-Mikroben Mutualismus unentbehrlich. Symbiosis Receptor-like kinase (SYMRK) enthält eine Malectin-artige Domäne (malectin-like domain; MLD) gefolgt von drei Leucin-reichen Wiederholungen (leucine-rich repeats; LRRs) in ihrer extracytoplasmischen Region. Im Gegensatz zu NFR1 und NFR5 ist SYMRK sowohl für die Ausbildung von AM als auch RNS notwendig. Die Mechanismen, die zwischen den beiden unterschiedlichen symbiotischen Entwicklungen unterscheiden, sowie die Verbindung zwischen SYMRK und den NFRs waren jedoch noch immer rätselhaft. Es ist uns gelungen zu zeigen, dass Volllängen SYMRK mit NFR1 oder NFR5 bei Überexpression in *Nicotiana benthamiana* Rezeptorkomplexe ausbildet. In Abhängigkeit von dem konservierten GDPC Motiv, das die LRRs mit der MLD verbindet – erfährt SYMRK eine konstitutive proteolytische Spaltung in der Pflanze. Diese Spaltung generiert SYMRK-ΔMLD, eine hoch instabile Version von SYMRK, welcher die MLD fehlt, die jedoch noch immer die LRRs beinhaltet. NFR5 interagierte spezifisch und stark mit SYMRK-ΔMLD in *N. benthamiana*; bei den Co-Immunoanreicherungs-Experimenten wurde Volllängen SYMRK auskonkurriert.

Interessanterweise führte die Expression der *RLK* Gene von dem starken *LjUbiquitin* Promoter in *L. japonicus* Wurzeln zur spontanen Ausbildung von Wurzelknöllchen und der Aktivierung RNS-spezifischer Promotoren und Gene in der Abwesenheit jeglicher

symbiotischer Stimulation. Dies zeigt klar, dass SYMRK eine aktive Rolle in der Symbiose-Signaltransduktion spielt. Dieses Phänomen wurde von der Assoziation von überexprimiertem NFR1 oder NFR5 mit endogenem SYMRK in *L. japonicus* Wurzeln in Ab- sowie Anwesenheit von Rhizobien begleitet. Wir stellen daher die Hypothese auf, dass Überexpression einer der drei *RLKs* zu spontaner Komplexbildung führt, die anschließend die Auto-Aktivierung der Symbiose-Signaltransduktion zur Folge hat. Außerdem konnten wir zeigen, dass das dominant-aktive *SYMRK* Allel für die Aktivierung der RNS-Signaltransduktion oberhalb von CSGs, die für die Erzeugung oder die Dechiffrierung des Calcium-Spikings in beiden Symbiosen zuständig sind, operiert und unabhängig von *NFR1* oder *NFR5* ist.

Die Beobachtung, dass nur die Überexpression von *SYMRK* - nicht aber die von *NFR1* oder *NFR5* - den AM-spezifischen *SbtM1* Promoter und die Expression AM-spezifischer Gene aktiviert, legt nahe, dass die Spezifität der Signaltransduktion im Hinblick darauf, welche der beiden Symbioseprogramme aktiviert wird, auf dem Level der Rezeptoren erreicht wird.

Arabidopsis thaliana gehört zu einer von fünf Pflanzenlinien, die die Fähigkeit AM auszubilden verloren haben. *Arabidopsis* kann jedoch von dem biotrophen Oomyceten *Hyaloperonospora arabidopsidis* kolonisiert werden, dessen Haustorien strukturelle Ähnlichkeit zu den Arbuskeln der AM Pilze aufweisen und innerhalb der Blattzellen der Pflanze beherbergt werden. Neben dem spezifischen Verlust bestimmter CSGs finden sich noch immer Homologe von *SYMRK (SYMRK-homologous Receptor-like Kinases; ShRKs)* im Genom von *Arabidopsis*. Wir berichten, dass Mutationen in diesen Genen die Anzahl der Reproduktionsorgane des Oomyceten reduziert und eine morphologische Veränderung der Haustorien zur Folge hat. Andererseits führten diese Mutationen jedoch nicht zu konstitutiven Resistenz- oder verstärkten Verteidigungs-Reaktionen durch die Pflanze. Aus diesem Grund postulieren wir, dass die genetischen Programme für die Entwicklung von Strukturen für die intrazellulärer Beherbergung von symbiontischen und pathogenen Mikroben Gemeinsamkeiten aufweisen und schlagen ein Modell vor, in dem Pathogene symbiontische Programme ausnutzen.

VI. INTRODUCTION

1. Endosymbioses of the plant root

Being stationary organisms, most land plants are fully dependent on the availability of water and nutrients (e.g. phosphorus and nitrogen) provided by the soil in which they root. In the course of evolution, however, plants have developed powerful strategies to circumvent nutrient deficiencies, such as the establishment of mutualistic endosymbioses with biotrophic fungi of the monophyletic phylum *Glomeromycota* or with nitrogen-fixing rhizobia and *Frankia* bacteria. The beneficial association between plant roots and *Glomeromycota* is called arbuscular mycorrhiza (AM) (Schüßler et al., 2001), and it is considered to have been an important prerequisite for the colonization of the land about 450 million years ago (Remy et al., 1994). The capacity to establish AM is found among 70 - 90 % of the land plant species (Smith and Read, 2008), rendering AM one of the most widespread symbioses (Fitter, 2005). AM is a form of endomycorrhiza that connects the root system of the plant with the extended extraradical mycelium of the AM fungus, a hyphal network specialized for the uptake of water and nutrients such as phosphorus (Parniske, 2008). Upon chemical and mechanical stimulation by the fungus, plant epidermis cells form the so-called prepenetration apparatus, an intracellular structure that forms prior to invasion by fungal hyphae and guides the fungus through the root epidermis into deeper cell layers (Genre et al., 2005). In the cortex, fungal hyphae enter the apoplastic space, where they grow longitudinally and branch to penetrate cells of the inner cortex in which they build tree-shaped structures called arbuscules (Genre et al., 2008). These cells are considered to be the main sites of nutrient exchange where AM fungi provide the root with water, phosphorus, nitrogen, sulphur and other inorganic nutrients and, in turn, receive carbohydrates from their host plant (Gutjahr and Parniske, 2013). Furthermore, it has been reported that *M. truncatula* plants colonized by AM fungi were more resistant to the virulent bacterial pathogen *Xanthomonas campestris* pv. *alfalfa* (Liu et al., 2007). There is a significant contribution of AM to global phosphate and carbon cycling, and the fact that up to 75 % of the phosphorus acquired by plants per year is provided by mycorrhizal fungi emphasizes the importance of this plant-microbe association for terrestrial ecosystems even more (Parniske, 2008, van der Heijden et al., 2008).

Root nodule symbiosis (RNS) with nitrogen-fixing bacteria evolved only 60 million years ago and, opposed to the widespread emergence of AM, only four related orders within the Eurosid I subclade, namely *Fabales*, *Fagales, Cucurbitales* and *Rosales,* are able to establish this form of plant-microbe mutualism (Doyle, 2011, Kistner and Parniske, 2002, Sprent, 2007). All RNSes have in common that the plant accommodates diazotrophic

bacteria in specialized plant derived organs, the root nodules, thus providing a favourable environment for nitrogen fixation. In doing so, the plant is efficiently supplied with ammonium by the microsymbiont in exchange for various nutrients, such as amino acids (aa) or dicarboxylates like malate (White et al., 2007). Members of the *Fagales*, *Cucurbitales* and *Rosales* establish RNS with gram-positive actinobacteria of the genus *Frankia*. In contrast, legumes that belong to the *Fabales*, and *Parasponia*, one exceptional member of the *Rosales*, engage in RNS with - from a phylogenetic point of view - rather diverse bacteria referred to as rhizobia (Pawlowski and Sprent, 2008, Sprent, 2007). The phylogenetic restriction of RNS to one subclade inside the Eurosids suggests that the common ancestor of these orders underwent a genetic predisposition for nodulation. The marked differences between RNSes put forward a model in which RNS evolved several times independently within the *Fabales*, *Fagales*, *Rosales* and *Cucurbitales* (Kistner and Parniske, 2002).

Most recent angiosperms possess a conserved genetic programme for the intracellular accommodation of phosphate-acquiring AM fungi that was recruited during the evolution of the nitrogen-fixing RNS, as evidenced by the so-called common symbiosis genes (CSGs) (Kistner et al., 2005). This core set of genes is required for both AM as well as RNS, and it was identified in extensive analyses of symbiosis-deficient mutants in the model legumes *Lotus japonicus* and *Medicago truncatula* (Venkateshwaran et al., 2013).

2. Chemical crosstalk, first contact and intracellular uptake of the microsymbiont by its plant host

A major prerequisite for the establishment of a mutualistic relationship is the proper communication between host plant and microsymbiont via chemical compounds. This is followed by the spatial approximation of the microbe towards the plant root. Once physical contact is made, the plant root proceeds to take up the microsymbiont intracellularly. As a result, the microbe gets accommodated in specialised root-derived organs inside the root cells, where the symbiotic programmes can finally be executed.

2.1 Arbuscular Mycorrhiza

Most plants are able to synthesize strigolactones, which are carotenoid-derived compounds that are released into the rhizosphere and can be perceived by AM fungi residing in the vicinity of the root (Gutjahr and Parniske, 2013). The perception of strigolactones by AM fungi initiates the pre-symbiotic stage, which is characterized by the induction of excessive hyphal branching (Akiyama et al., 2005), stimulation of cell proliferation and spore germination as well as by changes in mitochondria density, shape and movement (Besserer et al., 2006). Interestingly, strigolactones also activate the

germination of the parasitic plants *Striga* and *Orobanche* (Bouwmeester et al., 2003) and act as suppressors of shoot branching (Gomez-Roldan et al., 2008, Umehara et al., 2008). This suggests that, besides their function as a signal for AM, strigolactones are important and widespread endogenous plant hormones (Gutjahr, 2014).

AM fungi, on their part, release a mixture of different chemical compounds that are perceived by their putative host plant and induce responses including the activation of symbiosis-related genes (Czaja et al., 2012, Kosuta et al., 2003, Kuhn et al., 2010, Maillet et al., 2011, Ortu et al., 2012), lateral root formation (Maillet et al., 2011, Olah et al., 2005), rhythmic oscillation of cytosolic calcium concentrations in and around the nucleus (calcium spiking; Chabaud et al., 2011, Genre et al., 2013, Kosuta et al., 2008, Sieberer et al., 2012), and the accumulation of starch (Gutjahr et al., 2009). Over the last years, several of these compounds have been identified to be either sulphated and non-sulphated lipo-chitooligosaccharides (LCOs) that are capable of activating the symbiosis-related *ENOD11* promoter, of inducing lateral root formation and of stimulating the formation of AM in both leguminous and non-leguminous plants and are highly similar to rhizobia-derived nodulation factors (NF; Maillet et al., 2011), or short-chain chitin oligomers (COs) that trigger calcium spiking (Genre et al., 2013). While the perception of AM fungal LCOs appears to be dependent on the LysM-RLK gene *Medicago truncatula Nod Factor Perception* (*MtNFP)* and the common symbiosis pathway (Czaja et al., 2012, Maillet et al., 2011), responses induced by the AM fungal COs are dependent on the common symbiosis pathway but *MtNFP*-independent (Genre et al., 2013).

Upon physical contact between a fungal hypha and the plant root, mechanical as well as chemical cues induce differentiation into the so-called hyphopodium, which attaches to the root surfaces and marks the entry point for fungal invasion (Gutjahr and Parniske, 2013). One important prerequisite for successful hyphopodium formation is the synthesis of cutin monomers, in which the glycerol-3-phosphate acyl transferase Required for Arbuscular Mycorrhiza 2 (RAM2) is involved (Wang et al., 2012). The oomycete *Phytophthora palmivora* is a pathogen that infects *M. truncatula* roots and – in analogy to hyphopodia - forms appressoria. Strikingly, Wang and colleagues could demonstrate that the *ram2* mutation not only impairs AM symbiosis but also strongly decreases the number of oomycetal appressoria (Wang et al., 2012). This is in line with former discoveries that cutin monomers induce appressorium formation of virulent fungi. It suggests that cutin monomers might play an important and general role in plant interactions with fungi and oomycetes (Gutjahr and Parniske, 2013).

Once the hyphopodium is formed, the nucleus of the adjacent plant root epidermis cell first moves towards the contact site between plant root and AM fungus and then travels

through the plant cell vacuole, initiating the formation of the so-called prepenetration apparatus (PPA). The latter builds a cytoplasmic bridge across the vacuole connecting the nucleus with the site of fungal entry and guides the way through the plant rhizodermis and the cell layers of the outer root cortex (Genre et al., 2008, Genre et al., 2005, Parniske, 2008).

Interestingly, strong expression of a deregulated version of *Calcium and Calmodulin-dependent Kinase* (*CCaMK*) - a CSG that is implicated in the decoding of the calcium spiking in RNS as well as AM - is sufficient to spontaneously trigger the development of structures that resemble the PPA (Takeda et al., 2012). This phenomenon is accompanied by the spontaneous activation of the AM-specific Subtilisin-like Serine Protease *SbtM1* promoter, a characteristic also found in cells containing AM fungus-induced PPAs (Takeda et al., 2012).

When fungal hyphae reach the inner cortex, they enter the apoplastic space and proceed to grow longitudinally and branch to penetrate cells of the inner cortex, which will undergo massive rearrangement of the cytoskeleton (Blancaflor et al., 2001, Gutjahr and Parniske, 2013). Inside the host cells, the fungus builds highly branched tree-shaped structures that are called arbuscules. These arbuscules are surrounded by a plant-derived membrane, referred to as periarbuscular membrane, which, together with the fungal membrane and the periarbuscular space between both membranes, builds the symbiotic interface that is maintained for nutrient exchange between host and AM fungus (Parniske, 2008).

2.2 Legume root nodule symbiosis

Especially under nitrogen limiting conditions, legumes produce species-specific flavonoids and exude them into their rhizosphere to attract their cognate rhizobial symbiont (Weston and Mathesius, 2013). Rhizobia are able to perceive these compounds via NodD proteins of the LysR family (Peck et al., 2006), transcriptional regulators that activate the expression of bacterial *nod* genes (Fisher and Long, 1993). Subsequently, strain-specific NFs are synthesized.

NFs are highly similar to AM fungal LCOs and share significant similarity with chitin molecules. They consist of a chitin backbone built of β-(1-4)-linked N-acetylglucosamine residues, which is decorated with N-linked fatty acid moieties (e.g. fucosyl, acetyl, sulphuryl, methyl, carbamoyl or arabinosyl groups) that are attached to the non-reducing terminal sugar (Denarie, 1996). These strain-dependent modifications are essential for stringent host specificity (Downie and Walker, 1999). One hypothesis is that they have evolved to mask the chitin backbone to circumvent plant triggered immunity (Hamel and Beaudoin, 2010). Interestingly, it has been reported that two symbiotic, photosynthetic

Bradyrhizobium strains, BTAi1 and ORS278 - even though they can successfully colonize plant roots - do not contain canonical *nodABC* genes and are therefore believed to be unable to synthesize NFs. It is assumed that these rhizobia have developed an alternative signalling molecule, likely to be a purine derivative, which enables them to successfully establish RNS with their respective host plants (Giraud et al., 2007).

After NFs are synthesized, they are released into the soil and, once perceived by Nod Factor Receptors (NFRs) at the plant root hairs, trigger the first cellular responses within the rhizodermis and the root cortex (Oldroyd, 2013). Like chitin and AM fungal LCOs, NFs are potent elicitors of plant responses (Cooper, 2007). Some of the early responses to NFs in the rhizodermis that can be observed within the first hours after NF application include alkalisation, membrane depolarisation, an increase in the intracellular levels of calcium in root hairs and calcium spiking, modifications in the root hair cytoskeleton and root hair deformation including the formation of the so-called shepherd's crooks (Jones et al., 2007). Furthermore, the activation of symbiosis-related genes can be observed upon NF recognition (Horvath et al., 2002).

Early responses to NFs are not restricted to the epidermal cell layers but can also be found in the root cortex where they are thought to regulate nodule formation. An important prerequisite for organogenesis is the reactivation of the mitotic cell cycle in root cortical cells, which seems to be at least partly mediated by the inhibition of auxin transporters (Jones et al., 2007). The re-initiation of cell division results in the formation of a nodule primordium prone to develop into a mature nodule upon successful bacterial infection. It seems noteworthy that epidermal and cortical responses are at least partially independent, as bacterial infection can be observed in mutant plants impaired in organogenesis and vice-versa (Murray et al., 2007, Tirichine et al., 2006).

Once the rhizobia follow the plant's flavonoid track, they reach the root surface where physical attachment to the root hairs is either achieved via bacterial adhesins (Smit et al., 1992) or by the interaction of plant lectins and specific surface polysaccharides of the bacteria (Dazzo et al., 1984). Subsequent NF-induced root hair deformation is accompanied by polar growth resulting in root hair curling around the attached bacteria, which finally entraps them in an infection pocket (Esseling et al., 2003). Bacteria inside the infection pocked divide and form so-called infection foci (Oldroyd et al., 2011).

In response to diverse bacteria-derived stimuli, such as exopolysaccharides and NF concentration in the infection pocket, the root hair cell wall is hydrolysed and the plasma membrane invaginated. In analogy to the pre-penetration apparatus in AM, a pre-infection thread is formed in RNS. First, the nucleus traverses towards the site of bacterial infection and then guides through the cell the pre-infection thread that consists of ER-rich

cytoplasmic bridges aligned with the cytoskeleton. The infection thread progressively grows through the outer cortex towards the nodule primordium, allowing bacterial colonization (van Brussel et al., 1992, Yokota et al., 2009). When the infection thread finally reaches the cortex, bacteria covered with a plant-derived membrane are released into the nodule and differentiate into bacteroids, small biological fermenters capable of nitrogen fixation (Kereszt et al., 2011).

3. The role of LysM-RLKs in plant-microbe interactions

In 1986, a previously unknown motif consisting of a direct repeat of 44 aa separated by 7 aa was discovered in the C-terminus of the *Bacillus* phage ϕ29 lysozyme (Garvey et al., 1986). Already six years later, Joris and colleagues identified this motif as a modular cassette present in various bacterial proteins, and considered a particular involvement in ligand-binding (Joris et al., 1992). Because of its presence in bacterial lysins, this module was subsequently termed lysin motif (LysM) domain.

The LysM structure is composed of two α-helices located on one side of a two-stranded antiparallel β-sheet ($\beta\alpha\alpha\beta$) and was initially characterized by x-ray crystallography of two bacterial proteins (Bateman and Bycroft, 2000, Bielnicki et al., 2006). A typical LysM domain comprises one to six LysMs separated by a linker mainly consisting of serine (ser), threonine (thr), asparagine (asn) and proline (pro) residues (Buist et al., 2008). LysM domains are present in all kingdoms except archaea (Bateman and Bycroft, 2000) and can directly bind peptidoglycan (PGN) from different bacteria species (Steen et al., 2003). PGN is a major component of the bacterial cell wall and consists of linear chains of N-acetylmuramic acid cross-inked with β-(1-4)-linked N-acetylglucosamine. Besides, the LysM domain has a specific binding capacity for molecules structurally related to PGN (e.g. chitin and NF).

Various LysM domain-containing proteins can be found in a broad range of organisms, but the linkage of a LysM domain to a protein kinase so far appears to be unique to the plant kingdom comprising the family of plant LysM-type receptor-like kinases (LysM-RLKs) (Bateman and Bycroft, 2000). At least eleven early diverging clades of plant LysMs exist and only five of these have been found in LysM-RLKs so far. Furthermore, the LysM domain of a RLK does not contain more than three LysMs (Zhang et al., 2007). Based on the full-length proteins sequences of 76 LysM-RLKs from 10 species (At, Gm, Lj, Mt, Os, Pp, Pt, Sm, Vv, Zm), Zhang and associates grouped them into 6 multi-plant-family clades and a small group containing only MtLYK10 and 11 (Zhang et al., 2009). Clades I and VI are also called Nod Factor Receptor 5 (NFR5) and NFR1 clades, respectively, and most leguminous LysM-RLKs in subclades IA and VIA are putative NFRs (Zhang et al., 2009).

Interestingly, the NFR5 clade lacks LysM-RLKs from *Arabidopsis*, one out of five plant linages that has lost the ability to form AM (Zhang et al., 2009). In *L. japonicus* the number of *LysM-RLK* genes is higher (17) than in the non-leguminous plant *Arabidopsis* (5) (Lohmann et al., 2010, Zhang et al., 2009) consistent with that *LysM-RLK* duplicates have acquired new functions (e.g. NF perception) in legumes (Zhang et al., 2007).

3.1 LysM-type RLKs and RLPs in chitin signalling

Chitin, a long chain polymer of β-(1-4)-linked N-acetylglucosamine residues, is the main component of the fungal cell wall and functions as a potent elicitor in plant cells. Chitin-induced responses include lignification, expression of early chitin responsive and basic defence genes, and a biphasic generation of reactive oxygen species (ROS) (Shibuya and Minami, 2001). Interestingly, chitin also induces immune responses in mammalian cells, accounting for the existence of a common chitin-mediated defence system in higher eukaryotes (Reese et al., 2007).

In 1997, a 75 kDa plasma membrane protein with a high binding affinity for chitin oligosaccharides, hence called Chitin oligosaccharide Elicitor-Binding Protein (OsCEBiP), was identified in suspension-cultured rice cells via photoaffinity labelling and affinity crosslinking experiments (Ito et al., 1997). However, it took another nine years until part of the N-terminal protein sequence was identified and *OsCEB*iP could finally be cloned. *OsCEBiP* encodes a LysM domain protein (LYP) with a transmembrane spanning region, an extracellular LysM domain containing three LysMs, but, intriguingly, lacks any intracellular domains typically found in membrane receptors. Gene-specific knockdown of *CEBiP* via RNA-interference leads to impaired chitin responses (e.g. decrease in ROS production), which further substantiates the importance of OsCEBiP in chitin signalling (Kaku et al., 2006).

In *Arabidopsis*, the LysM-RLK Chitin Elicitor Receptor Kinase 1 (AtCERK1) was identified in a screen for chitin insensitive *Arabidopsis* mutants in LysM protein-encoding genes (Miwa et al., 2006) and, almost simultaneously, a *LysM-RLK* mutant characterized by the lack of induction of chitin responsive genes (e.g. *MPK3, WRKY22, WRKY33, WRKY53*) upon chitin octamers treatment was found (Wan et al., 2008). The affected gene encodes the LysM-RLK1 protein and turned out to be identical with *CERK1* (Wan et al., 2008). AtCERK1 contains an extracellular LysM domain comprising three LysMs, a transmembrane region, and a functional intracellular ser/thr protein kinase domain. In rice, OsCERK1 was identified based on its homology to AtCERK1 (54 % aa sequence identity) and the fact that it was upregulated in response to chitin treatment (Shimizu et al., 2010). In contrast to the *AtCERK1* knockout phenotype, knockout of *OsCERK1* did not lead to a complete block, but only resulted in a marked suppression of defence responses

(Shimizu et al., 2010), which is in line with the finding that OsCERK1 has no chitin binding capacity by itself (Shinya et al., 2012). As OsCEBiP does not contain a signalling module such as a protein kinase domain, the involvement of a RLK like OsCERK1 in chitin signalling appears likely. The extracellular domains of OsCEBiP and OsCERK1 specifically interact in the yeast-two hybrid system, and hetero-oligomerisation of OsCEBiP and OsCERK1 could be demonstrated in a ligand-dependent manner in rice cells (Shimizu et al., 2010).

The idea that AtCERK1 is directly involved in chitin binding and subsequent signal transduction was first substantiated when AtCERK1 could be purified from *Arabidopsis* leaf extracts with chitin magnetic beads (Petutschnig et al., 2010), and when AtRLK1-yEGFP was shown to specifically bind chitin beads and colloidal chitin (Iizasa et al., 2010). In 2012, Liu and colleagues could finally demonstrate that AtCERK1 directly interacts with chitin by providing the crystal structure of the extracytoplasmic domain of AtCERK1 in complex with a chitin pentamer (Liu et al., 2012). In this complex, the interaction between AtCERK1 and chitin was mediated by the second LysM of AtCERK1 and three N-acetylglucosamine units of the chitin pentamer (Liu et al., 2012). Liu and colleagues could further show that chitin octamers induce AtCERK1 dimerization, which is important for subsequent downstream signalling, while shorter chitin oligomers inhibited this dimerization and abolished AtCERK1-mediated signalling (Liu et al., 2012). Interestingly, it is also the second LysM of OsCEBiP that is involved in chitin binding and, similar to AtCERK1, two OsCEBiP molecules interact with chitin heptamers or octamers as a dimer (Hayafune et al., 2014).

3.2 LysM-type RLKs in plant root endosymbioses

Because of the structural similarity of NFs and AM fungal LCOs to chitin, it seems reasonable to assume that LysM-RLKs or LYPs are involved in the direct perception of these microsymbiont derived molecules.

In *L. japonicus*, two likely candidates for the NF receptors are the LysM-RLKs NFR1 and NFR5. *NFR1* consists of 12 exons and was isolated by positional cloning in 2003 (Radutoiu et al., 2003). Due to alternative splice donor sites at the 3‘ end of exon IV, two distinct *NFR1* mRNAs can be generated, resulting in two NFR1 gene products. One of them, NFR1a, consists of 621 aa and is predicted to have a molecular weight of 68.09 kDa. The other one, NFR1b, is a protein of 623 aa with a predicted molecular weight of 68.23 kDa (Radutoiu et al., 2003). Like AtCERK1 and OsCEBiP, NFR1 harbours three LysMs in its extracytoplasmic domain and has a functional intracellular protein kinase domain (Radutoiu et al., 2003). Moreover, *NFR1* gene activity is organ-regulated and root-specific (Radutoiu et al., 2003).

At the same time, Madsen and colleagues were able to identify another *Lotus* gene, *NFR5*, to be important at the very early stages of rhizobial infection (Madsen et al., 2003). Similarly, a map based cloning approach led to the isolation of *NFR5*, which is characterized by an intron-less gene structure. The predicted NFR5 protein contains 596 aa and has a predicted molecular weight of 65.3 kDa. Similar to NFR1, NFR5 comprises an extracellular region with three LysMs. Remarkably, the NFR5 kinase domain shows motifs associated with functional ser/thr kinases, except for motifs VII and VIII, whose modification results in a highly divergent or even absent activation loop (Madsen et al., 2003). NFR5 thus is a so-called pseudokinase, which lacks kinase activity and therefore resembles a signalling incompetent LYP like CEBiP (Madsen et al., 2011, Madsen et al., 2003). However, the cytosolic domain of NFR5 is likely to be important for protein-protein interactions as it can be phosphorylated by NFR1 and SYMRK *in vitro* (Madsen et al., 2011).

Any responses to rhizobia are completely abolished in *nfr1* or *nfr5* mutants (Madsen et al., 2003, Radutoiu et al., 2003). These data indicate that both NFRs are crucial components at the very early stages of rhizobial infection upstream of the common symbiosis pathway. The fact that NFR1 and NFR5 are both irreplaceable for the initiation of the earliest responses to purified NFs strongly accounts for their direct involvement in NF recognition (Madsen et al., 2003, Radutoiu et al., 2003).

Radutoiu and colleagues (2007) were able to demonstrate that NFR1 and NFR5 indeed confer host specificity by introducing both coding sequences into *M. truncatula* (Radutoiu et al., 2007). This resulted in *Medicago* plants that successfully established RNS with *Mesorhizobium loti*, the symbiont of *L. japonicus* (Radutoiu et al., 2007). Furthermore, domain swaps between *LjNFR5* and *NFR5* from *Lotus filicaulis*, or *MtNFP* (homolog of LjNFR5) and *NFR5* from pea, corroborate that it is the LysM domain of NFR5, especially LysM2, which is the major determinant in NF recognition (Bensmihen et al., 2011, Radutoiu et al., 2007). In 2012, direct binding of NFs to the LysM domains of NFR1 and NFR5 could finally be demonstrated (Broghammer et al., 2012). Nevertheless, the change in DZL specificity observed in *nfr1 x nfr5* double mutants complemented with chimerical genes of *LjNFR1* and *LjNFR5* containing the coding sequence of the LysM domains of *LfNFR1* and *LfNFR5* was incomplete. This suggests the involvement of other (cell-type specific) components in NF recognition The fact that co-expression of *NFR1* and *NFR5* or *MtNFP* (homolog of *LjNFR5*) and *MtLYK3* (homolog of *LjNFR1*) in *N. benthamiana* leaves leads to cell death responses, which are abolished if one of the RLKs is expressed alone or if NFR5 is co-expressed with a kinase inactive version of NFR1, first substantiated the idea of a NF receptor complex in RNS signalling (Madsen et al., 2011, Pietraszewska-Bogiel et

al., 2013). This could recently be corroborated by the finding that NFP and LYK3 form heteromeric complexes at the cell periphery in *M. truncatula* nodules (Moling et al., 2014).

In addition, domain swaps between NFR1 and AtCERK1 have led to the identification of two stretches in the NFR1 kinase domain to be essential for symbiosis signalling (Nakagawa et al., 2011). One of these stretches is located in the activation loop residues 467 – 470 and the other one is the YAQ (489 – 491) involved in the α-EF helix (Nakagawa et al., 2011). Only chimeric receptors consisting of the extracytoplasmic region of NFR1 fused to the kinase domain of AtCERK1 with the two stretches mentioned above included (NFR1-CERK1(AL-YAQ)), or the kinase domain of either CERK1 from *Ricinus communis*, rice, sorghum, or tomato, but not the unaltered kinase domains of AtCERK1 or CERK1 from *Brassica rapa,* fully rescued the *nfr1* phenotype (Miyata et al., 2014, Nakagawa et al., 2011). The sequence motif YAQ/YAR is well-conserved in non-leguminous dicots and indicative of symbiosis competence (De Mita et al., 2014). While it is present in the kinase domain of OsCERK1, it is absent from two members of the asymbiotic *Brassicaceae*, AtCERK1 and BrCERK1 (De Mita et al., 2014, Miyata et al., 2014, Nakagawa et al., 2011). Finally, NF-induced defence responses were observed in *AtCERK1* knock-out plants co-expressing chimeric receptors composed of the extracytoplasmic regions of either NFR1 or NFR5 and the kinase domain of AtCERK1, while chitin-induced RNS signalling was observed in *L. japonicus* roots co-expressing chimeric receptors composed of the extracytoplasmic regions of OSCERK1 and OsCEBiP fused to the kinase domains of NFR1 and NFR5 (Wang et al., 2014). Several interactors of NFR1 and NFR5 are shown in Figure 1.

The hypothesis that – similar to chitin and NFs - AM fungal LCOs and COs are perceived by LysM-RLKs or LYPs was substantiated with the identification of a NFR5-related LysM-RLK that is indispensable for RNS and AM in the non-legume *Parasponia andersonii* (Op den Camp et al., 2011). It has been a working model for years that in the course of a stepwise evolution of RNS several pre-existing modules such as receptors, have been co-opted from the ancient AM (Kistner and Parniske, 2002, Markmann et al., 2008). This is an idea that is further supported by a phylogenetic study, which suggests that the function of LysM-RLKs in AM predates their roles in chitin and NF perception (De Mita et al., 2014).

Intriguingly, also the LysM-RLK OsCERK1, which was originally described to be involved in chitin signalling together with its interaction partner and chitin receptor OsCEBiP, is indispensable for a proper establishment of AM in rice (Miyata et al., 2014). Inoculation of rice with *Rhizophagus irregularis* results in the exclusive expression of symbiosis-related genes in an *OsCERK1*-dependent manner, while chitin treatment only

induces the expression of defence-related genes (Gutjahr et al., 2008, Kouzai et al., 2014, Miyata et al., 2014). This indicates that specific interaction partners such as OsCEBiP might be crucial components in the mechanisms that enable the plant cell to discriminate between beneficial and pathogenic microbes and that ensure the activation of the appropriate signalling cascade.

4. One for all: The role of Symbiosis Receptor-like Kinase in plant root endosymbioses

The CSG *SYMRK* encodes a LRR I-RLK with an extracytoplasmic region comprising three LRRs that are connected to a MLD via the well-conserved GDPC motif and a functional intracellular ser/thr protein kinase domain (Kosuta et al., 2011, Stracke et al., 2002, Yoshida and Parniske, 2005, Antolín-Llovera et al., 2014). *LjSYMRK* consist of 15 exons that cover a full-length open reading frame of 2789 nucleotides. The encoded protein includes 923 aa and has a predicted molecular weight of 103 kDa.

SYMRK is not only required for AM as well as legume root nodule symbiosis but also for nodulation of the non-leguminous plants *Datisca glomerata* and *Casuarina glauca* with Frankia bacteria, suggesting a general involvement of *SYMRK* in actinorhizal symbiosis (Gherbi et al., 2008, Markmann et al., 2008).

Interestingly, while many CSGs like *CCaMK* show a conserved overall domain structure across angiosperm linages, at least three distinct domain compositions exist for the extracytoplasmic region of SYMRK (Markmann et al., 2008). Only the longest SYMRK version, which was exclusively found in nodulating as well as non-nodulating lineages of the Eurosid clade, fully complemented RNS (Markmann et al., 2008). SYMRK versions of reduced length that either lacked one LRR or one LRR and the extracytoplasmic extension, were still able to complement AM but did not restore nodulation in a *symrk-10* background (Markmann et al., 2008). These results suggest that the acquisition of a full-length SYMRK version might be part of the predisposition for nodulation event underwent by a common ancestor of the Eurosid I subclade.

Similar to *nfr1* or *nfr5* mutants, *symrk* mutants lack most cellular and physiological responses to rhizobia (Radutoiu et al., 2003), including NF-induced calcium spiking and the development of infection threads upon rhizobia inoculation (Miwa et al., 2006, Stracke et al., 2002). However, in contrast to *nfr1* and *nfr5* mutants, *symrk* mutants show exaggerated root hair swelling, and calcium influx can be measured in response to NF (Miwa et al., 2006, Stracke et al., 2002). Based on these phenotypic observations, SYMRK was positioned downstream of the very first responses towards NF recognition initiated by NFR1 and NFR5 but upstream of calcium spiking and activation of CCaMK (Miwa et al., 2006, Radutoiu et al., 2003).

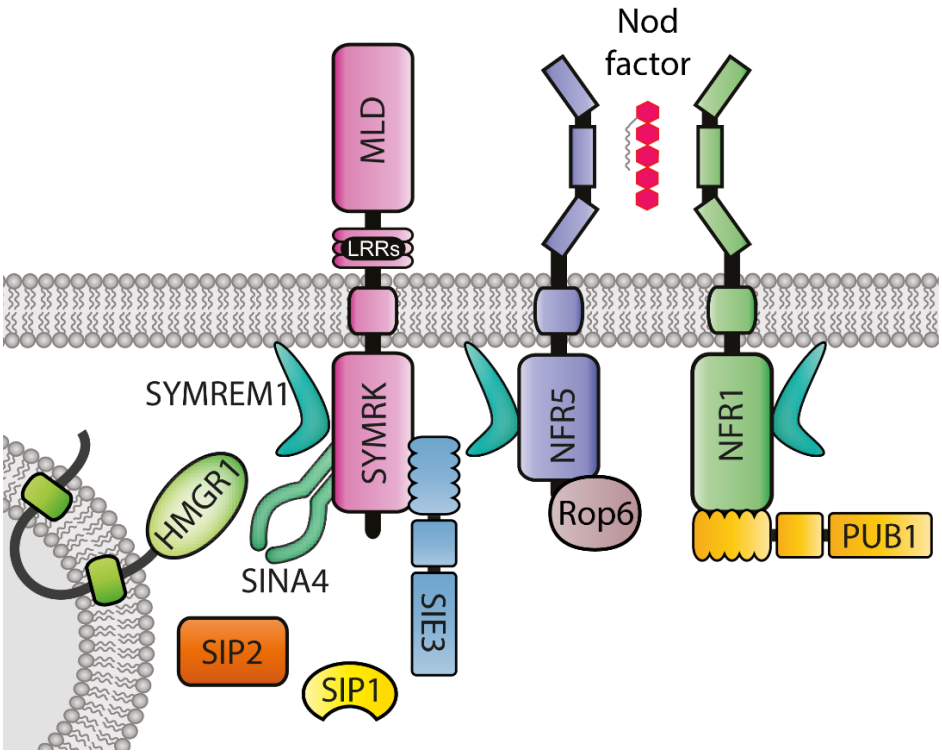


Figure 1: Interaction Network of the symbiotic RLKs NFR1, NFR5 and SYMRK. (Figure and legend modified from Antolín-Llovera et al. 2014b, New Phytologist).

Upon the perception of rhizobial NFs by the LysM-RLKs NFR1 and NFR5, a signalling cascade is initiated that results in nodule organogenesis and bacterial infection (Broghammer et al., 2012, Madsen et al., 2003, Radutoiu et al., 2003, Radutoiu et al., 2007). The membrane-attached remorin protein Symbiotic remorin 1 (SYMREM1) interacts with all three RLKs and has been shown to be highly upregulated during nodulation (Lefebvre et al., 2010, Toth et al., 2012). Remorin proteins are putative scaffold proteins involved in the organisation of microdomains, thus SYMREM1 might recruit a signalling platform including symbiotic RLKs (Jarsch et al., 2014, Jarsch and Ott, 2011). Several interactors of the symbiotic RLKs have been identified in independent yeast-two hybrid screens using the intracellular region of the respective RLK as bait. By these means an isoform of the 3-hydroxy-3-methylglutaryl CoA reductase 1 (MtHMGR1), an enzyme catalysing a key step in the production of isoprenoid compounds via the mevalonate pathway, was found to interact with the kinase domain of the Nodulation Receptor Kinase (MtNORK; homolog of SYMRK) (Kevei et al., 2007). It is still uncertain how this interaction links symbiosis signalling with secondary metabolism pathways. The E3 ubiquitin ligases Plant U-box protein 1 (MtPUB1) was identified as an interactor of the kinase domain of MtLYK3 and negative regulator of symbiosis signalling (Mbengue et al., 2010). Furthermore, the Rho-like small GTPase 6 (ROP6), a positive regulator of infection thread formation and nodulation and interactor of NFR5 (Ke et al., 2012), the ARID-containing transcription factor SYMRK-interacting protein 1 (SIP1) (Zhu et al., 2008), and the MAP kinase kinase SIP2 (Chen et al., 2012) were found. SIP1 could directly connect the kinase region of SYMRK with gene expression (Wang et al., 2013), while SIP2 provides a possible component for signalling from the plasma membrane to the nucleus via a SYMRK-induced phosphorylation / dephosphorylation cascade (Chen et al., 2012). Finally, two different E3 ubiquitin ligases have been found to associate with the intracellular region of SYMRK: SYMRK-interacting E3 ubiquitin ligase (SIE3) (Yuan et al., 2012) and SEVEN IN ABSENTIA 4 (SINA4) (Den Herder et al., 2012).

Interestingly, *NFR1* and *NFR5* but not *SYMRK* remain necessary for epidermal infection thread initiation in a deregulated *ccamk* background (Hayashi et al., 2010, Madsen et al., 2010). This finding strongly accounts for the existence of at least two interdependent pathways that employ alternative heterocomplexes consisting of symbiotic RLKs and other interacting proteins, and result in bacterial infection on the one hand and nodule organogenesis on the other hand. In addition, the involvement of alternative complex components might provide a mechanism to maintain a tight spatio-temporal regulation of symbiosis signalling. Some candidates for these proteins are illustrated in Figure 1.

Importantly, it has not been conclusively resolved whether SYMRK plays an active signalling role in symbiosis or, alternatively, is involved in mechanical stress desensitation (Esseling et al., 2004). This could explain the observation that almost all *symrk* mutant root hairs show aberrant responses to NF treatment, whereas only very few root hairs curl and develop infection threads in wild type plants (Stracke et al., 2002).

SYMRK undergoes constitutive proteolytic cleavage *in planta*, which is independent of symbiotic stimulation and which gives rise to a fragment that contains the MLD, and a membrane-bound SYMRK fragment, named SYMRK-ΔMLD, that retains the LRRs but lacks the MLD (Antolín-Llovera et al., 2014). Furthermore, mutations in the conserved GDPC motif like in the *symrk-14* mutant abolish the proper release of the MLD and impair symbiotic development in the epidermis (Antolín-Llovera et al., 2014, Kosuta et al., 2011). The fact that the release of the MLD could also be observed for SYMRK ectopically expressed in *Nicotiana benthamiana* leaves indicates that this phenomenon is tissue- and species-independent and yields two explanations: it is either caused by autocatalytic cleavage or by a conserved extracellular protease (Antolín-Llovera et al., 2014).

In contrast to full-length SYMRK and SYMRK-ΔEC (an artificial SYMRK version that lacks the whole extracytoplasmic region), SYMRK-ΔMLD is only detectable in very low abundance on western blots (Antolín-Llovera et al., 2014). This indicates that – upon MLD release - the presence of the LRRs destabilizes the protein which then is subject to high turn-over (Antolín-Llovera et al., 2014). The amount of receptor molecules and associated proteins at the plasma membrane is one crucial prerequisite for proper signalling, and therefore it is highly regulated. Overexpression of the E3 ubiquitin ligase SEVEN IN ABSENTIA 4 (SINA4), which interacts with the cytoplasmic region of SYMRK, reduces SYMRK abundance und negatively interferes with infection thread development (Den Herder et al., 2012). One hypothesis is that ectodomain cleavage is a mechanism to further regulate the abundance of SYMRK at the cell surface through its extracytoplasmic region

by generating SYMRK-ΔMLD, which might be implicated in self-clearance by providing a degron and might be the main target of SINA4 or other E3 ubiquitin ligases.

Intriguingly, the specific domain composition featuring a MLD followed by the GDPC motif and LRRs is not restricted to SYMRK but can also be found in 41 of the 50 members of LRR I-RLKs present in *Arabidopsis thaliana* (Hok et al., 2011). In addition, MLDs are present in members of other LRR-RLK subfamilies and the *Catharanthus roseus* RLK1-like (AtCrRLK1L) family (Boisson-Dernier et al., 2011). In *Arabidopsis*, the gene encoding the MLD-LRR-RLK Impaired Oomycete Susceptibility 1 (IOS1) is involved in defence-related signalling (Chen et al., 2014, Hok et al., 2011).

A mutation in the *IOS1* locus has a negative effect on the reproductive success of the oomycetal downy mildew pathogen *Hyaloperonospora arabidopsidis (Hpa),* presumably due to an impaired development of *Hpa* hyphae on the mutant (Hok et al., 2011). Moreover, IOS1 acts as a positive regulator of ligand-induced association of the flagellin receptor Flagellin-Sensing 2 (FLS2) and its co-receptor BRI1-Associated receptor Kinase 1 (BAK1), it constitutively interacts with FLS2, BAK1 as well as the EF-Tu receptor (EFR), and it is involved in the priming of pattern-triggered immunity (Chen et al., 2014). These results suggest that on the one hand, *IOS1* supports the infection of an obligate biotrophic oomycetal pathogen, but on the other hand, is important for the resistance to hemibiotrophic bacteria.

The involvement of a MLD-LRR-RLK in plant pathogen interactions in the asymbiotic Brassicaceae *Arabidopsis* poses the questions whether other MLD-LRR-RLKs are also implicated in plant pathogen interactions and whether MLD release is a common theme for this kind of proteins and is not restricted to SYMRK function in symbiosis. However, the mechanism of MLD release and its role for subsequent signalling have not been elucidated and shall be interesting targets for future research.

VII. AIM OF THE THESIS

Although the common symbiosis gene *SYMRK* had already been cloned in 2002, its precise role in symbiosis has remained enigmatic. Upon bacterial inoculation or NF application, *symrk* mutants show exaggerated root hair swelling and branching, but no proper root hair curling or infection thread development can be observed. In contrast, roots hairs of a *nfr1* or *nfr5* mutant lack most cellular and physiological responses to rhizobia. Based on these phenotypic mutant analyses and the fact that SYMRK is a plasma membrane localized RLK, it was hypothesized to act at early stages of symbiosis signalling, most likely directly downstream of NFR1 and NFR5. However, genetic evidence for this position and even for the involvement in the same signalling pathway as the NFRs was still missing. In 2004, it was reported that cytoplasmic streaming in root hairs of a *symrk-3* mutant did not resume after mechanical stimulation, which provided the possibility that the symbiotic defects observed in *symrk* mutants are a pleiotropic effect of the impaired touch desensitation in these mutants.

One major goal of this thesis was to **investigate whether *SYMRK* indeed plays an active signalling role in symbiosis**, and – if so – to **determine its precise position in the genetic RNS pathway**. To approach this issue, we built on the observation that overabundance of and specific mutations in mammalian receptor tyrosine kinases is associated with tumour development. This phenomenon is triggered by spontaneous receptor complex formation and inappropriate initiation of signalling. For this reason, we **studied the effect of overexpression of *SYMRK*, *NFR1* and *NFR5* on root development, promoter activation and gene expression** in hairy roots of *L. japonicus* wild type and several symbiosis-deficient mutants.

During the past few years, findings in the field of molecular plant research have pointed into the direction that plant RLKs, similar to animal RTKs, work together in highly dynamic receptor complexes. This provided the basis for the second major goal of this thesis, which was to **assess whether SYMRK is part of a symbiotic RLK complex in the context of RNS**. Interaction between SYMRK and NFR1 or NFR5 was studied in the heterologous system *Nicotiana benthamiana*, and several SYMRK deletion constructs were included in order to narrow down SYMRK domains involved in the interaction. Finally, *L. japonicus* hairy roots overexpressing either *NFR* were generated to verify the interactions in the homologous system in the presence and absence of symbiotic challenge.

Arabidopsis thaliana belongs to the Brassicaceae, one out of four plant lineages that have lost the ability to engage in plant root endosymbioses, which is accompanied by the erosion of specific genes from their genomes. However, *Arabidopsis* homologs of the symbiosis genes *SYMRK, POLLUX* and the NUP107-160 nuclear pore subcomplex genes

NUP133 and *SEC13* can still be found. The two RLK genes highly related to *SYMRK* were consequently named *SYMRK-homologous Receptor-like Kinase 1* (*ShRK1*) and *ShRK2*. The fact that the accommodation organs for AM fungi (arbuscules) and the biotrophic oomycetal pathogen *H. arabidopsidis* (haustoria) share striking structural and functional similarities, together with the observation that *Arabidopsis* retained homologs of common symbiosis genes (HCSGs) in its genome, raised the hypothesis that filamentous pathogens might exploit these genes for host infection.

The resulting third major goal of the thesis was to **explore whether *ShRK1* and *ShRK2* are involved in plant-pathogen interactions**, in particular, in the **accommodation of filamentous pathogens inside plant cells** in the asymbiotic host *Arabidopsis*. Therefore, we performed detailed analyses of the infection phenotypes of *H. arabidopsidis* on *Arabidopsis* wild type compared to mutants in HCSGs.

VIII. RESULTS

Paper I: Cleavage of the Symbiosis Receptor-like Kinase ectodomain promotes complex formation with Nod Factor Receptor 5.

Antolín-Llovera M, **Ried MK** & Parniske M. 2014b. Cleavage of the SYMBIOSIS RECEPTOR-LIKE KINASE ectodomain promotes complex formation with Nod Factor Receptor 5. *Curr. Biol.* **24**:422-7. doi: 10.1016/j.cub.2013.12.053.

Contributions of the author of this thesis to this manuscript are listed in detail under „**III. DECLARATION OF CONTRIBUTION AS CO-AUTHOR**" on page 7 of this thesis.

Paper II: Spontaneous symbiotic reprogramming of plant roots triggered by receptor-like kinases.

This chapter is based on the following manuscript:

Ried MK, Antolín-Llovera M, & Parniske M. (2014). Spontaneous symbiotic reprogramming of plant roots triggered by receptor-like kinases. *Elife***.** doi: 10.7554/eLife.03891.

Contributions of the author of this thesis to this manuscript are listed in detail under „**III. DECLARATION OF CONTRIBUTION AS CO-AUTHOR**" on pages 7 + 8 of this thesis.

Spontaneous symbiotic reprogramming of plant roots triggered by receptor-like kinases

1. Abstract

Symbiosis receptor-like kinase (SYMRK) is indispensable for the development of phosphate-acquiring Arbuscular Mycorrhiza (AM) as well as nitrogen-fixing root nodule symbiosis, but the mechanisms that discriminate between the two distinct symbiotic developmental fates have been enigmatic. Here we show that upon ectopic expression, the receptor-like kinases genes *Nod Factor Receptor 1* (*NFR1*), *NFR5* and *SYMRK* initiate spontaneous nodule organogenesis and nodulation-related gene expression in the absence of rhizobia. Furthermore, overexpressed NFR1 or NFR5 associates with endogenous SYMRK in roots of the legume *Lotus japonicus*. Epistasis tests revealed that the dominant active *SYMRK* allele initiates signalling independently of either the *NFR1* or *NFR5* gene and upstream of a set of genes required for the generation or decoding of calcium-spiking in both symbioses. Only *SYMRK* but not *NFR* overexpression triggered the expression of AM-related genes, indicating that the receptors play a key role in the decision between AM- or root nodule symbiosis-development.

2. Introduction

Plants circumvent nutrient deficiencies by establishing mutualistic symbioses with Arbuscular Mycorrhiza (AM) fungi or nitrogen-fixing rhizobia and *Frankia* bacteria (Gutjahr and Parniske, 2013, Oldroyd, 2013). One of the first steps in the reciprocal recognition between rhizobia and the legume *Lotus japonicus* is the perception of bacterial lipo-chitooligosaccharides, so called nodulation factors, by the two lysin motif (LysM)

receptor-like kinases (RLKs) Nod Factor Receptor 1 (NFR1) and NFR5 (Broghammer et al., 2012, Madsen et al., 2003, Radutoiu et al., 2003, Radutoiu et al., 2007). Nodulation factor application induces two genetically separable calcium signatures in root hair cells; an early transient influx into the cytoplasm and within minutes calcium-spiking - periodic calcium oscillations in and around plant cell nuclei (Ehrhardt et al., 1996, Miwa et al., 2006, Oldroyd, 2013).

(Lipo)-chitooligosaccharides have also been isolated from AM fungi (Genre et al., 2013, Maillet et al., 2011), and a NFR5-related LysM-RLK from *Parasponia* has been pinpointed as a likely candidate for their perception (Op den Camp et al., 2011). The common symbiosis genes of legumes are required for AM as well as root nodule symbiosis. A subset of these genes is essential for either the generation or the decoding of calcium-spiking. In *L. japonicus,* the former group encodes the RLK Symbiosis Receptor-like Kinase (SYMRK; (Antolín-Llovera et al., 2014, Stracke et al., 2002)), two cation-permeable ion channels CASTOR and POLLUX (Charpentier et al., 2008, Imaizumi-Anraku et al., 2005, Venkateshwaran et al., 2012) as well as the nucleoporins NUP85, NUP133 and NENA (Groth et al., 2010, Kanamori et al., 2006, Saito et al., 2007). The latter group encodes Calcium Calmodulin-dependent Protein Kinase (CCaMK; (Miller et al., 2013, Tirichine et al., 2006)) and CYCLOPS (Yano et al., 2008), which form a complex that has been implicated in the deciphering of calcium-spiking (Kosuta et al., 2008).

Phosphorylation by CCaMK activates CYCLOPS, a DNA-binding transcriptional activator of the *NODULE INCEPTION* gene (*NIN* (Schauser et al., 1999); (Singh et al., 2014)). NIN itself is a legume-specific and root nodule symbiosis-related transcription factor and regulates the *Nuclear Factor-Y subunit* genes *NF-YA1* and *NF-YB1* that control the cell division cycle (Soyano et al., 2013, Yoro et al., 2014). The paradigm of a common signalling pathway for both symbioses bears important open questions about the molecular mechanisms that ensure the appropriate cellular response for AM fungi on the one hand and for rhizobia on the other hand.

SYMRK carries an ectodomain composed of a malectin-like domain (MLD), and a leucine-rich repeat (LRR) region which experienced structural diversification during evolution (Markmann et al., 2008) and is cleaved to release the MLD (Antolín-Llovera et al., 2014). Although SYMRK has been cloned several years ago (Stracke et al., 2002) its precise function in symbiosis is still enigmatic. While *nfr* mutants lack most cellular and physiological responses to rhizobia (Radutoiu et al., 2003), including nodulation factor induced calcium influx and calcium spiking, root hairs of *symrk* mutants respond with calcium influx to nodulation factor but not with calcium spiking, and do not develop infection threads with rhizobia (Miwa et al., 2006, Stracke et al., 2002). Based on these

phenotypic observations, *SYMRK* was positioned downstream of the *NFRs* (Miwa et al., 2006, Radutoiu et al., 2003).

Importantly, it has not been conclusively resolved whether *SYMRK* plays an active signalling role in symbiosis or, alternatively, is involved in mechanical stress desensitation (Esseling et al., 2004). To approach this issue, we built on the observation that over-abundance or specific mutations of mammalian receptor tyrosine kinases on the cell surface is linked with the development of some cancers caused by spontaneous receptor complex formation and inappropriate initiation of signalling (Schlessinger, 2002, Shan et al., 2012, Wei et al., 2005). We hypothesized that similar behaviour could be triggered by overexpression of symbiosis-related plant RLKs, providing a tool to further dissect the specific signalling pathways they address.

3. Results

3.1 Symbiotic *RLKs* trigger spontaneous formation of root nodules

To achieve overexpression, we generated constructs expressing functional SYMRK (Antolín-Llovera et al., 2014), NFR5 or NFR1 under the control of the strong *L. japonicus Ubiquitin* promoter and added C-terminal mOrange fluorescent tags for detection purposes (*pUB:SYMRK-mOrange, pUB:NFR5-mOrange, pUB:NFR1-mOrange*). The functionality of the *NFR* constructs was confirmed by their ability to restore nodulation in the corresponding, otherwise nodulation deficient, *nfr* mutant roots to the level of *L. japonicus* wild-type roots transformed with the empty vector (Figure 2).

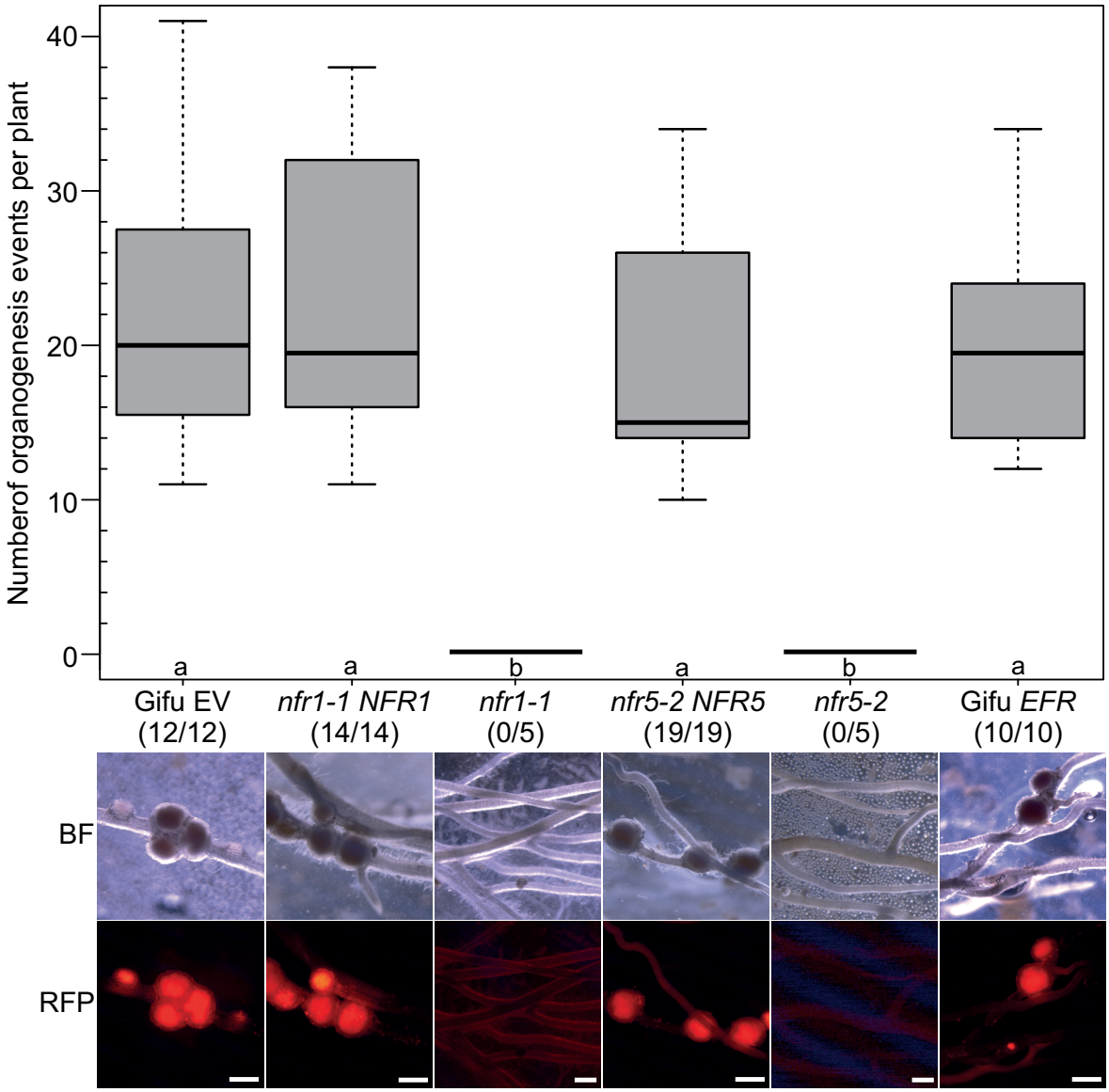


Figure 2: Expression of *NFR1* and *NFR5* from the *Ubiquitin* promoter restores nodulation in the *nfr1-1* and *nfr5-2* mutants, respectively.

Hairy roots of *L. japonicus* Gifu wild-type transformed with the empty vector (EV) or with *pUB:EFR-mOrange* (*EFR*), the *nfr1-1* mutant transformed with *pUB:NFR1-mOrange* (*NFR1*) or the *nfr5-2* mutant transformed with *pUB:NFR5-mOrange* (*NFR5*) were generated. Untransformed *nfr1-1* and *nfr5-2* mutant plants served as control. Plot represents the number of organogenesis events (nodules and nodule primordia) per plant formed 15 days post inoculation with *M. loti Ds*RED. Numbers below each line label indicate the number of nodulated plants per total analysed plants. Representative pictures are shown. BF, bright field; RFP, RFP filter. Bars, 1 mm. Bold black line, median; box, IQR; whiskers, lowest/highest data point within 1.5 IQR of the lower/upper quartile. A Kruskal-Wallis test followed by false discovery rate correction was performed. Different letters indicate significant differences. $p < 0.05$.

Intriguingly, transgenic expression of any of the three symbiotic RLK versions in *L. japonicus* roots was sufficient to spontaneously activate the entire nodule organogenesis pathway as evidenced by the formation of nodule-like structures in the absence of

rhizobia (Figure 3; Figure 4). The presence of peripheral vascular bundles instead of a central root vasculature unambiguously identified these lateral organs as spontaneous nodules (Figure 3C).

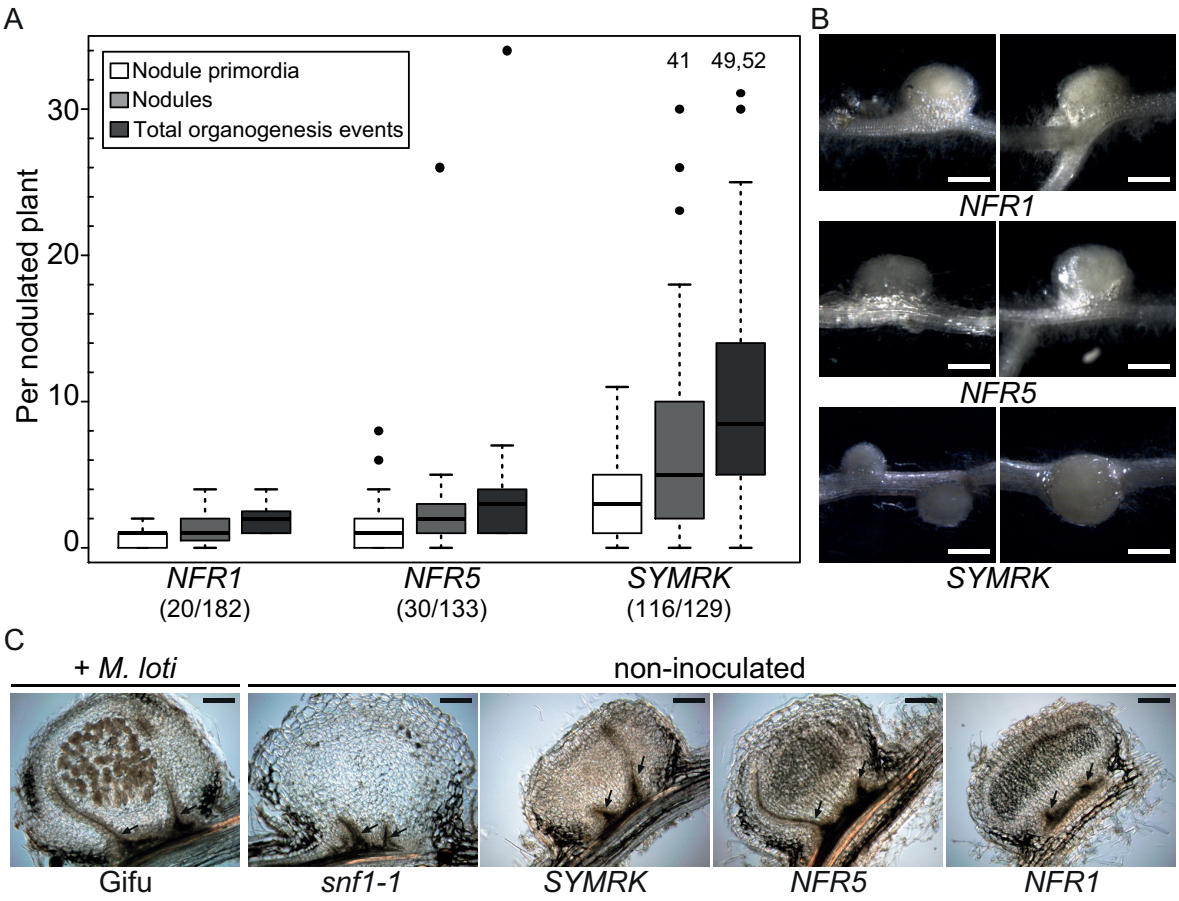


Figure 3: Symbiotic *RLKs* mediate spontaneous formation of root nodules.
Hairy roots of *L. japonicus* Gifu wild-type transformed with the empty vector (EV), *pUB:NFR1-mOrange* (*NFR1*), *pUB:NFR5-mOrange* (*NFR5*), or *pUB:SYMRK-mOrange* (*SYMRK*) were generated. A) Plot represents the numbers of nodules (white), nodule primordia (light grey) and organogenesis events (dark grey; nodules and nodule primordia) per nodulated plant formed in the absence of rhizobia at 60 dpt. Number of nodulated plants per total plants is specified under each line label. Black dots, data points outside 1.5 interquartile range (IQR) of the upper quartile; Numbers above upper whiskers indicate the values of individual data points outside of the plotting area. Bold black line, median; box, IQR; whiskers, lowest/highest data point within 1.5 IQR of the lower/upper quartile. Plants transformed with the empty vector did not develop spontaneous nodules. B) Pictures of spontaneous nodules on hairy roots expressing the indicated transgenes taken 60 dpt. Bars, 1mm. C) Micrographs of sections of spontaneous nodules on hairy roots expressing the indicated transgenes harvested at 60 dpt. Spontaneous nodules of 10 weeks old *snf1-1* mutant plants were used as controls. Nodules of 10 weeks old untransformed *L. japonicus* wild-type Gifu 6 weeks after inoculation with *M. loti* MAFF303099 *Ds*RED contained cortical cells filled with bacteria (brown colour) that are absent in spontaneous nodules. Arrows point to peripheral vascular bundles. Longitudinal 40 mm sections. Bars, 150 μm.

Spontaneous nodule primordia or nodules were present on 90 % (116 out of 129), 23 % (30 out of 133), 11 % (16 out of 182) and 0 % (0 out of 164) of *L. japonicus* root systems at 60 days post transformation (dpt) with, respectively, *pUB:SYMRK-mOrange*, *pUB:NFR5-mOrange*, *pUB:NFR1-mOrange*, or the empty vector (Figure 3A; Figure 4).

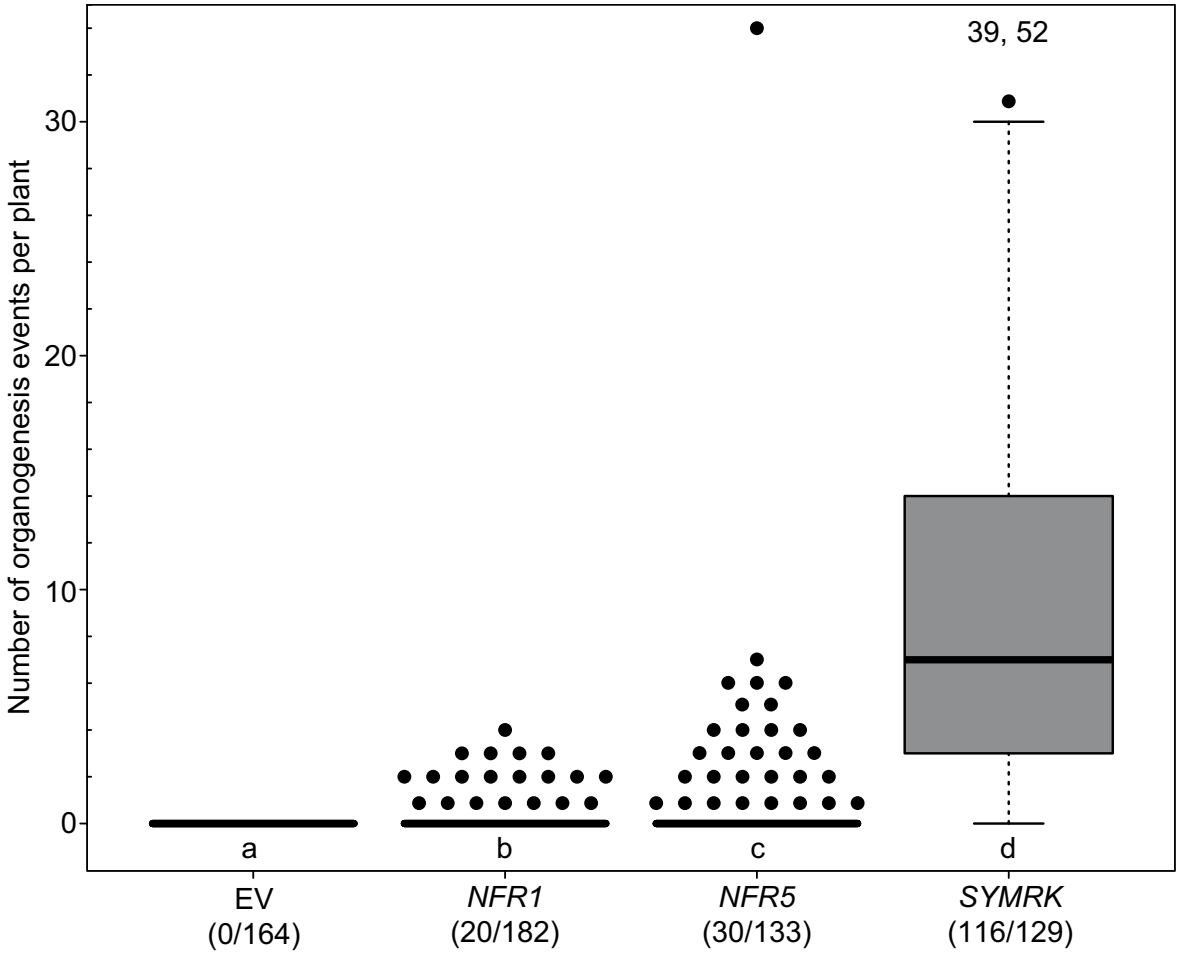


Figure 4: Statistical analysis of spontaneous root nodule formation.
Hairy roots of *L. japonicus* Gifu wild-type transformed with the empty vector (EV), *pUB:NFR1-mOrange* (*NFR1*), *pUB:NFR5-mOrange* (*NFR5*), or *pUB:SYMRK-mOrange* (*SYMRK*) were generated. Plot represents the numbers of organogenesis events (nodules and nodule primordia) per plant formed in the absence of rhizobia at 60 dpt. Number of nodulated plants per total plants is specified under each line label. Black dots, data points outside 1.5 IQR of the upper quartile; Numbers above upper whiskers indicate the values of individual data points outside of the plotting area. Bold black line, median; box, IQR; whiskers, lowest/highest data point within 1.5 IQR of the lower/upper quartile. A Kruskal-Wallis test followed by false discovery rate correction was performed. Different letters indicate significant differences. $p < 0.05$.

A total of 810 empty vector roots generated throughout the course of this study did not develop spontaneous nodules in any of the genetic backgrounds and time points tested. Roots expressing functional *SYMRK-RFP* from its native promoter (*pSYMRK:SYMRK-*

RFP; (Kosuta et al., 2011)) and grown in the absence of rhizobia did not develop spontaneous nodules, indicating that spontaneous nodulation was triggered by *SYMRK* expression from the *Ubiquitin* promoter and not by the addition of a C-terminal tag alone (Figure 5).

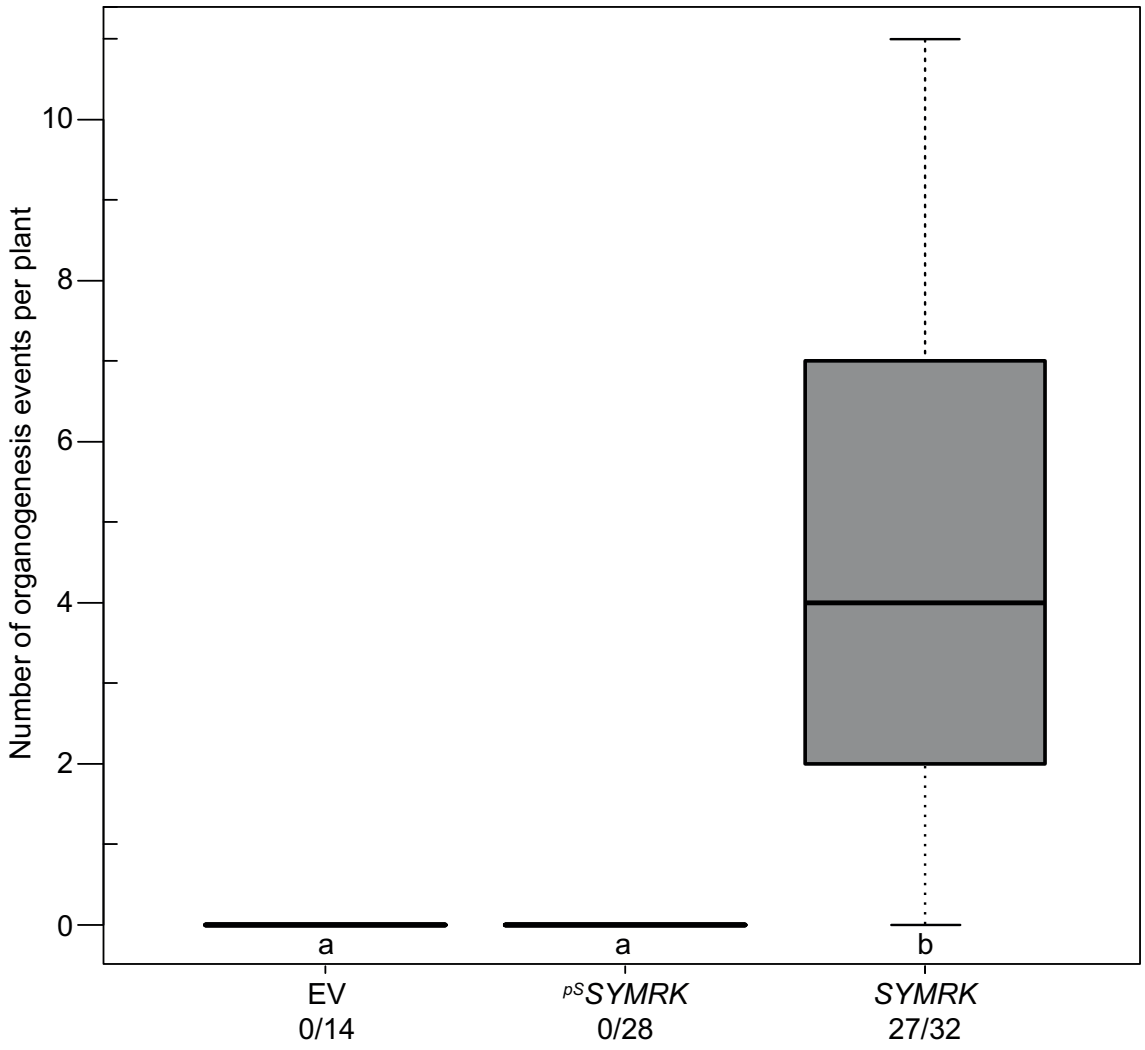


Figure 5: Expression of *SYMRK* from the native *SYMRK* promoter does not mediate spontaneous formation of root nodules.

Hairy roots of *L. japonicus symrk-3* transformed with the empty vector (EV), *pUB:SYMRK-mOrange* (*SYMRK*), or *pSYMRK:SYMRK-RFP* (*pSSYMRK*), were generated. Plot represents the numbers of total organogenesis events (nodules and nodule primordia) per plant formed in the absence of rhizobia at 21 dpt. Number of nodulated plants per total plants is specified under each line label. Bold black line, median; box, IQR; whiskers, lowest/highest data point within 1.5 IQR of the lower/upper quartile. A Kruskal-Wallis test followed by false discovery rate correction was performed. Different letters indicate significant differences. $p < 0.05$.

Moreover, the expression of non-tagged *SYMRK* under the control of the *Ubiquitin* promoter triggered the formation of spontaneous nodules. In comparison to roots transformed with the tagged *SYMRK* version, a lower number of roots transformed with non-tagged *SYMRK* contained spontaneous nodules (Figure 6).

One explanation for this observation is that the C-terminal mOrange tag might result in alterations in the relative amount of signalling-active SYMRK. Another possibility is that the presence of the tag improves homo- and/or hetero-dimerization, which subsequently leads to downstream signalling. Our results demonstrate that overexpression of *NFR1-mOrange*, *NFR5-mOrange*, or *SYMRK* results in the activation and execution of the nodule organogenesis pathway in the absence of external symbiotic stimulation.

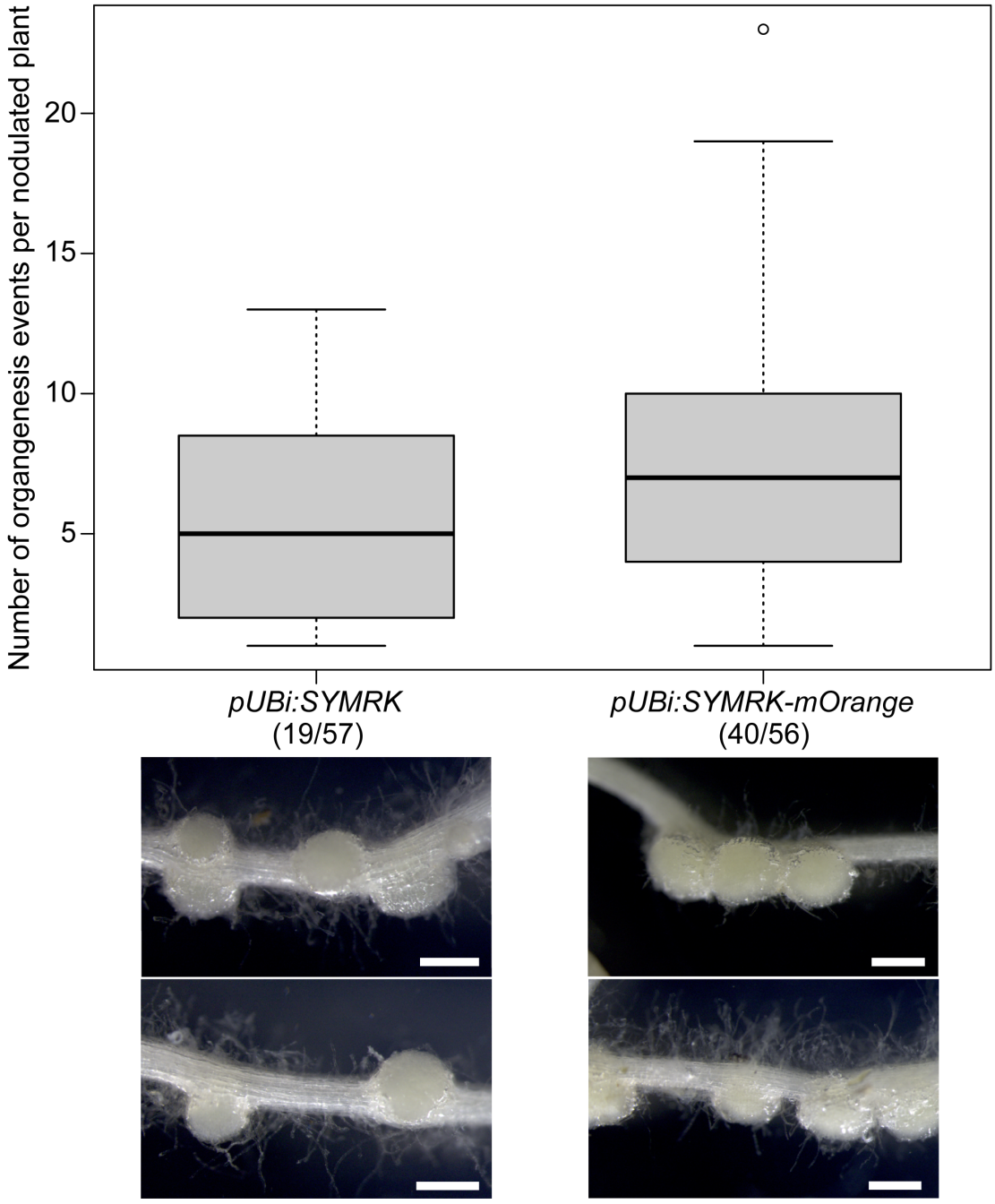


Figure 6: Expression of non-tagged *SYMRK* from the *Ubiquitin* promoter induces spontaneous formation of root nodules.

Hairy roots of *L. japonicus symrk-3* transformed with *pUBi:SYMRK* (untagged) or *pUBi:SYMRK-mOrange* (C-terminally tagged) were generated. Plot represents the numbers of total organogenesis events (nodules and primordia) per nodulated plant formed in the absence of rhizobia at 42 dpt. Number of nodulated plants per total plants is specified under each line label. Dot, data point outside 1.5 interquartile range of the upper quartile. Bold black line, median; box, IQR; whiskers, lowest/highest data point within 1.5 IQR of the lower/upper quartile. Plants non-transformed or transformed with the empty vector did not develop spontaneous nodules. A Kruskal-Wallis test followed by false discovery rate correction was performed for total organogenesis events per nodulated root (p-value of 0.16) and for total organogenesis events per transformed root system (p-value of 1.2e-05). Numbers below each line label indicate the number of nodulated plants per total analysed plants. Representative pictures are shown. Bars, 0.5 mm.

3.2 Symbiotic *RLKs* trigger spontaneous nodulation-related signal transduction

To establish whether the development of nodule-like structures was associated with nodulation-related gene activation, we analysed the expression behaviour of marker genes induced during root nodule symbiosis (*NIN* and *SbtS*; (Kistner et al., 2005)) via quantitative real-time PCR (qRT-PCR; Figure 7A).

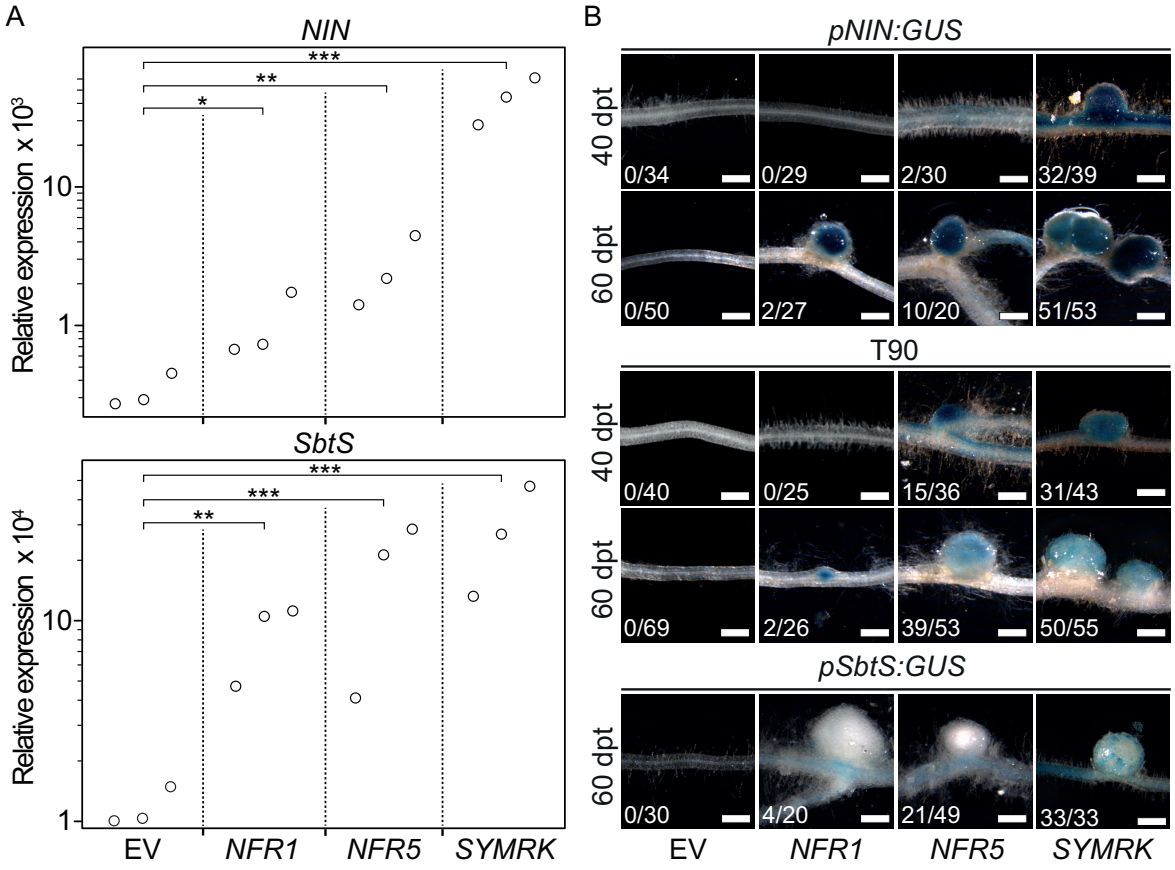


Figure 7: Symbiotic *RLKs* mediate spontaneous symbiosis-related signal transduction.
Hairy roots of *L. japonicus* Gifu wild-type (A) or of three stable transgenic *L. japonicus* Gifu reporter lines (B) - carrying either the T90 reporter fusion, a *NIN* promoter:*GUS* fusion (*pNIN:GUS*), or a *SbtS* promoter:*GUS* fusion (*pSbtS:GUS*) – transformed with the empty vector (EV), *pUB:NFR1-mOrange* (*NFR1*), *pUB:NFR5-mOrange* (*NFR5*), or *pUB:SYMRK-mOrange* (*SYMRK*) were generated. A) Relative expression of *NIN* or *SbtS* at 40 dpt was determined in three biological replicates for each treatment via qRT-PCR. Transcript levels in each replicate were determined through technical duplicates. Expression was normalized with the house keeping genes *EF1alpha* and *Ubiquitin*. Circles indicate expression relative to the *EF1alpha* gene. A Dunnett's test as performed comparing the transcript levels of *NIN* or *SbtS* detected for each treatment with those detected in the empty vector samples. Stars indicate significant differences from the EV control. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. B) β-glucuronidase (GUS) activity was analysed by histochemical staining with 5-bromo-4-chloro-3-indolyl glucuronide (X-Gluc) 40 and 60 dpt. Representative root sections are shown. Number of plants with detectable GUS activity per number of total plants is indicated. Bars, 500 μm.

The *SbtS* gene is also induced during AM symbiosis (Kistner et al., 2005). In comparison to control roots transformed with the empty vector, the *SYMRK* construct resulted in a highly significant increase in *NIN* and *SbtS* transcript levels (mean fold increase of 137 and 24, respectively). A slighter but statistically significantly increase in transcript levels could be observed in roots overexpressing either *NFR1-mOrange* (*NIN*, mean fold increase 3; *SbtS*, mean fold increase 7) or *NFR5-mOrange* (*NIN*, mean fold increase 8; *SbtS*, mean fold increase 15) (Figure 7A).

To monitor the spontaneous activation of *NIN* and *SbtS* by an independent and histochemical method, we made use of stable transgenic *L. japonicus* reporter lines carrying either a *NIN* promoter:*β-glucuronidase (GUS)* fusion (*pNIN:GUS;* (Radutoiu et al., 2003)) or a *SbtS* promoter:*GUS* fusion (*pSbtS:GUS*; (Takeda et al., 2009)) (Figure 7B). In addition, we employed the symbiosis-reporter line T90 that was isolated in a screen for symbiosis-specific *GUS* expression from a promoter-tagging population (Webb et al., 2000) (Figure 7B). The T90 reporter is activated in roots treated with nodulation factor or inoculated with *Mesorhizobium loti*, and - similar to *pSbtS:GUS* - also shows *GUS* expression during AM (Kistner et al., 2005, Radutoiu et al., 2003). GUS activity was determined in roots by histochemical staining with 5-bromo-4-chloro-3-indolyl glucuronide (X-Gluc; Figure 7B). Either of the three symbiotic *RLKs* but not the empty vector activated the *pNIN:GUS,* the *pSbtS:GUS,* as well as the T90 reporter in the absence of *M. loti* or AM fungi (Figure 7B).

This histochemical analysis of GUS activity, in combination with the qRT-PCR results, provide strong evidence that overexpression of symbiotic *RLKs* leads to the activation of nodulation-related genes in the absence of external symbiotic stimulation (Figure 7). However, the three *RLK* genes were not equally effective in inducing the symbiotic program: *NFR5* or *NFR1* overexpression resulted in a lower percentage of root systems showing promoter activation and formation of spontaneous nodules when compared to *SYMRK* overexpression (Figure 3; Figure 4; Figure 7B). Interestingly, *SYMRK*- as well as *NFR5*-mediated T90 or *NIN* promoter activation was first observed in the root and retracted to nodule primordia and nodules over time, while *NFR1*-mediated T90 or *NIN* promoter activation could only be detected in nodule primordia or in nodules (Figure 7B). The *Ubiquitin* promoter drives expression of the receptors in all cells of the root (Maekawa et al., 2008), which is in marked contrast to the highly specific and developmentally controlled expression patters of the marker genes observed. These incongruences thus reveal the presence of additional layers of regulation, operating downstream of the receptors, which dictate the precise expression patterns of the reporters.

3.3 *SYMRK* triggers spontaneous AM-related signal transduction

Since *SYMRK* is not only required for nodulation but also for AM symbiosis, we investigated the potential of dominant active *RLK* alleles to spontaneously activate AM-related marker genes or a promoter:*GUS* reporter (Figure 9). *Blue copper-binding protein 1* (*Bcp1*) as well as the subtilisin-like serine protease gene *SbtM1* are induced during AM symbiosis (Takeda et al., 2009, Kistner et al., 2005, Liu et al., 2003) and both genes are predominantly expressed in arbuscule-containing and adjacent cortical cells (Hohnjec et al., 2005, Takeda et al., 2012, Takeda et al., 2009). Furthermore, in *L. japonicus*, *SbtM1* expression marks root cells that contain an AM fungi-induced prepenetration apparatus (Takeda et al., 2012) – an intracellular structure that forms prior to invasion by fungal hyphae (Genre et al., 2005). Transcript levels of *SbtM1* as well as *Bcp1* were determined via qRT-PCR and both were significantly increased in roots transformed with *pUB:SYMRK-mOrange* compared to the empty vector control (Figure 8A).

To determine *SbtM1* activation by an independent, histochemical approach, we employed a stable transgenic *L. japonicus* line harbouring a *SbtM1* promoter:*GUS* fusion (*pSbtM1:GUS;* (Takeda et al., 2009)). In line with the results from the qRT-PCR experiments, overexpression of *SYMRK-mOrange* in roots of the *pSbtM1:GUS* reporter line resulted in activation of the *SbtM1* promoter at 40 and 60 dpt (Figure 8B).

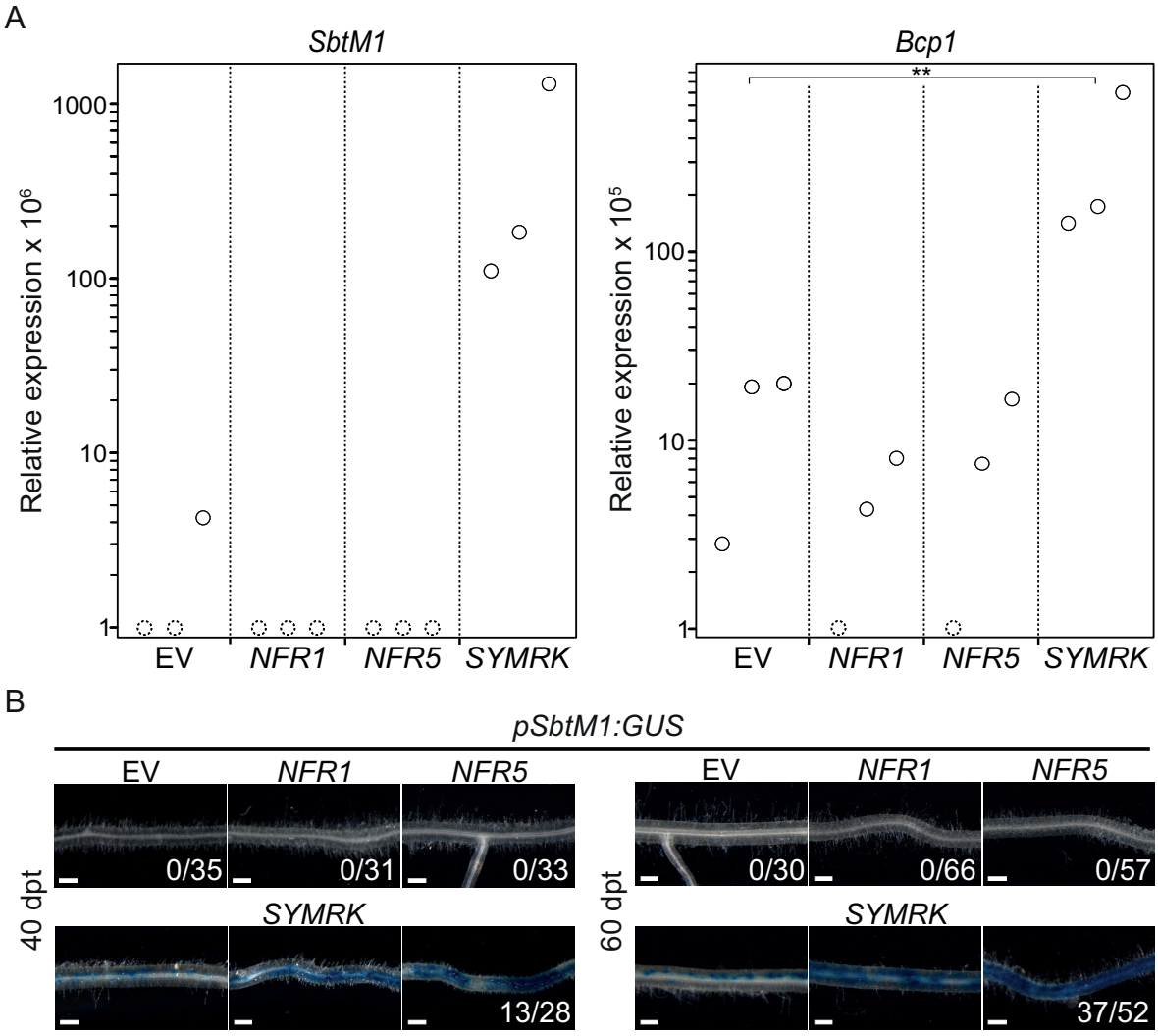


Figure 8: *SYMRK* mediates spontaneous AM-related signal transduction.
Hairy roots of *L. japonicus* Gifu wild-type (A) or a stable transgenic *L. japonicus* MG20 reporter line carrying a *SbtM1* promoter:*GUS* fusion (*pSbtM1:GUS*) (B) transformed with the empty vector (EV), *pUB:NFR1-mOrange* (*NFR1*), *pUB:NFR5-mOrange* (*NFR5*), or *pUB:SYMRK-mOrange* (*SYMRK*) were generated. A) Relative expression of *SbtM1* or *Bcp1* at 40 dpt was determined in three biological replicates for each treatment via quantitative real time PCR. Transcript levels in each replicate were determined through technical duplicates. Expression was normalized with the house keeping genes *EF1alpha* and *Ubiquitin*. Circles indicate expression relative to the *EF1alpha* gene. Dashed circles indicate that no transcripts could be detected for this sample. Samples in which the indicated transcript could not be detected were floored to 1. A Dunnett's test was performed comparing the transcript levels of *Bcp1* detected for each treatment with those detected in the empty vector samples. Stars indicate significant differences. **, $p < 0.01$. B) GUS activity was analysed by histochemical staining with X-Gluc 40 and 60 dpt. Representative root sections are shown. Number of plants with detectable GUS activity per total plants is indicated. Bars, 500 µm.

In contrast, no *SbtM1* promoter activation or AM-related gene induction could be detected upon overexpression of either of the *NFRs* (Figure 8). The absence of AM-related gene expression in *NFR5* expressing roots is not a consequence of the overall lower induction power of the *NFR5* construct. In *SYMRK* versus *NFR5* expressing roots, the relative ratio of transcripts was 1.6 : 1 for *SbtS* and 17 : 1 for *NIN* (Figure 7A). In contrast, *SbtM1* was undetectable in *NFR5*- but more than 1100-fold above detection limit in *SYMRK*-overexpressing roots (Figure 8A). These data clearly demonstrate a strong difference in the gene repertoire activated by *SYMRK* versus *NFR5*. Together with the spontaneous nodulation, these results demonstrate that overexpression of *NFR1-mOrange, NFR5-mOrange,* or *SYMRK-mOrange* activates the nodulation pathway as evidenced by spontaneous organogenesis and gene expression results at the level of endogenous transcripts as well as promoter:*GUS* expression. In contrast, only the *SYMRK* construct but neither of the *NFR* constructs induced AM-related gene expression. This suggests that signalling specificity towards the two different symbiotic programs is achieved at the level of the receptors.

3.4 SYMRK associates with NFR1 and NFR5 in *Lotus japonicus* roots

Spontaneous receptor complex formation caused by overexpression offers itself as a likely explanation for the observed activation of symbiosis signalling in the absence of an external trigger or ligand. This is a scenario described in the context of cancer formation, where receptor tyrosine kinase overexpression or specific mutations in the receptor lead to receptor dimerization in the absence of a ligand, which results in ectopic cell proliferation (Akiyama et al., 2005, Schlessinger, 2002, Shan et al., 2012, Wei et al., 2005). Upon expression in *Nicotiana benthamiana* leaves in the absence of symbiotic stimulation, we observed previously weak association between full-length SYMRK and NFR1 as well as NFR5, but not between SYMRK and the functionally unrelated RLK Brassinosteroid Insensitive 1 (BRI1, (Li and Chory, 1997); (Antolín-Llovera et al., 2014); Figure 9).

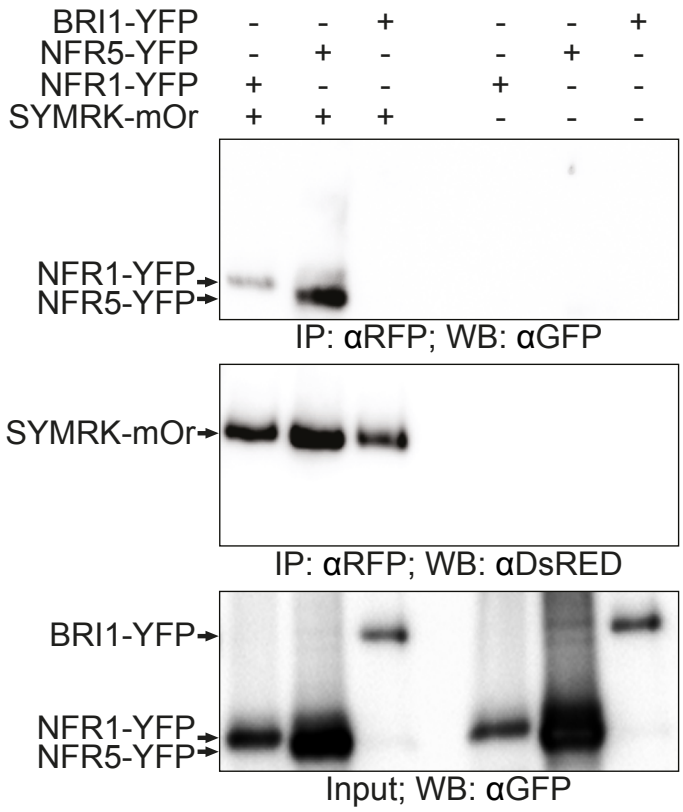


Figure 9: Full length SYMRK associates with NFR1 and NFR5 in *Nicotiana benthamiana* leaves.

N. benthamiana leaves were transiently co-transformed with constructs expressing NFR1-YFP, NFR5-YFP or BRI1-YFP together with SYMRK-mOrange under the control of the CaMV 35S promoter. Leaf discs expressing the respective constructs were extracted 3 dpt. SYMRK-mOrange was immuno-enriched with RFP magnetotrap and monitored by immunoblot with an anti*Ds*RED antibody. Co-enrichment of NFR1-YFP, NFR5-YFP or BRI1-YFP was monitored by immunoblot with an antiGFP antibody. mOr, mOrange; IE, immuno-enrichment; WB, western blot.

To test whether overexpression is associated with receptor complex formation in *L. japonicus* roots, we employed the overexpression constructs of *NFR1*, *NFR5*, or the unrelated *EF-Tu receptor kinase* (*EFR*, (Zipfel et al., 2006)) for co-immuno-enrichment experiments. The *EFR* construct did not interfere with nodulation in wild-type plants (Figure 2). Endogenous full-length SYMRK was co-enriched with NFR1 and NFR5, but not with EFR demonstrating association of SYMRK and both NFRs (Figure 10).

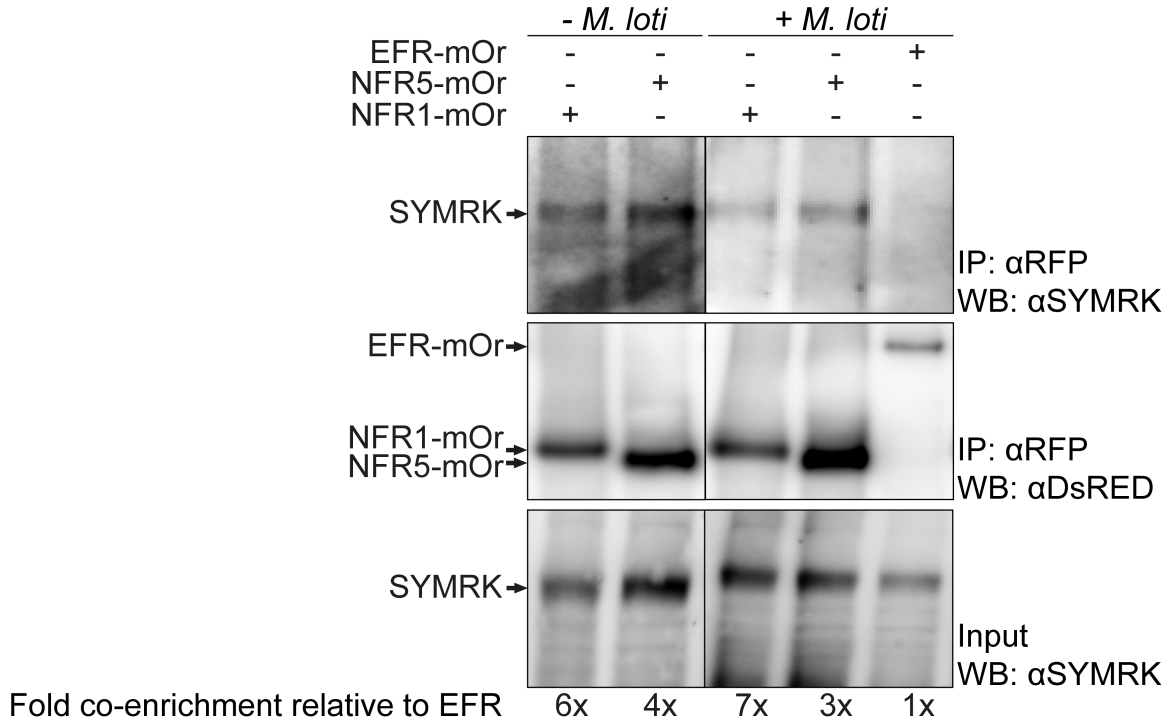
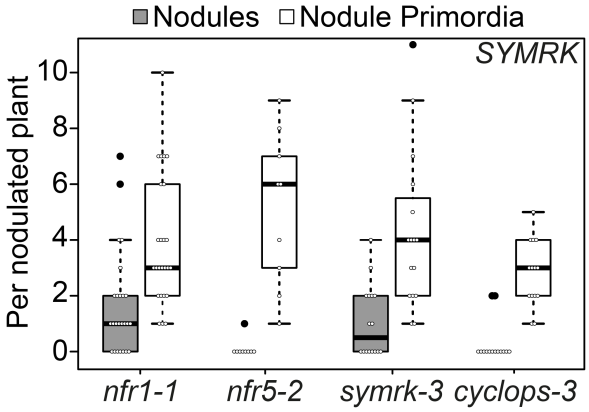


Figure 10: SYMRK associates with NFR1 and NFR5 in *Lotus japonicus* roots.
Hairy roots of *L. japonicus* Gifu wild-type roots expressing *NFR1-mOrange* (NFR1-mOr), *NFR5-mOrange* (NFR5-mOr) or *EFR-mOrange* (EFR-mOr) under the control of the *Ubiquitin* promoter were extracted 10 days post inoculation with *M. loti Ds*RED or mock treatment. mOrange fusions were affinity bound with RFP magneto trap and immuno-enrichment was monitored by immunoblot with and anti*Ds*RED antibody. Co-enrichment of endogenous SYMRK protein was monitored by immunoblot with an antiSYMRK antibody. Numbers below the western blot panels indicate the fold co-enrichment of SYMRK by NFR1 or NFR5 relative to the amount of SYMRK co-enriched with EFR. mOr, mOrange; IE, immuno-enrichment; WB, western blot.

However, it should be noted that the expression strength of EFR was lower than that of NFR1 and NFR5. SYMRK-NFR association was detected in the absence of nodulation factor. We did not observe an effect of *M. loti* on this association at 10 days post inoculation (Figure 10).

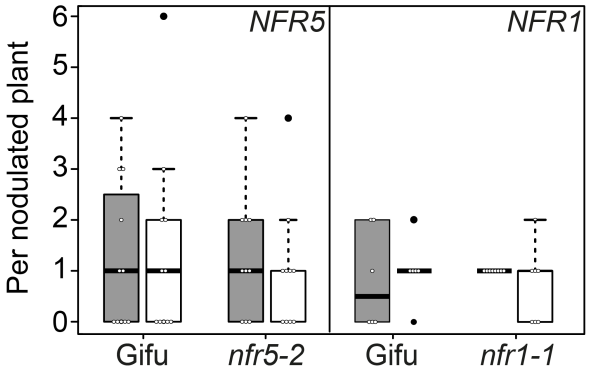
3.5 Epistatic relationships between *SYMRK* and other common symbiosis genes

The availability of dominant active receptor gene alleles offers an attractive tool for their positioning in the genetic pathway required for nodule organogenesis and symbiosis-related gene expression. We asked whether the *pUB:SYMRK-mOrange* construct induced spontaneous nodules or the symbiosis-specific T90 reporter in mutants of common symbiosis genes (Figure 11 - 13).



Nodulated / total number of plants

Background	*SYMRK*	*^{pS}SYMRK*
nfr1-1	25/30	*0/12*
nfr5-2	9/12	*0/13*
symrk-3	16/21	*0/8*
nup133-1	0/14	-
castor-12	0/9	-
pollux-2	0/17	-
ccamk-13	0/9	-
cyclops-3	14/29	*0/16*



Background	*NFR5*	EV	*NFR1*	EV
Gifu	11/55	0/22	6/60	0/24
nfr1-1	0/42	0/12	7/56	0/23
nfr5-2	10/39	0/10	0/17	0/22
symrk-3	0/30	0/16	0/40	0/19
symrk-10	0/30	0/10	0/57	0/19
castor-12	0/25	0/14	-	-
ccamk-13	0/32	0/12	-	-
cyclops-3	0/22	0/15	-	-

Figure 11: Epistatic relationships between symbiotic *RLK* genes and common symbiosis genes.

Hairy roots of *L. japonicus* Gifu wild-type and different symbiosis defective mutants transformed with *pUB:SYMRK-mOrange* (*SYMRK*) or *pSYMRK:SYMRK-RFP* (*^{pS}SYMRK*) (upper panel), or the empty vector (EV), *pUB:NFR1-mOrange* (*NFR1*) or *pUB:NFR5-mOrange* (*NFR5*) (lower panel), were generated. Plots represent the numbers of nodules (white) and nodule primordia (grey) per nodulated plant formed in the absence of rhizobia at 40 (*SYMRK*) and 60 (*NFR5* + *NFR1*) dpt. White circles indicate individual organogenesis events. Black dots, data points outside 1.5 IQR of the upper/lower quartile; bold black line, median; box, IQR; whiskers, lowest/highest data point within 1.5 IQR of the lower/upper quartile. Table, fraction of nodulated per total number of plants. Plants transformed with *pSYMRK:SYMRK-RFP* or the empty *pUB* vector did not develop spontaneous nodules.

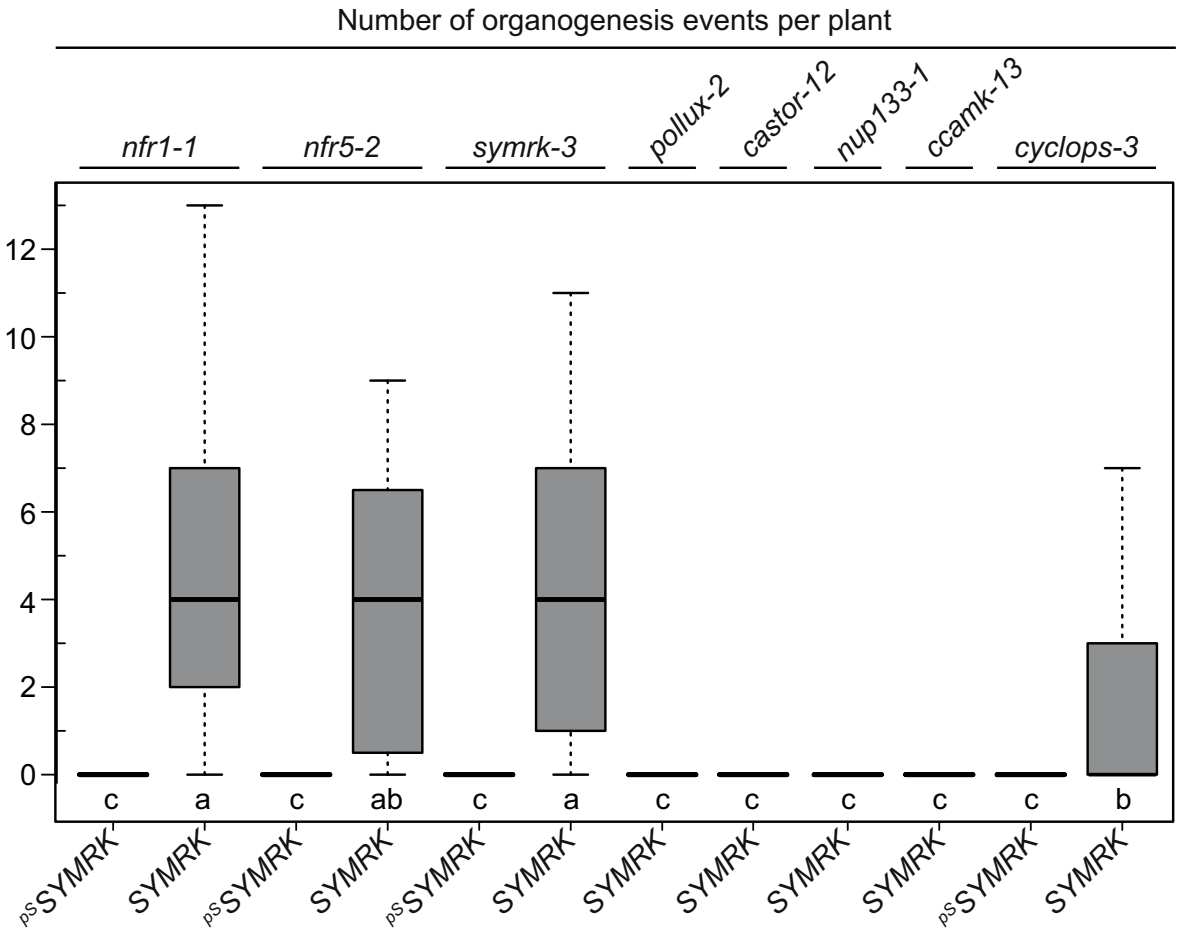


Figure 12: *SYMRK*-mediated spontaneous organogenesis events in *nfr1-1*, *nfr5-2*, and common symbiosis mutants.

Hairy roots of different symbiosis defective mutants transformed with *pUB:SYMRK-mOrange* (*SYMRK*) or *pSYMRK:SYMRK-RFP* (*pSSYMRK*) were generated. Plot represents the numbers of organogenesis events (nodules and nodule primordia) per plant formed in the absence of rhizobia at 40 dpt. Bold black line, median; box, IQR; whiskers, lowest/highest data point within 1.5 IQR of the lower/upper quartile. A Kruskal-Wallis test followed by false discovery rate correction was performed. Different letters indicate significant differences. $p < 0.05$.

SYMRK-induced spontaneous nodules were absent from *pollux-2, castor-12, nup133-1* or *ccamk-13* mutant roots. Likewise T90 reporter (GUS) activation was not detectable in the *castor-2* x T90 (Kistner et al., 2005) or *ccamk-2* x T90 (Gossmann et al., 2012) lines (Figure 13).

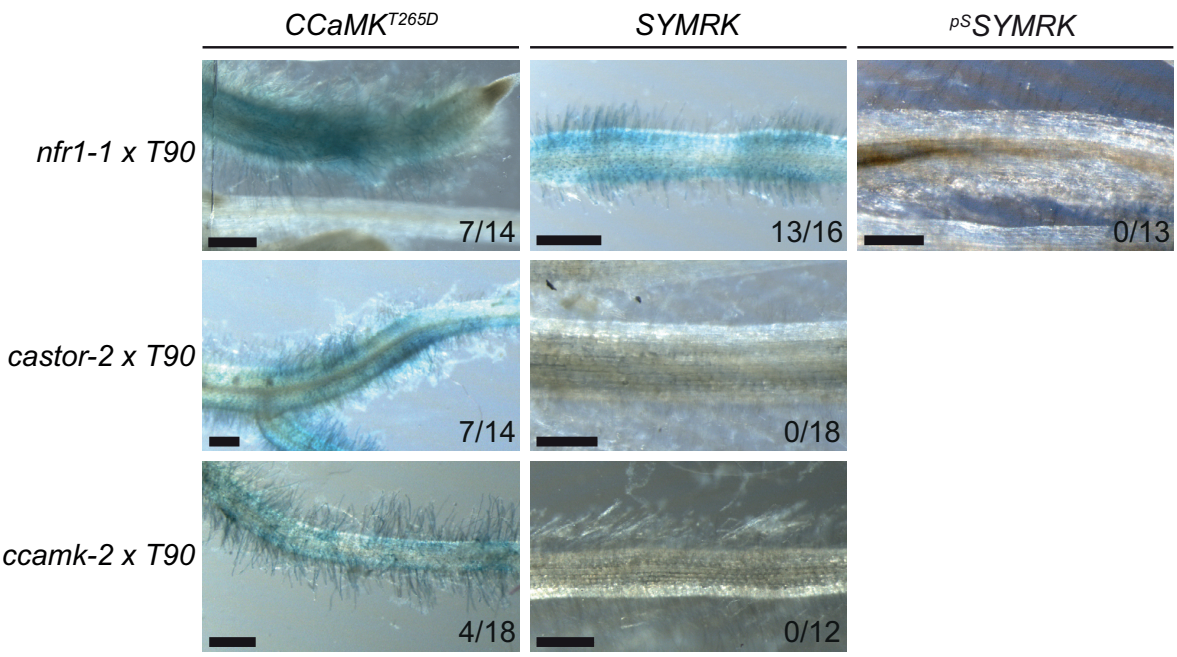


Figure 13: *SYMRK*-mediated activation of the symbiosis-specific T90 reporter in symbiosis-defective mutants.

Hairy roots of three stable transgenic *L. japonicus* Gifu reporter lines homozygous for the T90 reporter fusion and the indicated mutant alleles transformed with *pUB:CCaMK*^{T265D} (*CCaMK*^{T265D}, a deregulated version of CCaMK), *pUB*:SYMRK-mOrange (*SYMRK*) or *pSYMRK*:SYMRK-RFP (^{pS}*SYMRK*) were generated and kept on agar plates for a total of 38 dpt (see methods). The vast majority of transgenic root systems did not develop spontaneous nodules at this time point under these growth conditions. GUS activity was analysed by histochemical staining with X-Gluc at 38 dpt. Representative root sections are shown. Number of plants with detectable GUS activity per total plants is indicated. Bars, 500 μm.

This epistasis revealed that the ion channel genes *CASTOR* and *POLLUX*, the nucleoporin gene *NUP133*, and the calcium- and calmodulin dependent protein kinase gene *CCaMK*, operate downstream of *SYMRK* in a pathway leading to spontaneous nodulation and activation of T90 (Figure 11 - 13). In contrast, *SYMRK* induced spontaneous nodules on *cyclops-3* mutant roots (Figure 11 + 12). Spontaneous nodule formation on the *cyclops-3* mutant (Figure 11 + 12) corresponds to the formation of bump-like structures upon inoculation with *M. loti* on *cyclops* mutants (Yano et al., 2008). While bacterial infection is strongly impaired in *L. japonicus cyclops* or *M. truncatula ipd3* mutants, nodule primordia or nodules, respectively, develop upon rhizobia inoculation (Horvath et al., 2011, Ovchinnikova et al., 2011, Yano et al., 2008). Furthermore, an auto-active version of CCaMK is able to induce the formation of mature spontaneous nodules in *cyclops* mutants (Yano et al., 2008). The ability of *SYMRK* to mediate spontaneous nodule organogenesis in the *cyclops* mutant is consistent with these results and points towards the

existence of redundancies in the genetic pathway leading to organogenesis at the level of *CYCLOPS* (Singh et al., 2014).

3.6 Epistatic relationships between symbiotic *RLK* genes

We used the dominant active alleles to determine the hierarchy of the symbiotic *RLK* genes in the spontaneous nodulation and T90 activation pathways. Control roots of mutant lines transformed with the empty vector (218 root systems) or *SYMRK* driven by its own promoter (33 root systems) did not carry spontaneous nodules or nodule primordia (Figure 11 + 12; Figure 14 + 15). Expression of *pUB:SYMRK-mOrange* spontaneously activated the nodulation program in *nfr1-1*, *nfr5-2* and *symrk-3* mutant roots (Figure 11 + 12). Spontaneous nodules on *nfr1-1* or *nfr5-2* roots overexpressing *SYMRK-mOrange* indicate that the simultaneous presence of both *NFRs* is not necessary for spontaneous *SYMRK*-mediated nodulation (Figure 11 + 12). Consistent with this result, overexpression of *SYMRK-mOrange* resulted in spontaneous GUS expression in the *nfr1-1* x T90 line (Gossmann et al., 2012) (Figure 13). *NFR*-mediated formation of spontaneous nodules could only be observed in the wild-type or the respective *nfr* mutant (Figure 11; Figure 14 + 15).

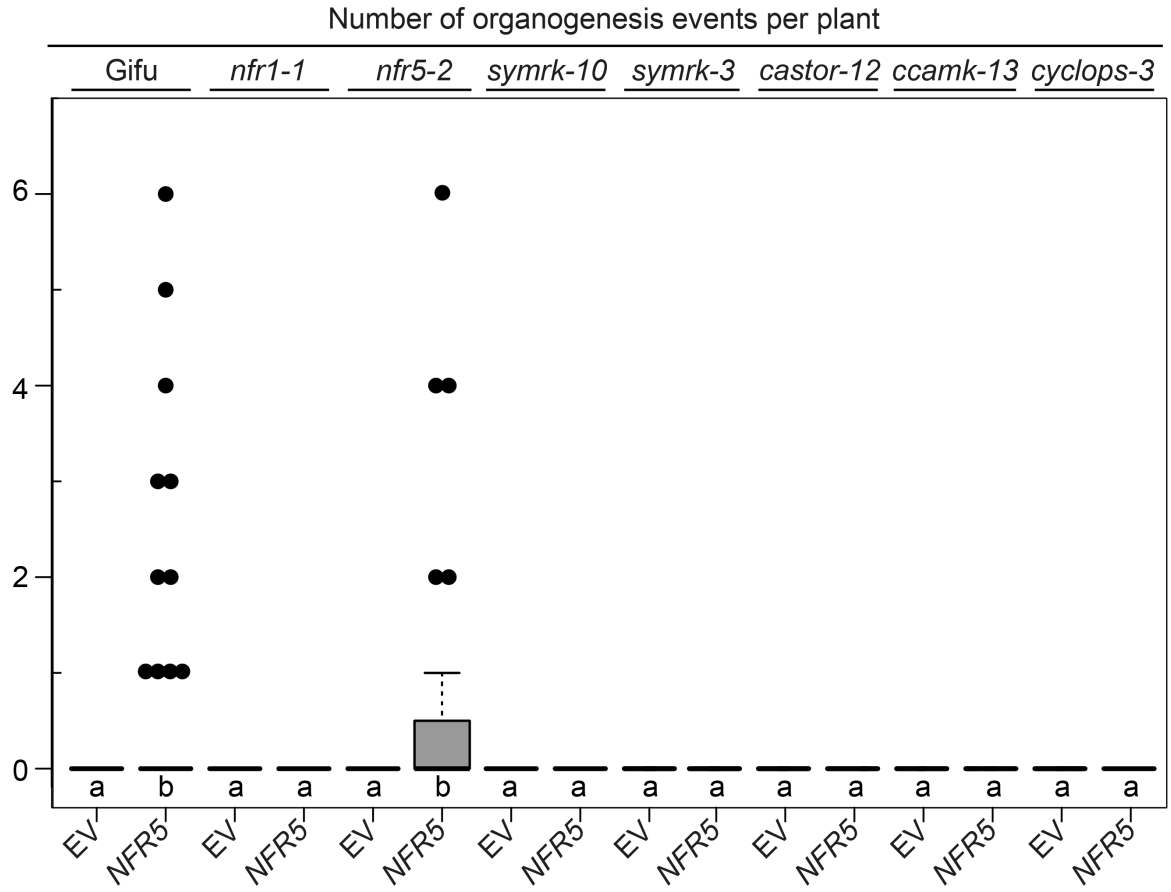


Figure 14: *NFR5*-mediated spontaneous organogenesis events in Gifu wild-type, *nfr1-1*, *nfr5-2*, and common symbiosis mutants.

Hairy roots of *L. japonicus* Gifu wild-type and different symbiosis defective mutants transformed with the empty vector (EV) or *pUB:NFR5-mOrange* (*NFR5*) were generated. Plot represents the numbers of organogenesis events (nodules and nodule primordia) per plant formed in the absence of rhizobia at 60 dpt. Black dots, data points outside 1.5 IQR of the upper quartile; bold black line, median; box, IQR; whiskers, lowest/highest data point within 1.5 IQR of the lower/upper quartile. A Kruskal-Wallis test followed by false discovery rate correction was performed. Different letters indicate significant differences. $p < 0.05$.

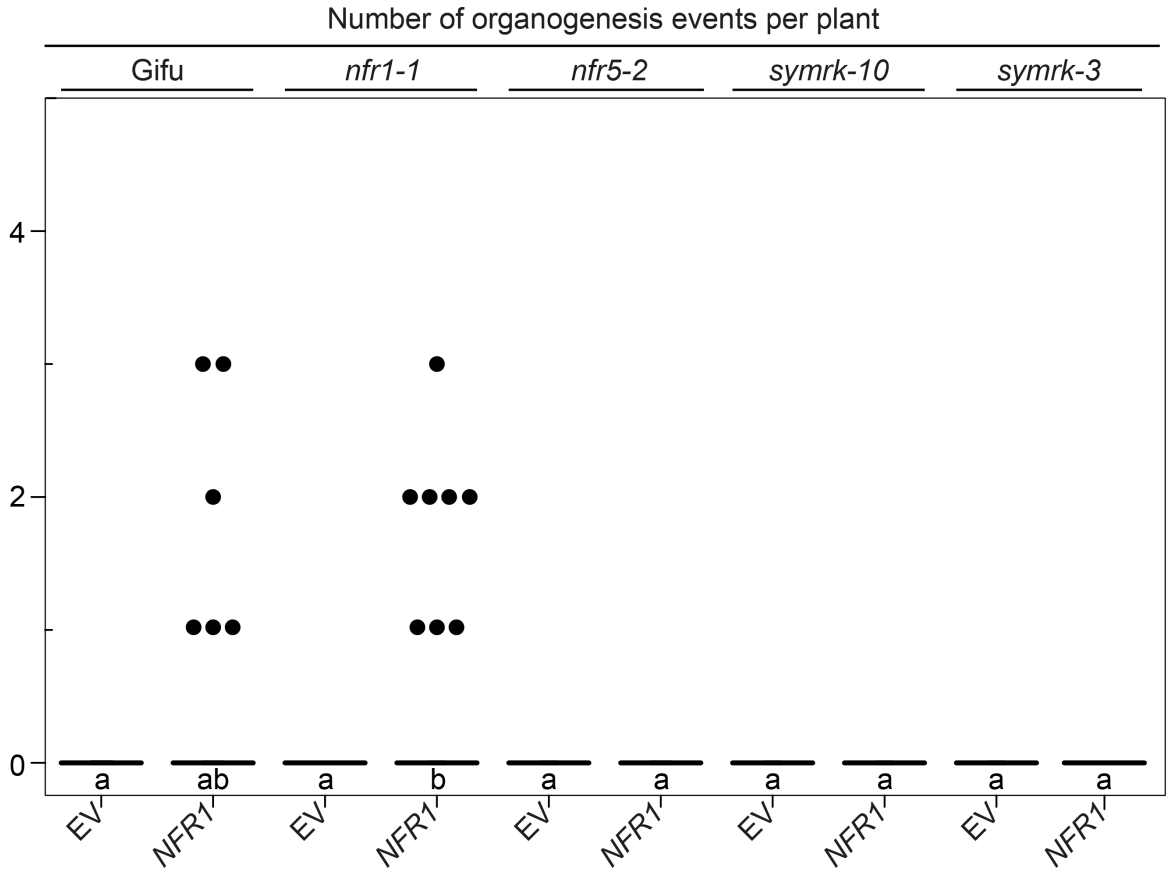


Figure 15: *NFR1*-mediated spontaneous organogenesis events in Gifu wild-type, *nfr1-1*, *nfr5-2*, *symrk-10* and *symrk-3*.

Hairy roots of *L. japonicus* Gifu wild-type and different symbiosis defective mutants transformed with the empty vector (EV) or *pUB:NFR1-mOrange* (*NFR1*) were generated. Plot represents the numbers of organogenesis events (nodules and nodule primordia) per plant formed in the absence of rhizobia at 60 dpt. Black dots, data points outside 1.5 IQR of the upper quartile; bold black line, median. A Kruskal-Wallis test followed by false discovery rate correction was performed. Different letters indicate significant differences. $p < 0.05$.

Neither *NFR* construct spontaneously induced nodule organogenesis in a *symrk-3* (null mutant) or *symrk-10* (kinase dead mutant) background, indicating that the formation of nodules is depended on the presence of kinase-active SYMRK (Figure 11; Figure 14 + 15). Spontaneous *NFR5*-mediated nodulation was completely abolished in the *nfr1-1* mutant, demonstrating that *NFR1* is essential for this *NFR5* function (Figure 11; Figure 14). This dependence of *NFR5* on *NFR1* is further supported by the observation that overexpression of *NFR1-mOrange* and *SYMRK-mOrange* but not of *NFR5-mOrange* activated the T90 reporter in the *nfr1-1* mutant background (Figure 16).

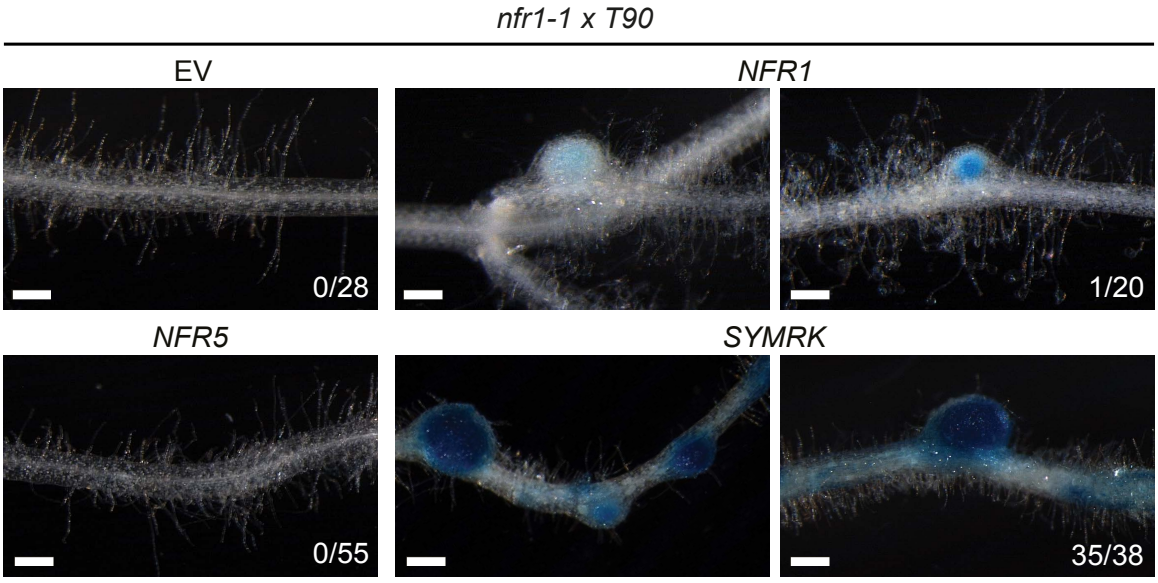


Figure 16: *NFR*-mediated activation of the symbiosis-specific T90 reporter in the *nfr1-1* mutant background.

Hairy roots a stable transgenic *L. japonicus* Gifu reporter line homozygous for the T90 reporter fusion and the *nfr1-1* mutant allele transformed with the empty vector (EV), *pUB:NFR1-mOrange* (*NFR1*), *pUB:NFR5-mOrange* (*NFR5*), or *pUB:SYMRK-mOrange* (*SYMRK*) were generated. GUS activity was analysed by histochemical staining with X-Gluc at 60 dpt. Representative root sections are shown. Number of plants with detectable GUS activity per total number of plants is indicated. Bars, 500 μm.

These results position *SYMRK* downstream of or at the same hierarchical level as *NFRs*. Moreover, while *SYMRK*-mediated spontaneous signalling does not require the simultaneous presence of *NFR1* and *NFR5*, *NFR5*-mediated spontaneous signalling is dependent on the presence of *NFR1*.

4. Discussion

4.1 Spontaneous signalling induced by receptor overexpression

A hallmark of the nitrogen-fixing symbiosis of legumes is the accommodation of rhizobia inside plant root cells in specialized organs - the nodules - that provide a favourable environment for nitrogen fixation. Given that the common symbiosis pathway is operating in AM symbiosis in most land plants, the discovery that expression of either of the three symbiotic *RLK* constructs from the strong *Ubiquitin* promoter leads to the spontaneous formation of nodules in transgenic *L. japonicus* roots (Figure 3 + 4; Figure 6; Figure 7; Figure 11 - 16) could pave the way towards the synthetic transfer of nitrogen-fixing root nodules to important non-leguminous crop species. As the symbiotic RLKs act at the entry level of root nodule symbiosis signalling, auto-active versions provide a

valuable tool to study the entire nodulation pathway uncoupled from bacterial infection. Furthermore, dominant active *RLK* versions could be useful for probing and dissecting the symbiotic signalling pathway, also in those plant lineages that are presently unable to develop nitrogen fixing root nodule symbiosis.

4.2 *SYMRK* has an active and direct role in symbiosis signalling

It has been observed that cytoplasmic streaming in root hairs of a *symrk-3* mutant did not resume after mechanical stimulation, which raised the possibility that the absence of calcium-spiking upon injection of calcium sensitive dyes into mutant root hair cells was a pleiotropic effect of this increased touch sensitivity (Miwa et al., 2006, Esseling et al., 2004). If touch desensitation was the only function of *SYMRK*, its overexpression would not lead to spontaneous nodule formation. We therefore unambiguously demonstrated a direct role of *SYMRK* in symbiosis signalling, while eliminating the possibility that the symbiosis defects of *symrk* mutants are due to pleiotropic effects only.

4.3 SYMRK is positioned upstream of genes involved in calcium-spiking

Mutants defective for either of the common symbiosis genes *SYMRK, CASTOR, POLLUX, NENA, NUP85* or *NUP133* produce very similar phenotypes in symbiosis, in that they abort infection at the epidermis and are impaired in calcium-spiking (Miwa et al., 2006, Kistner et al., 2005, Groth et al., 2010), which placed them at the same hierarchical level. Consequently, a genetic resolution of the relative position of the common symbiosis genes upstream of calcium-spiking was missing. Epistasis tests revealed that *SYMRK* initiates signalling upstream of other common symbiosis genes implicated in the generation and interpretation of nuclear calcium signatures (Figure 11 - 13). These findings support the conceptual framework in which SYMRK activates the calcium-spiking machinery and consequently the CCaMK/CYCLOPS complex, a central regulator of symbiosis-related gene expression and nodule organogenesis (Gleason et al., 2006, Singh et al., 2014, Singh and Parniske, 2012, Tirichine et al., 2006). This is in line with the observation that dominant-active variants of CCaMK were able to restore nodulation and infection in *symrk* mutant backgrounds, indicating that a main function of SYMRK in symbiosis is the activation of CCaMK (Hayashi et al., 2010, Madsen et al., 2010).

4.4 Interaction between SYMRK and the NFRs

We observed association between SYMRK and either NFR1 or NFR5 upon *NFR* overexpression in *L. japonicus* roots (Figure 10). Interestingly, under these conditions, the SYMRK-NFR association was detected in the absence of nodulation factor (Figure 10). In mammalian receptor tyrosine kinases as well as plant RLKs, ligand-induced receptor dimerization is the single most critical step in signal initiation (Chinchilla et al., 2007, Li et

al., 2002, Liu et al., 2012, Nam and Li, 2002, Schlessinger, 2002, Schulze et al., 2010, Sun et al., 2013a, Sun et al., 2013b). However, ligand-independent dimerization of receptor tyrosine kinases mediated by specific mutations in the kinase domain (Shan et al., 2012) or by overabundance of receptor tyrosine kinases (Wei et al., 2005) results in signalling activation and is a scenario well described in the context of cancer formation (Schlessinger, 2002). Similarly, overexpression of symbiotic *RLKs* might trigger ligand-independent receptor complex formation and activation of downstream signalling, thus providing an explanation why the interaction was also detected in the absence of external symbiotic stimulation. Unfortunately, we could not address the question whether SYMRK-NFR interaction is ligand-induced at endogenous levels of *NFR* expression since NFR1 and NFR5 were difficult to detect under these conditions.

4.5 The relationship between NFR1, NFR5 and SYMRK

We observed that *NFR5* requires *NFR1* as well as *SYMRK* for the spontaneous initiation of symbiosis signalling. This provides support for a model first put forward by Radutoiu (2003), in which NFR1 and NFR5 engage in a nodulation factor perception complex. This model has received additional support through their synergistic effect on promoting cell death in *N. benthamiana* (Madsen et al., 2011, Pietraszewska-Bogiel et al., 2013). The finding that NFR1 as well as NFR5 interact with SYMRK upon overexpression suggests that the three RLKs engage in a receptor complex ((Antolín-Llovera et al., 2014); Figure 9 + 10), and that this interaction might activate SYMRK for signal transduction. The observation that *SYMRK* operates independently of *NFR1* or *NFR5* brings about a new twist into current models of the signalling pathway (Downie, 2014) (Figure 11 - 13). *NFR1* and *NFR5* are only essential in the epidermis (Hayashi et al., 2014, Madsen et al., 2010), and it is likely that – at least partially - other members of the *LysM-RLK* gene family of *L. japonicus* (Lohmann et al., 2010) take over their role in the root cortex. *NFR1* or *NFR5* dispensability may be explained by other LysM-RLKs that might engage in alternative receptor complexes with SYMRK. Alternatively, spontaneous SYMRK-mediated signalling might be independent of any LysM-RLK, however, given the large number of LysM-RLKs in legumes (17 in *L. japonicus*; (Lohmann et al., 2010)), it is difficult to test the latter hypothesis conclusively.

SYMRK undergoes cleavage of its ectodomain, resulting in a truncated RLK molecule called SYMRK-ΔMLD (Antolín-Llovera et al., 2014). In competition experiments in *N. benthamiana* leaves, NFR5 binds preferentially to SYMRK-ΔMLD, which experiences rapid turnover in *N. benthamiana* as well as in *L. japonicus* (Antolín-Llovera et al., 2014). As our SYMRK antibody does not recognize endogenous SYMRK-ΔMLD, we were not able to assess whether overexpressed NFR1 or NFR5 also associates with this truncated SYMRK

variant in *L. japonicus* roots. In a hypothetical scenario, the SYMRK-ΔMLD complex with NFR5 forms constitutively to prevent inappropriate signalling, for example in the absence of rhizobia. The recruitment of NFR1, a hypothetical signal initiation event, would be promoted by the presence of nodulation factor. Our observation that upon overexpression in *L. japonicus* both NFR1 and NFR5 seem to interact with full-length SYMRK (Figure 10) suggests the formation of a ternary complex. This hypothetical complex has dual functionality: it signals through SYMRK on one hand to activate CCaMK and through the NFR1-NFR5 complex on the other hand to trigger the infection-related parallel pathways discovered by Madsen et al. (2010) and Hayashi et al. (2010). It is possible that SYMRK has a dual - positive and negative - regulatory role: on the one hand SYMRK promotes signalling but on the other hand SYMRK-ΔMLD may be involved in preventing inappropriate signalling. A negative regulatory role would explain the exaggerated root hair response of *symrk* mutants to nodulation factor (Stracke et al., 2002), since NFR1-NFR5 interaction is no longer under governance by SYMRK-ΔMLD. It has been demonstrated recently that expression of the intracellular kinase domain of *SYMRK* (*SYMRK-KD*) from *Medicago truncatula* or *Arachis hypogaea* in *M. truncatula* roots from the CaMV 35S promoter induces nodule organogenesis in the absence of rhizobia (Saha et al., 2014). However, in the presence of *Sinorhizobium meliloti*, nodules on plants overexpressing *AhSYMRK-KD* were poorly colonized and bacteria were rarely released from infection threads, highlighting the role of the extracytoplasmic domain of SYMRK in root nodule symbiosis (Saha et al., 2014).

4.6 Heterocomplexes between SYMRK and alternative LysM-RLKs may govern nodulation- versus mycorrhiza signalling

The origin of AM dates back to the earliest land plants (~ 400 mya) and recent angiosperms maintained a conserved genetic program for the intracellular accommodation of AM fungi (Gutjahr and Parniske, 2013). During the evolution of the nitrogen-fixing root nodule symbiosis, this ancient genetic program has been co-opted, as evidenced by the common symbiosis genes (Kistner et al., 2005). The discovery that the ancient SYMRK might act as a docking site for the recently evolved nodulation factor perception system ((Antolín-Llovera et al., 2014); Figure 9 + 10), highlights the role of this putative interface during the recruitment of the ancestral AM signalling pathway for root nodule symbiosis. Since a LysM-RLK closely related to NFR5 has been implicated in AM signalling (Op den Camp et al., 2011), this finding also provides a conceptual mechanism for the integration of signals from the rhizobial and fungal microsymbiont through alternative complex formation between SYMRK and NFRs or AM factor receptors.

4.7 Specificity originates from the receptors

One question that has puzzled the community since the postulate of a common symbiosis pathway is how the decision between the developmental pathways of AM or root nodule symbiosis is made when the signalling employs identical signalling components. Models proposed involved different calcium-spiking signatures with symbiosis-specific information content (Kosuta et al., 2008) or additional yet unidentified pathways that operate in parallel to the common symbiosis pathway to mediate exclusive and appropriate signalling (Takeda et al., 2011). Our observation of differential gene activation triggered by *NFR*s and *SYMRK* provides evidence that an important decision point is directly at the level of the receptors (Figure 7 + 8). Moreover, the observation that the dominant active *SYMRK* allele activates both pathways, which is not detected by stimulation with AM fungi or rhizobia, implies the existence of negative regulatory mechanisms that prevent the activation of the inappropriate pathway upon contact with either bacterial or fungal microsymbiont. The *SYMRK*-mediated loss of signalling specificity may be explained by simultaneous complex formation of SYMRK with NFR1 and NFR5, and related LysM-RLKs that mediate recognition of signals from the AM fungus (Maillet et al., 2011, Op den Camp et al., 2011), which results in the release of both negative regulatory mechanisms, or by an unbalanced stoichiometry of SYMRK and putative specific negative regulators of AM- and root nodule symbiosis signalling. Candidates for such regulators include the identified interactors of the kinase domains of NFR1, NFR5 and SYMRK (Chen et al., 2012, Den Herder et al., 2012, Ke et al., 2012, Kevei et al., 2007, Lefebvre et al., 2010, Mbengue et al., 2010, Toth et al., 2012, Yuan et al., 2012, Zhu et al., 2008). The loss of signalling specificity upon *SYMRK* overexpression is reminiscent of expression of the deregulated $CCaMK_{314}$ deletion mutant that also induces spontaneous nodules and AM-related gene activation (Takeda et al., 2012). It is therefore possible that *SYMRK* overexpression imposes a deregulated state on CCaMK that is otherwise attainable artificially through the deletion of its regulatory domain.

5. Materials and methods

5.1 DNA constructs and primers

For a detailed description of the constructs and primers used in this study, please see Supplemental File 1.

5.2 Agrobacterium tumefaciens-mediated transient transformation of Nicotiana benthamiana leaves

Transient transformation of *N. benthamiana* leaves was performed as described previously (Antolín-Llovera et al., 2014).

5.3 Plant growth, hairy root transformation and inoculation

L. japonicus seed germination (Groth et al., 2010) and hairy root transformation (Charpentier et al., 2008) were performed as described previously. Plants with emerging hairy roots systems were transferred to Fahraeus medium (FP) plates containing 0.1 µM of the ethylene biosynthesis inhibitor L-*α*-(2-aminoethoxyvinyl)-glycine at 2.5 weeks after transformation. For spontaneous nodulation experiments, promoter activation assays, or qRT-PCR experiments, plants were transferred to sterile Weck jars containing 300 mL dried sand/vermiculite and 25 mL FP medium at 23 dpt. For co-enrichment experiments, plants were transferred to sterile Weck jars containing 300 mL dried sand/vermiculite at 23 dpt, mock treated with 20 mL FP medium or inoculated with 20 mL of a *M. loti* MAFF303099 *Ds*RED suspension in FP medium set to an OD_{600} of 0.05, and incubated for 10 days. Plants for the SYMRK- and $CCaMK^{T265D}$-mediated T90 activation in the *nfr1-1*, *cyclops-2* and *ccamk-2* mutants were transferred to FP plates containing 0.1 µM of the ethylene biosynthesis inhibitor L-*α*-(2-aminoethoxyvinyl)-glycine at 21 dpt and kept on FP plates for 17 days. Transformants of the *pSbtM1:GUS* line were directly transferred to Weck jars containing 300 mL dried sand/vermiculite and approximately 25 mL ddH_2O at 2.5 weeks after transformation. It is important to avoid free water at the bottom of the Weck jar. Plants were grown in Weck jars in a growth chamber (16 hours light / 8 hours dark; 24 °C) for 1.5 - 6 weeks. For complementation experiments, plants were transferred from FP plates to open pots containing 300 mL dried sand/vermiculite and 75 mL FP medium at 23 dpt. After one week, plants were inoculated with 25 mL per pot of a *M. loti* MAFF303099 *Ds*RED suspension in FP medium set to an OD_{600} of 0.05. Roots were phenotyped 15 days after inoculation.

5.4 Non-denaturing protein extraction from *Nicotiana benthamiana* leaves and immunoprecipitation experiments

Protein extraction and immunoprecipitation was performed as described previously (Antolín-Llovera et al., 2014).

5.5 Non-denaturing protein extraction from *Lotus japonicus* hairy roots and immuno-enrichment experiments

Plant tissue was ground to a fine powder in liquid nitrogen with mortar and pestle. Proteins were extracted by adding 200 µL extraction buffer per 100 mg root tissue (50 mM Hepes, pH 7.5, 10 mM EDTA, 150 mM NaCl, 10 % sucrose, 2 mM DTT, 0.5 mg/mL Pefabloc, 1 % Triton-X 100, PhosSTOP [Roche], Plant Protease Inhibitor [P9599; Sigma-Aldrich], 1% polyvinylpolypyrrolidone). Samples were incubated for 10 minutes at 4 °C with 20 rpm end-over mixing, and subsequently centrifuged for 15 minutes at 4°C and 16000 RCF. 30 µL of each protein extract was mixed with 10 µL 4x SDS-PAGE sample

buffer (input; 25 % (v/v) 0.5 M Tris-HCl (pH 6.8), 35 % (v/v) 20 % SDS, 40 % (v/v) 100 % Glycerol, 0.03 g/mL DTT, dash of Bromphenol blue). For immuno-enrichment procedures, 30 µL RFP binder coupled to magnetic particles (Chromotek, rtm-20) were washed in wash buffer (WB; 50 mM Hepes, pH 7.5, 10 mM EDTA, 150 mM NaCl, 1 % Triton-X 100). Between 500 and 1000 µL of the protein extract was added to the beads and immuno-enrichment was performed for 4 hours at 4 °C with 20 rpm end-over mixing, followed by 15 minutes magnetic separation at 4 °C. Supernatant was removed and beads were washed twice with WB. 40 µL 2x SDS-PAGE sample buffer was added to the beads and both beads and input were incubated 10 minutes at 56 °C. After heating, beads were magnetically collected at the tube wall for 5 minutes and 40 µL of the supernatant (eluate) was taken. For SDS-PAGE, 20 µL of the input or eluate were loaded on each gel.

5.6 Western blot analysis

Western blot analysis was performed as described previously (Antolín-Llovera et al., 2014).

5.7 T90, *NIN*, *SbtM1* and *SbtS* promoter analysis in *Lotus japonicus*

GUS activity originating from the activation of promoter:*GUS* reporters was visualized by X-Gluc staining as described previously (Groth et al., 2010).

5.8 Expression Analysis

Transgenic root systems of *L. japonicus* plants were harvested 40 dpt. 80 mg root fresh weight per sample was applied for total RNA extraction using the Spectrum™ Plant Total RNA kit (Sigma-Aldrich). For removal of genomic DNA, RNA was treated with DNase I (amplification grade DNase I, Invitrogen™). RNA integrity was verified on an agarose gel and the absence of genomic DNA was confirmed by PCR. First strand cDNA synthesis was performed in 20 µL reactions with 600 ng total RNA using the SuperScript® III First-Strand Synthesis SuperMix (Invitrogen™) with oligo(dT) primers. qRT-PCR was performed in 20 µL reactions containing 1 x SYBR Green I (Invitrogen™) in a CFX96 Real-time PCR detection system (Bio-Rad). PCR program: 95 °C - 2 min, 45 x (95 °C - 30 sec; 60 °C - 30 sec; 72 °C – 20 sec; plate read), 95 °C - 10 sec, melt curve 60 °C to 95 °C: increment 0.5 °C per 5 sec. Expression was normalized to the reference genes *EF-1alpha* and *Ubiquitin* and *EF-1alpha* was used as a reference to calculate the relative expression of the target genes. The empty vector samples were used as negative control. Three biological replicates were analyzed in technical duplicates per treatment. A primer list can be found in the supplemental files (Supplemental File 1B).

5.9 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" (R-Team, 2013) and the packages "Hmisc" (Harrell, 2014), "agricolae" (de Mendiburu, 2014), "car" (Fox and Weisberg, 2011), "multcompView" (Graves et al., 2012) and "multcomp" (Hothorn et al., 2008). For statistical analysis of the numbers of nodules, nodule primordia or total organogenesis events, a Kruskal-Wallis test was applied followed by false discovery rate correction. Quantitative real-time PCR data was power transformed with the Box-Cox transformation and a one-way ANOVA followed by a Dunnett's test was performed, in which every treatment was compared to the empty vector samples.

6. Supplemental files

6.1 Supplemental File 1 A: Constructs.

Constructs labelled with "GG" were generated via Golden Gate cloning (Binder et al., 2014).

Table 1: Entry clones / Golden Gate Level I & Level II plasmids (LI & LII).

Name	Description
pENTR:NFR1	Phusion PCR product amplified from *p35S:NFR1-YFPv* with caccNFR1_fwd and NFR1_rev; cloned into pENTR/D-TOPO (Invitrogen) via TOPO reaction
pENTR:NFR5	Phusion PCR product amplified with caccNFR5_fwd and NFR5_rev; cloned into pENTR/D-TOPO (Invitrogen) via TOPO reaction
pENTR:NFR1-mOrange	Phusion PCR product amplified from *p35S:NFR1-mOrange* with caccNFR1_fwd and mOrange_STOP; cloned into pENTR/D-TOPO (Invitrogen) via TOPO reaction
pENTR:NFR5-mOrange	Phusion PCR product amplified from *p35S:NFR5-mOrange* with caccNFR5_fwd and mOrange_STOP; cloned into pENTR/D-TOPO (Invitrogen) via TOPO reaction
pENTR:EFR-mOrange	Phusion PCR product amplified from *p35S:EFR-mOrange* with EFR_SP_fwd and mOrange_STOP
LI C-D *SYMRK (GG)*	LI element containing *SYMRK*
LII F 2-3 *pUBi:SYMRK (GG)*	Assembled by BsaI cut ligation from: LI A-B pUBi + LI dy B-C + LI C-D *SYMRK* + LI dy D-E + LI E-F nos-T + LI dy F-G + LII F 2-3

LII F 2-3 *pUBi:SYMRK-mOrange (GG)*	Assembled by BsaI cut ligation from: LI A-B pUBi + LI dy B-C + LI C-D *SYMRK* + LI D-E mOrange + LI E-F nos-T + LI dy F-G + LII F 2-3

Table 2: Plasmids for *N. benthamiana* transformation and cloning.

Name	Description
p35S:GW-mOrange	(Bayle et al., 2008)
p35S:SYMRK-mOrange	(Den Herder et al., 2012)
p35S:NFR1-mOrange	LR-reaction of *pENTR:NFR1* and *p35S:GW-mOrange*
p35S:NFR1-YFPv	(Antolín-Llovera et al., 2014)
p35S:NFR5-mOrange	LR reaction of *pENTR:NFR5* and *p35S:GW-mOrange*
p35S:NFR5-YFPv	(Antolín-Llovera et al., 2014)
p35S:BRI1-YFPv	(Mbengue et al., 2010)

Table 3: Plasmids for hairy root transformation of *L. japonicus.*

Name	Description
pUB:GW-GFP	(Maekawa et al., 2008)
pUB:SYMRK-mOrange	(Antolín-Llovera et al., 2014)
pUB:EFR-mOrange	LR reaction of *pENTR:gEFR-mOrange* and *pUB:GW_GFP*
pUB:NFR1-mOrange	LR reaction of *pENTR:NFR1-mOrange* (cut with ApaL1) and *pUB:GW-GFP*
pUB:NFR5-mOrange	LR reaction of *pENTR:NFR5-mOrange* (cut with ApaL1) and *pUB:GW-GFP*
pSYMRK:SYMRK-RFP	(Kosuta et al., 2011)
pUBi:SYMRK (GG)	Assembled by BpiI cut ligation from: LII dy 1-2 + LII F 2-3 *pUBi:SYMRK* + LII dy 3-4 + LII F 5-6 *p35S:GFP* + LIII β F A-B
pUBi:SYMRK-mOrange (GG)	Assembled by BpiI cut ligation from: LII dy 1-2 + LII F 2-3 *pUBi:SYMRK:mOrange* + LII dy 3-4 + LII F 5-6 *p35S:GFP* + LIII β F A-B

6.2 Supplemental File 1B: Oligonucleotides

Table 4: Expression analysis.

Target sequence	Primer sequence
Ubiquitin *EF1alpha* *NIN* *SbtS*	(Takeda et al., 2009)
SbtM1 *Bcp1*	(Groth et al., 2013)

Table 5: Plasmid construction.

Name		Primer sequence
caccNFR1_fwd	forward	5'-caccATGAAGCTAAAAACTGGTCTACTT-3'
NFR1_rev	reverse	5'-TCTCACAGACAGTAAATTTATGA-3'
caccNFR5_fwd	forward	5'-caccATGGCTGTCTTCTTTCTTACCTCT-3'
NFR5_rev	reverse	5'-ACGTGCAGTAATGGAAGTCACA-3'
mOrange_STOP	reverse	5'-TTACTTGTACAGCTCGTCCATGC-3'
EFR_SP_fwd	forward	5'-caccATGAAGCTGTCCTTTTCACTTG-3'

Paper III: Symbiosis-related genes sustain the development of a downy mildew pathogen on *Arabidopsis thaliana*.

This chapter is based on the following manuscript:

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 *Arabidopsis thaliana*. Under review.

* These authors contributed equally to the work

Contributions of the author of this thesis to this manuscript are listed in detail under „**III. DECLARATION OF CONTRIBUTION AS CO-AUTHOR**" on pages 8 + 9 of this thesis.

Symbiosis-related genes sustain the development of a downy mildew pathogen on *Arabidopsis thaliana*

1. Summary

An ancient genetic program for intracellular infection of plant roots by symbiotic arbuscular mycorrhizal (AM) fungi is conserved among angiosperms. *Arabidopsis* accommodates haustoria of the oomycete *Hyaloperonospora arabidopsidis*, intracellular feeding organs structurally similar to fungal arbuscules. We report that, without constitutive resistance or exacerbated defence activation, *H. arabidopsidis* produces less sporangiophores and more morphologically altered haustoria on *Arabidopsis* mutants for homologs of the symbiosis genes *SYMRK, POLLUX* and the NUP107-160 nuclear pore subcomplex genes *NUP133* and *SEC13*. These findings reveal genetic commonalities between the host plant's programs for the development of intracellular accommodation structures in symbiosis and disease. While such exploitation of symbiotic programs by pathogens might explain the consistent deletion of symbiosis genes from five independent plant lineages after the loss of AM symbiosis, it raises the question which evolutionary drives retained the symbiosis core gene set in an otherwise AM-asymbiotic plant.

2. Introduction

Most land plant species feed carbon sources to arbuscular mycorrhizal (AM) fungi, which in turn deliver phosphate and other nutrients via finely branched intracellular structures called arbuscules (Gutjahr, 2014, Gutjahr and Parniske, 2013). The accommodation of fungal symbionts inside living plant cells involves substantial developmental

reprogramming of the host plant cell, initiated by the formation of the prepenetration apparatus, a transcellular tubular structure formed in anticipation of fungal infection (Genre et al., 2008, Genre et al., 2005). The oomycetal downy mildew pathogen *Hyaloperonospora arabidopsidis* (*Hpa*) and the fungal powdery mildew pathogen *Erysiphe cruciferarum* develop intracellular feeding organs, so called haustoria, which are, like the arbuscules of symbiotic fungi, entirely surrounded by a plant-derived membrane, and thus kept physically outside the host cytoplasm (Mims et al., 2002, Pumplin and Harrison, 2009, Huckelhoven and Panstruga, 2011). Structural and functional similarities between accommodation organs for microbes in symbiotic and pathogenic associations raised the hypothesis that both types of plant-microbe interactions rely on a shared genetic program (Parniske, 2000). This would imply that filamentous hyphal pathogens exploit an Achilles heel, the presence of the symbiotic program in most land plant species, for their own parasitic lifestyle (Evangelisti et al., 2014, Parniske, 2000).

Here we test this hypothesis by focusing on an ancient genetic program comprising the "common symbiosis genes" (CSGs), conserved among angiosperms for the intracellular accommodation of AM fungi (Kistner and Parniske, 2002, Markmann et al., 2008). In legumes, the CSGs are also required for root nodule symbiosis with nitrogen-fixing bacteria (Kistner et al., 2005). In the legume *Lotus japonicus*, the products of some of the "classical" CSGs - the Symbiosis Receptor-like Kinase SYMRK (Markmann et al., 2008, Stracke et al., 2002, Antolín-Llovera et al., 2014), the nucleoporins NUP85, NUP133 and SEC13 homolog (SEH1) of the NUP107-160 subcomplex (Alber et al., 2007, Groth et al., 2010, Kanamori et al., 2006, Saito et al., 2007), as well as the nuclear-envelope localized cation channel POLLUX (Charpentier et al., 2008, Venkateshwaran et al., 2012) - are implicated in a signal transduction pathway leading from the perception of microbial signalling molecules at the plasma membrane to the induction of symbiosis-related genes in the nucleus (Gutjahr and Parniske, 2013, Oldroyd, 2013).

H. arabidopsidis and *E. cruciferarum* form haustoria on *Arabidopsis*, which belongs to the Brassicaceae, a plant lineage that lost the ability to establish AM symbiosis after the divergence of the Brassicales. This asymbiotic state correlates with the loss of a specific set of CSGs (Delaux et al., 2013, Delaux et al., 2014), indicating a strong selection for the genome-wide loss of symbiosis-related genes. Importantly, the nuclear complex comprising CCaMK and CYCLOPS, which is of central importance for transcriptional regulation in symbiosis (Singh et al., 2014, Singh and Parniske, 2012), is absent from *Arabidopsis* (Delaux et al., 2013). This finding indicates that the complete common symbiosis gene set is not required for the compatible interaction with biotrophic pathogens. However, *Arabidopsis* retained vestiges of the symbiosis program as homologs

of CSGs (HCSGs). Transgenic complementation of a symbiosis-defective legume mutant with *AtPOLLUX* versions indicated overall functional conservation (Venkateshwaran et al., 2012). The retention of HCSGs in the *Arabidopsis* genome led us to investigate whether this vestigial symbiotic gene set plays a role in the interaction of *Arabidopsis* with the oomycete *Hpa,* a powdery mildew fungus and an extracellular bacterial pathogen.

3. Results and discussion

3.1 *Arabidopsis* HCSG mutants reduce the reproductive success of the oomycete *Hyaloperonospora arabidopsidis*

We inspected the *Arabidopsis* genome for the presence of HCSG and identified candidate orthologs of *POLLUX* and of the nucleoporins of the NUP107-160 subcomplex (Figure 17 + 18).

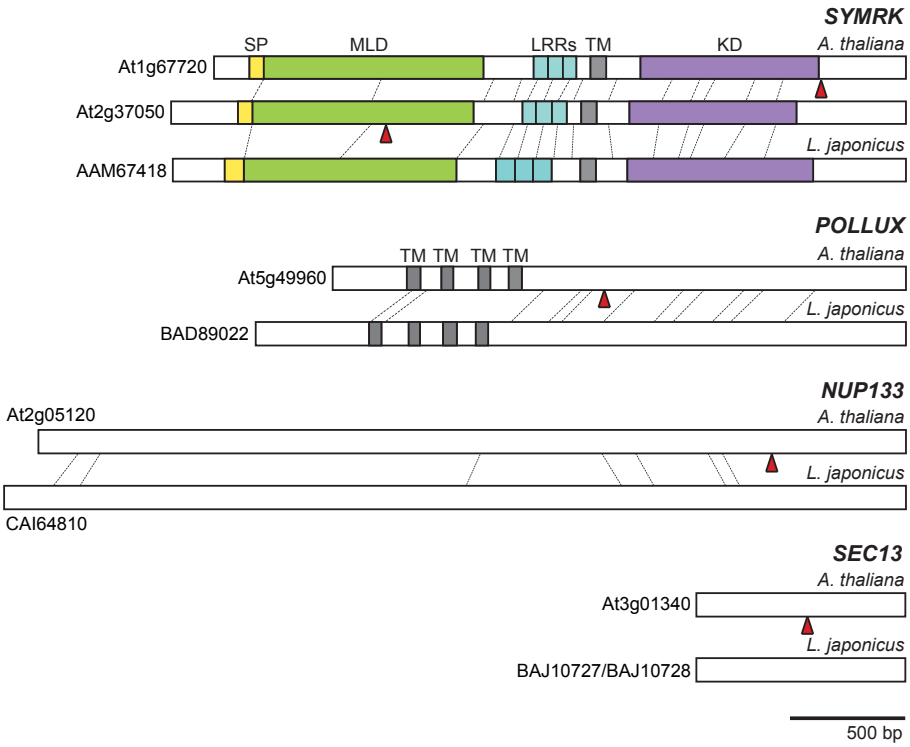
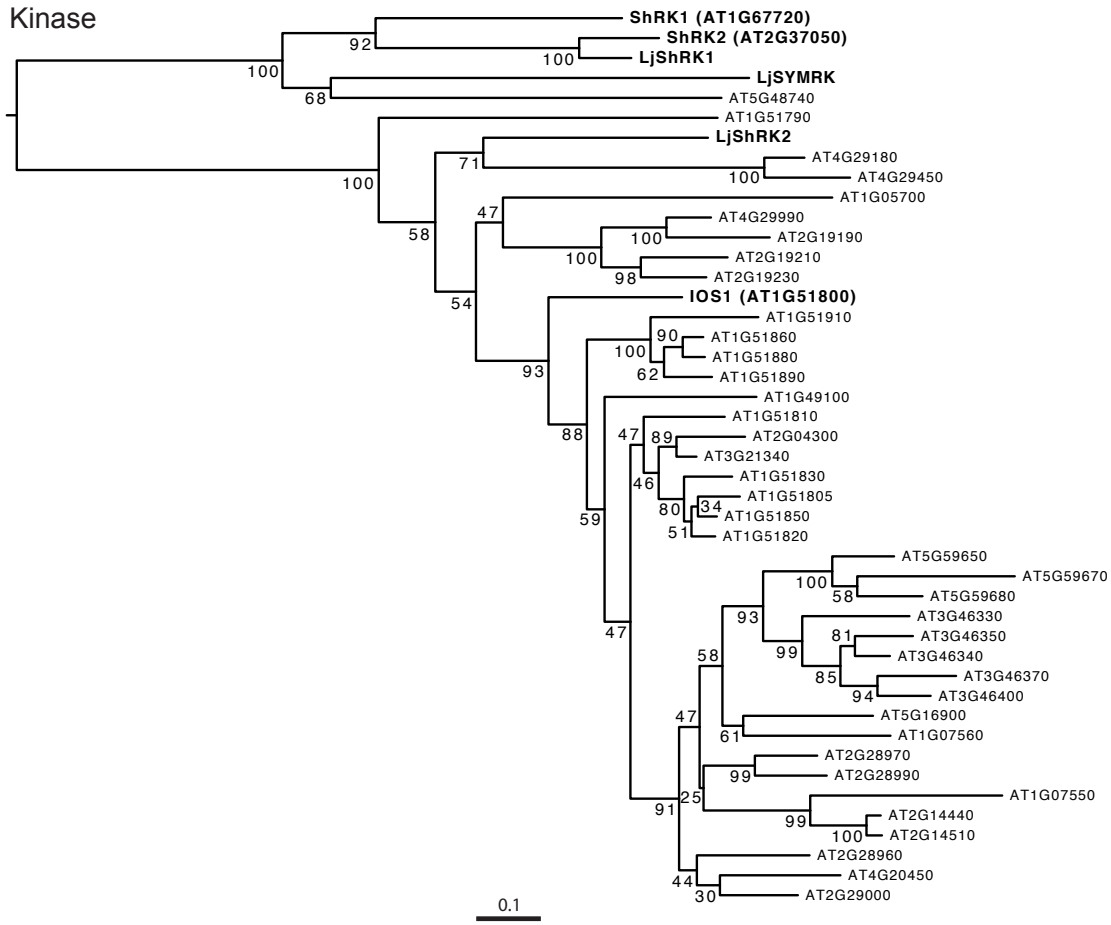


Figure 17: Comparison of gene structures and protein domains of *L. japonicus* common symbiosis genes (CSGs) and their closest homologs (HCSGs) in *Arabidopsis*.
Complete annotated genomic sequences were obtained from The *Arabidopsis* Information Resource (TAIR – www.arabidopsis.org) for *A. thaliana*, and from the KDRI website (Kazusa DNA Research Institute, Japan; http://www.kazusa.or.jp/lotus/) and the GenBank for *L. japonicus*. The protein domain organization and the exon-intron structure of the *Arabidopsis* homologs of *POLLUX*, *SEC13*, *NUP133* (GenBank accession number: KM269292), *ShRK1* and *ShRK2* are identical to that of their *L. japonicus* counterparts. By sequencing a PCR product amplified from *Arabidopsis* 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 TAIR. 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 18: *Arabidopsis* 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 *Arabidopsis* 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.

A direct ortholog of *SYMRK*, a malectin-like domain leucine-rich repeat receptor-like kinase (MLD-LRR-RLK) (Antolín-Llovera et al., 2014), was deleted from the *Arabidopsis* genome (Kevei et al., 2005), but SYMRK-homologous Receptor-like Kinases (ShRKs) that belong to the same gene family could be identified (Figure 18 + 19), and corresponding insertion mutant lines were analysed for their phenotype in the interaction with *Hpa*.


Kinase
ShRK1 (AT1G67720)
ShRK2 (AT2G37050)
LjShRK1
LjSYMRK
AT5G48740
AT1G51790
LjShRK2
AT4G29180
AT4G29450
AT1G05700
AT4G29990
AT2G19190
AT2G19210
AT2G19230
IOS1 (AT1G51800)
AT1G51910
AT1G51860
AT1G51880
AT1G51890
AT1G49100
AT1G51810
AT2G04300
AT3G21340
AT1G51830
AT1G51805
AT1G51850
AT1G51820
AT5G59650
AT5G59670
AT5G59680
AT3G46330
AT3G46350
AT3G46340
AT3G46370
AT3G46400
AT5G16900
AT1G07560
AT2G28970
AT2G28990
AT1G07550
AT2G14440
AT2G14510
AT2G28960
AT4G20450
AT2G29000
0.1

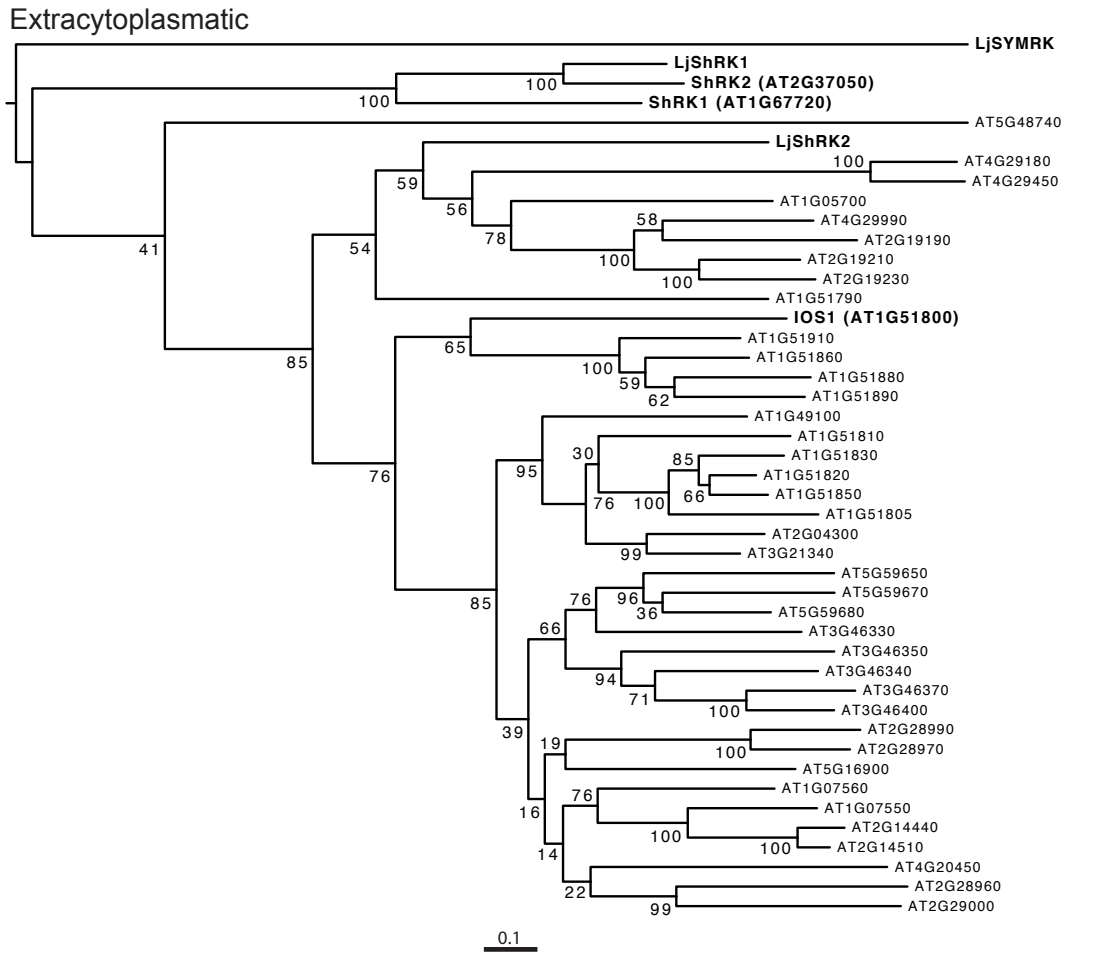

Extracytoplasmatic
LjSYMRK
LjShRK1
ShRK2 (AT2G37050)
ShRK1 (AT1G67720)
AT5G48740
LjShRK2
AT4G29180
AT4G29450
AT1G05700
AT4G29990
AT2G19190
AT2G19210
AT2G19230
AT1G51790
IOS1 (AT1G51800)
AT1G51910
AT1G51860
AT1G51880
AT1G51890
AT1G49100
AT1G51810
AT1G51830
AT1G51820
AT1G51850
AT1G51805
AT2G04300
AT3G21340
AT5G59650
AT5G59670
AT5G59680
AT3G46330
AT3G46350
AT3G46340
AT3G46370
AT3G46400
AT2G28990
AT2G28970
AT5G16900
AT1G07560
AT1G07550
AT2G14440
AT2G14510
AT4G20450
AT2G28960
AT2G29000
0.1

Figure 19: Maximum likelihood phylogenetic trees of MLD-LRR-RLKs from *Arabidopsis* and *L. japonicus*.
Maximum likelihood phylogenetic trees were constructed using the highly conserved kinase domains (upper tree) or the extracytoplasmic regions minus signal peptide (lower tree) of MLD-LRR-RLK proteins from *Arabidopsis*, where this family underwent a recent expansion (Shiu and Bleecker, 2003), and *L. japonicus*. 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 extracytoplasmic 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 *Arabidopsis* 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)). Interestingly, like in *IOS1*, which is also important for the interaction with *Hpa* (Hok et al., 2011), the extracytoplasmic regions of both proteins (but not the one encoded by At5g48740) contain the conserved gly-asp-pro-cys (GDPC) motif, known to be required for the establishment of the symbiotic program in the root epidermis (Antolín-Llovera et al., 2014, Kosuta et al., 2011), suggesting that this region might also be important for the interaction between *Arabidopsis* and *Hpa*. Numbers on each node represent the respective bootstrap values. Bar = relative genetic distance (arbitrary unit).

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 (Figure 20 + 21).

It has been speculated that the symbiotic phenotypes of *Lotus* CSG nucleoporin mutants *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 (Binder and Parniske, 2013, Capoen et al., 2011). 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 (Binder and Parniske, 2013, Gonzalez-Aguilera and Askjaer, 2012). To capture such potential structural or functional shifts during evolution, we included a wider 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 (Figure 20), while *seh1*, *nup43*, *nup85* and *nup160* did not (Figure 22). This pattern is not congruent with the

observation in *L. japonicus*, in which *seh1*, *nup85* and *nup133* impaired symbiosis (Groth et al., 2010, Kanamori et al., 2006, Saito et al., 2007), and may be explained by species-specific adaptations of the NUP107-160 subcomplex.

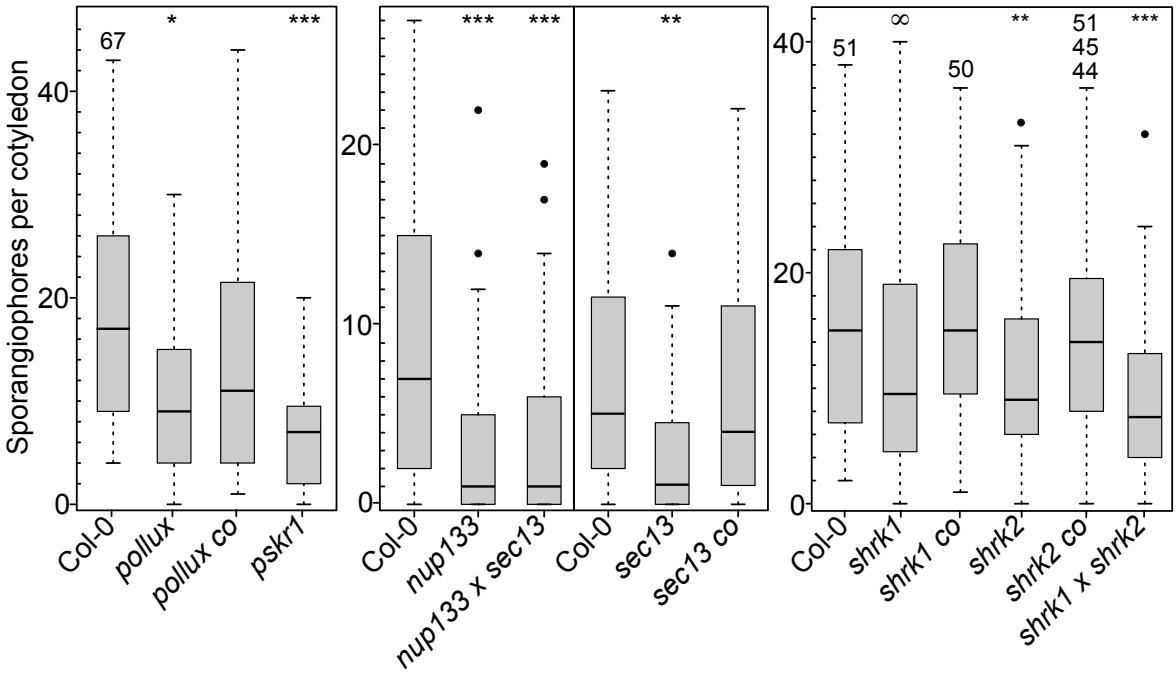


Figure 20: Mutation of *Arabidopsis* HCSGs reduces the reproductive success of *H. arabidopsidis*.

Plots show the number of sporangiophores per infected cotyledon on ca. 50 cotyledons of *Arabidopsis* 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). ∞ , $p = 0.067$, *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

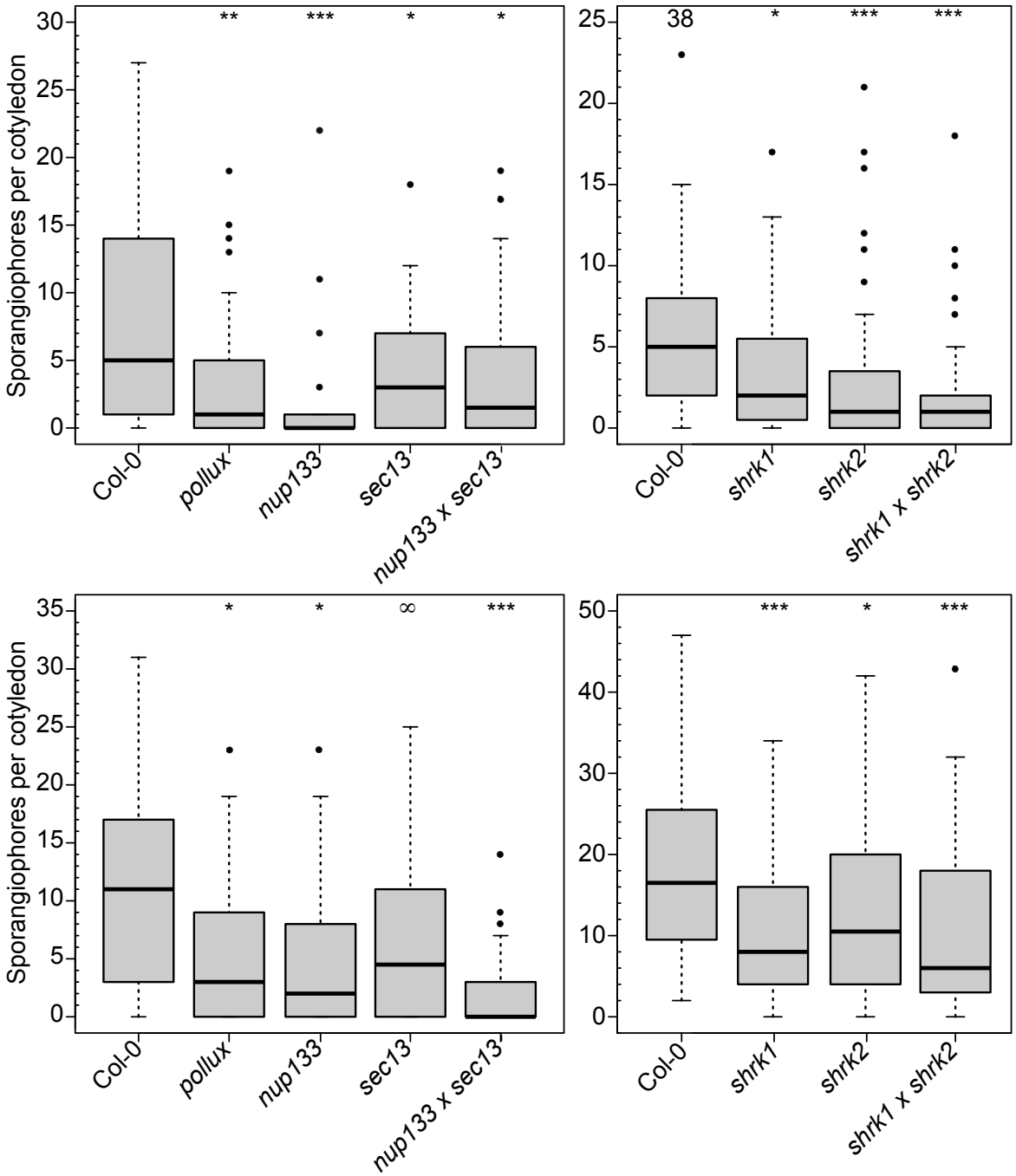


Figure 21: Reproductive success of *H. arabidopsidis* is reduced in the HSCG mutants.
Plots represent the number of sporangiophores per infected cotyledon on ca. 50 cotyledons of *Arabidopsis* wild-type (Col-0) and the HSCGs mutants 4 dpi with *Hpa* isolate NoCo2. Two replicates are shown for each mutant 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 test with Bonferroni-Holm correction), except for *sec13* in the lower set, for which p was 0,065 (indicated with ∞). $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

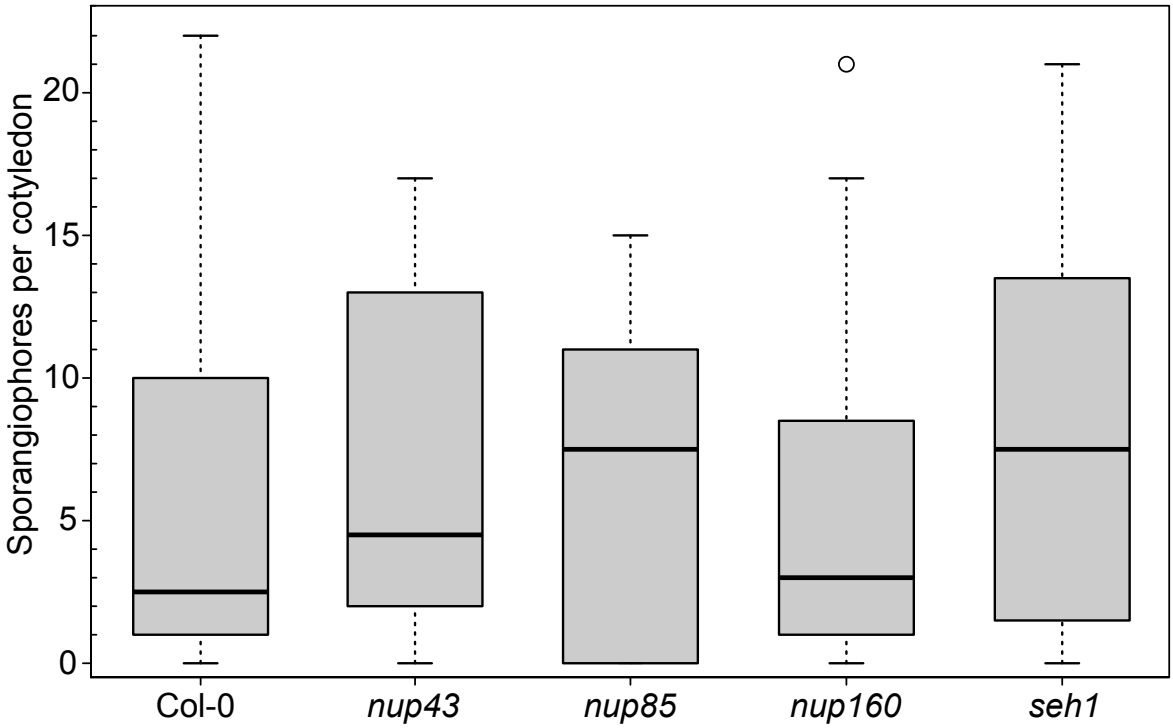


Figure 22: Reproductive success of *H. arabidopsidis* is not affected in the *seh1*, *nup43*, *nup85* and *nup160* mutants.

Plots represent the number of sporangiophores per infected cotyledon on ca. 50 cotyledons of *Arabidopsis* 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).

3.2 Haustorial development of *Hyaloperonospora arabidopsidis* is impaired in *Arabidopsis* 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 23).

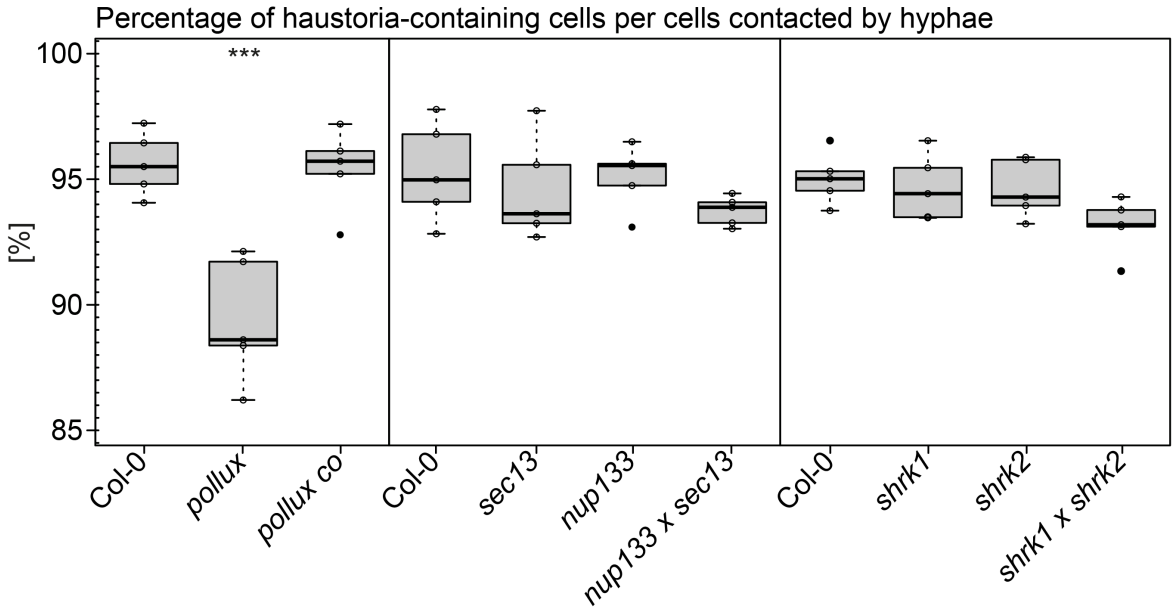


Figure 23: *H. arabidopsidis* haustoria formation on *Arabidopsis* HCSG mutants.
Plots represent the mean percentage of haustoria-containing cells per cells contacted by hyphae on 5 leaves of *Arabidopsis* wild-type (Col-0), the indicated mutants and transgenic complementation lines (co) at 5 dpi with *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). *** $p < 0.001$.

However, all *Arabidopsis* 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 (Figure 24 - 27). In contrast to the HCSG mutants, the disease resistant reference mutant *pskr1* did not show any signs of altered haustorial development (Figure 24).

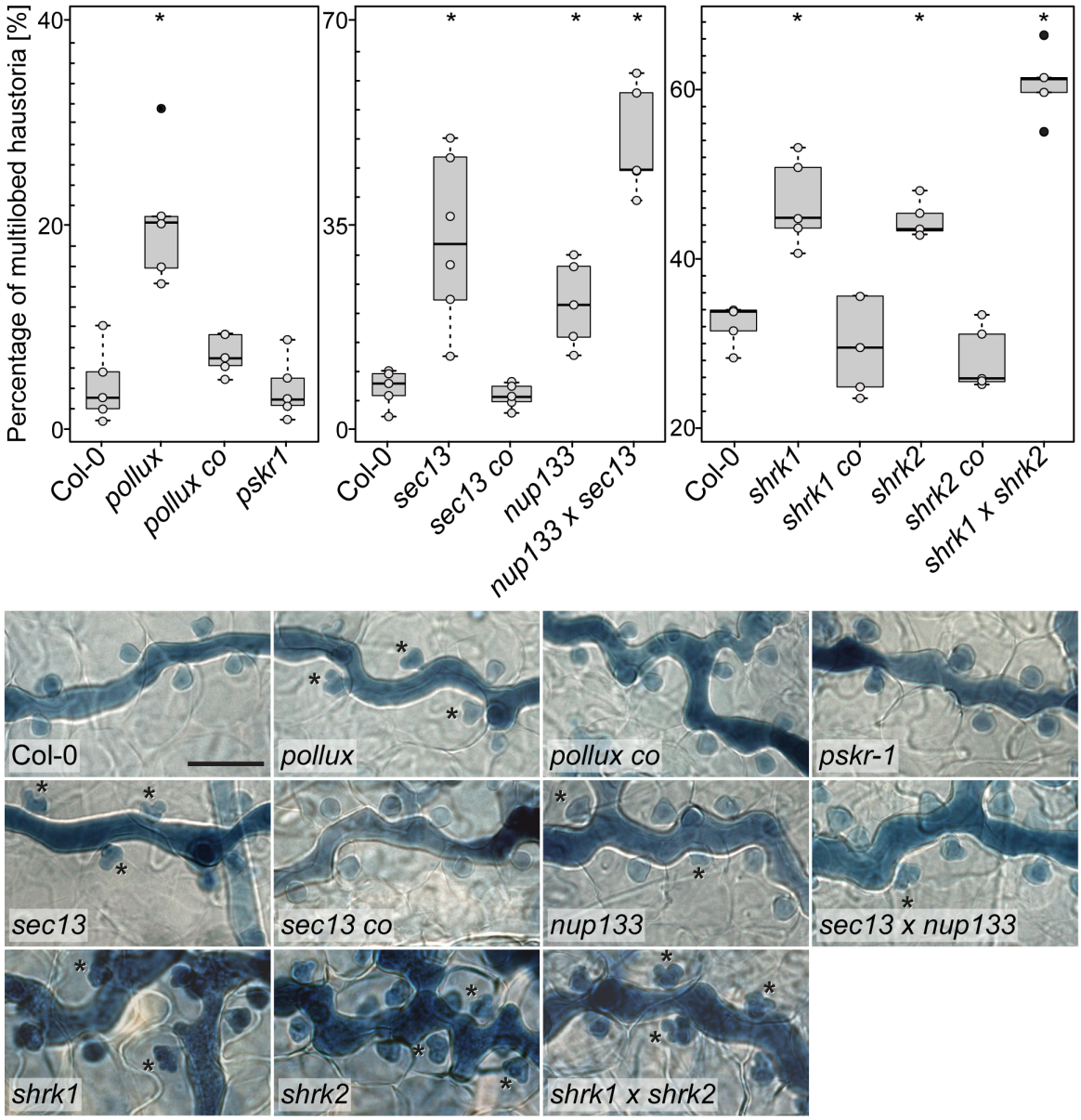


Figure 24: *H. arabidopsidis* haustorium morphology is altered in *Arabidopsis* HCSG mutants.
Arabidopsis wild-type (Col-0), the indicated HCSG mutants, and transgenic complementation lines (co) were analysed 5 dpi with *Hpa* isolate NoCo2. Upper panel: Plots show the percentage of multilobed haustoria 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. Bars = 25 µm.

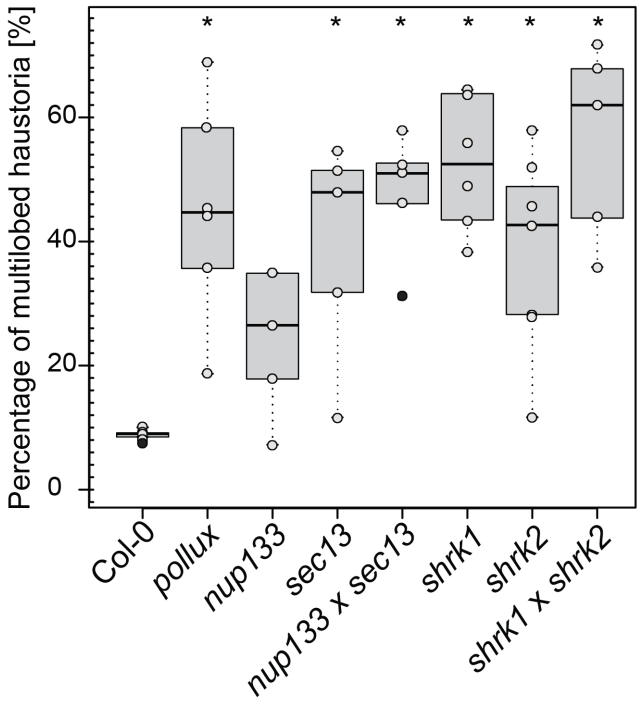


Figure 25: Frequency of multilobed haustoria on *Arabidopsis* wild-type and on the HSCG mutants.

Arabidopsis 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*: $p = 0{,}151$. *, $p < 0.05$.

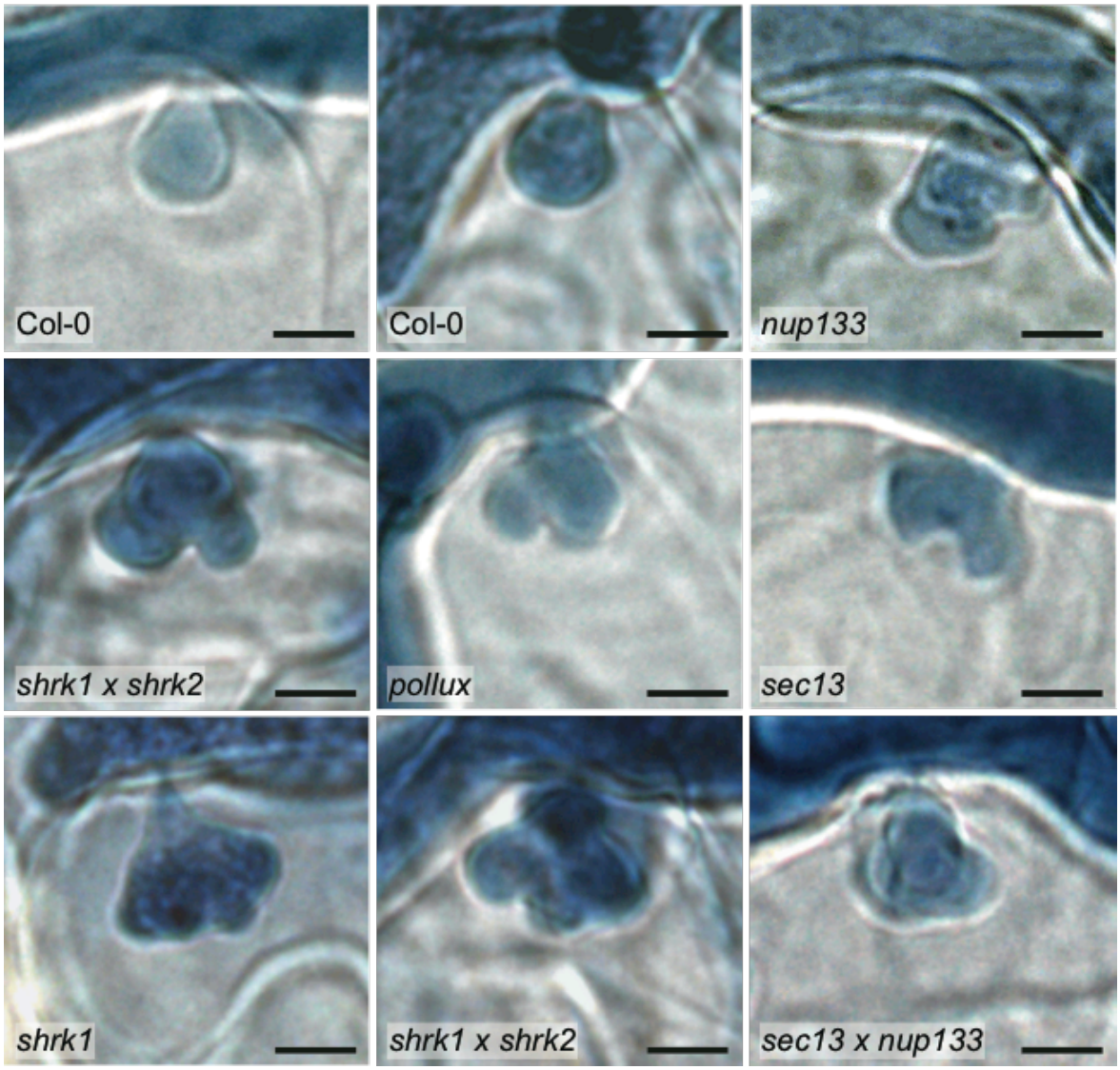


Figure 26: *H. arabidopsidis* haustorium morphology is altered in *Arabidopsis* HCSG mutants.

Differential interference contrast microscopy of leaves of *Arabidopsis* wild-type (Col-0) and HCSG mutants stained with trypan-blue lactophenol 5 dpi with *Hpa* isolate NoCo2. Bar = 25 μm.

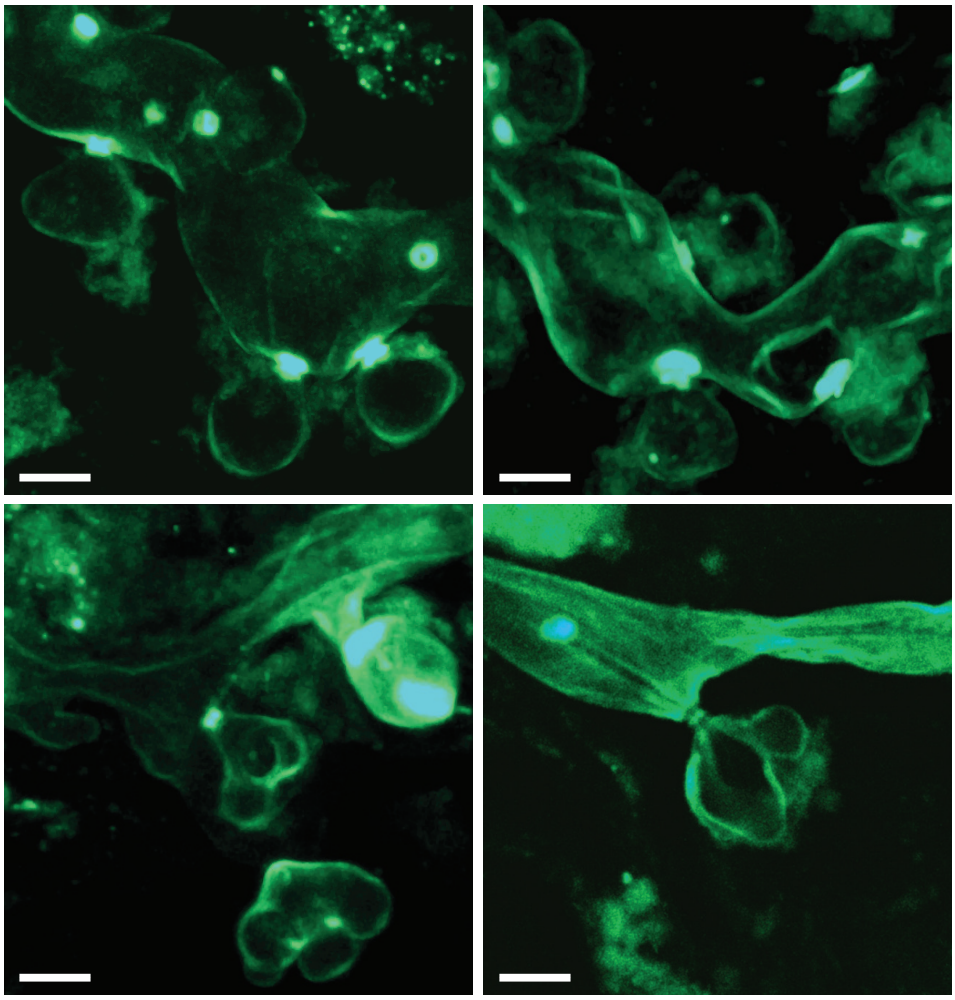


Figure 27: *H. arabidopsidis* haustoria morphology.
Hpa haustoria on wild-type *Arabidopsis* (Col-0) leaves (upper row) 5 dpi, with the regular globular or pear-like morphology, and examples of multilobed haustoria observed in the *shrk1* x *shrk2* double mutant (lower row). Leaves were stained with aniline-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. Bar: 25 μm.

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 28), suggesting early haustoria aging in the double mutant.

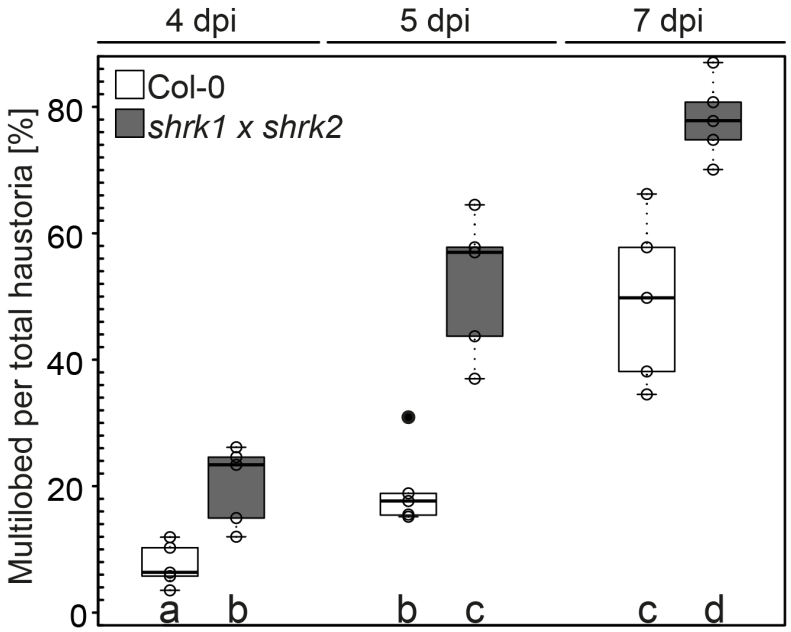
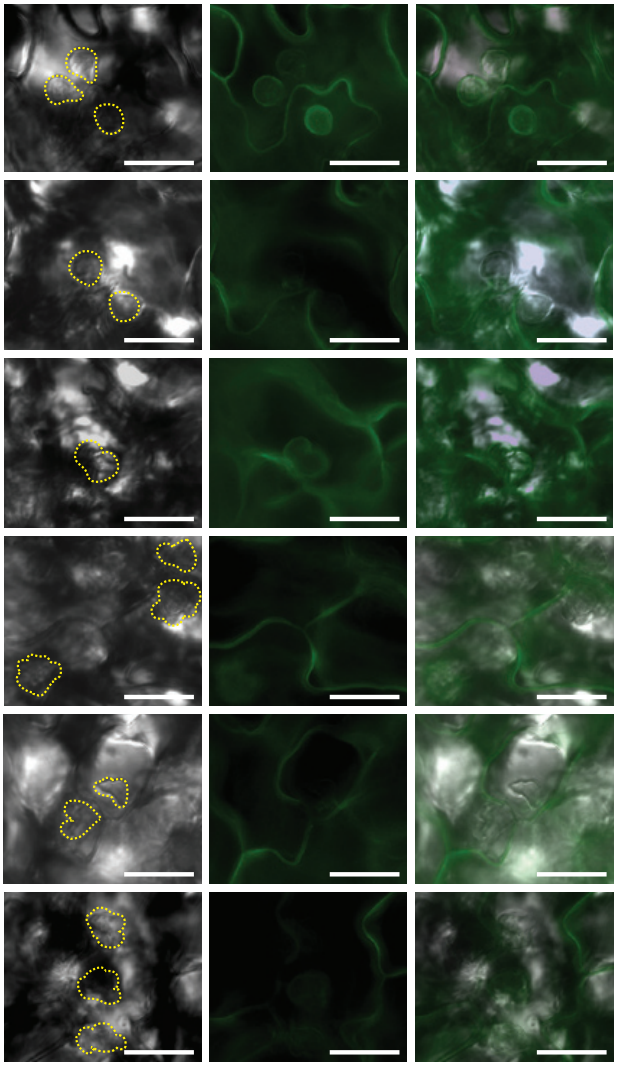


Figure 28: Time-course of haustoria development in *Arabidopsis* wild-type and in *shrk1* x *shrk2*.

Plots represent the mean percentage of multilobed haustoria per total haustoria on 5 leaves of *Arabidopsis* 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). **, $p < 0.01$.

An altered morphology was also observed for *Hpa* haustoria in leaves of transgenic *Arabidopsis* 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 was localized 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 29).



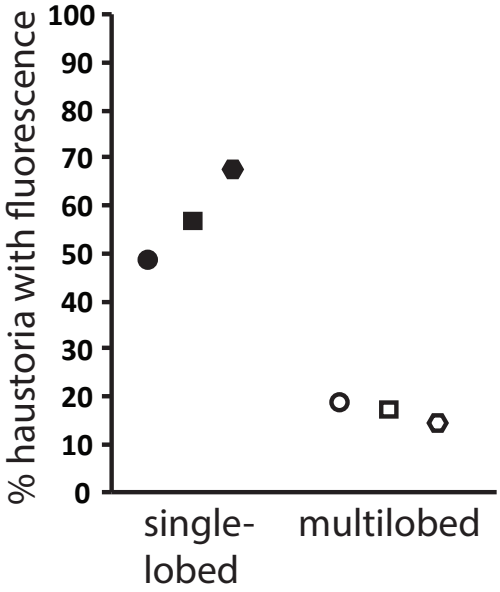


Figure 29: Quantification of haustoria with fluorescence on *ProAt3g61260:YFP-At3g61260* plants.

Arabidopsis 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.

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 *Arabidopsis* 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 30), 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.

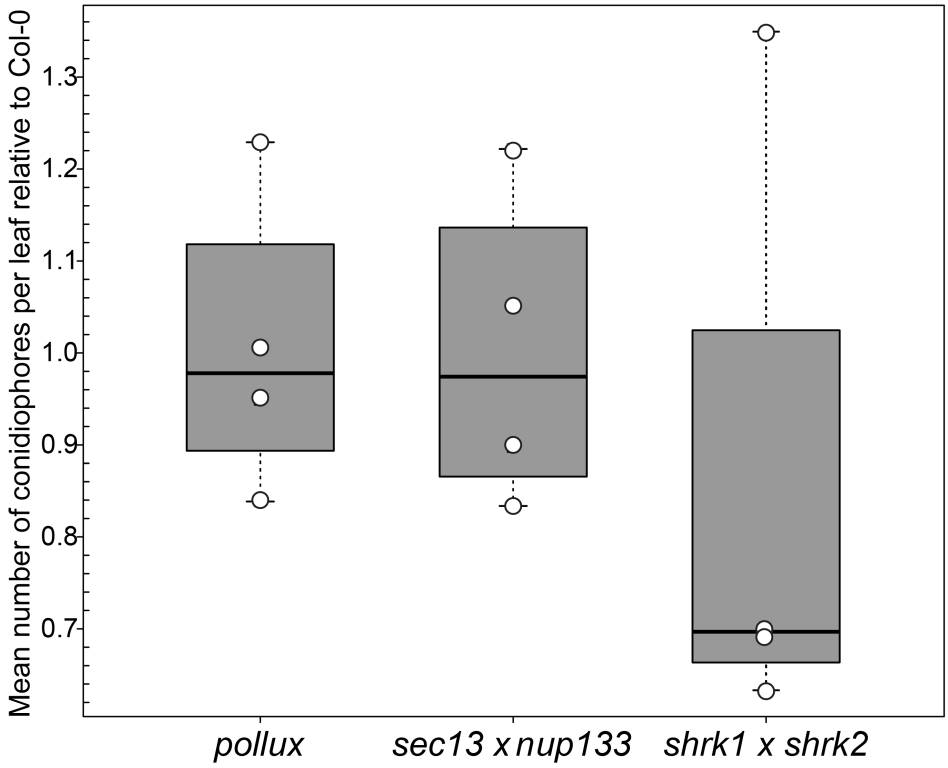


Figure 30: Conidiophores per leaf on HCSG mutants relative to the wild-type.
Box-plots represent a compilation from four independent replicates and show mean number of conidiophore on HCSG mutant relative to the wild-type (Col-0) leaves 5 dai with 3-4 *E. cruciferarum* spores/mm². For each replicate, 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).

3.3 *Arabidopsis* HCSG mutants do not exhibit constitutive or enhanced defence responses

The decreased susceptibility of HCSG mutants to *Hpa* is not the result of constitutively exacerbated activation of pathogen-associated molecular pattern (PAMP)-triggered plant immunity (Jones and Dangl, 2006), since the basal transcript levels of six PAMP-induced marker genes in the HCSG mutants did not differ from the wild-type or the *FLAGELLIN SENSING 2* (*FLS2*; (Gomez-Gomez and Boller, 2000)) mutant levels. Moreover, 6 hours after flg22 treatment these genes were all upregulated in the HCSG mutants to values similar to the wild-type (Figure 31 + 32). Any deviations observed for individual mutants were not consistent through the gene set and are thus unlikely responsible for the increased pathogen resistance.

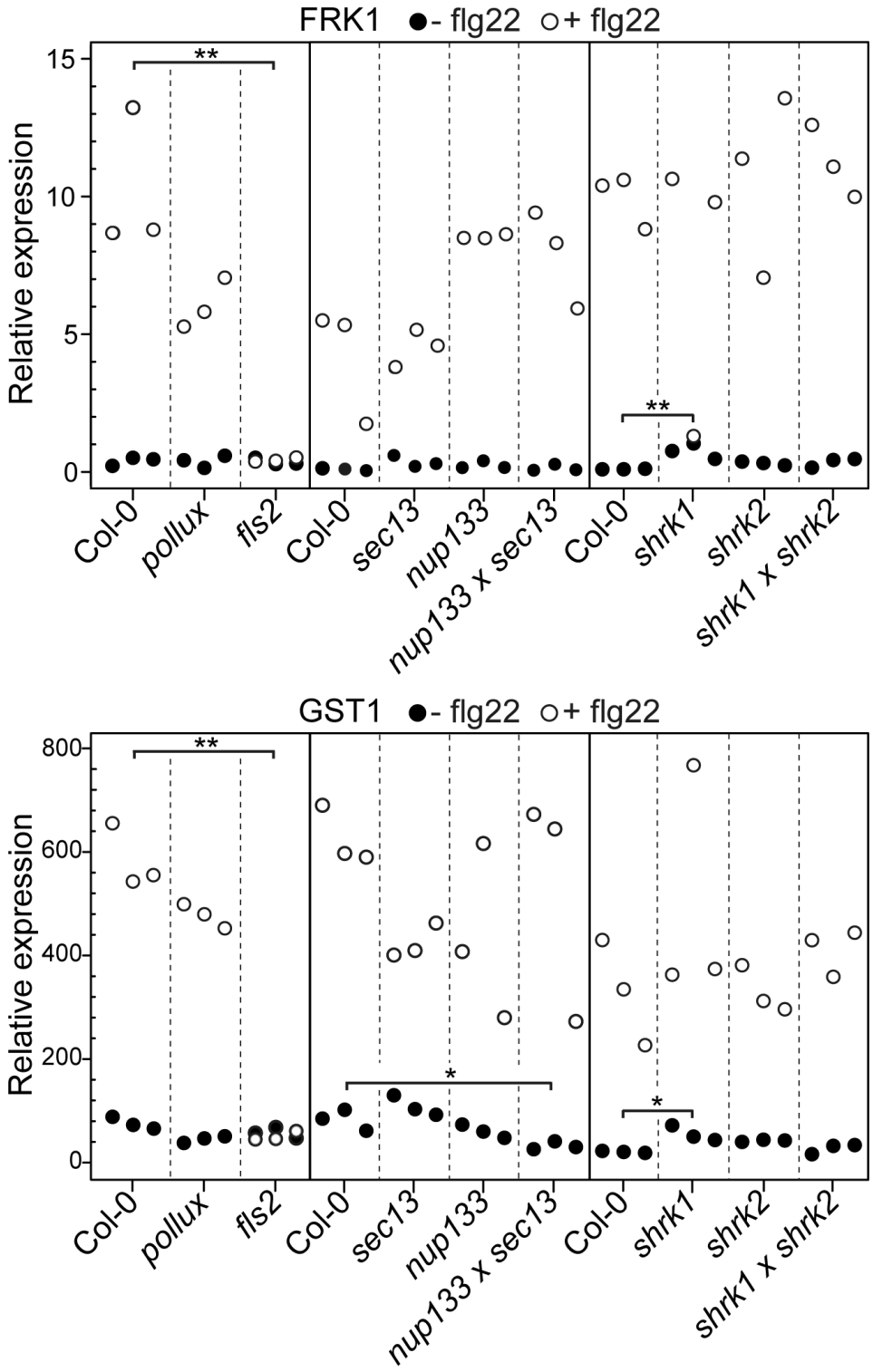


Figure 31: Basal expression levels and flg22-mediated induction of PAMP-responsive genes *FRK1* and *GST1* in *Arabidopsis* HCSG mutants are not different from the wild-type.

The relative expression of *FLG22-INDUCED RECEPTOR-LIKE KINASE 1* (*FRK1;* (Asai et al., 2002)) and *GLUTATHIONE S-TRANSFERASE 1* (*GST1;* (Grant et al., 2000)) 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). *, $p < 0.01$; **, $p < 0.001$.

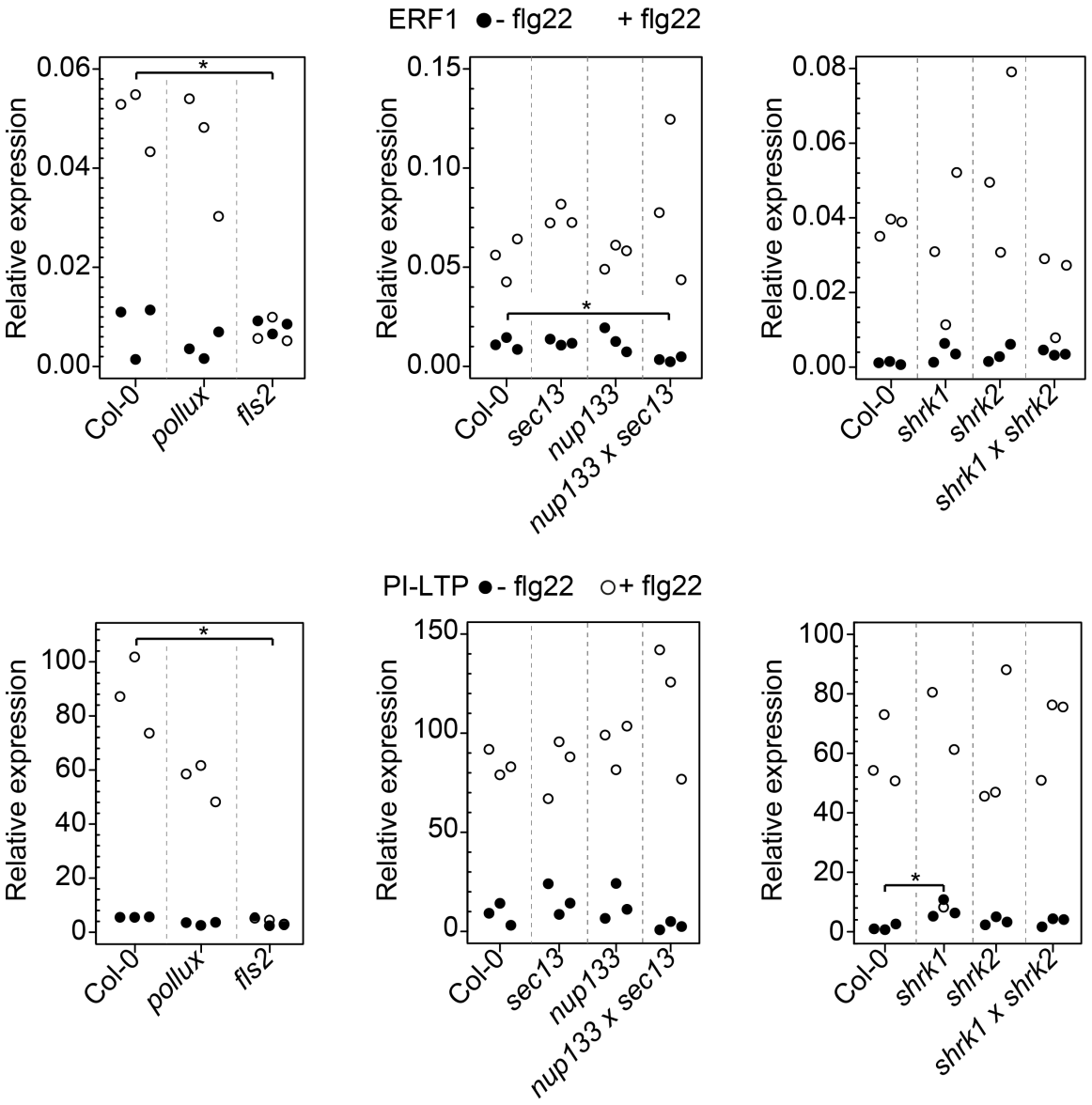


Figure 32: Basal expression levels and flg22-mediated induction of the PAMP-responsive genes *ERF1* and *PI-LTP* in *Arabidopsis* HCSG mutants are not different from the wild-type.
Relative expression of *ETHYLENE RESPONSE FACTOR 1* (*ERF1*; (Solano et al., 1998)) and *PHOSPHATYDILINOSITOL-LIPID TRANSFER PROTEIN* (*PI-LTP;* (Denoux et al., 2008)) in mock-treated samples (-) or in samples treated with 1 μM 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). *, $p < 0.01$.

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 33).

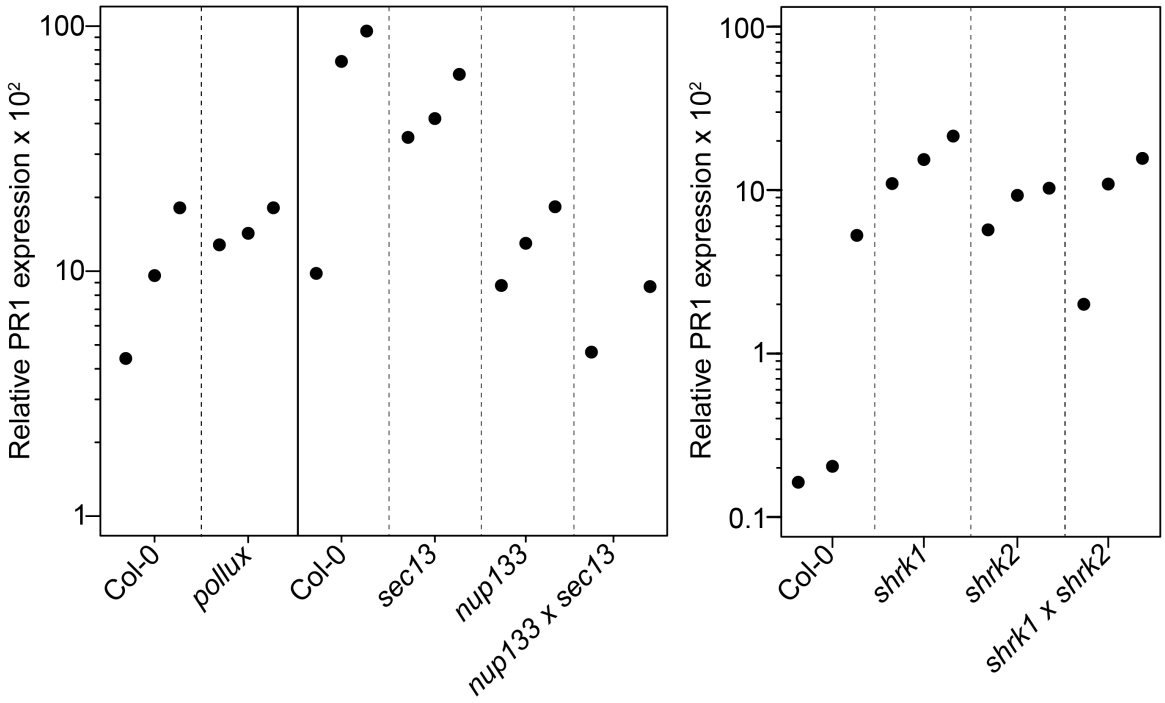


Figure 33: Transcript levels of the defence marker gene *PR1* in *Arabidopsis* HCSG mutants. Relative expression of *PR1* (Ryals et al., 1996) was determined in three mock-treated biological replicates for each genotype via qRT-PCR. Transcript levels for every plant genotype were determined through technical duplicates. No statistical differences to the wild-type (Col-0) were obtained at the 1 % significance level (Dunnett's Test with Bonferroni correction).

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 *Arabidopsis* HCSG mutants (Figure 34 + 35).

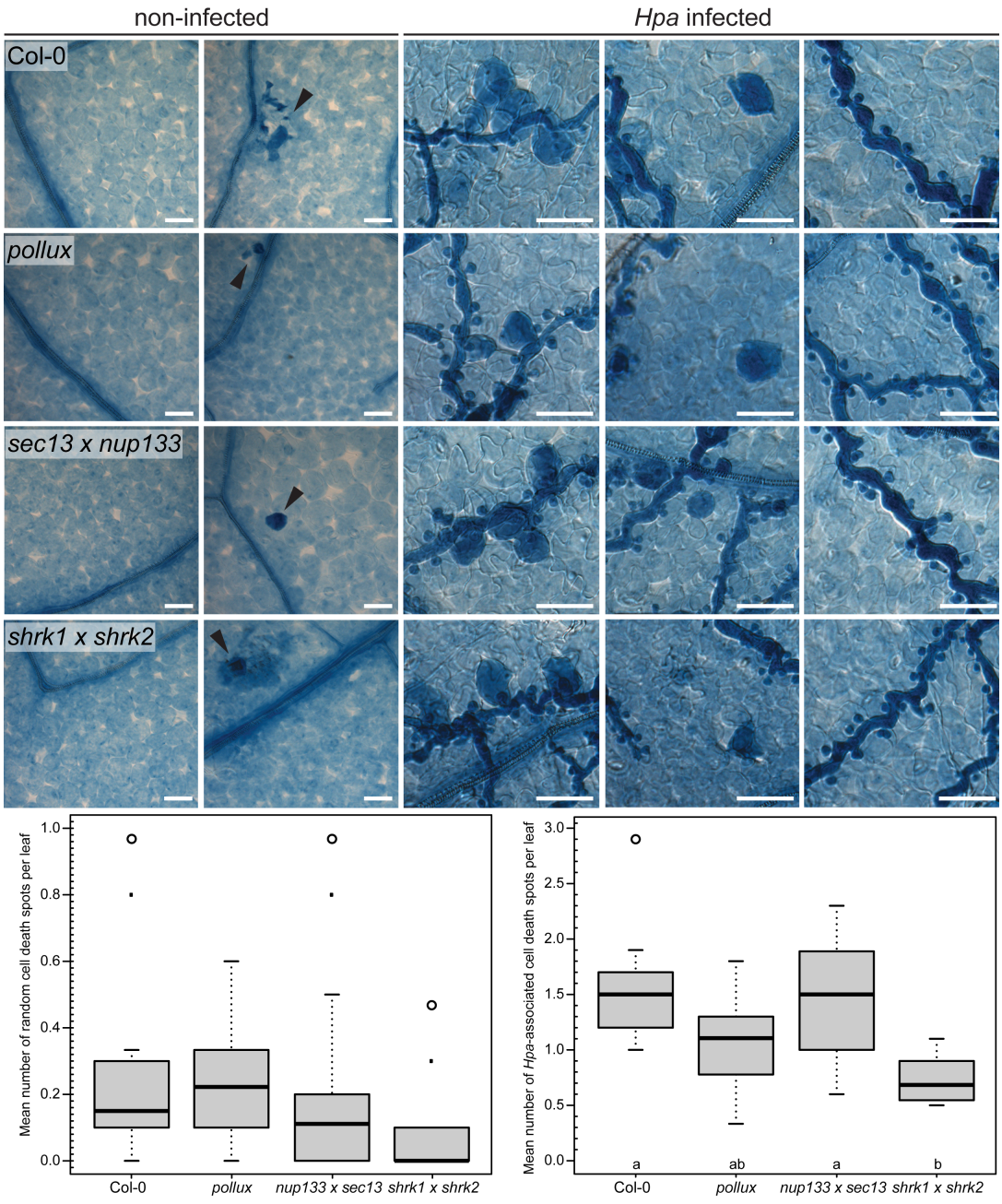


Figure 34: *Arabidopsis* HCSG mutants do not show increased levels of spontaneous or pathogen-induced cell death.

Upper panel: differential interference contrast microscopy of representative *Arabidopsis* 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). 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 of *Arabidopsis* wild-type (Col-0) and indicated mutants 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.

Figure 35: *Arabidopsis* HCSG mutants do not show developmental or growth defects.
3-week-old *Arabidopsis* wild-type (Col-0) plants grown alongside the indicated mutant lines under long day conditions (16 h 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.

In addition, the ability of *Hpa* to suppress callose deposition around the haustorial neck region (Sohn et al., 2007) was not disturbed (Figure 36).

The NUP107-160 subcomplex has also been implicated in plant defence; mutations in *NUP96*, *NUP160* and *SEH1* impair basal and resistance-gene mediated immunity (Wiermer et al., 2012, Zhang and Li, 2005). However, our mutants did not show such deregulation, possibly due to distinct roles of individual subcomplex components in compatibility and defence.

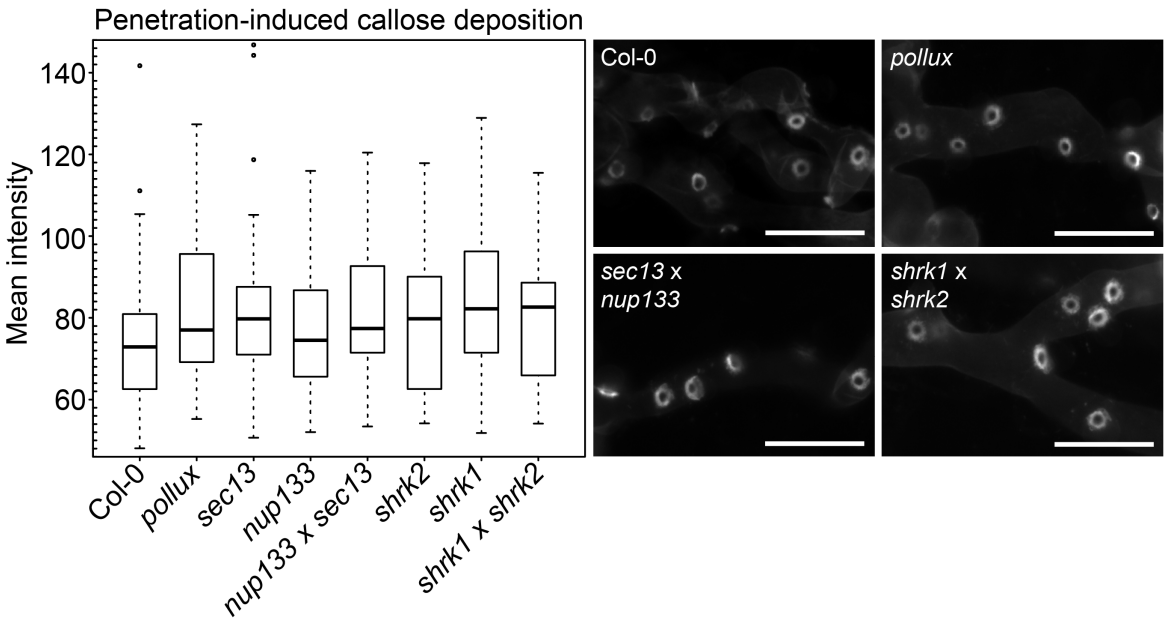


Figure 36: *H. arabidopsidis* haustoria-associated callose deposition is not reduced in the *Arabidopsis* HCSG mutants.

Plot shows the mean intensity of callose deposition on a total of ca. 50 oomycete penetration sites from 5 different leaves of *Arabidopsis* 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. Bar = 25 μm.

3.4 Growth kinetics of *Pseudomonas syringae* are not altered on the *Arabidopsis* HCSG mutants

The bacterial pathogen *Pseudomonas syringe* DC3000 induces the activation of the SA- and the jasmonic acid (JA)-dependent defence signalling in the host (Farmer et al., 2003, Thaler et al., 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 37) was unaltered on the *Arabidopsis* HCSG mutants, providing further evidence that they do not exhibit constitutive or enhanced activation of SA- and JA-dependent defences.

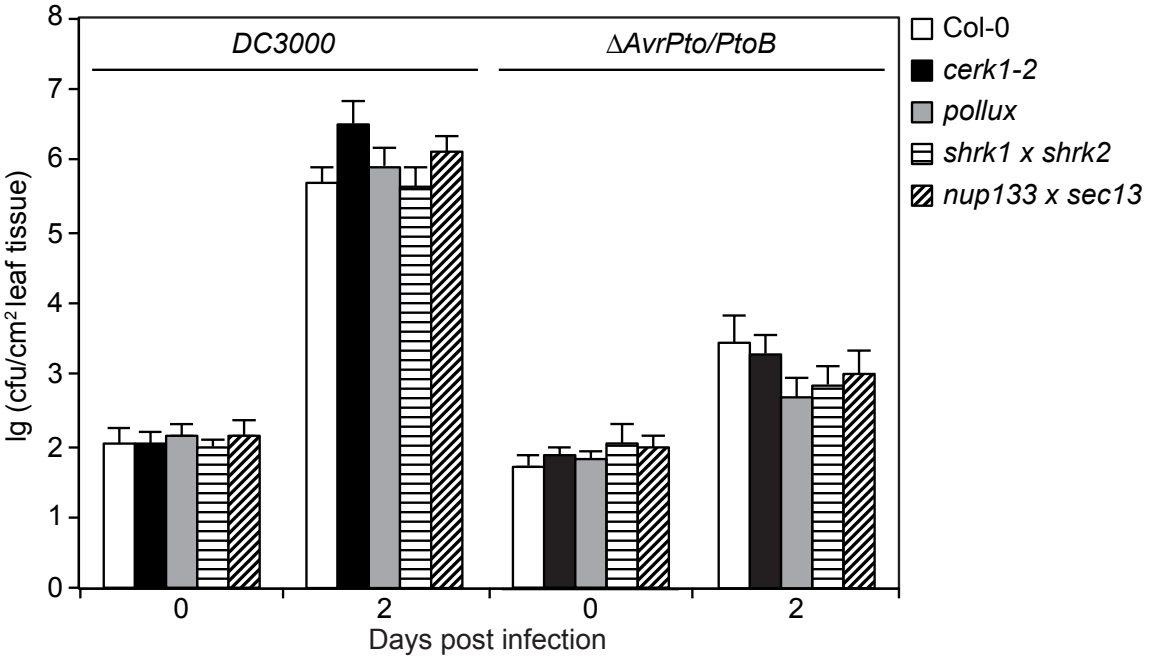


Figure 37: *Arabidopsis* HCSG mutants support similar bacterial growth curves as the wild-type.

Growth of Pto DC3000 (left) or Pto DC3000 ΔAvrPto/AvrPtoB (right) in *Arabidopsis* 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 104 colony forming units ml-1 (cfu/cm2). Data represent means ± S.D. of six replicate measurements/genotype/data point. Results from one representative of at least four independent experiments are shown.

3.5 *Arabidopsis* HCSGs are involved in the intracellular accommodation of *Hyaloperonospora arabidopsidis*

In the present work, we show that *Hpa* performs poorly on *Arabidopsis* HCSG mutants with no abnormal activation of defence pathways, obtaining evidence that these genes promote the colonization of plant cells by biotrophic oomycetes. In contrast to the vast knowledge on genes contributing to disease resistance, relatively few genes have been identified that facilitate pathogen colonization on *Arabidopsis*, such as *PMR4*, *PMR5*, *PMR6* (Vogel and Somerville, 2000, Vogel et al., 2002, Vogel et al., 2004), *DMR1* (Van Damme et al., 2005, van Damme et al., 2009), *MYB3R4* (Chandran et al., 2010), and *IOS1* (Hok et al., 2011). In legumes, the *RAM2* gene encoding a glycerol-phosphate acyl-transferase (GPAT) has been proposed to be involved in the production of cutin monomers, which act as common host-derived signals for both AM fungi and the pathogenic oomycete *Phytophthora palmivora* to form infection organs (appressoria or hyphopodia) at the plant root surface (Wang et al., 2012). However, the initial cell infection process of *Hpa* appears unaltered in the HCSG mutants of *Arabidopsis*.

Our study revealed a common requirement of common symbiosis genes or their homologs in *Arabidopsis* for the formation of intracellular feeding organs by both

symbiotic and pathogenic microbes. Although strongly suggested by the function of the legume common symbiosis genes, it remains unclear whether the *Arabidopsis* homologs are similarly involved in a signal transduction pathway directly supporting oomycetal development. It will be therefore interesting to identify the mechanistic commonalities between symbiotic and pathogenic interactions that are controlled by the HCSGs.

The loss of AM symbiosis in *Arabidopsis* and in four other independent plant lineages was correlated with the absence of more than 100 genes with potential roles in AM symbiosis (Delaux et al., 2013, Delaux et al., 2014). The requirement of HCSGs for full *Hpa* reproductive success indicates that pathogens may exploit that ancient symbiotic program, and the selection pressure resulting from this scenario provides a plausible explanation for the observed convergent pattern of symbiosis-related gene loss. However, the evolutionary forces leading to their specific retention in the genome remain completely obscure. A housekeeping function was not revealed since no pleiotropic developmental phenotypes were observed in the mutants. This leaves us with the unexpected finding that the only detected role for the HCSGs in *Arabidopsis* is the support of an oomycete. It will be interesting to find out whether ecological conditions exist, under which oomycetal colonization might provide a selective advantage to the host plant.

4. Experimental procedures

4.1 Seed sterilization and plant growth

Seeds were obtained from "The Nottingham Arabidopsis Stock Centre" – NASC (Scholl et al., 2000) or the GABI-DUPLO double mutant collection (Bolle et al., 2013). 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, *Arabidopsis* plants were grown in a 2:1 soil/sand mixture. Seeds were stratified (4 ° C for 48 h) 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 treatment, seeds were placed on half-strength MS plates (Murashige and Skoog, 1962) stratified for 48 h at 4 ° C in the dark, and grown under long day conditions (16 h light, 23 ° C, 85 µmol m^{-2} s^{-1}) for 8 days.

4.2 *Arabidopsis* stable transformation

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

4.3 Pathogen assays and phenotypic analyses

Seven days after infection, *Arabidopsis* leaves with sporulating *H. arabidopsidis* isolate NoCo2 were harvested into 15 mL reaction tubes containing 10 mL dH_2O, and vortexed for 2 s. 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 (16 h 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 5 h at room temperature, followed by overnight clearing in saturated chloral hydrate (2.5 g/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. *Arabidopsis* 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 *Arabidopsis* plants as described previously (Kemmerling et al., 2007, Lin and Martin, 2005).

4.4 Observation of fluorescently labelled haustoria

For fluorescent analysis of haustoria morphology, leaves of *Arabidopsis* 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% aniline blue in 0.067 M 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 *Arabidopsis* (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 and emission at 535/30). 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).

4.5 Analysis of oomycete-associated callose deposition

Oomycete-associated callose deposition was analysed on cotyledons of *Arabidopsis* wild-type and HCSG mutants. Leaves were harvested at 4 dpi, cleared in 10M KOH for 5 min, stained with 0.05 % aniline blue in 0.067 M 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 and emission 465/30). 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.

4.6 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 and 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 and 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.

4.7 RNA Extraction and qRT-PCR analysis

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. qRT-PCR 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.

4.8 Gene structure and phylogenetic analyses

Analyses of gene structures and protein domain organization were performed using online databases TAIR (http://www.arabidopsis.org/) for *Arabidopsis*, and the KDRI website (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 *Arabidopsis* 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.

4.9 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" (R-Team, 2013) and the packages "Hmisc" (Harrell, 2014) "car" (Fox and Weisberg, 2011), "multcompView" (Graves et al., 2012) and "multcomp" (Hothorn et al., 2008). 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.

5. Supplemental tables

5.1 Constructs

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.

Table 6: 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)

Table 7: Plasmids for stable transformation of *Arabidopsis.*

Name	Description
pSEC13:SEC13 + free mCherry	LR reaction of pENTR-pSEC13:SEC13 and PMDC99 (Curtis and Grossniklaus, 2003); hygromycin resistance
pNUP133:NUP133	LR reaction of pENTR-pNUP133:NUP133 and pMDC99 (Curtis and Grossniklaus, 2003); 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

5.2 Oligonucleotides

Table 8: Expression analysis.

Target	Reference
PP2A	(Czechowski et al., 2005)
TIP41-like	(Czechowski et al., 2005)
FRK1	(Asai et al., 2002)
GST1	(Grant et al., 2000)
ERF1	(Solano et al., 1998)
PI-LTP	(Denoux et al., 2008)
PR1	(Onate-Sanchez et al., 2007)

Table 9: 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_RW	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

6. Supplemental files

Supplemental files 1 and 2 can be found in "**XV. APPENDIX**" on pages 135 - 150.

Supplemental File 1: Pruned alignment of kinase domain of LjSYMRK (aa 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].

Supplemental File 2: Pruned alignment of extracytoplasmic region of LjSYMRK (aa 30-517) and homologous stretches of related MLD-LRR-RLKs.

IX. GENERAL DISCUSSION

1. Overexpression of symbiotic receptors induces spontaneous symbioses-related signalling in the absence of external stimulation

In 2004, Esseling and colleagues reported a non-symbiotic root hair phenotype for *symrk* mutants of three legume species associated with an enhanced touch sensitivity of these mutants (Esseling et al., 2004). They could demonstrate that root hairs of a *symrk* mutant respond to NF treatment morphologically like the wild type as long as they are not subjected to mechanical stress (Esseling et al., 2004). This finding raised the question whether the absence of calcium spiking in *symrk* mutants is a pleiotropic effect of an enhanced touch responsiveness triggered by the injection of calcium sensitive dyes. For this reason, it has been a hypothesis for the last ten years that a main function of *SYMRK* is the desensitation to mechanical stimulation and experimental proof for *SYMRK* being actively involved in symbiosis signalling was still lacking. One main objective of this study was to clarify whether *SYMRK* plays an active signalling role in symbiosis. To tackle this question we made use of an observation from the mammalian field: Overabundance of specific receptors at the cell surface is linked with spontaneous or exaggerated activation of downstream signalling even in the absence of the respective ligand, a scenario that can finally results in cancer formation (Schlessinger, 2002, Shan et al., 2012, Wei et al., 2005).

In order to investigate whether this behaviour could also be observed for plant RLKs, which might provide a useful tool to elucidate the respective pathways RLKs are involved in, we analysed transgenic *L. japonicus* roots expressing *NFR1*, *NFR5* or *SYMRK* from the strong *L. japonicus Ubiquitin* promoter for spontaneous activation of symbiosis signalling. Intriguingly, overexpression of either of the three symbiotic RLKs spontaneously triggered the activation of RNS-related promoters, the expression of RNS-related genes, and the formation of root nodules in the absence of rhizobia or NF. This is in line with recent results from Saha and colleagues, who discovered that expression of the intracellular kinase domain of *SYMRK* (*SYMRK-KD*) from *Medicago truncatula* or *Arachis hypogaea* in *M. truncatula* roots from the CaMV 35S promoter induces nodule organogenesis in the absence of rhizobia (Saha et al., 2014). However, in contrast to overexpression of full-length *SYMRK* in *L. japonicus* roots, which results in normal nodule numbers, overexpression of the *SYMRK-KD* in *M. truncatula* roots resulted in hypernodulation, indicating that this *SYMRK* version circumvents the mechanisms of autoregulation of nodulation (Saha et al., 2014).

On the one hand, our discovery that overexpression of structurally diverse RLKs results in spontaneous initiation of specific downstream signalling offers a promising

approach to shed light on the roles of the many hundreds of orphan receptors residing in plant genomes for which we neither know the ligand nor the triggered pathway.

On the other hand, we could, for the first time, clearly demonstrate that *SYMRK* plays an active signalling role in RNS, while demoting the possibility that the symbiosis defects of *symrk* mutants are due to pleiotropic effects.

2. *SYMRK* acts independently of *NFR1* or *NFR5* and upstream of other common symbiosis genes involved in the generation or decoding of calcium spiking

To further investigate whether *SYMRK* acts at the same hierarchical level as *NFR1* and *NFR5* in the nodule organogenesis pathway, we expressed the dominant-active *SYMRK* allele in *nfr1* or *nfr5* mutants. For both mutant backgrounds, roots overexpressing *SYMRK* formed spontaneous nodules in the absence of rhizobia, indicating that *SYMRK* does not need the simultaneous presence of both *NFRs* to trigger nodulation. One explanation might be redundancy with other members of the LysM-RLK family of *L. japonicus* (Lohmann et al., 2010), as additional NFRs appear to be important at later developmental stages (Madsen et al., 2010). Another possibility is that the dominant-active *SYMRK* allele operates independently of the *NFRs*.

The CSGs encoding SYMRK, the ion channels CASTOR and POLLUX, or the nucleoporins NUP85, NUP133 and NENA have all been placed at the same hierarchical level as mutants in these genes share striking phenotypic characteristics: they are impaired in calcium spiking and bacterial infection is aborted at the epidermis (Groth et al., 2010, Kistner et al., 2005, Miwa et al., 2006). To position *SYMRK* in the genetic pathway relative to other CSGs, we performed epistasis analyses demonstrating that *SYMRK* acts upstream of other CSGs implicated in the generation or decoding of calcium spiking. These results confirm the assumed but unproven genetic position of *SYMRK* and support the idea that a main function of SYMRK in symbiosis is the activation of CCaMK, which consequently leads to the expression of symbiosis-related genes and nodule organogenesis (Hayashi et al., 2010, Madsen et al., 2010, Singh and Parniske, 2012, Gleason et al., 2006, Tirichine et al., 2006).

3. Dominant-active alleles of symbiotic receptors could pave the way for the engineering of nodulating non-leguminous crop species

The Haber-Bosch process is the predominant way of ammonium production worldwide, but it consumes a significant amount of fossil energy sources. Biological nitrogen fixation by bacteria is a sustainable alternative to the Haber-Bosch process, but unfortunately nitrogen-fixing root nodule symbiosis is restricted to plant species of the Eurosid I

subclade. However, since the oil crisis in the 1970s, research into biological nitrogen fixation aims at alleviating the dependence of food production on fossil energy sources and oil prices. During the last decade, a number of legume genes have been identified that are required for RNS including the CSGs that are shared between AM and RNS. One important characteristic of legume RNS is the formation of root nodules, in which rhizobia are accommodated and which provide a favourable environment for nitrogen fixation. Considering the functional conservation of a core symbiosis pathway in most angiosperms represented by the CSGs, and the important discovery that functional CSGs are present in important crops such as rice, our discovery that overexpression of symbiotic RLKs confers spontaneous induction of root nodule organogenesis could pave the way for biotechnological attempts to transfer nitrogen-fixing root nodules to important non-leguminous crop species.

Furthermore, the symbiotic RLKs constitute the entry point for symbiosis signalling and dominant-active alleles activate the entire nodulation pathway in the absence of rhizobia. This feature opens the possibility to further dissect and analyse the nodulation pathway uncoupled from bacterial infection.

4. Cleavage of the extracytoplasmic domain of SYMRK generates a receptor version that specifically interacts with NFR5 in *Nicotiana benthamiana*

Similar to RTKs, plant RLKs function in highly dynamic receptor complexes. To investigate whether the symbiotic RLKs also follow this pattern, we conducted targeted protein-protein interaction studies in *N. benthamiana* employing the full-length proteins and several deletion variants of SYMRK.

We have recently demonstrated that the extracytoplasmic domain of SYMRK undergoes constitutive proteolytic cleavage *in planta*, which results in the release of the MLD and gives rise to SYMRK-ΔMLD, a very unstable SYMRK variant that still contains the three LRRs and resembles the structure of the co-receptor BAK1 (Antolín-Llovera et al., 2014). To study the contribution of the SYMRK domains to its capacity to interact with the NFRs, we made use of a synthetic *SYMRK-ΔMLD* construct that mimics SYMRK after MLD release and included SYMRK-ΔED and SYMRK-ΔKD (SYMRK version that lacks the intracellular kinase domain) in the co-immunoenrichment experiments (Antolín-Llovera et al., 2014).

Both NFRs could be co-enriched with either full-length SYMRK or SYMRK-ΔED, but no interaction was observed with SYMRK-ΔKD, suggesting that the kinase domain of SYMRK contributes to the association with NFR1 and NFR5 (Antolín-Llovera et al., 2014).

Intriguingly, SYMRK-ΔMLD strongly and specifically interacted with NFR5, and co-expression of SYMRK, NFR5 and the Brassinolide receptor 1 (BRI1) resulted in a strong co-enrichment of the native SYMRK-ΔMLD variant upon immuno-purification of NFR5 that outcompeted SYMRK full-length for NFR5 interaction (Antolín-Llovera et al., 2014).

These results suggest that the presence of the MLD has an impeding effect on NFR5-SYMRK association which is released upon ectodomain cleavage. The fact that the association between NFR5 and SYMRK-ΔED was markedly weaker than between NFR5 and SYMRK-ΔMLD further implies that the LRRs contribute to this interaction. However, all SYMRK/NFR associations were observed in the absence of any symbiotic stimuli, calling for further research into whether the perception of NF influences these interactions in *L. japonicus* roots.

Interestingly, while SYMRK-ΔMLD is subject to high turnover, SYMRK-ΔED stability was comparable to that of full-length SYMRK, indicating that the altered accessibility of the LRRs in SYMRK-ΔMLD is important for rapid degradation of this SYMRK version. Taken together, the release of the MLD one the one hand promotes association with NFR5, but on the other hand results in SYMRK degradation. One explanation for this dual role of the MLD release would be that, after ectodomain cleavage, SYMRK functions as a co-receptor of NFR5 initiating symbiosis signalling and that the tight regulation of SYMRK-ΔMLD abundance is a mechanism to control the amount of active receptor complexes at the plasma membrane. Conversely, our results hint at the possibility that SYMRK-ΔMLD is not part of the active signalling complex but rather provides a degron. In this context, it will be interesting to investigate whether the specific association between SYMRK-ΔMLD and NFR5 plays a role in receptor clearance from the plasma membrane. The association between SYMRK versions and NFRs along with the regulation of SYMRK via cleavage of its extracytoplasmic domain are illustrated in Figure 38.

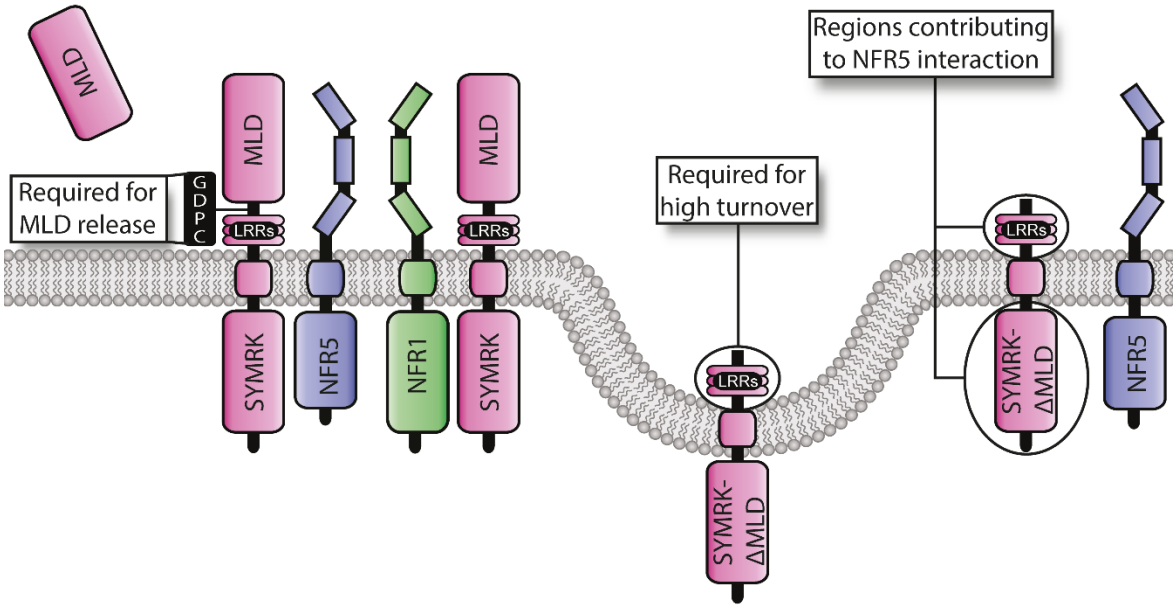


Figure 38: Interaction between SYMRK versions und NFRs and the regulation of SYMRK via cleavage of its extracytoplasmic region. (Figure and legend modified from Antolín-Llovera et al. 2014b, New Phytologist).

SYMRK contains an extracellular domain that is partitioned in two modules: three LRRs and a MLD. Both elements are connected via the conserved GDPC motif, which is required for the release of the MLD (Antolín-Llovera et al., 2014, Kosuta et al., 2011). The resulting membrane-bound SYMRK version containing the LRRs – referred to as SYMRK-ΔMLD – is subject to high turn-over, and the presence of the LRRs seems to destabilize the protein, as a SYMRK deletion construct that lacks the whole extracellular region appears to be more stable than SYMRK-ΔMLD (Antolín-Llovera et al., 2014). Moreover, SYMRK-ΔMLD has been found to strongly and specifically associate with NFR5 in *N. benthamiana* and it has been demonstrated that the kinase domain as well as the LRRs contribute to this association (Antolín-Llovera et al., 2014).

5. Heterocomplex formation as possible explanation for spontaneous induction of symbiosis-related signalling

To investigate whether SYMRK also associates with NFR1 and NFR5 in *L. japonicus* roots, we made use of our *NFR* constructs and performed co-immunoenrichment experiments on transgenic roots overexpressing either of the *NFRs*. We observed that upon overexpression of either NFR1 or NFR5 in *L. japonicus* roots we could co-enrich native full-length SYMRK in the absence of symbiotic challenge. Due to the fact that our SYMRK antibody does not recognize native SYMRK-ΔMLD, we were not able to study a putative NFR5 / SYMRK-ΔMLD interaction in *L. japonicus* roots. Still, it remains an open question whether NFR / SYMRK interaction is modulated by NF recognition at endogenous levels of gene expression. However, these results identify the LRR-MLD-RLK SYMRK as the founder member of a new class of potential co-receptors for LysM-RLKs which are represented by large gene families in all plant genomes.

Typically, RLK interaction is ligand-induced and the single most critical step in signal initiation (Chinchilla et al., 2007, Li et al., 2002, Liu et al., 2012, Nam and Li, 2002, Schulze et al., 2010, Sun et al., 2013a, Sun et al., 2013b). However, in the context of cancer formation it has been reported that overexpression of specific RTKs leads to receptor dimerization in the absence of a ligand, which results in ectopic cell proliferation (Schlessinger, 2002, Shan et al., 2012, Wei et al., 2005).

This scenario provides a plausible explanation for the SYMRK / NFR association in the absence of NF and the spontaneous activation of symbiosis signalling observed for NFR1, NFR5 and SYMRK: Overabundance of one of the symbiotic RLKs might lead to spontaneous receptor complex formation in the absence of ligands and subsequently initiate downstream signalling.

Based on our observation, we suggest a model in which NF recognition results in the formation of a ternary complex including NFR1, NFR5 and SYMRK. One role of this complex would be the activation of CCaMK via SYMRK, while a parallel infection pathway would be activated by the NFRs (Hayashi et al., 2010, Madsen et al., 2010).

6. Specificity towards AM and root nodule symbiosis is achieved at the level of the receptors

AM and RNS both rely on the presence of the CSGs, and it is a well-established model that this conserved genetic program for the intracellular accommodation of AM fungi has been co-opted during the more recent evolution of RNS. Our epistasis revealed that SYMRK acts upstream of all other tested CSGs and associates with NFR1 and NFR5, thus directly connecting the recognition of the microsymbiont at the plasma membrane via LysM-RLKs with the activation of the common symbiosis pathway. This is particularly interesting since *PaNFP*, a gene encoding a LysM-RLK closely related to LjNFR5 in the non-legume Celtidaceae *Parasponia andersonii,* is indispensable for AM as well as RNS, putting forward a model in which SYMRK acts as general docking site for the perception systems of AM fungi on the one hand and nitrogen-fixing bacteria on the other hand. Together with our results, this suggests that SYMRK can engage in different heterocomplexes featuring either NFRs or receptors for AM fungal (L)COs.

However, while this hypothesis provides a mechanism for how different signals at the plasma membrane are integrated into common symbiosis signalling, it does not answer the question how the plant cell consequently decides between the activation of the developmental pathways for AM or RNS. Several strategies of the plant to discriminate between the two symbioses have been suggested, including calcium-spiking signatures that differ for AM and RNS (Kosuta et al., 2008), or the existence of additional signalling

pathways that ensure the exclusive activation of the appropriate developmental responses (Takeda et al., 2011).

Intriguingly, SYMRK overexpression – similarly to the expression of the deregulated CCaMK$_{314}$ deletion mutant (Takeda et al., 2012) - resulted in the activation of AM as well as RNS signalling, a phenomenon that is not observed when the plant is inoculated with either AM fungi or rhizobia. However, overexpression of *NFR1* or *NFR5* – even though the spontaneous activation of symbiosis signalling is dependent on SYMRK - exclusively led to the activation of RNS-related genes. One the one hand, these results imply that signalling specificity is already achieved at the level of the receptors. On the other hand they show that overexpressed *SYMRK* can escape the proposed regulatory mechanisms that normally ensure the activation of only the appropriate pathway. This could be caused by an unbalanced stoichiometry of SYMRK and specific negative regulators or simultaneous association of SYMRK with the NFRs and receptors for AM fungal (L)COs, which consequently leads to the activation of AM and RNS signalling.

7. The *Arabidopsis* homologs of *SYMRK, ShRK1* and *ShRK2,* are putative compatibility factors for the accommodation of the oomycete *Hyaloperonospora arabidopsidis*

In the course of evolution, plants have come up with several strategies to defend themselves against microbial pathogens. One integral part of the plant innate immune system and the first layer of pathogen-induced resistance employs RLKs or RLPs that belong to the group of the so-called pattern recognition receptors (PRRs) (Antolín-Llovera et al., 2012). PRR reside at the plasma membrane, where they perceive pathogen-associated molecular patterns (PAMPs) such as the conserved bacterial flagellum protein flagellin, or plant-derived damage-associated molecular patterns (DAMPs), such as *Arabidopsis* Pep peptides that are produced upon pathogen attack (Antolín-Llovera et al., 2012). The recognition of PAMPs or DAMPs initiates PAMP-triggered immunity (PTI), which results in plant responses including the production of ROS, callose deposition, or the secretion of hydrolytic enzymes (van Schie and Takken, 2014). As a consequence, microbes have evolved effector proteins that undermine PTI. To counteract the effector-mediated suppression of PTI, plants, in turn, have utilised the so-called resistance genes (*R* genes) (Antolín-Llovera et al., 2012). *R* genes mainly code for intracellular resistance proteins that belong to the group of nucleotide-binding site-LRR (NBL-LRR) proteins and activate effector-triggered immunity (ETI), which constitutes a second layer of defence (Antolín-Llovera et al., 2012). ETI induces HR and other defence responses highly similar to PTI, suggesting a substantial overlap in the signalling pathways involved in PTI and ETI (van Schie and Takken, 2014). Effectors as well as *R* genes are subject to strong natural

selection. This evolutionary arms race between plants and pathogens is known as the zig-zag model (Jones and Dangl, 2006).

However, particularly for biotrophic pathogens that depend on living host cells for their own survival and reproduction, it is not enough to simply escape plant immunity. Rather, they have to engage in a compatible interaction with their respective host plant, which allows for their successful intracellular accommodation and the formation of specialised feeding structures for nutrient uptake. To accomplish this, pathogenic microbes exploit or manipulate the function of specific plant genes to promote their biotrophic life-style. Genes that serve this purpose are consequently named susceptibility genes (*S* genes) that encode compatibility factors (van Schie and Takken, 2014). *S* genes are involved in various processes in the host plant including metabolite biosynthesis, vesicle trafficking, cytoskeleton dynamics, or determining the composition of the cell wall. Van Schie and Takken (2014) assigned them to three different groups depending on their mechanism of pathogen support (van Schie and Takken, 2014).

The first group acts at the very early stages of plant-pathogen interaction and facilitates host recognition and penetration (van Schie and Takken, 2014). A famous member of this group, *Mildew resistance Locus O* (*MLO)*, was already discovered in 1942 by Freisleben and colleagues (Freisleben and Lein, 1942). Since its molecular identification (Büschges et al., 1997), MLO has been demonstrated to act as compatibility factor for powdery mildew infection in barley, *Arabidopsis*, pea, wheat, pepper, tomato, and strawberry (Bai et al., 2008, Consonni et al., 2006, Humphry et al., 2011, Jiwan et al., 2013, Pavan et al., 2011, van Schie and Takken, 2014, Varallyay et al., 2012, Zheng et al., 2013). In the *Arabidopsis*/*Golovinomyces orontii* interaction, *MLO* is required for the penetration of the epidermis cells and *mlo*-mediated resistance depends on actin polarization, vesicular trafficking and the suppression of programmed cell death (Consonni et al., 2006, Miklis et al., 2007). RAM2, another potential group one compatibility factor, is involved in the production of cutin monomers (Wang et al., 2012). Interestingly, *RAM2* is not only important for the formation of appressoria on *M. truncatula* during colonisation by the oomycetal pathogen *Phytophthora palmivora*, but it also plays a role for the development of AM fungal hyphopodia and arbuscules during AM symbiosis (Wang et al., 2012). However, it is unclear whether the changes in cutin compositions in the *ram2* mutant are the main cause for the reduced colonization by the oomycete and the AM fungus. Nevertheless, this observation implies that one compatibility factor can be exploited by beneficial symbionts and, likewise, by microbial pathogens.

The second group of *S* genes codes for proteins that negatively regulate plant immune responses (van Schie and Takken, 2014). One example is the callose synthase gene *Powdery*

Mildew Resistant 4 (*PMR4*), which is implicated in the susceptibility of *Arabidopsis* to powdery as well as downy mildew infection possibly by suppressing PTI (Jacobs et al., 2003, Nishimura et al., 2003). Interestingly, also overexpression of *PMR4* results in powdery mildew resistance in *Arabidopsis* (Ellinger et al., 2013). However, while the reduced susceptibility of the *pmr4* mutant is accompanied by elevated levels of defence gene transcripts and dependent on SA (Nishimura et al., 2003), the increased resistance upon PMR4 overexpression is independent from PTI suppression and can be explained by elevated callose deposition that blocks pathogen entry (Ellinger et al., 2013).

The last group of *S* genes comes into play after the initial plant-pathogen association has been established, and it is important for its maintenance and progression (van Schie and Takken, 2014). One interesting set of group three *S* genes encodes the plasma membrane localised sugar efflux transporters SWEET. SWEET11 and SWEET13 from rice have been shown to contribute to susceptibility to the bacterial blight *Xanthomonas oryzae* (Chen et al., 2010). They provide the pathogen with nutrients by exporting sugar from the cell into the apoplast, a mechanism that is most likely also utilised by several pathogens during *Arabidopsis* infection (Chen et al., 2010). *Downy Mildew Resistance 1* (*DMR1*) codes for a homoserine kinase, and *hsk* mutants accumulate homoserine (Van Damme et al., 2005). However, homoserine at the concentrations tested appears not to be directly toxic to the pathogen (Van Damme et al., 2005). This leaves open the possibilities that other metabolites that might be underrepresented or overabundant in the *DMR1* mutant are either required by or toxic to the downy mildew, or that perturbations of amino acid homeostasis in the host have an effect on biosynthesis pathways in the pathogen (van Schie and Takken, 2014). *DMR1*, together with several other genes involved in amino acid metabolism, contributes to the sustained compatibility between *Arabidopsis* and *H. arabidopsidis* (Van Damme et al., 2005, van Damme et al., 2009).

Arabidopsis, a member of the Brassicaceae, belongs to one out of five plant lineages that lost the ability to engage in AM (Delaux et al., 2013, Delaux et al., 2014). Even though this asymbiotic state is accompanied with the specific loss of CSGs from its genome, *Arabidopsis* retained certain HCSG, including *ShRK1* and *ShRK2*, two genes encoding MLD-LRR-RLKs closely related to the CSG product SYMRK. In the present study, we investigated whether these genes play a role in plant defence or susceptibility, particularly in the intracellular accommodation of the biotrophic oomycetal pathogen *H. arabidopsidis* and of the biotrophic fungal pathogen *Erysiphe cruciferarum*, or in the interaction with the extracellular bacterial pathogen *Pseudomonas syringae* pv. tomato *DC3000*.

While the number of *Hpa* sporangiophores per cotyledon – a direct measure for reproductive success of the oomycete – was significantly decreased in *shrk1*, *shrk2* and a

shrk1 x *shrk2* double mutant compared to the wild type, we could not detect any constitutive or increased pathogen-induced defence responses, ruling out one possible cause for the reduced sporangiophore count.

In analogy to AM fungal arbuscules, the oomycetal haustoria are believed to constitute the site of nutrient exchange between the microorganism and its host plant (Mendgen and Hahn, 2002). We investigated whether the reduced reproductive success was linked to changes in haustorial development, however, the overall number of cells containing haustoria per hyphal strand was unaltered in the *shrk* mutants. Intriguingly, the amount of multilobed haustoria in the *shrk* mutants was significantly higher than in the wild type. A time course experiment comparing the haustorial morphology in the *shrk1* x *shrk2* double mutant and in the wild type revealed that in both backgrounds the number of multilobed haustoria increased over time. Yet, at all tested time points, the number of altered haustoria in the double mutant was significantly higher than in the wild type. These results suggest that altered haustorial morphology is connected with the aging of the haustoria, a process that is seemingly accelerated in the HCSG mutants. It is therefore tempting to speculate that early senescence of haustoria might be directly associated with lower nutrient availability for the oomycete resulting in a reduced amount of sporangiophores. Furthermore, we could not observe signs of early haustorial senescence in the disease resistant *pskr1* mutant, which exhibits constitutive defence responses (Mosher et al., 2013), pointing towards the HCSGs playing a distinct role in the intracellular accommodation of *H. arabidopsidis*. The hypothesis that the haustoria phenotype obtained for the *shrk* mutants is specific to the interaction with biotrophic pathogens that form intracellular accommodation structures, was also corroborated by the finding that the colonization by the extracellular bacterial pathogen *P. syringae* was unaltered in the *shrk* mutants. In addition, this provides evidence that the activation of SA- and JA-dependent defense responses is not deregulated in the *shrk* mutants.

To investigate whether the HCSGs are specifically involved in the intracellular accommodation of *H. arabidopsidis*, or are part of a general pathway for the development of intracellular accommodation structures in plant-pathogen interactions, we infected the *shrk* mutants with the haustoria-forming fungal pathogen *E. cruciferarum*. We could not detect any changes in the reproductive success of *E. cruciferarum* in the HCSG mutants. The morphology of the fungal haustoria is highly variable, which impeded the analysis of differences in haustorial development in the *shrk* mutants compared to the wild type. We concluded that the tested HCSGs are not involved in the interaction with *E. cruciferarum*. One explanation for this could be the different infection styles of *H. arabidopsidis* and *E. cruciferarum*: while *H. arabidopsidis* infects the epidermal cells and then progresses to the

mesophyll (Slusarenko and Schlaich, 2003), *E. cruciferarum* only penetrates cells in the epidermis (Micali et al., 2008). This leaves open the possibility of the existence of two different infection pathways, one in the epidermal cells and one in the mesophyll cells with the tested HCSGs only being important for the latter. This is a scenario reminiscent of the *MLO* gene, which is expressed in the epidermis and important for the penetration of *Arabidopsis* epidermal cells by *E. cruciferarum*, but which has not been implicated in *H. arabidopsidis* infection (Consonni et al., 2006). With at least 42 *ShRKs* in *Arabidopsis* (Hok et al., 2011), there is also the possibility of the existence of an epidermis *ShRK*, like *SYMRK* in *L. japonicus*. Another possibility is that the HCSGs are not at all involved in the intracellular accommodation of fungal pathogens, and it will be interesting to investigate whether the *ShRKs* play a role in other intracellular oomycete-plant associations or are exclusively exploited by *H. arabidopsidis*.

As a whole, our results clearly demonstrate that *shrk1*, *shrk2* and the *shrk1* x *shrk2* double mutant are less susceptible to infection with *H. arabidopsidis* and constitute important genetic components for proper intracellular accommodation and haustorial development. The effects on the reproductive success of *H. arabidopsidis* and the development of haustoria were not linked to deregulated activation of the plant immune system, which renders these symbiosis-related genes likely candidates for compatibility factors in the interaction with *H. arabidopsidis*.

Another symbiosis-related gene, *RAM2*, has already been described to play a dual role in plant microbe interactions with beneficial as well as pathogenic microorganisms (Wang et al., 2012). However, in the *ram2* mutant, the interaction with the oomycete *P. palmivora* was already impaired at the stage of appressoria formation (Wang et al., 2012). As the *ShRKs* are not important for the initial penetration of *Arabidopsis* leaf cells and rather seem to be crucial for the maintenance of compatibility in the *Hpa*/*Arabidopsis* association, they fall into group three *S* genes. Thus, in contrast to Wang and colleagues, we were able to demonstrate genetic commonalities of symbiosis and disease in the formation and maintenance of intracellular accommodation structures at a later developmental stage of the plant-microbe association.

Interestingly, the MLD-LRR-RLK gene *IOS1* also contributes to *Hpa* resistance in *Arabidopsis* (Hok et al., 2011) and very likely encodes another RLK-type compatibility factor. IOS1, ShRK1, ShRK2 and SYMRK share striking structural similarities. All four MLD-LRR-RLKs carry the conserved GDPC motif, which is important for ectodomain cleavage in SYMRK, in their extracytoplasmic domains (Antolín-Llovera et al., 2014, Hok et al., 2011, Kosuta et al., 2011). Impaired ectodomain cleavage in the *symrk-14* mutant compromises symbiotic development in the epidermis of *Lotus japonicus* roots during RNS

and AM (Antolín-Llovera et al., 2014, Kosuta et al., 2011). It will be an interesting question for future research whether ectodomain release is a general feature of MLD-LRR-RLKs, including the *Arabidopsis* RLKs *IOS1* and the *ShRKs*. If this hypothesis holds true, *Arabidopsis* provides a model plant to study the role of ectodomain cleavage for MLD-LRR-RLK function in plant-microbe interactions.

X. REFERENCES

Akiyama K, Matsuzaki K & Hayashi H. 2005. Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. *Nature* **435**:824-7. doi: 10.1038/nature03608.

Alber F, Dokudovskaya S, Veenhoff LM, Zhang W, Kipper J, Devos D, Suprapto A, Karni-Schmidt O, Williams R, Chait BT, Sali A & Rout MP. 2007. The molecular architecture of the nuclear pore complex. *Nature* **450**:695-701. doi: 10.1038/nature06405.

Antolín-Llovera M, Ried MK, Binder A & Parniske M. 2012. Receptor kinase signaling pathways in plant-microbe interactions. *Annu Rev Phytopathol* **50**:451-73. doi: 10.1146/annurev-phyto-081211-173002.

Antolín-Llovera M, Ried MK & Parniske M. 2014. Cleavage of the SYMBIOSIS RECEPTOR-LIKE KINASE ectodomain promotes complex formation with Nod Factor Receptor 5. *Curr Biol* **24**:422-7. doi: 10.1016/j.cub.2013.12.053.

Asai T, Tena G, Plotnikova J, Willmann MR, Chiu WL, Gomez-Gomez L, Boller T, Ausubel FM & Sheen J. 2002. MAP kinase signalling cascade in *Arabidopsis* innate immunity. *Nature* **415**:977-83. doi: 10.1038/415977a.

Bai Y, Pavan S, Zheng Z, Zappel NF, Reinstadler A, Lotti C, De Giovanni C, Ricciardi L, Lindhout P, Visser R, Theres K & Panstruga R. 2008. Naturally occurring broad-spectrum powdery mildew resistance in a Central American tomato accession is caused by loss of *mlo* function. *Mol Plant Microbe Interact* **21**:30-9. doi: 10.1094/MPMI-21-1-0030.

Bapteste E, Charlebois RL, Macleod D & Brochier C. 2005. The two tempos of nuclear pore complex evolution: highly adapting proteins in an ancient frozen structure. *Genome Biol* **6**:R85. doi: 10.1186/gb-2005-6-10-r85.

Bateman A & Bycroft M. 2000. The structure of a LysM domain from *E. coli* membrane-bound lytic murein transglycosylase D (MltD). *J Mol Biol* **299**:1113-9. doi: 10.1006/jmbi.2000.3778.

Bayle V, Nussaume L & Bhat RA. 2008. Combination of novel green fluorescent protein mutant TSapphire and DsRed variant mOrange to set up a versatile *in planta* FRET-FLIM assay. *Plant Physiol* **148**:51-60. doi: 10.1104/pp.108.117358.

Bensmihen S, De Billy F & Gough C. 2011. Contribution of NFP LysM domains to the recognition of Nod factors during the *Medicago truncatula*/*Sinorhizobium meliloti* symbiosis. *PLoS One* **6**:e26114. doi: 10.1371/journal.pone.0026114.

Besserer A, Puech-Pages V, Kiefer P, Gomez-Roldan V, Jauneau A, Roy S, Portais JC, Roux C, Becard G & Sejalon-Delmas N. 2006. Strigolactones stimulate arbuscular mycorrhizal fungi by activating mitochondria. *PLoS Biol* **4**:e226. doi: 10.1371/journal.pbio.0040226.

Bielnicki J, Devedjiev Y, Derewenda U, Dauter Z, Joachimiak A & Derewenda ZS. 2006. *B. subtilis* ykuD protein at 2.0 A resolution: insights into the structure and function of a novel, ubiquitous family of bacterial enzymes. *Proteins* **62**:144-51. doi: 10.1002/prot.20702.

Binder A, Lambert J, Morbitzer R, Popp C, Ott T, Lahaye T & Parniske M. 2014. A modular plasmid assembly kit for multigene expression, gene silencing and silencing rescue in plants. *PLoS One* **9**:e88218. doi: 10.1371/journal.pone.0088218.

Binder A & Parniske M. 2013. Analysis of the *Lotus japonicus* nuclear pore NUP107-160 subcomplex reveals pronounced structural plasticity and functional redundancy. *Front Plant Sci* **4**:552. doi: 10.3389/fpls.2013.00552.

Blancaflor EB, Zhao L & Harrison MJ. 2001. Microtubule organization in root cells of *Medicago truncatula* during development of an arbuscular mycorrhizal symbiosis with *Glomus versiforme*. *Protoplasma* **217**:154-65. doi: 10.1007/BF01283396

Boisson-Dernier A, Kessler SA & Grossniklaus U. 2011. The walls have ears: the role of plant CrRLK1Ls in sensing and transducing extracellular signals. *J Exp Bot* **62**:1581-91. doi: 10.1093/jxb/erq445.

Bolle C, Huep G, Kleinbolting N, Haberer G, Mayer K, Leister D & Weisshaar B. 2013. GABI-DUPLO: a collection of double mutants to overcome genetic redundancy in *Arabidopsis thaliana*. *Plant J* **75**:157-71. doi: 10.1111/tpj.12197.

Bouwmeester HJ, Matusova R, Zhongkui S & Beale MH. 2003. Secondary metabolite signalling in host-parasitic plant interactions. *Curr Opin Plant Biol* **6**:358-64. doi: 10.1016/S1369-5266(03)00065-7.

Bowling SA, Clarke JD, Liu Y, Klessig DF & Dong X. 1997. The *cpr5* mutant of *Arabidopsis* expresses both *NPR1*-dependent and *NPR1*-independent resistance. *Plant Cell* **9**:1573-84. doi: 10.1105/tpc.9.9.1573.

Bozkurt TO, Richardson A, Dagdas YF, Mongrand S, Kamoun S & Raffaele S. 2014. The plant membrane-associated REMORIN1.3 accumulates in discrete perihaustorial domains and enhances susceptibility to *Phytophthora infestans*. *Plant Physiol* **165**:1005-1018. doi: 10.1104/pp.114.235804.

Broghammer A, Krusell L, Blaise M, Sauer J, Sullivan JT, Maolanon N, Vinther M, Lorentzen A, Madsen EB, Jensen KJ, Roepstorff P, Thirup S, Ronson CW, Thygesen MB & Stougaard J. 2012. Legume receptors perceive the rhizobial lipochitin oligosaccharide signal molecules by direct binding. *Proc Natl Acad Sci U S A* **109**:13859-64. doi: 10.1073/pnas.1205171109.

Buist G, Steen A, Kok J & Kuipers OP. 2008. LysM, a widely distributed protein motif for binding to (peptido)glycans. *Mol Microbiol* **68**:838-47. doi: 10.1111/j.1365-2958.2008.06211.x.

Büschges R, Hollricher K, Panstruga R, Simons G, Wolter M, Frijters A, Van Daelen R, Van Der Lee T, Diergaarde P, Groenendijk J, Topsch S, Vos P, Salamini F & Schulze-Lefert P. 1997. The barley *Mlo* gene: a novel control element of plant pathogen resistance. *Cell* **88**:695-705. doi: 10.1016/S0092-8674(00)81912-1.

Capoen W, Sun J, Wysham D, Otegui MS, Venkateshwaran M, Hirsch S, Miwa H, Downie JA, Morris RJ, Ane JM & Oldroyd GE. 2011. Nuclear membranes control symbiotic calcium signaling of legumes. *Proc Natl Acad Sci USA* **108**:14348-53. doi: 10.1073/pnas.1107912108.

Chabaud M, Genre A, Sieberer BJ, Faccio A, Fournier J, Novero M, Barker DG & Bonfante P. 2011. Arbuscular mycorrhizal hyphopodia and germinated spore exudates trigger Ca2+ spiking in the legume and nonlegume root epidermis. *New Phytol* **189**:347-55. doi: 10.1111/j.1469-8137.2010.03464.x.

Chandran D, Inada N, Hather G, Kleindt CK & Wildermuth MC. 2010. Laser microdissection of *Arabidopsis* cells at the powdery mildew infection site reveals site-specific processes and regulators. *Proc Natl Acad Sci U S A* **107**:460-5. doi: 10.1073/pnas.0912492107.

Charpentier M, Bredemeier R, Wanner G, Takeda N, Schleiff E & Parniske M. 2008. *Lotus japonicus* CASTOR and POLLUX are ion channels essential for perinuclear calcium spiking in legume root endosymbiosis. *Plant Cell* **20**:3467-79. doi: 10.1105/tpc.108.063255.

Chen CW, Panzeri D, Yeh YH, Kadota Y, Huang PY, Tao CN, Roux M, Chien SC, Chin TC, Chu PW, Zipfel C & Zimmerli L. 2014. The *Arabidopsis* malectin-like leucine-rich repeat receptor-like kinase IOS1 associates with the pattern recognition receptors FLS2 and EFR and is critical for priming of pattern-triggered immunity. *Plant Cell* **26**:3201-19. doi: 10.1105/tpc.114.125682.

Chen LQ, Hou BH, Lalonde S, Takanaga H, Hartung ML, Qu XQ, Guo WJ, Kim JG, Underwood W, Chaudhuri B, Chermak D, Antony G, White FF, Somerville SC, Mudgett MB & Frommer WB. 2010. Sugar transporters for intercellular exchange and nutrition of pathogens. *Nature* **468**:527-32. doi: 10.1038/nature09606.

Chen T, Zhu H, Ke D, Cai K, Wang C, Gou H, Hong Z & Zhang Z. 2012. A MAP kinase kinase interacts with SymRK and regulates nodule organogenesis in *Lotus japonicus*. *Plant Cell* **24**:823-38. doi: 10.1105/tpc.112.095984.

Chinchilla D, Zipfel C, Robatzek S, Kemmerling B, Nürnberger T, Jones JD, Felix G & Boller T. 2007. A flagellin-induced complex of the receptor FLS2 and BAK1 initiates plant defence. *Nature* **448**:497-500. doi: 10.1038/nature05999.

Clough SJ & Bent AF. 1998. Floral dip: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. *Plant J* **16**:735-43. doi: 10.1385/1-59259-827-7:091.

Consonni C, Humphry ME, Hartmann HA, Livaja M, Durner J, Westphal L, Vogel J, Lipka V, Kemmerling B, Schulze-Lefert P, Somerville SC & Panstruga R. 2006. Conserved requirement for a plant host cell protein in powdery mildew pathogenesis. *Nat Genet* **38**:716-20. doi: 10.1038/ng1806.

Cooper JE. 2007. Early interactions between legumes and rhizobia: disclosing complexity in a molecular dialogue. *J Appl Microbiol* **103**:1355-65. doi: 10.1111/j.1365-2672.2007.03366.x.

Curtis MD & Grossniklaus U. 2003. A gateway cloning vector set for high-throughput functional analysis of genes *in planta*. *Plant Physiol* **133**:462-9. doi: 10.1104/pp.103.027979.

Czaja LF, Hogekamp C, Lamm P, Maillet F, Martinez EA, Samain E, Denarie J, Kuster H & Hohnjec N. 2012. Transcriptional responses toward diffusible signals from symbiotic microbes reveal *MtNFP*- and *MtDMI3*-dependent reprogramming of host gene expression by arbuscular mycorrhizal fungal lipochitooligosaccharides. *Plant Physiol* **159**:1671-85. doi: 10.1104/pp.112.195990.

Czechowski T, Stitt M, Altmann T, Udvardi MK & Scheible WR. 2005. Genome-wide identification and testing of superior reference genes for transcript normalization in *Arabidopsis*. *Plant Physiol* **139**:5-17. doi: 10.1104/pp.105.063743.

Dazzo FB, Truchet GL, Sherwood JE, Hrabak EM, Abe M & Pankratz SH. 1984. Specific phases of root hair attachment in the *Rhizobium trifolii*-clover symbiosis. *Appl Environ Microbiol* **48**:1140-50. doi.

De Mendiburu F 2014. agricolae: Statistical Procedures for Agricultural Research.

De Mita S, Streng A, Bisseling T & Geurts R. 2014. Evolution of a symbiotic receptor through gene duplications in the legume-rhizobium mutualism. *New Phytol* **201**:961-72. doi: 10.1111/nph.12549.

Delaux PM, Sejalon-Delmas N, Becard G & Ane JM. 2013. Evolution of the plant-microbe symbiotic 'toolkit'. *Trends Plant Sci* **18**:298-304. doi: 10.1016/j.tplants.2013.01.008.

Delaux PM, Varala K, Edger PP, Coruzzi GM, Pires JC & Ane JM. 2014. Comparative phylogenomics uncovers the impact of symbiotic associations on host genome evolution. *PLoS Genet* **10**:e1004487. doi: 10.1371/journal.pgen.1004487.

Den Herder G, Yoshida S, Antolín-Llovera M, Ried MK & Parniske M. 2012. *Lotus japonicus* E3 ligase SEVEN IN ABSENTIA4 destabilizes the symbiosis receptor-like kinase SYMRK and negatively regulates rhizobial infection. *Plant Cell* **24**:1691-707. doi: 10.1105/tpc.110.082248.

Denoux C, Galletti R, Mammarella N, Gopalan S, Werck D, De Lorenzo G, Ferrari S, Ausubel FM & Dewdney J. 2008. Activation of defense response pathways by OGs and Flg22 elicitors in *Arabidopsis* seedlings. *Mol Plant* **1**:423-45. doi: 10.1093/mp/ssn019.

Downie JA. 2014. Legume nodulation. *Curr Biol* **24**:R184-90. doi: 10.1016/j.cub.2014.01.028.

Downie JA & Walker SA. 1999. Plant responses to nodulation factors. *Curr Opin Plant Biol* **2**:483-9. doi: doi:10.1016/S1369-5266(99)00018-7.

Doyle JJ. 2011. Phylogenetic perspectives on the origins of nodulation. *Mol Plant Microbe Interact* **24**:1289-95. doi: 10.1094/MPMI-05-11-0114.

Ehrhardt DW, Wais R & Long SR. 1996. Calcium spiking in plant root hairs responding to Rhizobium nodulation signals. *Cell* **85**:673-81. doi: 10.1016/S0092-8674(00)81234-9.

Ellinger D, Naumann M, Falter C, Zwikowics C, Jamrow T, Manisseri C, Somerville SC & Voigt CA. 2013. Elevated early callose deposition results in complete penetration resistance to powdery mildew in *Arabidopsis*. *Plant Physiol* **161**:1433-44. doi: 10.1104/pp.112.211011.

Esseling JJ, Lhuissier FG & Emons AM. 2003. Nod factor-induced root hair curling: continuous polar growth towards the point of nod factor application. *Plant Physiol* **132**:1982-8. doi: 10.1104/pp.103.021634.

Esseling JJ, Lhuissier FG & Emons AM. 2004. A nonsymbiotic root hair tip growth phenotype in *NORK*-mutated legumes: implications for nodulation factor-induced signaling and

formation of a multifaceted root hair pocket for bacteria. *Plant Cell* **16**:933-44. doi: 10.1105/tpc.019653.

Evangelisti E, Rey T & Schornack S. 2014. Cross-interference of plant development and plant-microbe interactions. *Curr Opin Plant Biol* **20C**:118-126. doi: 10.1016/j.pbi.2014.05.014.

Farmer EE, Almeras E & Krishnamurthy V. 2003. Jasmonates and related oxylipins in plant responses to pathogenesis and herbivory. *Curr Opin Plant Biol* **6**:372-8. doi: 10.1016/S1369-5266(03)00045-1.

Fisher RF & Long SR. 1993. Interactions of NodD at the *nod* Box: NodD binds to two distinct sites on the same face of the helix and induces a bend in the DNA. *J Mol Biol* **233**:336-48. doi: 10.1006/jmbi.1993.1515.

Fitter AH. 2005. Darkness visible: reflections on underground ecology. *Journal of Ecology* **93**:231-243. doi: 10.1111/j.0022-0477.2005.00990.x.

Fox J & Weisberg S 2011. An {R} Companion to Applied Regression. Thousand Oaks {CA}: Sage.

Freisleben R & Lein A. 1942. Über die Auffindung einer mehltauresistenten Mutante nach Röntgenbestrahlung einer anfälligen reinen Linie von Sommergerste. *Naturwissenschaften* **30**:608-608. doi: 10.1007/BF01488231.

Garvey KJ, Saedi MS & Ito J. 1986. Nucleotide sequence of *Bacillus* phage *phi 29* genes 14 and 15: homology of gene 15 with other phage lysozymes. *Nucleic Acids Res* **14**:10001-8. doi: 10.1093/nar/14.24.10001.

Genre A, Chabaud M, Balzergue C, Puech-Pages V, Novero M, Rey T, Fournier J, Rochange S, Becard G, Bonfante P & Barker DG. 2013. Short-chain chitin oligomers from arbuscular mycorrhizal fungi trigger nuclear Ca^{2+} spiking in *Medicago truncatula* roots and their production is enhanced by strigolactone. *New Phytol.* **198**:190-202. doi: 10.1111/nph.12146.

Genre A, Chabaud M, Faccio A, Barker DG & Bonfante P. 2008. Prepenetration apparatus assembly precedes and predicts the colonization patterns of arbuscular mycorrhizal fungi within the root cortex of both *Medicago truncatula* and *Daucus carota*. *Plant Cell* **20**:1407-20. doi: 10.1105/tpc.108.059014.

Genre A, Chabaud M, Timmers T, Bonfante P & Barker DG. 2005. Arbuscular mycorrhizal fungi elicit a novel intracellular apparatus in *Medicago truncatula* root epidermal cells before infection. *Plant Cell* **17**:3489-99. doi: 10.1105/tpc.105.035410.

Gherbi H, Markmann K, Svistoonoff S, Estevan J, Autran D, Giczey G, Auguy F, Peret B, Laplaze L, Franche C, Parniske M & Bogusz D. 2008. *SymRK* defines a common genetic basis for plant root endosymbioses with arbuscular mycorrhiza fungi, rhizobia, and *Frankia* bacteria. *Proc Natl Acad Sci U S A* **105**:4928-32. doi: 10.1073/pnas.0710618105.

Giraud E, Moulin L, Vallenet D, Barbe V, Cytryn E, Avarre JC, Jaubert M, Simon D, Cartieaux F, Prin Y, Bena G, Hannibal L, Fardoux J, Kojadinovic M, Vuillet L, Lajus A, Cruveiller S, Rouy Z, Mangenot S, Segurens B, Dossat C, Franck WL, Chang WS, Saunders E, Bruce D, Richardson P, Normand P, Dreyfus B, Pignol D, Stacey G, Emerich D, Vermeglio A, Medigue C & Sadowsky M. 2007. Legumes symbioses: absence of *Nod* genes in photosynthetic bradyrhizobia. *Science* **316**:1307-12. doi: 10.1126/science.1139548.

Glazebrook J. 2005. Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. *Annu Rev Phytopathol* **43**:205-27. doi: 10.1146/annurev.phyto.43.040204.135923.

Glazebrook J, Rogers EE & Ausubel FM. 1996. Isolation of *Arabidopsis* mutants with enhanced disease susceptibility by direct screening. *Genetics* **143**:973-82. doi.

Gleason C, Chaudhuri S, Yang T, Munoz A, Poovaiah BW & Oldroyd GE. 2006. Nodulation independent of rhizobia induced by a calcium-activated kinase lacking autoinhibition. *Nature* **441**:1149-52. doi: 10.1038/nature04812.

Gomez-Gomez L & Boller T. 2000. FLS2: an LRR receptor-like kinase involved in the perception of the bacterial elicitor flagellin in *Arabidopsis*. *Mol Cell* **5**:1003-11. doi: 10.1016/S1097-2765(00)80265-8.

Gomez-Roldan V, Fermas S, Brewer PB, Puech-Pages V, Dun EA, Pillot JP, Letisse F, Matusova R, Danoun S, Portais JC, Bouwmeester H, Becard G, Beveridge CA, Rameau C & Rochange

SF. 2008. Strigolactone inhibition of shoot branching. *Nature* **455**:189-94. doi: 10.1038/nature07271.

Gonzalez-Aguilera C & Askjaer P. 2012. Dissecting the NUP107 complex: multiple components and even more functions. *Nucleus* **3**:340-8. doi: 10.4161/nucl.21135.

Gossmann JA, Markmann K, Brachmann A, Rose LE & Parniske M. 2012. Polymorphic infection and organogenesis patterns induced by a *Rhizobium leguminosarum* isolate from *Lotus* root nodules are determined by the host genotype. *New Phytol* **196**:561-73. doi: 10.1111/j.1469-8137.2012.04281.x.

Grant JJ, Yun BW & Loake GJ. 2000. Oxidative burst and cognate redox signalling reported by luciferase imaging: identification of a signal network that functions independently of ethylene, SA and Me-JA but is dependent on MAPKK activity. *Plant J* **24**:569-82. doi: 10.1046/j.1365-313x.2000.00902.x.

Graves S, Piepho HP, Selzer L & With Help from Dorai-Raj S 2012. multcompView: Visualizations of paired comparisons.

Groth M, Kosuta S, Gutjahr C, Haage K, Hardel SL, Schaub M, Brachmann A, Sato S, Tabata S, Findlay K, Wang TL & Parniske M. 2013. Two *Lotus japonicus* symbiosis mutants impaired at distinct steps of arbuscule development. *Plant J* **75**:117-29. doi: 10.1111/tpj.12220.

Groth M, Takeda N, Perry J, Uchida H, Draxl S, Brachmann A, Sato S, Tabata S, Kawaguchi M, Wang TL & Parniske M. 2010. *NENA*, a *Lotus japonicus* homolog of *Sec13*, is required for rhizodermal infection by arbuscular mycorrhiza fungi and rhizobia but dispensable for cortical endosymbiotic development. *Plant Cell* **22**:2509-26. doi: 10.1105/tpc.109.069807.

Gutjahr C. 2014. Phytohormone signaling in arbuscular mycorhiza development. *Curr Opin Plant Biol* **20**:26-34. doi: 10.1016/j.pbi.2014.04.003.

Gutjahr C, Banba M, Croset V, An K, Miyao A, An G, Hirochika H, Imaizumi-Anraku H & Paszkowski U. 2008. Arbuscular mycorrhiza-specific signaling in rice transcends the common symbiosis signaling pathway. *Plant Cell* **20**:2989-3005. doi: 10.1105/tpc.108.062414.

Gutjahr C, Novero M, Guether M, Montanari O, Udvardi M & Bonfante P. 2009. Presymbiotic factors released by the arbuscular mycorrhizal fungus *Gigaspora margarita* induce starch accumulation in *Lotus japonicus* roots. *New Phytol* **183**:53-61. doi: 10.1111/j.1469-8137.2009.02871.x.

Gutjahr C & Parniske M. 2013. Cell and developmental biology of arbuscular mycorrhiza symbiosis. *Annu Rev Cell Dev Biol* **29**:593-617. doi: 10.1146/annurev-cellbio-101512-122413.

Hamel LP & Beaudoin N. 2010. Chitooligosaccharide sensing and downstream signaling: contrasted outcomes in pathogenic and beneficial plant-microbe interactions. *Planta* **232**:787-806. doi: 10.1007/s00425-010-1215-9.

Harrell FE 2014. Hmisc: Harrell Miscellaneous.

Hayafune M, Berisio R, Marchetti R, Silipo A, Kayama M, Desaki Y, Arima S, Squeglia F, Ruggiero A, Tokuyasu K, Molinaro A, Kaku H & Shibuya N. 2014. Chitin-induced activation of immune signaling by the rice receptor CEBiP relies on a unique sandwich-type dimerization. *Proc Natl Acad Sci U S A* **111**:E404-13. doi: 10.1073/pnas.1312099111.

Hayashi T, Banba M, Shimoda Y, Kouchi H, Hayashi M & Imaizumi-Anraku H. 2010. A dominant function of CCaMK in intracellular accommodation of bacterial and fungal endosymbionts. *Plant J* **63**:141-54. doi: 10.1111/j.1365-313X.2010.04228.x.

Hayashi T, Shimoda Y, Sato S, Tabata S, Imaizumi-Anraku H & Hayashi M. 2014. Rhizobial infection does not require cortical expression of upstream common symbiosis genes responsible for the induction of Ca^{2+} spiking. *Plant J* **77**:146-59. doi: 10.1111/tpj.12374.

Hoelz A, Debler EW & Blobel G. 2011. The structure of the nuclear pore complex. *Annu Rev Biochem* **80**:613-43. doi: 10.1146/annurev-biochem-060109-151030.

Hohnjec N, Vieweg MF, Puhler A, Becker A & Kuster H. 2005. Overlaps in the transcriptional profiles of *Medicago truncatula* roots inoculated with two different *Glomus* fungi provide insights into the genetic program activated during arbuscular mycorrhiza. *Plant Physiol* **137**:1283-301. doi: 10.1104/pp.104.056572.

Hok S, Danchin EG, Allasia V, Panabieres F, Attard A & Keller H. 2011. An *Arabidopsis* (malectin-like) leucine-rich repeat receptor-like kinase contributes to downy mildew disease. *Plant Cell Environ* **34**:1944-57. doi: 10.1111/j.1365-3040.2011.02390.x.

Horvath B, Heidstra R, Lados M, Moerman M, Spaink HP, Prome JC, Van Kammen A & Bisseling T. 2002. Lipo-oligosaccharides of *Rhizobium* induce infection-related early *nodulin* gene expression in pea root hairs. *Plant J* **4**:727-33. doi: 10.1046/j.1365-313X.1993.04040727.x.

Horvath B, Yeun LH, Domonkos A, Halasz G, Gobbato E, Ayaydin F, Miro K, Hirsch S, Sun J, Tadege M, Ratet P, Mysore KS, Ane JM, Oldroyd GE & Kalo P. 2011. *Medicago truncatula IPD3* is a member of the common symbiotic signaling pathway required for rhizobial and mycorrhizal symbioses. *Mol Plant Microbe Interact* **24**:1345-58. doi: 10.1094/MPMI-01-11-0015.

Hothorn T, Bretz F & Westfall P. 2008. Simultaneous inference in general parametric models. *Biometrical Journal* **50**:346-363. doi: 10.1002/bimj.200810425.

Huckelhoven R & Panstruga R. 2011. Cell biology of the plant-powdery mildew interaction. *Curr Opin Plant Biol* **14**:738-46. doi: 10.1016/j.pbi.2011.08.002.

Humphry M, Reinstadler A, Ivanov S, Bisseling T & Panstruga R. 2011. Durable broad-spectrum powdery mildew resistance in pea *er1* plants is conferred by natural loss-of-function mutations in *PsMLO1*. *Mol Plant Pathol* **12**:866-78. doi: 10.1111/j.1364-3703.2011.00718.x.

Iizasa E, Mitsutomi M & Nagano Y. 2010. Direct binding of a plant LysM receptor-like kinase, LysM RLK1/CERK1, to chitin *in vitro*. *J Biol Chem* **285**:2996-3004. doi: 10.1074/jbc.M109.027540.

Imaizumi-Anraku H, Takeda N, Charpentier M, Perry J, Miwa H, Umehara Y, Kouchi H, Murakami Y, Mulder L, Vickers K, Pike J, Downie JA, Wang T, Sato S, Asamizu E, Tabata S, Yoshikawa M, Murooka Y, Wu GJ, Kawaguchi M, Kawasaki S, Parniske M & Hayashi M. 2005. Plastid proteins crucial for symbiotic fungal and bacterial entry into plant roots. *Nature* **433**:527-31. doi: 10.1038/nature03237.

Ito Y, Kaku H & Shibuya N. 1997. Identification of a high-affinity binding protein for N-acetylchitooligosaccharide elicitor in the plasma membrane of suspension-cultured rice cells by affinity labeling. *Plant J* **12**:347-56. doi: 10.1046/j.1365-313X.1997.12020347.x.

Jacobs AK, Lipka V, Burton RA, Panstruga R, Strizhov N, Schulze-Lefert P & Fincher GB. 2003. An *Arabidopsis* Callose Synthase, GSL5, is required for wound and papillary callose formation. *Plant Cell* **15**:2503-13. doi: 10.1105/tpc.016097.

Jarsch IK, Konrad SS, Stratil TF, Urbanus SL, Szymanski W, Braun P, Braun KH & Ott T. 2014. Plasma membranes are subcompartmentalized into a plethora of coexisting and diverse microdomains in *Arabidopsis* and *Nicotiana benthamiana*. *Plant Cell* **26**:1698-1711. doi: 10.1105/tpc.114.124446.

Jarsch IK & Ott T. 2011. Perspectives on remorin proteins, membrane rafts, and their role during plant-microbe interactions. *Mol Plant Microbe Interact* **24**:7-12. doi: 10.1094/MPMI-07-10-0166.

Jiwan D, Roalson EH, Main D & Dhingra A. 2013. Antisense expression of peach *mildew resistance locus O* (*PpMlo1*) gene confers cross-species resistance to powdery mildew in *Fragaria* x *ananassa*. *Transgenic Res* **22**:1119-31. doi: 10.1007/s11248-013-9715-6.

Jones JD & Dangl JL. 2006. The plant immune system. *Nature* **444**:323-9. doi: 10.1038/nature05286.

Jones KM, Kobayashi H, Davies BW, Taga ME & Walker GC. 2007. How rhizobial symbionts invade plants: the *Sinorhizobium-Medicago* model. *Nat Rev Microbiol* **5**:619-33. doi: 10.1038/nrmicro1705.

Joris B, Englebert S, Chu CP, Kariyama R, Daneo-Moore L, Shockman GD & Ghuysen JM. 1992. Modular design of the *Enterococcus hirae muramidase-2* and *Streptococcus faecalis autolysin*. *FEMS Microbiol Lett* **70**:257-64. doi: 10.1016/0378-1097(92)90707-U.

Kaku H, Nishizawa Y, Ishii-Minami N, Akimoto-Tomiyama C, Dohmae N, Takio K, Minami E & Shibuya N. 2006. Plant cells recognize chitin fragments for defense signaling through a

plasma membrane receptor. *Proc Natl Acad Sci U S A* **103**:11086-91. doi: 10.1073/pnas.0508882103.

Kanamori N, Madsen LH, Radutoiu S, Frantescu M, Quistgaard EM, Miwa H, Downie JA, James EK, Felle HH, Haaning LL, Jensen TH, Sato S, Nakamura Y, Tabata S, Sandal N & Stougaard J. 2006. A nucleoporin is required for induction of Ca^{2+} spiking in legume nodule development and essential for rhizobial and fungal symbiosis. *Proc Natl Acad Sci U S A* **103**:359-64. doi: 10.1073/pnas.0508883103.

Katoh K, Misawa K, Kuma K & Miyata T. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Res* **30**:3059-66. doi: 10.1093/nar/gkf436.

Ke D, Fang Q, Chen C, Zhu H, Chen T, Chang X, Yuan S, Kang H, Ma L, Hong Z & Zhang Z. 2012. The small GTPase ROP6 interacts with NFR5 and is involved in nodule formation in *Lotus japonicus*. *Plant Physiol* **159**:131-43. doi: 10.1104/pp.112.197269.

Kemmerling B, Schwedt A, Rodriguez P, Mazzotta S, Frank M, Qamar SA, Mengiste T, Betsuyaku S, Parker JE, Mussig C, Thomma BP, Albrecht C, De Vries SC, Hirt H & Nürnberger T. 2007. The BRI1-associated kinase 1, BAK1, has a brassinolide-independent role in plant cell-death control. *Curr Biol* **17**:1116-22. doi: 10.1016/j.cub.2007.05.046.

Kereszt A, Mergaert P & Kondorosi E. 2011. Bacteroid development in legume nodules: evolution of mutual benefit or of sacrificial victims? *Mol Plant Microbe Interact* **24**:1300-9. doi: 10.1094/MPMI-06-11-0152.

Kevei Z, Lougnon G, Mergaert P, Horvath GV, Kereszt A, Jayaraman D, Zaman N, Marcel F, Regulski K, Kiss GB, Kondorosi A, Endre G, Kondorosi E & Ane JM. 2007. 3-hydroxy-3-methylglutaryl coenzyme a reductase 1 interacts with NORK and is crucial for nodulation in *Medicago truncatula*. *Plant Cell* **19**:3974-89. doi: 10.1105/tpc.107.053975.

Kevei Z, Seres A, Kereszt A, Kalo P, Kiss P, Toth G, Endre G & Kiss GB. 2005. Significant microsynteny with new evolutionary highlights is detected between *Arabidopsis* and legume model plants despite the lack of macrosynteny. *Mol Genet Genomics* **274**:644-57. doi: 10.1007/s00438-005-0057-9.

Kistner C & Parniske M. 2002. Evolution of signal transduction in intracellular symbiosis. *Trends Plant Sci* **7**:511-8. doi: 10.1016/S1360-1385(02)02356-7.

Kistner C, Winzer T, Pitzschke A, Mulder L, Sato S, Kaneko T, Tabata S, Sandal N, Stougaard J, Webb KJ, Szczyglowski K & Parniske M. 2005. Seven *Lotus japonicus* genes required for transcriptional reprogramming of the root during fungal and bacterial symbiosis. *Plant Cell* **17**:2217-29. doi: 10.1105/tpc.105.032714.

Kosuta S, Chabaud M, Lougnon G, Gough C, Denarie J, Barker DG & Becard G. 2003. A diffusible factor from arbuscular mycorrhizal fungi induces symbiosis-specific *MtENOD11* expression in roots of *Medicago truncatula*. *Plant Physiol* **131**:952-62. doi: 10.1104/pp.011882.

Kosuta S, Hazledine S, Sun J, Miwa H, Morris RJ, Downie JA & Oldroyd GE. 2008. Differential and chaotic calcium signatures in the symbiosis signaling pathway of legumes. *Proc Natl Acad Sci U S A* **105**:9823-8. doi: 10.1073/pnas.0803499105.

Kosuta S, Held M, Hossain MS, Morieri G, Macgillivary A, Johansen C, Antolín-Llovera M, Parniske M, Oldroyd GE, Downie AJ, Karas B & Szczyglowski K. 2011. *Lotus japonicus symRK-14* uncouples the cortical and epidermal symbiotic program. *Plant J* **67**:929-40. doi: 10.1111/j.1365-313X.2011.04645.x.

Kouzai Y, Mochizuki S, Nakajima K, Desaki Y, Hayafune M, Miyazaki H, Yokotani N, Ozawa K, Minami E, Kaku H, Shibuya N & Nishizawa Y. 2014. Targeted gene disruption of *OsCERK1* reveals its indispensable role in chitin perception and involvement in the peptidoglycan response and immunity in rice. *Mol Plant Microbe Interact* **27**:975-82. doi: 10.1094/MPMI-03-14-0068-R.

Kuhn H, Kuster H & Requena N. 2010. Membrane steroid-binding protein 1 induced by a diffusible fungal signal is critical for mycorrhization in *Medicago truncatula*. *New Phytol* **185**:716-33. doi: 10.1111/j.1469-8137.2009.03116.x.

Lefebvre B, Timmers T, Mbengue M, Moreau S, Herve C, Toth K, Bittencourt-Silvestre J, Klaus D, Deslandes L, Godiard L, Murray JD, Udvardi MK, Raffaele S, Mongrand S, Cullimore J, Gamas P, Niebel A & Ott T. 2010. A remorin protein interacts with symbiotic receptors and regulates bacterial infection. *Proc Natl Acad Sci U S A* **107**:2343-8. doi: 10.1073/pnas.0913320107.

Li J & Chory J. 1997. A putative leucine-rich repeat receptor kinase involved in brassinosteroid signal transduction. *Cell* **90**:929-38. doi: 10.1016/S0092-8674(00)80357-8.

Li J, Wen J, Lease KA, Doke JT, Tax FE & Walker JC. 2002. BAK1, an *Arabidopsis* LRR receptor-like protein kinase, interacts with BRI1 and modulates brassinosteroid signaling. *Cell* **110**:213-22. doi: 10.1016/S0092-8674(02)00812-7.

Li X, Clarke JD, Zhang Y & Dong X. 2001. Activation of an *EDS1*-mediated R-gene pathway in the *snc1* mutant leads to constitutive, *NPR1*-independent pathogen resistance. *Mol Plant Microbe Interact* **14**:1131-9. doi: 10.1094/MPMI.2001.14.10.1131.

Lin NC & Martin GB. 2005. An avrPto/avrPtoB mutant of *Pseudomonas syringae* pv. *tomato* DC3000 does not elicit Pto-mediated resistance and is less virulent on tomato. *Mol Plant Microbe Interact* **18**:43-51. doi: 10.1094/MPMI-18-0043.

Liu J, Blaylock LA, Endre G, Cho J, Town CD, Vandenbosch KA & Harrison MJ. 2003. Transcript profiling coupled with spatial expression analyses reveals genes involved in distinct developmental stages of an arbuscular mycorrhizal symbiosis. *Plant Cell* **15**:2106-23. doi: 10.1105/tpc.014183.

Liu J, Maldonado-Mendoza I, Lopez-Meyer M, Cheung F, Town CD & Harrison MJ. 2007. Arbuscular mycorrhizal symbiosis is accompanied by local and systemic alterations in gene expression and an increase in disease resistance in the shoots. *Plant J* **50**:529-44. doi: 10.1111/j.1365-313X.2007.03069.x.

Liu T, Liu Z, Song C, Hu Y, Han Z, She J, Fan F, Wang J, Jin C, Chang J, Zhou JM & Chai J. 2012. Chitin-induced dimerization activates a plant immune receptor. *Science* **336**:1160-4. doi: 10.1126/science.1218867.

Lohmann GV, Shimoda Y, Nielsen MW, Jorgensen FG, Grossmann C, Sandal N, Sorensen K, Thirup S, Madsen LH, Tabata S, Sato S, Stougaard J & Radutoiu S. 2010. Evolution and regulation of the *Lotus japonicus LysM receptor* gene family. *Mol Plant Microbe Interact* **23**:510-21. doi: 10.1094/MPMI-23-4-0510.

Madsen EB, Antolín-Llovera M, Grossmann C, Ye J, Vieweg S, Broghammer A, Krusell L, Radutoiu S, Jensen ON, Stougaard J & Parniske M. 2011. Autophosphorylation is essential for the in vivo function of the *Lotus japonicus* Nod factor receptor 1 and receptor-mediated signalling in cooperation with Nod factor receptor 5. *Plant J* **65**:404-17. doi: 10.1111/j.1365-313X.2010.04431.x.

Madsen EB, Madsen LH, Radutoiu S, Olbryt M, Rakwalska M, Szczyglowski K, Sato S, Kaneko T, Tabata S, Sandal N & Stougaard J. 2003. A receptor kinase gene of the LysM type is involved in legume perception of rhizobial signals. *Nature* **425**:637-40. doi: 10.1038/nature02045.

Madsen LH, Tirichine L, Jurkiewicz A, Sullivan JT, Heckmann AB, Bek AS, Ronson CW, James EK & Stougaard J. 2010. The molecular network governing nodule organogenesis and infection in the model legume *Lotus japonicus*. *Nat Commun* **1**:10. doi: 10.1038/ncomms1009.

Maekawa T, Kusakabe M, Shimoda Y, Sato S, Tabata S, Murooka Y & Hayashi M. 2008. *Polyubiquitin* promoter-based binary vectors for overexpression and gene silencing in *Lotus japonicus*. *Mol Plant Microbe Interact* **21**:375-82. doi: 10.1094/MPMI-21-4-0375.

Maillet F, Poinsot V, Andre O, Puech-Pages V, Haouy A, Gueunier M, Cromer L, Giraudet D, Formey D, Niebel A, Martinez EA, Driguez H, Becard G & Denarie J. 2011. Fungal lipochitooligosaccharide symbiotic signals in arbuscular mycorrhiza. *Nature* **469**:58-63. doi: 10.1038/nature09622.

Markmann K, Giczey G & Parniske M. 2008. Functional adaptation of a plant receptor-kinase paved the way for the evolution of intracellular root symbioses with bacteria. *PLoS Biol* **6**:e68. doi: 10.1371/journal.pbio.0060068.

Mbengue M, Camut S, De Carvalho-Niebel F, Deslandes L, Froidure S, Klaus-Heisen D, Moreau S, Rivas S, Timmers T, Herve C, Cullimore J & Lefebvre B. 2010. The *Medicago truncatula* E3 ubiquitin ligase PUB1 interacts with the LYK3 symbiotic receptor and negatively regulates infection and nodulation. *Plant Cell* **22**:3474-88. doi: 10.1105/tpc.110.075861.

Mendgen K & Hahn M. 2002. Plant infection and the establishment of fungal biotrophy. *Trends Plant Sci* **7**:352-6. doi: 10.1016/S1360-1385(02)02297-5.

Micali C, Gollner K, Humphry M, Consonni C & Panstruga R. 2008. The powdery mildew disease of *Arabidopsis*: A paradigm for the interaction between plants and biotrophic fungi. *Arabidopsis Book* **6**:e0115. doi: 10.1199/tab.0115.

Miklis M, Consonni C, Bhat RA, Lipka V, Schulze-Lefert P & Panstruga R. 2007. Barley *MLO* modulates actin-dependent and actin-independent antifungal defense pathways at the cell periphery. *Plant Physiol* **144**:1132-43. doi: 10.1104/pp.107.098897.

Miller JB, Pratap A, Miyahara A, Zhou L, Bornemann S, Morris RJ & Oldroyd GE. 2013. Calcium/Calmodulin-dependent protein kinase is negatively and positively regulated by calcium, providing a mechanism for decoding calcium responses during symbiosis signaling. *Plant Cell* **25**:5053-66. doi: 10.1105/tpc.113.116921.

Mims CW, Rodriguez-Lother C & Richardson EA. 2002. Ultrastructure of the host-pathogen interface in daylily leaves infected by the rust fungus *Puccinia hemerocallidis*. *Protoplasma* **219**:221-6. doi: 10.1007/s007090200023.

Miwa H, Sun J, Oldroyd GE & Downie JA. 2006. Analysis of Nod-factor-induced calcium signaling in root hairs of symbiotically defective mutants of *Lotus japonicus*. *Mol Plant Microbe Interact* **19**:914-23. doi: 10.1094/MPMI-19-0914.

Miyata K, Kozaki T, Kouzai Y, Ozawa K, Ishii K, Asamizu E, Okabe Y, Umehara Y, Miyamoto A, Kobae Y, Akiyama K, Kaku H, Nishizawa Y, Shibuya N & Nakagawa T. 2014. Bifunctional plant receptor, OsCERK1, regulates both chitin-triggered immunity and arbuscular mycorrhizal symbiosis in rice. *Plant Cell Physiol*. doi: 10.1093/pcp/pcu129.

Moling S, Pietraszewska-Bogiel A, Postma M, Fedorova E, Hink MA, Limpens E, Gadella TW & Bisseling T. 2014. Nod Factor Receptors form heteromeric complexes and are essential for intracellular infection in *Medicago* nodules. *Plant Cell*. doi: 10.1105/tpc.114.129502.

Mosher S, Seybold H, Rodriguez P, Stahl M, Davies KA, Dayaratne S, Morillo SA, Wierzba M, Favery B, Keller H, Tax FE & Kemmerling B. 2013. The tyrosine-sulfated peptide receptors PSKR1 and PSY1R modify the immunity of *Arabidopsis* to biotrophic and necrotrophic pathogens in an antagonistic manner. *Plant J* **73**:469-82. doi: 10.1111/tpj.12050.

Murashige T & Skoog F. 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiol Plant* **15**:473-497. doi: 10.1111/j.1399-3054.1962.tb08052.x.

Murray JD, Karas BJ, Sato S, Tabata S, Amyot L & Szczyglowski K. 2007. A cytokinin perception mutant colonized by *Rhizobium* in the absence of nodule organogenesis. *Science* **315**:101-4. doi: 10.1126/science.1132514.

Nakagawa T, Kaku H, Shimoda Y, Sugiyama A, Shimamura M, Takanashi K, Yazaki K, Aoki T, Shibuya N & Kouchi H. 2011. From defense to symbiosis: limited alterations in the kinase domain of LysM receptor-like kinases are crucial for evolution of legume-*Rhizobium* symbiosis. *Plant J* **65**:169-80. doi: 10.1111/j.1365-313X.2010.04411.x.

Nam KH & Li J. 2002. BRI1/BAK1, a receptor kinase pair mediating brassinosteroid signaling. *Cell* **110**:203-12. doi: 10.1016/S0092-8674(02)00814-0.

Nishimura MT, Stein M, Hou BH, Vogel JP, Edwards H & Somerville SC. 2003. Loss of a callose synthase results in salicylic acid-dependent disease resistance. *Science* **301**:969-72. doi: 10.1126/science.1086716.

Olah B, Briere C, Becard G, Denarie J & Gough C. 2005. Nod factors and a diffusible factor from arbuscular mycorrhizal fungi stimulate lateral root formation in *Medicago truncatula* via the *DMI1*/*DMI2* signalling pathway. *Plant J* **44**:195-207. doi: 10.1111/j.1365-313X.2005.02522.x.

Oldroyd GE. 2013. Speak, friend, and enter: signalling systems that promote beneficial symbiotic associations in plants. *Nat Rev Microbiol* **11**:252-63. doi: 10.1038/nrmicro2990.

Oldroyd GE, Murray JD, Poole PS & Downie JA. 2011. The rules of engagement in the legume-rhizobial symbiosis. *Annu Rev Genet* **45**:119-44. doi: 10.1146/annurev-genet-110410-132549.

Onate-Sanchez L, Anderson JP, Young J & Singh KB. 2007. AtERF14, a member of the ERF family of transcription factors, plays a nonredundant role in plant defense. *Plant Physiol* **143**:400-9. doi: 10.1104/pp.106.086637.

Op Den Camp R, Streng A, De Mita S, Cao Q, Polone E, Liu W, Ammiraju JS, Kudrna D, Wing R, Untergasser A, Bisseling T & Geurts R. 2011. LysM-type mycorrhizal receptor recruited for rhizobium symbiosis in nonlegume *Parasponia*. *Science* **331**:909-12. doi: 10.1126/science.1198181.

Ortu G, Balestrini R, Pereira PA, Becker JD, Kuster H & Bonfante P. 2012. Plant genes related to gibberellin biosynthesis and signaling are differentially regulated during the early stages of AM fungal interactions. *Mol Plant* **5**:951-4. doi: 10.1093/mp/sss027.

Ovchinnikova E, Journet EP, Chabaud M, Cosson V, Ratet P, Duc G, Fedorova E, Liu W, Den Camp RO, Zhukov V, Tikhonovich I, Borisov A, Bisseling T & Limpens E. 2011. IPD3 controls the formation of nitrogen-fixing symbiosomes in pea and *Medicago* Spp. *Mol. Plant Microbe Interact.* **24**:1333-44. doi: 10.1094/MPMI-01-11-0013.

Parniske M. 2000. Intracellular accommodation of microbes by plants: a common developmental program for symbiosis and disease? *Curr Opin Plant Biol* **3**:320-8. doi: 10.1016/S1369-5266(00)00088-1.

Parniske M. 2008. Arbuscular mycorrhiza: the mother of plant root endosymbioses. *Nat Rev Microbiol* **6**:763-75. doi: 10.1038/nrmicro1987.

Pavan S, Schiavulli A, Appiano M, Marcotrigiano AR, Cillo F, Visser RG, Bai Y, Lotti C & Ricciardi L. 2011. Pea powdery mildew *er1* resistance is associated to loss-of-function mutations at a *MLO* homologous locus. *Theor Appl Genet* **123**:1425-31. doi: 10.1007/s00122-011-1677-6.

Pawlowski K & Sprent JI 2008. Comparison between actinorhizal and legume symbiosis. *In:* PAWLOWSKI, K. & NEWTON, W. (eds.) *Nitrogen-fixing Actinorhizal Symbioses.* Springer Netherlands.

Peck MC, Fisher RF & Long SR. 2006. Diverse flavonoids stimulate NodD1 binding to *nod* gene promoters in *Sinorhizobium meliloti*. *J Bacteriol* **188**:5417-27. doi: 10.1128/JB.00376-06.

Petutschnig EK, Jones AM, Serazetdinova L, Lipka U & Lipka V. 2010. The lysin motif receptor-like kinase (LysM-RLK) CERK1 is a major chitin-binding protein in *Arabidopsis thaliana* and subject to chitin-induced phosphorylation. *J Biol Chem* **285**:28902-11. doi: 10.1074/jbc.M110.116657.

Pietraszewska-Bogiel A, Lefebvre B, Koini MA, Klaus-Heisen D, Takken FL, Geurts R, Cullimore JV & Gadella TW. 2013. Interaction of *Medicago truncatula* lysin motif receptor-like kinases, NFP and LYK3, produced in *Nicotiana benthamiana* induces defence-like responses. *PLoS One* **8**:e65055. doi: 10.1371/journal.pone.0065055.

Pumplin N & Harrison MJ. 2009. Live-cell imaging reveals periarbuscular membrane domains and organelle location in *Medicago truncatula* roots during arbuscular mycorrhizal symbiosis. *Plant Physiol* **151**:809-19. doi: 10.1104/pp.109.141879.

R-Team 2013. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.

Radutoiu S, Madsen LH, Madsen EB, Felle HH, Umehara Y, Gronlund M, Sato S, Nakamura Y, Tabata S, Sandal N & Stougaard J. 2003. Plant recognition of symbiotic bacteria requires two LysM receptor-like kinases. *Nature* **425**:585-92. doi: 10.1038/nature02039.

Radutoiu S, Madsen LH, Madsen EB, Jurkiewicz A, Fukai E, Quistgaard EM, Albrektsen AS, James EK, Thirup S & Stougaard J. 2007. LysM domains mediate lipochitin-oligosaccharide

recognition and *Nfr* genes extend the symbiotic host range. *EMBO J* **26**:3923-35. doi: 10.1038/sj.emboj.7601826.

Reese TA, Liang HE, Tager AM, Luster AD, Van Rooijen N, Voehringer D & Locksley RM. 2007. Chitin induces accumulation in tissue of innate immune cells associated with allergy. *Nature* **447**:92-6. doi: 10.1038/nature05746.

Remy W, Taylor TN, Hass H & Kerp H. 1994. Four hundred-million-year-old vesicular arbuscular mycorrhizae. *Proc Natl Acad Sci U S A* **91**:11841-3. doi: 10.1073/pnas.91.25.11841.

Ryals JA, Neuenschwander UH, Willits MG, Molina A, Steiner HY & Hunt MD. 1996. Systemic acquired resistance. *Plant Cell* **8**:1809-1819. doi: 10.1105/tpc.8.10.1809.

Saha S, Dutta A, Bhattacharya A & Dasgupta M. 2014. Intracellular catalytic domain of Symbiosis Receptor Kinase (SYMRK) hyperactivates spontaneous nodulation in absence of rhizobia. *Plant Physiol*. doi: 10.1104/pp.114.250084.

Saito K, Yoshikawa M, Yano K, Miwa H, Uchida H, Asamizu E, Sato S, Tabata S, Imaizumi-Anraku H, Umehara Y, Kouchi H, Murooka Y, Szczyglowski K, Downie JA, Parniske M, Hayashi M & Kawaguchi M. 2007. NUCLEOPORIN85 is required for calcium spiking, fungal and bacterial symbioses, and seed production in *Lotus japonicus*. *Plant Cell* **19**:610-24. doi: 10.1105/tpc.106.046938.

Schauser L, Roussis A, Stiller J & Stougaard J. 1999. A plant regulator controlling development of symbiotic root nodules. *Nature* **402**:191-5. doi: 10.1038/46058.

Schlessinger J. 2002. Ligand-induced, receptor-mediated dimerization and activation of EGF receptor. *Cell* **110**:669-72. doi: 10.1016/S0092-8674(02)00966-2.

Schneider CA, Rasband WS & Eliceiri KW. 2012. NIH Image to ImageJ: 25 years of image analysis. *Nat Methods* **9**:671-5. doi: 10.1038/nmeth.2089.

Scholl RL, May ST & Ware DH. 2000. Seed and molecular resources for *Arabidopsis*. *Plant Physiol* **124**:1477-80. doi: 10.1104/pp.124.4.1477

Schulze B, Mentzel T, Jehle AK, Mueller K, Beeler S, Boller T, Felix G & Chinchilla D. 2010. Rapid heteromerization and phosphorylation of ligand-activated plant transmembrane receptors and their associated kinase BAK1. *J Biol Chem* **285**:9444-51. doi: 10.1074/jbc.M109.096842.

Schüßler A, Schwarzott D & Walker C. 2001. A new fungal phylum, the *Glomeromycota*: phylogeny and evolution. *Mycological Research* **105**:1413-1421. doi: 10.1017/s0953756201005196.

Shan Y, Eastwood MP, Zhang X, Kim ET, Arkhipov A, Dror RO, Jumper J, Kuriyan J & Shaw DE. 2012. Oncogenic mutations counteract intrinsic disorder in the EGFR kinase and promote receptor dimerization. *Cell* **149**:860-70. doi: 10.1016/j.cell.2012.02.063.

Shibuya N & Minami E. 2001. Oligosaccharide signalling for defence responses in plant. *Physiological and Molecular Plant Pathology* **59**:223-233. doi: http://dx.doi.org/10.1006/pmpp.2001.0364.

Shimizu T, Nakano T, Takamizawa D, Desaki Y, Ishii-Minami N, Nishizawa Y, Minami E, Okada K, Yamane H, Kaku H & Shibuya N. 2010. Two LysM receptor molecules, CEBiP and OsCERK1, cooperatively regulate chitin elicitor signaling in rice. *Plant J* **64**:204-14. doi: 10.1111/j.1365-313X.2010.04324.x.

Shinya T, Motoyama N, Ikeda A, Wada M, Kamiya K, Hayafune M, Kaku H & Shibuya N. 2012. Functional characterization of *CEBiP* and *CERK1* homologs in *Arabidopsis* and rice reveals the presence of different chitin receptor systems in plants. *Plant Cell Physiol* **53**:1696-706. doi: 10.1093/pcp/pcs113.

Shiu SH & Bleecker AB. 2003. Expansion of the receptor-like kinase/Pelle gene family and receptor-like proteins in *Arabidopsis*. *Plant Physiol* **132**:530-43. doi: 10.1104/pp.103.021964.

Sieberer BJ, Chabaud M, Fournier J, Timmers AC & Barker DG. 2012. A switch in Ca2+ spiking signature is concomitant with endosymbiotic microbe entry into cortical root cells of *Medicago truncatula*. *Plant J* **69**:822-30. doi: 10.1111/j.1365-313X.2011.04834.x.

Singh S, Katzer K, Lambert J, Cerri M & Parniske M. 2014. CYCLOPS, a DNA-binding transcriptional activator, orchestrates symbiotic root nodule development. *Cell Host Microbe* **15**:139-52. doi: 10.1016/j.chom.2014.01.011.

Singh S & Parniske M. 2012. Activation of calcium- and calmodulin-dependent protein kinase (CCaMK), the central regulator of plant root endosymbiosis. *Curr Opin Plant Biol* **15**:444-53. doi: 10.1016/j.pbi.2012.04.002.

Slusarenko AJ & Schlaich NL. 2003. Downy mildew of *Arabidopsis thaliana* caused by *Hyaloperonospora parasitica* (formerly *Peronospora parasitica*). *Mol Plant Pathol* **4**:159-70. doi: 10.1046/j.1364-3703.2003.00166.x.

Smit G, Swart S, Lugtenberg BJ & Kijne JW. 1992. Molecular mechanisms of attachment of *Rhizobium* bacteria to plant roots. *Mol Microbiol* **6**:2897-903. doi: 10.1111/j.1365-2958.1992.tb01748.x

Smith SE & Read DJ. 2008. *Mycorrhizal Symbiosis* **Academic, London,**. doi.

Sohn KH, Lei R, Nemri A & Jones JD. 2007. The downy mildew effector proteins ATR1 and ATR13 promote disease susceptibility in *Arabidopsis thaliana*. *Plant Cell* **19**:4077-90. doi: 10.1105/tpc.107.054262.

Solano R, Stepanova A, Chao Q & Ecker JR. 1998. Nuclear events in ethylene signaling: a transcriptional cascade mediated by ETHYLENE-INSENSITIVE3 and ETHYLENE-RESPONSE-FACTOR1. *Genes Dev* **12**:3703-14. doi: 10.1101/gad.12.23.3703.

Soyano T, Kouchi H, Hirota A & Hayashi M. 2013. Nodule inception directly targets *NF-Y* subunit genes to regulate essential processes of root nodule development in *Lotus japonicus*. *PLoS Genet* **9**:e1003352. doi: 10.1371/journal.pgen.1003352.

Sprent JI. 2007. Evolving ideas of legume evolution and diversity: a taxonomic perspective on the occurrence of nodulation. *New Phytol* **174**:11-25. doi: 10.1111/j.1469-8137.2007.02015.x.

Stamatakis A, Hoover P & Rougemont J. 2008. A rapid bootstrap algorithm for the RAxML Web servers. *Syst Biol* **57**:758-71. doi: 10.1080/10635150802429642.

Steen A, Buist G, Leenhouts KJ, El Khattabi M, Grijpstra F, Zomer AL, Venema G, Kuipers OP & Kok J. 2003. Cell wall attachment of a widely distributed peptidoglycan binding domain is hindered by cell wall constituents. *J Biol Chem* **278**:23874-81. doi: 10.1074/jbc.M211055200.

Stracke S, Kistner C, Yoshida S, Mulder L, Sato S, Kaneko T, Tabata S, Sandal N, Stougaard J, Szczyglowski K & Parniske M. 2002. A plant receptor-like kinase required for both bacterial and fungal symbiosis. *Nature* **417**:959-62. doi: 10.1038/nature00841.

Sun Y, Han Z, Tang J, Hu Z, Chai C, Zhou B & Chai J. 2013a. Structure reveals that BAK1 as a co-receptor recognizes the BRI1-bound brassinolide. *Cell Res* **23**:1326-9. doi: 10.1038/cr.2013.131.

Sun Y, Li L, Macho AP, Han Z, Hu Z, Zipfel C, Zhou JM & Chai J. 2013b. Structural basis for flg22-induced activation of the *Arabidopsis* FLS2-BAK1 immune complex. *Science* **342**:624-8. doi: 10.1126/science.1243825.

Takeda N, Haage K, Sato S, Tabata S & Parniske M. 2011. Activation of a *Lotus japonicus* subtilase gene during arbuscular mycorrhiza is dependent on the common symbiosis genes and two *cis*-active promoter regions. *Mol Plant Microbe Interact* **24**:662-70. doi: 10.1094/MPMI-09-10-0220.

Takeda N, Maekawa T & Hayashi M. 2012. Nuclear-localized and deregulated calcium- and calmodulin-dependent protein kinase activates rhizobial and mycorrhizal responses in *Lotus japonicus*. *Plant Cell* **24**:810-22. doi: 10.1105/tpc.111.091827.

Takeda N, Sato S, Asamizu E, Tabata S & Parniske M. 2009. Apoplastic plant subtilases support arbuscular mycorrhiza development in *Lotus japonicus*. *Plant J* **58**:766-77. doi: 10.1111/j.1365-313X.2009.03824.x.

Thaler JS, Owen B & Higgins VJ. 2004. The role of the jasmonate response in plant susceptibility to diverse pathogens with a range of lifestyles. *Plant Physiol* **135**:530-8. doi: 10.1104/pp.104.041566.

Tirichine L, Imaizumi-Anraku H, Yoshida S, Murakami Y, Madsen LH, Miwa H, Nakagawa T, Sandal N, Albrektsen AS, Kawaguchi M, Downie A, Sato S, Tabata S, Kouchi H, Parniske M, Kawasaki S & Stougaard J. 2006. Deregulation of a Ca^{2+}/calmodulin-dependent kinase leads to spontaneous nodule development. *Nature* **441**:1153-6. doi: 10.1038/nature04862.

Toth K, Stratil TF, Madsen EB, Ye J, Popp C, Antolín-Llovera M, Grossmann C, Jensen ON, Schussler A, Parniske M & Ott T. 2012. Functional domain analysis of the Remorin protein LjSYMREM1 in *Lotus japonicus*. *PLoS One* **7**:e30817. doi: 10.1371/journal.pone.0030817.

Umehara M, Hanada A, Yoshida S, Akiyama K, Arite T, Takeda-Kamiya N, Magome H, Kamiya Y, Shirasu K, Yoneyama K, Kyozuka J & Yamaguchi S. 2008. Inhibition of shoot branching by new terpenoid plant hormones. *Nature* **455**:195-200. doi: 10.1038/nature07272.

Van Brussel AA, Bakhuizen R, Van Spronsen PC, Spaink HP, Tak T, Lugtenberg BJ & Kijne JW. 1992. Induction of pre-infection thread structures in the leguminous host plant by mitogenic lipo-oligosaccharides of *Rhizobium*. *Science* **257**:70-2. doi: 10.1126/science.257.5066.70.

Van Damme M, Andel A, Huibers RP, Panstruga R, Weisbeek PJ & Van Den Ackerveken G. 2005. Identification of *Arabidopsis* loci required for susceptibility to the downy mildew pathogen *Hyaloperonospora parasitica*. *Mol Plant Microbe Interact* **18**:583-92. doi: 10.1094/MPMI-18-0583.

Van Damme M, Zeilmaker T, Elberse J, Andel A, De Sain-Van Der Velden M & Van Den Ackerveken G. 2009. Downy mildew resistance in *Arabidopsis* by mutation of *HOMOSERINE KINASE*. *Plant Cell* **21**:2179-89. doi: 10.1105/tpc.109.066811.

Van Der Heijden MG, Bardgett RD & Van Straalen NM. 2008. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecol Lett* **11**:296-310. doi: 10.1111/j.1461-0248.2007.01139.x.

Van Schie CC & Takken FL. 2014. Susceptibility genes 101: how to be a good host. *Annu Rev Phytopathol* **52**:551-81. doi: 10.1146/annurev-phyto-102313-045854.

Varallyay E, Giczey G & Burgyan J. 2012. Virus-induced gene silencing of *Mlo* genes induces powdery mildew resistance in *Triticum aestivum*. *Arch Virol* **157**:1345-50. doi: 10.1007/s00705-012-1286-y.

Venkateshwaran M, Cosme A, Han L, Banba M, Satyshur KA, Schleiff E, Parniske M, Imaizumi-Anraku H & Ane JM. 2012. The recent evolution of a symbiotic ion channel in the legume family altered ion conductance and improved functionality in calcium signaling. *Plant Cell* **24**:2528-45. doi: 10.1105/tpc.112.098475.

Venkateshwaran M, Volkening JD, Sussman MR & Ané J-M. 2013. Symbiosis and the social network of higher plants. *Curr Opin Plant Biol* **16**:118-127. doi: http://dx.doi.org/10.1016/j.pbi.2012.11.007.

Vogel J & Somerville S. 2000. Isolation and characterization of powdery mildew-resistant *Arabidopsis* mutants. *Proc Natl Acad Sci U S A* **97**:1897-902. doi: 10.1073/pnas.030531997.

Vogel JP, Raab TK, Schiff C & Somerville SC. 2002. *PMR6*, a pectate lyase-like gene required for powdery mildew susceptibility in *Arabidopsis*. *Plant Cell* **14**:2095-106. doi: 10.1105/tpc.003509

Vogel JP, Raab TK, Somerville CR & Somerville SC. 2004. Mutations in *PMR5* result in powdery mildew resistance and altered cell wall composition. *Plant J* **40**:968-78. doi: 10.1111/j.1365-313X.2004.02264.x.

Wan J, Zhang XC, Neece D, Ramonell KM, Clough S, Kim SY, Stacey MG & Stacey G. 2008. A LysM receptor-like kinase plays a critical role in chitin signaling and fungal resistance in Arabidopsis. *Plant Cell* **20**:471-81. doi: 10.1105/tpc.107.056754.

Wang C, Zhu H, Jin L, Chen T, Wang L, Kang H, Hong Z & Zhang Z. 2013. Splice variants of the *SIP1* transcripts play a role in nodule organogenesis in *Lotus japonicus*. *Plant Mol Biol* **82**:97-111. doi: 10.1007/s11103-013-0042-3.

Wang E, Schornack S, Marsh JF, Gobbato E, Schwessinger B, Eastmond P, Schultze M, Kamoun S & Oldroyd GE. 2012. A common signaling process that promotes mycorrhizal and oomycete colonization of plants. *Curr Biol* **22**:2242-6. doi: 10.1016/j.cub.2012.09.043.

Wang W, Xie ZP & Staehelin C. 2014. Functional analysis of chimeric lysin motif domain receptors mediating Nod factor-induced defense signaling in *Arabidopsis thaliana* and chitin-induced nodulation signaling in *Lotus japonicus*. *Plant J* **78**:56-69. doi: 10.1111/tpj.12450.

Webb KJ, Skot L, Nicholson MN, Jorgensen B & Mizen S. 2000. *Mesorhizobium loti* increases root-specific expression of a calcium-binding protein homologue identified by promoter tagging in *Lotus japonicus*. *Mol Plant Microbe Interact* **13**:606-16. doi: 10.1094/MPMI.2000.13.6.606.

Wei X, Ni S & Correll PH. 2005. Uncoupling ligand-dependent and -independent mechanisms for mitogen-activated protein kinase activation by the murine Ron receptor tyrosine kinase. *J Biol Chem* **280**:35098-107. doi: 10.1074/jbc.M505737200.

Weston LA & Mathesius U. 2013. Flavonoids: their structure, biosynthesis and role in the rhizosphere, including allelopathy. *J Chem Ecol* **39**:283-97. doi: 10.1007/s10886-013-0248-5.

White J, Prell J, James EK & Poole P. 2007. Nutrient sharing between symbionts. *Plant Physiol* **144**:604-14. doi: 10.1104/pp.107.097741.

Wiermer M, Cheng YT, Imkampe J, Li M, Wang D, Lipka V & Li X. 2012. Putative members of the *Arabidopsis* Nup107-160 nuclear pore sub-complex contribute to pathogen defense. *Plant J* **70**:796-808. doi: 10.1111/j.1365-313X.2012.04928.x.

Yano K, Yoshida S, Muller J, Singh S, Banba M, Vickers K, Markmann K, White C, Schuller B, Sato S, Asamizu E, Tabata S, Murooka Y, Perry J, Wang TL, Kawaguchi M, Imaizumi-Anraku H, Hayashi M & Parniske M. 2008. CYCLOPS, a mediator of symbiotic intracellular accommodation. *Proc Natl Acad Sci U S A* **105**:20540-5. doi: 10.1073/pnas.0806858105.

Yokota K, Fukai E, Madsen LH, Jurkiewicz A, Rueda P, Radutoiu S, Held M, Hossain MS, Szczyglowski K, Morieri G, Oldroyd GE, Downie JA, Nielsen MW, Rusek AM, Sato S, Tabata S, James EK, Oyaizu H, Sandal N & Stougaard J. 2009. Rearrangement of actin cytoskeleton mediates invasion of *Lotus japonicus* roots by *Mesorhizobium loti*. *Plant Cell* **21**:267-84. doi: 10.1105/tpc.108.063693.

Yoro E, Suzaki T, Toyokura K, Miyazawa H, Fukaki H & Kawaguchi M. 2014. A positive regulator of nodule organogenesis, *NODULE INCEPTION*, acts as a negative regulator of rhizobial infection in *Lotus japonicus*. *Plant Physiol*. doi: 10.1104/pp.113.233379.

Yoshida S & Parniske M. 2005. Regulation of plant Symbiosis Receptor Kinase through serine and threonine phosphorylation. *J Biol Chem* **280**:9203-9. doi: 10.1074/jbc.M411665200.

Yuan S, Zhu H, Gou H, Fu W, Liu L, Chen T, Ke D, Kang H, Xie Q, Hong Z & Zhang Z. 2012. A ubiquitin ligase of Symbiosis Receptor Kinase involved in nodule organogenesis. *Plant Physiol* **160**:106-17. doi: 10.1104/pp.112.199000.

Zhang XC, Cannon SB & Stacey G. 2009. Evolutionary genomics of LysM genes in land plants. *BMC Evol Biol* **9**:183. doi: 10.1186/1471-2148-9-183.

Zhang XC, Wu X, Findley S, Wan J, Libault M, Nguyen HT, Cannon SB & Stacey G. 2007. Molecular evolution of lysin motif-type receptor-like kinases in plants. *Plant Physiol* **144**:623-36. doi: 10.1104/pp.107.097097.

Zhang Y & Li X. 2005. A putative Nucleoporin 96 Is required for both basal defense and constitutive resistance responses mediated by suppressor of *npr1-1,constitutive 1*. *Plant Cell* **17**:1306-16. doi: 10.1105/tpc.104.029926.

Zheng Z, Nonomura T, Appiano M, Pavan S, Matsuda Y, Toyoda H, Wolters AM, Visser RG & Bai Y. 2013. Loss of function in *Mlo* orthologs reduces susceptibility of pepper and tomato to powdery mildew disease caused by *Leveillula taurica*. *PLoS One* **8**:e70723. doi: 10.1371/journal.pone.0070723.

Zhu H, Chen T, Zhu M, Fang Q, Kang H, Hong Z & Zhang Z. 2008. A novel ARID DNA-binding protein interacts with SymRK and is expressed during early nodule development in *Lotus japonicus*. *Plant Physiol* **148**:337-47. doi: 10.1104/pp.108.119164.

Zipfel C, Kunze G, Chinchilla D, Caniard A, Jones JD, Boller T & Felix G. 2006. Perception of the bacterial PAMP EF-Tu by the receptor EFR restricts *Agrobacterium*-mediated transformation. *Cell* **125**:749-60. doi: 10.1016/j.cell.2006.03.037.

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XII. LIST OF TABLES

XIII. ACKNOWLEDGEMENTS

First of all, I would like to express my deepest gratitude to my PhD supervisor, Prof Martin Parniske, who has supported me throughout my thesis with his encouragement, guidance and knowledge. Martin has been a constant source of inspiration, always being ready to discuss and develop new ideas on the spot. Throughout the years, he has not only given me the freedom to pursue "my own" side projects but has also encouraged my participation in international scientific conferences and collaborations, which greatly helped me in finding my own place and role in the scientific community.

I also want to thank the remaining members of my thesis advisory committee:
Prof Thorsten Nürnberger and Dr Meritxell Antolín-Llovera for our insightful discussions and the valuable advice they provided.
Before my PhD studies, Meri took me in as a student helper and continued to give me support and excellent supervision during my diploma thesis. I want to thank her for the highly inspiring dedication to her research all the times we have worked together and the many invaluable things she has taught me about the research into RLKs.

Many thanks go to the reviewers of this thesis, who agreed to take on this job on such short notice, for taking the time and effort to evaluate this work.

This thesis would not have been possible without the great help of a number of colleagues and friends with whom I had the pleasure of collaborating on various projects:

I would like to thank our collaborators on the *Arabidopsis* project Dr Andrea Gust and Prof Thorsten Nürnberger for performing *P. syringae* assays, Dr Corina Weis, Dr Caroline Höfle, Dr Ruth Eichmann, and Prof Ralph Hückelhoven for performing *E. cruciferarum* assays, and Dr Erik Kemen and Dr Ulla Neumann for performing electron microscopy on *Hpa*-inoculated *Arabidopsis* samples.
Special thanks go to Prof Didier Reinhardt for his collaboration on the *Petunia* project.

I want to thank Aline Banhara Pereira for the great time we had while working together on the *Arabidopsis* project, for sharing ideas on our research, for all the exciting and fruitful discussions, as well as the fun moments with Mickey Mouse and the vacuum cleaner in the recycling room. I also want to acknowledge Aline for critically reading my thesis. Many thanks go to Andreas Binder for the great collaboration on the *Arabidopsis* project, joining in productive nighttime writing sessions and for always cheering me up and being incredibly helpful (even after answering the 1000th question).

I want to deeply thank Dr Caroline Gutjahr for sharing her expertise on qRT-PCR, for always listening and giving me insightful answers and advice, as well as for the inspirational and deep scientific discussions we had.
Dr Sylvia Singh never grew tired of answering my countless questions with patience and competence, and I really enjoyed all the fun we had when it was our turn to prepare Wednesday lunch.
Special thanks go to Dr Axel Strauß, who taught in the R course and who could be consulted for expert advice on statistics at all times.
I want to thank Dr Marion Cerri for teaching me how to make nodule sections and for always being helpful and eager to embark on scientific questions and discussions.

It was a great pleasure and rewarding experience to supervise and work with Michael Bartoschek, who was an excellent Bachelor student, great assistant in the lab and a good guy.

I want to thank Joana Bittencourt Silvestre for the great company, the many conversations and lunches we had together as well as the long hours in the microscopy room that otherwise would have been very lonely.
I really enjoyed having worked with Dr Iris Jarsch, who was a fabulous lab colleague and always helpful and with whom I was very happy to share both fun moments and difficult situations in the daily PhD life.
I especially want to thank Jayne Lambert and Petra Winterholler for their invaluable support both in and outside the lab, for cheering me up in difficult times, the fun moments and all the times they kept me from starving by sharing their lunch or coffee break with me.

I would also like to thank Claudia Popp, Tom Stratil and Janett Elsässer, who were lovely and hospitable colleagues I very much enjoyed sharing an office with.

Many thanks go to all past and present members of the Parniske, Gutjahr, Ott, Brachmann, Lahaye and Schüssler group I had the pleasure of working with and who made the lab a friendly and collaborative workplace.

Finally yet importantly, I would like to express my deepest gratitude to my family, for without their unwavering support and love I would not have come this far.

XIV. CURRICULUM VITAE

MARTINA KATHARINA RIED
Martina.Saja.Ried@lmu.de

EDUCATION

Ph.D., Biology, University of Munich (LMU), Germany. 04/2011 – present.

Title: "Symbiosis Receptor-like Kinase and its *Arabidopsis* homologs in the interaction with beneficial and pathogenic microbes".

Advisor: Prof. Dr. Martin Parniske.

Diploma in Biology, University of Munich (LMU), Germany. 10/2006 – 03/2011.

Subjects: Genetics (major); Plant Molecular Biology, Physics (minors).

Diploma thesis: "Interaction between Symbiosis Receptor-like Kinase SYMRK and Nod Factor Receptor 1 and 5".

Advisor: Prof. Dr. Martin Parniske. 07/2010 – 03/2011.

Grade: 1.0 (highest grade).

PROFESSIONAL EXPERIENCE

Ph.D. student, University of Munich (LMU), Germany. 04/2011 – present.

Advisor: Prof. Dr. Martin Parniske.

Research Experience

Optimized the protocol for co-immunoenrichment experiments on protein extracts from *Lotus japonicus* roots. Investigated the relevance of different parts of the SYMRK ectodomain and the kinase domain for interaction with NFR1 and NFR5 in co-immunoenrichment experiments on protein extracts from *Nicotiana benthamiana* leaves. Analysed the effect of overexpression of symbiotic receptors on symbiosis-related promoter activation histochemically in stable promoter:reporter lines, on the expression of symbiosis-related genes via qRT-PCR and on nodule organogenesis. Conceived and developed an assay for the cultivation of transgenic roots in liquid culture in petri dishes for the analysis of lateral root formation. Studied the role of specific *Arabidopsis thaliana* receptor-like kinases in pathogen assays with *Hyaloperonospora arabidopsidis, Pseudomonas syringae* and *Erysiphe cruciferarum*, and their requirement for several defence-associated read-outs such as gene induction, ROS burst, callose deposition, or the accumulation of secondary metabolites.

Supervisory Experience

Supervised a Bachelor student. Assisted in the advanced practical course "Genetics II". Organised tutorial courses for the lecture "Genetics I".

Diploma Research, University of Munich (LMU), Germany. 07/2010 – 03/2011.

Advisor: Prof. Dr. Martin Parniske.

Performed interaction studies on symbiotic receptor-like kinases via the yeast two- and three-hybrid system. Established a protocol for co-immunoenrichment experiments and used this method along with mass spectrometry approaches to further characterize the interaction between the receptors and to investigate phosphorylation sites on SYMRK associated with complex formation and symbiosis signalling.

Research Internship, UCSD, USA. 08/2009 - 10/2009.

Advisors: Prof. Dr. Julian Schroeder and Prof. Dr. Martin Parniske.

Electrophysiologically characterised the ion channel SLAC1 and the effect of OST1 and ABI1 on SLAC1 function via two-electrode patch clamp on *Xenopus laevis* oocytes. Studied SLAC1-OST1 interaction via BiFC in *Nicotiana benthamiana*.

Student Research and Teaching Assistant, University of Munich (LMU), Germany. 08/2008 – 03/2010.

Research Experience

Assisted Dr. Meritxell Antolín-Llovera in the lab of Prof. Dr. Martin Parniske in her research into interactions between receptor-like kinases from *Lotus japonicus*.

Supervisory Experience

Assisted in the beginners' practical courses "Genetics I" and "Genetik für Nebenfächer". Prepared media and reagents and evaluated the students' protocols. Organised tutorial courses for the lecture "Genetics I".

FURTHER EDUCATION

- Language course "English for Biologists - Communicating scientifically". 09 - 12/2013.
- Seminar "Structured training for tutors, Part 1". 04 – 07/2013.
- Soft skill course "Media Training". 05/2013.
- Methods course "Analysing Quantitative Genetic Data - Applied Statistics". 01/2013.

- Soft skill course "PhD Management". 12/2009.

CONFERENCES

- Gordon Research Conference „Posttranslational modifications", The Hong Kong University of Science and Technology, China. Poster presentation. 08/2013.
- Symposium „Frontiers in legume symbiosis - A symposium in memory of Adam Kondorosi", IMAGIF, Gif-sur-Yvette, France. 12/2012.
- „10th European Nitrogen Fixation Congress (ENFC)", University of Munich (LMU). Best poster price. 09/2012.
- Symposium „Plant Protein Phosphorylation", University of Tübingen, Poster presentation. 09/2011.
- Conference „Plant Calcium Signalling", WWU Münster. 09/2010.

PUBLICATIONS AND MANUSCRIPTS IN PREPARATION

Research papers and manuscripts

- 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 *Arabidopsis thaliana.* Under review.
  * These authors contributed equally to the work
- **Ried MK**, Antolín-Llovera M & Parniske M. (2014). Spontaneous symbiotic reprogramming of plant roots triggered by receptor-like kinases. *Elife*. doi: 10.7554/eLife.03891
- Antolín-Llovera M, **Ried MK** & Parniske M. 2014b. Cleavage of the SYMBIOSIS RECEPTOR-LIKE KINASE ectodomain promotes complex formation with Nod Factor Receptor 5. *Curr. Biol.* **24**:422-7. doi: 10.1016/j.cub.2013.12.053.
- Den Herder G, Yoshida S, Antolín-Llovera M, **Ried MK** & Parniske M. 2012. *Lotus japonicus* E3 ligase SEVEN IN ABSENTIA4 destabilizes the symbiosis receptor-like kinase SYMRK and negatively regulates rhizobial infection. *Plant Cell* **24**:1691-707. doi: 10.1105/tpc.110.082248.

Reviews

- Antolín-Llovera M, Petutschnig EK, **Ried MK**, Lipka V, Nürnberger T, Robatzek S & Parniske M. 2014a. Knowing your friends and foes - plant receptor-like kinases as initiators of symbiosis or defence. *New Phytol* **204**:791-802. doi: 10.1111/nph.13117.

- Antolín-Llovera M, **Ried MK**, Binder A & Parniske M. 2012. Receptor kinase signalling pathways in plant-microbe interactions. *Annu. Rev. Phytopathol.* **50**:451-73. doi: 10.1146/annurev-phyto-081211-173002.

SCHOLARSHIPS

- Graduate School LSM (Fast Track Student). 10/2009 – present.
- Max Weber Programme (German National Academic Foundation / Bavarian promotion of elites). 10/2008 – 03/2011.

XV. APPENDIX

Supplemental File 1: Pruned alignment of kinase domain of LjSYMRK (aa 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
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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
AT2G28970/1-275    59 SLVGYCDEGDHLALIYEYMPNGDLKQHLSGKRGG-FVLSWESRLRVAVDAALGLEYLHTG
AT2G28990/1-275    59 SLVGYCDEGEHLALIYEYMPNGDLKQHLSGKHGG-FVLSWESRLKIVLDAALGLEYLHTG
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AT1G51810/1-275    59 GLVGYCEEGDKLALIYEYMANGDLDEHMSGKRGG-SILNWGTRLKIALEAAQGLEYLHNG
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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---
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AT1G51860/1-274   117 CRPPMVHRDVKTTNILLNERCGAKLADFGLSRSFPIDG-ECHVSTVVAGTPGYLDPEYYR
AT1G51880/1-274   117 CTPPMVHRDVKTTNILLNERYGAKLADFGLSRSFPVDG-ESHVSTVVAGTPGYLDPEYYR
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AT2G14440/1-274   117 CKPPMVHRDVKSTNILLGLRFEAKLADFGLSRSFLVGS-QTHVSTNVAGTLGYLDPEYYQ
AT2G14510/1-274   117 CQPPMVHRDVKSTNILLGLRFEAKLADFGLSRSFLVGS-QAHVSTNVAGTLGYLDPEYYL
AT3G46350/1-275   118 CRPSMVHRDVKSTNILLDENFMAKIADFGLSRSFILGG-ESHVSTVVAGSLGYLDPEYYR
AT3G46340/1-276   119 CRPSMVHRDVKSTNILLDDQFTAKMADFGLSRSFQLGD-ESQVSTVVAGTPGYLDPEYYR
AT3G46370/1-275   118 CRPSIVHRDVKSTNILLDDQLMAKIADFGLSRSFKLGE-ESQASTVVAGTLGYLDPEYYR
AT3G46400/1-275   118 CRPSMVHRDVKSTNILLDDQFMAKIADFGLSRSFKVGD-ESEISTVVAGTPGYLDPEYYR
AT3G46330/1-276   118 CKPAMVHRDVKSTNILLDEEFKAKIADFGLSRSFQVGGDQSQVSTVVAGTLGYLDPEYYL
AT5G59670/1-275   118 CTPPMVHRDVKTANILLDENFKAKLADFGLSRSFQGEG-ESQESTTIAGTLGYLDPECYH
AT5G59680/1-275   118 CTPPMVHRDVKTTNILLDEHYKAKLADFGLSRSFPVGG-ESHVSTVIAGTPGYLDPEYYH
AT5G59650/1-275   118 CTPPIVHRDIKTTNILLDEQLKAKLADFGLSRSFPIGG-ETHISTVVAGTPGYLDPEYYQ
AT5G16900/1-275   118 CKPLMVHRDVKSMNILLDEHFQAKLADFGLSRSFSVGE-ESHVSTGVVGTPGYLDPEYYR
AT1G07560/1-272   115 CKPLIVHRDVKSSNILLDDQLQAKLADFGLSRSFPIGD-ESHVSTLVAGTFGYLDHEYYQ
AT4G20450/1-275   118 CKPPMIHRDIKSMNILLDNNFQAKLGDFGLSRSFPVGS-ETHVSTNVAGSPGYLDPEYYR
AT2G28960/1-275   118 CKPPMVHRDVKTTNILLDEHFQAKLADFGLSRSFPVGG-ETHVSTAVAGTPGYLDPEYYR
AT2G29000/1-275   118 CEPPMIHRDVKTTNILLDEHFHAKLADFGLSRSFPVGV-ESHVSTNVAGTPGYLDPEYYR
AT2G28970/1-275   118 CKPPMVHRDIKSTNILLDERFQAKLADFGLSRSFPTEN-ETHVSTVVAGTPGYLDPEYYQ
AT2G28990/1-275   118 CVPPMVHRDIKTTNILLDQHLQAKLADFGLSRSFPIGN-EKNVSTVVAGTPGYLDPEYYQ
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 CKPPIVQRDVKPANILLINEKLQAKIADFGLSRSVALDG-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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Supplemental File 2: Pruned alignment of extracytoplasmic region of LjSYMRK (aa 30-517) and homologous stretches of related MLD-LRR-RLKs.

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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
ShRK1/1-533         1 ------MGLCLAQLAVTCLFLVPFVLS-QVT-EFVSIDCGCS----SNYTDPRTGLGWVS
ShRK2/1-529         1 --MVRISLLLLCLLVSTCLFTSS--SA-QAP-GFVSLDCGGA----EPFTD-ELGLKWSP
AT5G48740/1-492     1 ---------MLFWVLLSSFCVFCF----SSPDGFLSLSCGG-----SSYT-AAYNISWVS
AT1G51790/1-518     1 --MMTSKAKALTF--ICCVALLNLAIA-QDQSGFISIDCGLQPEN-SSYTETSTDIKYVS
IOS1/1-513          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 --MERSLELLLL--IRTLAIIHISQA-QSQQGFISLDCGLPANEPSPYTEPRTGLQFSS
AT5G59650/1-509     1 --MDSPCWLLLL--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
ShRK1/1-533        49 DSEIIKQGKPVTL------ANTNWNSMQYRRRRDFPTDNKKYCYRLSTKERR--RYIVRT
ShRK2/1-529        50 DNHLI-YGETANI------SSVNETRTQYTTLRHFPADSRKYCYTLNVTSRN--RYLIRA
AT5G48740/1-492    42 DNDYIETGNTTTVTYAEGNSTSSV------PIRLFPDPQGRQCYKLPVRKDLS-SVLIRA
AT1G51790/1-518    55 DSSYTDTGTSYFV---APENRQNM-KQSMWSVRSFP-EGIRNCYTIAVNSST--KYLIRA
IOS1/1-513         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
ShRK1/1-533       101 TFLYGGLG-SEEAYPKFQLYLDATKWATVT-----IQ----EVSRVYVEELIVRATSSYV
ShRK2/1-529       101 TFLYGNFDNSNNVYPKFDISLGATHWATIV-----IS----ETYIIETAELVFLASSPTV
AT5G48740/1-492    95 TFVYRNYD-SQNSPPAFHVSLGRRITSTVD-----LR-----TNDPWIEELVWPVNNDSL
AT1G51790/1-518   108 DFMYGNYD-SRNEIPGFDLHLGPNKWDTVELVSPLQT---------VSKEIIYYVLTDTI
IOS1/1-513        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
ShRK1/1-533       151 DVCVCC-AITGSPFMSTLELRPLNLSMYATDY-EDNFFLKVAARVNFGAPN----MDALR
ShRK2/1-529       152 SVCLSN-ATTGQPFISTLELRQLSGSMYGSMLSEDRFYLSVAARINFGAES----EASVR
AT5G48740/1-492   144 LLCLLAVKGRGIPVISSLEVRPLPLGSYKYSLEG---SPDIILRRSYRINSGYT-NGTIR
AT1G51790/1-518   158 QVCLVN-TGNGTPFISVLELRQLPNSSYAAQS-E---SLQLFQRLDFGSTT----NLTVR
IOS1/1-513        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
ShRK1/1-533       205 YPDDPYDRIWESDINKRPNYLVGVAPGTTRINTSKTINT---LTREYPPMKVMQTAVVGT
ShRK2/1-529       207 YPDDPYDRIWESDLQKKPNYLVDVAAGTVRVSTTLPIES---RVDDRPPQKVMQTAVVGT
AT5G48740/1-492   200 YPSDPFDRIWDPDQSYSPFHASWSFNGLTKLN--------SFNITENPPASVLKTARILA
AT1G51790/1-518   209 YPNDVFDRIWFP-------ATPNGTKPLSDPSTSLTSNS-TGN--FRLPQVVMRTGIVPD
IOS1/1-513        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------------
ShRK1/1-533       262 QGL------ISYRLNLEDFPANARAYAYFAEIE--ELGANETRKFKLVQPYFPDYSNAVV
ShRK2/1-529       264 NGS------LTYRMNLDGFPGFGWAFTYFAEIE--DLAEDESRKFRLVLPEQPEYSKSVV
AT5G48740/1-492   252 RKE-S----LSYTLSLHTPGD-YYIILYFAGIL--SL----SPSFSVT------------
AT1G51790/1-518   259 NPR-GF---VDFGWIPDDPSLEFFFYLYFTELQQPNSGTVETREFVIL------------
IOS1/1-513        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
ShRK1/1-533       314 NIAENANGSYTLYEPSYMNVTLDFVLTF-----------------SFGKTKDS-TQGPLL
ShRK2/1-529       316 NIKENTQRPYRVYAPGYPNITLPFVLNF-----------------RFAKTADS-SRGPIL
AT5G48740/1-492   288 -INDEVK--QSDYTVTSSEAGTLYFTQKG--------------ISKLNITLRKIKFNPQV
AT1G51790/1-518   303 -LNGKSF--GEPLSLNYFRTLALFTSNPL--------KAESFQF-SLRQTQSS-SLPPLI
IOS1/1-513        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
ShRK1/1-533       356 NAIEISKYLPISVKTDRS-DVSVLDAIRSMS---PDSDWASEGGDPCIPVLWSW--VNCS
ShRK2/1-529       358 NAMEISKYLR---KSDGSVDATVMANVASLY---SSTEWAQEGGDPCSPSPWSW--VQCN
AT5G48740/1-492   331 SALEVYEILQIPPE-ASSTTVSALKVIEQFTG--QDLGWQ---DDPCTP--LPWNHIECE
AT1G51790/1-518   350 NAMETYFVNKLPQSSTDPNDLSAMRNIKSAYKV-KR-NWE---GDVCVPQAYTWEGLNCS
IOS1/1-513        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
ShRK1/1-533        410 ---STSPPRVTKIALSRKNLRGEIPPGINYMEALTELWLDDNELTG-TLPDM-SKLVNLK
ShRK2/1-529        410 ---SDPQPRVVAIKLSSMNLTGNIPSDLVKLTGLVELWLDGNSFTG-PIPDF-SRCPNLE
AT5G48740/1-492    383 ------GNRVTSLDLHNTSLTGAIQSELEDLVNLEVLDLQNNSLQG-SVPETLGKLKKLR
AT1G51790/1-518    405 -FNGTNMPRVIALNLSSAGLTGEITSDISRLSQLQILDLSNNNLSGPAVPAFLAQLQFLR
IOS1/1-513         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-
ShRK1/1-533       465 IMHLENN-QLS-GSLPPYLAHL---PNLQELSIENNSFKGKIPSALLKGKVLFKYNNNPE
ShRK2/1-529       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
IOS1/1-513        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----------
ShRK1/1-533       520 LQNEAQRKHFWQIL—
ShRK2/1-529       519 LEKSGDKGKKL-----
AT5G48740/1-492   477 SSTIDTPQVTIPINKK
AT1G51790/1-518   503 SQNRSKKNKLPSFVIP
IOS1/1-513        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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