Title

Organ-specific NLR resistance gene expression varies with plant symbiotic status

Authors

David Munch[1], Vikas Gupta[1], Asger Bachmann[1], Wolfgang Busch[2], Simon Kelly[1], Terry Mun[1], and Stig Uggerhøj Andersen[1*]

Affiliations

1: Department of Molecular Biology and Genetics, Aarhus University, Gustav Wieds Vej 10, 8000 Aarhus C, Denmark.

2: Salk Institute for Biological Studies, Plant Molecular and Cellular Biology Laboratory, 10010 N Torrey Pines Rd, La Jolla, CA 92037, USA

Abbreviations

Arabidopsis, AM, CC, CNL, ETI, *Lotus*, LRR, MAMP, *Medicago*, NB-ARC, NBD, NF, NLR, PRRs, RNL, TIR, TNL, XNL.

Author for correspondence

Stig Uggerhøj Andersen, sua@mbg.au.dk.

Keywords

Resistance genes, NB-LRR, NLR, symbiosis, plant immunity, *Brassicaceae*, *Arabidopsis*

Abstract

Nucleotide-binding site leucine-rich repeat resistance genes (NLRs) allow plants to detect microbial effectors. We hypothesized that NLR expression patterns would reflect organ-specific differences in effector challenge and tested this by carrying out a meta-analysis of expression data for 1,235 NLRs from 9 plant species. We found stable NLR root/shoot expression ratios within species, suggesting organ-specific hardwiring of NLR expression patterns in anticipation of distinct challenges. Most monocot and dicot plant species preferentially expressed NLRs in roots. In contrast, *Brassicaceae* species, including oilseed rape and the model plant *Arabidopsis thaliana*, were unique in showing NLR expression skewed towards the shoot across multiple phylogenetically distinct groups of NLRs. The *Brassicaceae* NLR expression shift coincides with loss of the endomycorrhization pathway, which enables intracellular root infection by symbionts. We propose that its loss offer two likely explanations for the unusual *Brassicaceae* NLR expression pattern: loss of NLR-guarded symbiotic components and elimination of constraints on general root defences associated with exempting symbionts from targeting. This hypothesis is consistent with the existence of *Brassicaceae*-specific receptors for conserved microbial molecules and suggests that *Brassicaceae* species are rich sources of unique antimicrobial root defences.

Introduction

The sessile nature of vascular plants has spurred development of mechanisms for coping with biotic and abiotic stresses and for optimizing uptake of inorganic compounds under low nutrient availability. In response to these challenges, plant roots and shoots have evolved specialized functions above and below ground, where they have also adapted to interact with the distinct microbial communities of the phyllo- or rhizosphere. These diverse plant-microbe interactions range from symbiosis over parasitism to pathogenic infection (Bulgarelli et al. 2013; Fatima et al. 2015; Vandenkoornhuyse et al. 2015).

Reflecting the different characteristics of plant roots and shoots, distinct host-microbe combinations have been used to unravel the molecular components required for trans-species interaction and communication. In plant shoots, the focus has almost exclusively been on pathogenic interactions, where work in the model plant *Arabidopsis thaliana* (*Arabidopsis*) from the Brassicaceae family has provided great insight into plant immunity (Jones and Dangl 2006; Nishimura et al. 2010). Passive defences, such as the waxy cuticle on epidermal cells, cell walls and preformed anti-microbial chemicals form the first barriers for microbes and are often sufficient for deterring would-be pathogens (Thordal-Christensen 2003). Microbes that successfully evade these obstacles encounter a large repertoire of resistance (R) proteins in the form of trans-membrane receptor-like proteins and receptor-like kinases on the surface of plant cells, which recognize conserved microbe-associated molecular patterns (MAMPs). Upon activation, these pattern-recognition receptors (PRRs) trigger complex intracellular signalling cascades, such as phytohormone perturbations, accumulation of ions, mitogen-activated protein kinase activation and production of reactive oxygen species, ultimately leading to transcriptional and translational changes that promote the production of defence compounds (Pel et al. 2012; Muthamilarasan et al. 2013).

To escape this MAMP-triggered immunity (MTI), microbes have evolved effectors that are injected into the plant cell cytoplasm using specialized secretion systems that penetrate the plant cell

membrane. Upon translocation, these effectors target components of the defence machinery, suppressing immune signalling and gene expression through degradation, allosteric or covalent modification of host molecules, thus adapting the local environment to be more suitable for microbial growth and improving the chances of successful tissue colonization (Jones and Dangl 2006; Xin et al. 2013; Le Fevre et al. 2015). In response, plant cells employ a family of intracellular R proteins that recognize effectors either by direct interaction, or indirectly through detection of modifications made to host proteins (Khan et al. 2015). Effector-triggered immunity (ETI) activation by an intracellular R protein leads to a stronger immune response than that of MTI and is often associated with localized cell death to limit the spread of biotrophic pathogens (Jones and Dangl 2006; Hofius et al. 2007).

The majority of intracellular R proteins share a similar structure with an amino-terminal signalling domain, followed by a highly conserved nucleotide binding domain (NBD) and a carboxy-terminal leucine-rich repeat (LRR) domain of variable length (van der Biezen et al. 1998; Takken et al. 2012). This class of R proteins are referred to as nucleotide-binding site leucine-rich repeat (NLR) proteins. The NBD domain class is shared by Apaf1, plant R proteins and CED4 (NB-ARC) and is highly conserved among all NLR proteins. It acts as a molecular switch, and cycles between active ATP-bound and inactive ADP-bound states depending on the activity of the LRR domain. The LRR domain is believed to be directly involved in protein-protein interactions with microbial effectors or host proteins and to function by auto-suppressing the NBD domain of the NLR (Jones and Jones 1997; Takken et al. 2006; Marquenet et al. 2007; Lukasik et al. 2009; Takken et al. 2012). The amino-terminal signalling domain is generally divided into two separate classes based on homology to either the signalling domain of Toll/Interleukin-1 Receptors (TIR) or the presence of a coiled-coil (CC) domain. These two distinct signalling components share common downstream signalling pathways, however both classes have also been observed to activate separate downstream components (Aarts et al. 1998; Falk et al. 1999; Meyers et al. 1999; Pan et al. 2000; Takken et al. 2006; Hofius et al. 2009). While both CC and TIR type NLRs (CNLs and TNLs, respectively) are widely distributed in dicots,

canonical TNLs appear to be absent in monocots (Meyers et al. 1999; Pan et al. 2000; Meyers et al. 2002; Tarr et al. 2009). In addition, variations of the signalling domain-NBD-LRR (NLR) structure can be found in most plant species, with NBD-containing proteins lacking either the amino-terminal signalling domain or the carboxy-terminal LRR domain, or having juxtaposed non-canonical domains, extending their flexibility as signalling components or effector decoys for host proteins (Bonardi et al. 2012; Kroj et al. 2016).

Whilst many NLRs play important roles in *Arabidopsis* shoot immunity, little is known about how *Arabidopsis* roots mount immune response against microbes, or what role NLRs play. However, the PRR FLAGELLIN-SENSITIVE2 is fully functional in roots and activates similar downstream MAP-kinase cascades in both root and shoot (Millet et al. 2010). There are reported differences between roots and shoots for the phytohormone salicylic acid, which is considered a requirement for basal defence in leaves against biotrophic pathogens, but does not appear to be as important in root immune responses (Jones and Dangl 2006; Millet et al. 2010).

Unlike the work on *Arabidopsis* pathogen responses, studies of root-microbe interactions have focused on endosymbiosis. Up to 90% of all terrestrial plants are believed to associate with arbuscular mycorrhizal (AM) fungi to enhance their acquisition of phosphorus and other nutrients. Plant associations with nitrogen-fixing bacteria contained within nodules is restricted to around 10 families, including the agriculturally important Fabaceae (legume) family (Doyle 1998; Gualtieri et al. 2000; Parniske 2008). *Arabidopsis* belongs to the *Brassicaceae* family which is one of the few plant families that has lost the capacity for root endosymbiosis with mycorrhizal fungi that is ancestral to the Angiospermae (flowering plants) (Gualtieri et al. 2000; Smith et al. 2010; Delaux et al. 2014). Two model plants from the legume family, *Lotus japonicus* (*Lotus*) and *Medicago truncatula* (*Medicago*), have been extensively studied for unravelling the genetic pathways required for root nodulation through their symbiotic association with gram-negative soil bacteria collectively referred to as rhizobia (Barker et al. 1990; Handberg and Stougaard 1992). This work has led to the discovery of

nodulation factors (NF), a key signal molecule secreted by rhizobia, and several host receptors that perceive and transduce the signal through regulatory components to modulate downstream transcriptional regulation and coordinate nodule organogenesis and infection of these by nitrogen-fixing rhizobia (Long 1989; Schauser et al. 1999; Limpens et al. 2003; E. B. Madsen et al. 2003; Radutoiu et al. 2003; Lévy et al. 2004; Kalo et al. 2005; Smit et al. 2005; Tirichine et al. 2006; Kouchi et al. 2010; Madsen et al. 2010). Similar to NF produced by rhizobia, AM fungi secrete Myc factors to activate symbiotic signalling in the host. Despite their distinct phenotypic characteristics, AM and nodulation pathways share conserved genetic components, likely owing to their common evolutionary origin (Oldroyd and Downie 2006; Parniske 2008; Banba et al. 2008; Singh and Parniske 2012; Guillotin, Couzigou, and Combier 2016).

Despite the history of focusing on pathogenic plant-microbe interactions in plant shoots and on symbiotic interactions in roots, both organs are prone to pathogen infection and would presumably be protected by NLR proteins present in cells subject to effector challenge. Currently, little is known about the expression characteristics of NLRs and, unless they are ubiquitously expressed across all plant organs, NLR gene expression patterns could provide indications about differences in pathogen effector pressures between plant tissues and across plant species. Here we present a meta-analysis of NLR gene expression data, including plant species with and without the capacity for mycorrhizal and/or root nodule symbiosis. The analysis revealed stable root to shoot NLR gene expression ratios within species, with all of the endomycorrhizal plant species examined predominantly expressing NLRs in roots. In contrast, large differences were found between species, with the Brassicaceae family displaying an aberrant shoot-skewed expression, which suggested an unusual mode of plant-microbe interaction for this plant family.

Results

NLR gene expression varies between tissues in a species-specific manner

Individual plant organs have evolved to function in specific environments, where they interact with distinct microbiota (Vandenkoornhuyse et al. 2015). To investigate if NLR expression patterns reflected these tissue differences we identified all putative NLRs in *Lotus* and *Arabidopsis*, where expression atlas data was available for multiple tissues (Schmid et al. 2005; Høgslund et al. 2009; Verdier et al. 2013) (**Supplemental table 1-2 and Supplemental file 1**). We then examined the available expression data and identified genes predominantly expressed in reproductive, shoot, root or root nodule tissues. NLR expression in *Lotus* shoot and nodule tissues did not show significant differences compared to overall gene expression, but reproductive tissues showed strong depletion of NLR expression and *Lotus* roots displayed a significant enrichment of NLR expression (**Figure 1A-B**). For *Arabidopsis*, reproductive tissues also showed a significant depletion of expressed NLR genes, but *Arabidopsis* roots did not show enriched NLR gene expression. Instead, *Arabidopsis* shoots displayed a significant enrichment of NLR gene expression (**Figure 1C-D**).

To investigate if the contrasting root/shoot NLR gene expression ratios were general for the two species, we examined additional data sets. For *Arabidopsis*, we quantified NLR root/shoot expression ratios based on two recent RNA-seq experiments including both root and shoot samples in the same experimental series (van Veen et al. 2016; Liu et al. 2016). Both RNA-seq data sets showed a clear shoot skew for *Arabidopsis* NLRs relative to the average expression ratio for all genes (**Figure 1E**), and the NLR expression ratios were strongly correlated across array and RNA-seq experiments (**Figure 1F**). Since no equivalent data sets were available for *Lotus*, we carried out an RNA-seq experiment including mock and rhizobium inoculated root and shoot samples. For *Lotus*, the RNA-seq data was also consistent with the array data in showing a pronounced root skewed NLR expression (**Figure 1G-H**). Since bacterial inoculation could potentially influence NLR root/shoot expression ratios, we compared *Lotus* inoculated and uninoculated samples, but found no significant differences

in the root/shoot NLR expression ratios for neither the array nor the RNA-seq experiment (**Supplemental figure 1**). NLR root/shoot expression ratios thus showed clear differences between *Lotus* and *Arabidopsis*, and these differences were consistent across independent experiments carried out using either array or RNA-seq methodology for transcript quantification, indicating that regulation of NLR gene expression varied between organs in a species-specific manner.

The *Brassicaceae* family shows aberrant shoot-skewed NLR gene expression

To determine which of these contrasting patterns of NLR gene expression was predominant among flowering plants, we analysed additional species for which root and shoot tissues had been subjected to global expression profiling in the same experiment. These included three legume species (*Medicago*, *Glycine max*, *Lupinus albus*), two *Brassicaceae* family members (*Brassica rapa* ssp. *pekinensis*, *Brassica napus*) and two monocots (*Zea mays, Oryza sativa*) (**Figure 1I and Supplemental figure 2**). We calculated root/shoot expression ratios for whole transcriptomes, including only samples where root and shoot tissues had been analysed in the same experimental series (**Supplemental tables 1-2**). We identified a total of 2,167 NLR genes across the selected species, and expression data was available for 1,235 out of the 2,167 NLRs (**Supplemental table 3**).

Like *Lotus*, the three other dicot legumes and the two monocots displayed NLR gene expression skewed towards the root when compared to the overall gene expression pattern (**Figure 1I, Supplemental figure 2 and Supplemental table 4**). In comparison, the three *Brassicaceae* species stood out by displaying shoot-skewed NLR gene expression (**Figure 1I and Supplemental table 4)**. Comparisons within either the legume, *Brassicaceae* or monocot groups did not show any statistically significant differences. However, when we compared between species groups, many comparisons showed significant differences, with the differences between *Brassicaceae* versus both legumes and monocots highly significant (**Figure 1I and Supplemental table 5**). Among the flowering plants investigated, shoot-skewed expression of NLR genes was a feature exclusive to the dicot *Brassicaceae* family, while the remaining monocots and dicot species all displayed root-skewed expression.

The *Brassicaceae* expression shift is seen across multiple NLR clades

We speculated if the *Brassicaceae* expression shift could have been caused by the loss of a specialized set of phylogenetically related NLRs evolved specifically to guard the root endosymbiotic machinery or other root specific pathways. To test this hypothesis, we categorized all identified NLRs by aligning their NBDs and constructing a phylogenetic tree based on 2,033 sequences (**Figure 2A)**. In addition to the previously mentioned species, we included the carnivorous and submerged aquatic bladderwort *Utricularia gibba* from the Asterids clade, which lacks a true root (Ibarra-Laclette et al. 2013). The phylogenetic analysis allowed us to identify five well-supported major NLR clades (**Figure 2B, Supplemental file 2, and Supplemental table 7**). We also categorized the NLRs based on the presence of TIR, CC or CC_R amino terminal signalling domains (Xiao et al. 2001; Meyers et al. 2003; Shao et al. 2016) and compared these results to our phylogenetic analysis (**Supplemental figure 3 and Supplemental table 6**). Hereafter, we refer to NLRs containing TIR, CC and CC_R domains as TNLs, CNLs and RNLs, respectively. NLRs containing neither of the three described domains are referred to as XNLs. Clade 1 was highly enriched in TNLs (708/806), CNLs dominated clade 2 (307/510) and clade 4 (326/385), clade 5 was enriched for RNLs (62/87), and clade 3 contained mainly XNLs (211/245) (**Supplemental table 7**). The clear correlation between domain structure and the NBD-based phylogeny indicated that the NBD sequences contained sufficient information for inferring the evolutionary history of the plant NLR family, as previously suggested (Pan et al. 2000).

In accordance with previous studies, we did not observe any sequences from monocots in the TNL-enriched clade 1, but among all sequences analysed we did find 7 monocot NLRs that had an identifiable TIR-like domain, which has previously been observed to be juxtaposed irregularly compared to the normal TIR domain (Meyers et al. 2002; Caplan et al. 2013). We did not recover any TIR or TIR-related domain containing NLR sequences from *U. gibba* either, despite it being a dicot

(Pan et al. 2000; Fluhr 2001; Tarr et al. 2009; Ibarra-Laclette et al. 2013). In fact, *U. gibba* sequences were only found in clades 2 and 4 (**Figure 2B and Supplemental table 7**).

We then plotted NLR root/shoot ratios for the five NLR clades. Across data from all species, we observed highly significant root skews for the CNL-enriched clade 2 and for the RNL-enriched clade 5 (**Figure 2C and Supplemental table 8**). When examining the *Brassicaceae*, legume and monocot species groups separately, we found significant shoot skews for *Brassicaceae* clades 2, 3 and 4, and significant root skews for monocot clades 2 and 4 and for all legume NLR clades. The mean *Brassicaceae* root/shoot expression ratios deviated significantly from those of legumes for clades 1-4, and from monocots for clades 3 and 4 (**Figure 2D and Supplemental table 8**). In contrast, we did not find significant deviations between *Brassicaceae* and legumes for the RNL-enriched clade 5, where both species groups showed root-skewed expression. Since we observed significant *Brassicaceae* deviations for multiple NLR clades, a monophyletic group of NLRs was not responsible for the *Brassicaceae* expression shift. However, there were differences between the NLR clades in the severity of the shift, with the smallest effect seen for the TNL-enriched clade 1.

Comparing the species tree (**Figure 2A**) to the NBD-based NLR tree (**Figure 2B**), we noted that clade 1 and 5 in the NBD tree contained mainly dicot members, whereas clades 3 and 4 comprised monocot and dicot members from all species, in line with the species tree. In contrast, clade 2 from the NBD-tree was depleted in dicot *Brassicaceae* members, while both legume and monocot members were well-represented, indicating a family-specific depletion of a major NLR clade in the *Brassicaceae* family (**Figure 2B and Supplemental table 7**).

NLR Clade 2 depletion is not generally associated with loss of mycorrhization

Although the NLR clade 2 depletion observed in the *Brassicaceae* family (**Supplemental table 7**) could not explain the *Brassicaceae* expression shift, it remained possible that NLR clade 2 would generally be depleted across non-mycorrhizal plants, pointing to a potentially specialized function in guarding the endomycorrhizal signalling machinery. To test this hypothesis, we identified and

extracted NLR protein sequences from 8 additional non-mycorrhizal plant species and constructed a new phylogenetic tree containing a total of 2,448 NLR sequences (**Figure 3A-B, Supplemental table 9, and Supplemental file 3**). We found that 120 out of the 415 new NLR sequences were present in clade 2, leading us to reject our hypothesis that this clade had evolved specifically for guarding root endosymbiotic symbiotic components (**Figure 3C and Supplemental table 9**). After including three additional *Brassicaceae* species, we still observed a pronounced family-specific *Brassicaceae* depletion in clade 2, as we only found 20 out of 544 *Brassicaceae* NLRs belonging to this family (**Supplemental table 9**). In conclusion, NLR clade 2 depletion is likely *Brassicaceae* family specific and is not generally associated with loss of the endomycorrhizal pathway.

Discussion

Cytoplasmic NLRs make up the last line of defence against potentially pathogenic microbes that have evaded physical barriers and membrane-localized PRRs to successfully deliver effectors into plant cells. The stable root/shoot NLR expression ratios observed here are consistent with a defence system in which NLR expression patterns are hardwired to match organ-specific effector challenges, in anticipation of microbial challenge, similar to that observed for the plant circadian cycle (Ingle 2011; Wang et al. 2011). Indeed, we also found that rhizobium inoculation of the nodulating legume *Lotus* did not alter the overall pattern of NLR expression, further underlining the stability within species of NLR root/shoot expression ratios. It was striking that we found an overall root-skew in NLR expression in the majority of plant species. This suggested that roots generally experience a higher level of effector pressure than shoots, despite the fact that NLR function has mainly been characterized in the context of shoot-pathogen interactions (Erb et al. 2009; Nishimura and Dangl 2010). It might not be surprising given the complexity of soil microbial communities, but our data does underline the need for establishing new root pathosystems and for understanding the role of NLRs in root-microbe interactions.

Plants from the *Brassicaceae* family made up a very conspicuous group of outliers that displayed shoot- rather than root-skewed NLR expression. The *Brassicaceae* are also outliers in the sense that they have lost the capacity for root endomycorrhization, which remains functional in 80-90% of land plants (Parniske 2008; Delaux et al. 2014). This symbiotic interaction between plant roots and arbuscular mycorrhizal fungi has existed for around 400 million years, coinciding with the appearance of terrestrial plants, and parts of the mycorrhization signalling machinery have been recruited in the ~110 million year old symbiotic interaction between plants and nitrogen fixing rhizobia (Parniske 2000; Deguchi et al. 2007). NLRs are also found in early land plant species, such as Bryophytes and lycophytes (Xue et al. 2012; Yue et al. 2012; Jacob et al. 2013; Tanigaki et al. 2014), meaning that endomycorrhizal signalling has co-evolved with NLRs through hundreds of millions of years.

It is conceivable that a specialized set of phylogenetically related NLRs could have evolved specifically to guard the root endosymbiotic machinery or other root specific pathways, and that the *Brassicaceae* NLR expression shift might be caused by the loss of such a group of NLRs. Here, we tested this hypothesis by grouping NLRs according to the sequence homology of their NBD domains, identifying five major clades. While the CNL-enriched NLR clade 2 was strongly depleted in the *Brassicaceae*, it was well-represented in other non-mycorrhizal plants. In addition, we observed a *Brassicaceae* shoot skew for all NLR clades, with the smallest shift observed for TNLs, which are absent in the endomycorrhizal monocots rice and maize, and therefore cannot be generally required for protecting the endomycorrhizal signalling machinery. The shift in *Brassicacae* NLR expression could thus not be attributed to the loss of a single NLR clade, and our data did not support the existence of a specific group of phylogenetically distinct NLRs guarding the root endosymbiotic machinery.

The general expression shift towards the shoot across four major NLR clades suggests a reduced anticipation of effector challenge to root cells relative to shoot cells in the *Brassicaceae*. We envisage two scenarios, which are not mutually exclusive, that could account for the shift. First, our data is consistent with a model where NLRs were randomly recruited from an expanding NLR complement,

regardless of phylogenetic origin, for guarding root specific components. When the guarded pathways became defunct in the *Brassicaceae* family, it gradually lost the associated root-expressed NLRs across the different NLR clades, leading to the overall shoot skew in NLR expression. Second, rather than passively reducing the effector challenge level to roots by loss of a potentially exposed pathway, the *Brassicaceae* could have developed family-specific active measures that efficiently deter putative soil pathogens before they have a chance to deploy their effectors, reducing the requirement for NLR protection. One possibility is that the *Brassicaceae* maintain high levels of antimicrobial glucosinolates in the root apoplasm, and there are indications that root have higher constitutive glucosinolate levels than shoots (Van Dam, Tytgat, and Kirkegaard 2009). Another is that the *Brassicaceae* have evolved a unique set of highly efficient pattern recognition receptors that quickly eliminate putative root pathogens. For instance, the Ef-Tu and lipopolysaccharide PRRs are thought to be *Brassicaceae*-specific (Kunze et al. 2004; Ranf et al. 2015).

The root endosymbiosis signalling pathway allows intracellular accommodation of symbiotic mycorrhizal fungi and rhizobia (Madsen et al. 2010; Oldroyd 2013). This could impose severe constraints on the general defence mechanisms employed in roots of plant species that rely on symbiotic interactions for nutrient acquisition, compelling these symbiotic species to depend to a greater extent on NLR effector recognition in roots. We propose that the loss of root endomycorrhizal signalling in the *Brassicaceae* family offers the most parsimonious explanation for the *Brassicaceae* NLR expression shift. Its loss would both have removed a potentially heavily NLR-guarded pathway and eliminated constraints impeding development of more effective general root defence systems. This hypothesis is consistent with both scenarios described above, agrees with the discovery of apparently *Brassicaceae*-specific PRRs (Kunze et al. 2004; Ranf et al. 2015), and suggests that *Brassicaceae*, and perhaps other non-mycorrhizal plants, may be rich sources of unique PRRs and antimicrobial root metabolites.

Materials and methods

Identification of putative NLR genes

To allow identification of putative NLR genes, protein sequences were downloaded as indicated **(Supplemental table 1**). Annotation versions were chosen for compatibility with the available microarray or RNA-seq data to allow subsequent expression analysis. This is why the latest versions were not used in all cases. NLR genes were then identified in a three-step procedure. First, candidate genes were selected using HMMER 3.1b1 (Eddy 2011) based on the NB-ARC PFAM protein domain PF00931. Second, the candidate list was filtered by performing a search for conserved protein domains using CDD (Marchler-Bauer et al. 2011), requiring that the selected putative NLR genes contain, in addition to the NB-ARC domain, either LRR, TIR, PLN00113, PLN03194, or PLN03210 domains. Third, all NLR gene sequences were manually curated to identify and remove false positives. The total number of identified NLR genes in each of the 18 species is shown in **Supplemental table 3,** with sequences available in **Supplemental file 4**.

Lotus RNA-seq

L. japonicus ecotype Gifu (Handberg and Stougaard 1992) seeds were surface sterilized, germinated and grown in conditions as described previously (Kawaharada et al. 2015). Three biological replicates per sample were analyzed with each consisting of 10 seedlings grown on 1/4 B&D plates for 10 days before inoculation of the roots with 750 μL of an *M. loti* R7A suspension (OD_{600} = 0.02) or water. Three days post-inoculation roots and shoots were separated and total RNA was isolated using a NucleoSpin® RNA Plant kit (Machery-Nagel) according to the manufacturer's instructions. RNA quality was assessed with on an Agilent 2100 Bioanalyser and samples were sent to GATC Biotech (http://gatc-biotech.com/) for library preparation and sequencing. Sequencing data have been deposited at the NCBI Short Read Archive with BioProject ID PRJNA384655 and are available for analysis on *Lotus* Base (Mun et al. 2016).

Analysis of NLR gene expression data

For tissues-specific gene expression enrichment analysis (**Figure 1 A-D**), we classified genes as being enriched in a specific tissue group, if the average expression level in a that group was higher than the average of all other tissue groups, and at least two times higher than that of at least one other tissue group.

In order to evaluate root/shoot expression ratios, available expression data was downloaded as indicated in **Supplemental table 2**. Samples IDs along with expression values are available in **Supplemental file 1**. For *Lotus* and *B. rapa*, probes were reassigned to the updated annotation using BLAST to match probe and cDNA sequences (e-value cut-off 0.001), assigning only the best matching probe to a gene. For *Lotus*, *Medicago* and soybean, samples representing identical or closely related plant accessions were used in the analysis. For rice and maize, data from a number of different accessions were used, but only data where both root and shoot samples had been assayed within the same experiment were used to ensure the comparability of samples from the two tissues. For *B. napus*, the analysis was based on raw RNA-seq reads. RNA-seq data files were downloaded from the NCBI short read archive (https://www.ncbi.nlm.nih.gov/sra) and reads from each library were assembled using Trinity (--full_cleanup) (Haas et al. 2013) followed by clustering using cd-hit-est v.4.6.6 (-M 16000 -T 8) (Fu et al. 2012). Next, the longest open reading frames were identified for each transcript and the corresponding protein sequences were used for identification of NLRs as described. Reads were mapped back to the gene set output from cd-hit-est using STAR (--runMode genomeGenerate --genomeChrBinNbits 14) parameters for index generation and standard options for mapping (Dobin et al. 2013). Finally reads mapping to multiple locations were filtered out followed by summarizing read counts per gene for each sample. For all species, expression data from the genes with the 15% lowest expression levels were filtered out, and the log2 NLR root/shoot expression ratios were normalized by subtracting the mean value for all genes. Expression ratios were plotted using ggplot2 in R version 3.1.2.

The significance of differences in mean expression ratios between all genes and NLR genes were evaluated using Student's t-test (**Supplemental table 4**). Next, the significances of interspecies differences in root/shoot expression ratios were evaluated using one-way ANOVA followed by Tukey's multiple comparison test as implemented in GraphPad Prism 6 (**Supplemental table 5**). Differences in the average root/shoot expression by NLR gene clade or domain based on the phylogenetic tree shown in Figure 2B, were evaluated using one-way ANOVA followed by Tukey's multiple comparison test, or Student's t-test, as implemented in GraphPad Prism 6 (**Supplemental tables 6 and 8**).

Construction of NLR protein phylogeny

Sequences of the NB-ARC domains of identified R genes were extracted using a python script, based on domains as identified by the CCD search, and aligned using Clustal Omega v1.2.3 (Sievers et al. 2011). Sequences were then filtered for low coverage positions (50% cut-off) and sequences lacking more than 50% of the aligned NB-ARC domain were removed. Phylogenetic trees were constructed in IQ-Tree v.1.5.2 and evaluated using the ultrafast bootstrap approximation approach (UFBoot) implemented the software package (Minh et al. 2013; L.-T. Nguyen et al. 2015). The resulting tree was colored by species using colorTree v1.1 and visualized using Dendroscope v3.5.7 (Chen et al. 2009; Huson et al. 2012). See **Supplemental files 2 and 3** for NB-ARC domain alignments of the trees described in **Figures 2B and 3B** respectively, along with bootstrap analysis. See **Supplemental file 4** for sequences for all NLRs used to construct the phylogenetic trees, and **Supplemental file 5** for a general overview of all NLRs used in the study.

Author contributions

DM, VG, AB, TM, WB, and SUA analysed data. SK carried out the *Lotus* RNA-seq experiment. SUA designed and supervised the study. DM and SUA wrote the manuscript.

Acknowledgements

This work was supported by the Danish National Research Foundation grant no. DNRF79. The authors wish to acknowledge all research groups contributing expression data used in our meta-analysis.

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Figures and Figure Legends

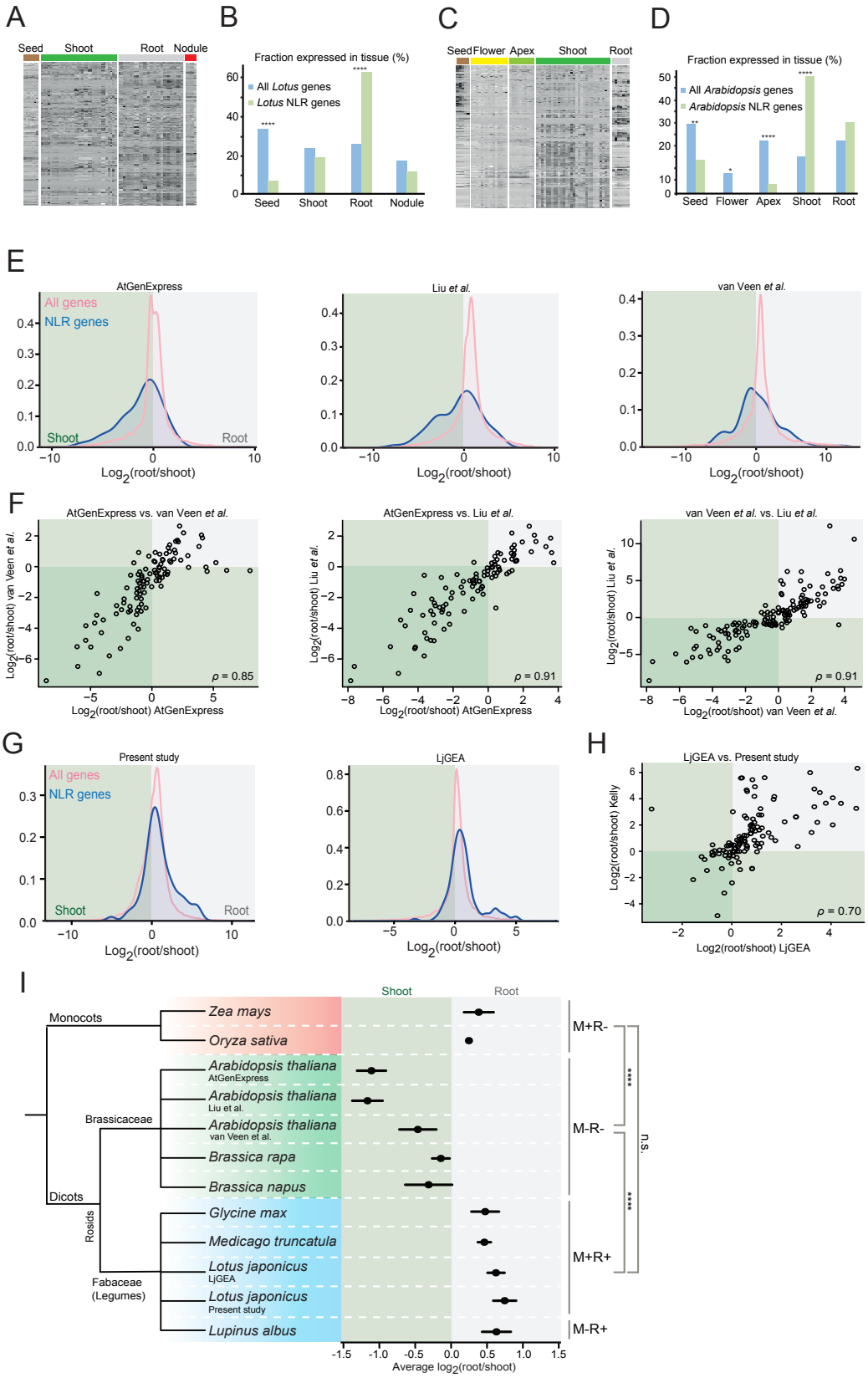


Figure 1. NLR gene expression patterns. A) Expression patterns of 198 putative *Lotus* NLR genes. **B)** Enrichment of *Lotus* NLR genes by tissue type. The fraction of all genes and NLR genes with enriched expression in the given tissue are shown. **C)** Expression patterns of 160 putative *Arabidopsis* NLR genes. **D)** Enrichment of *Arabidopsis* NLR genes by tissue type. The fraction of all genes and NLR genes with enriched expression in the given tissue are shown. *P*-values indicate the probability that the fraction of NLR genes showing enriched expression in a specific tissue is identical to that of all genes. **E)** Density plots displaying the distribution of the logarithm of the root/shoot expression ratios of all genes and NLR genes for each *Arabidopsis* expression data set indicated. See main text for sources. **F)** Root/shoot expression correlations for *Arabidopsis* NLR genes from the three data sets shown in Figure 1E. Each circle represents one NLR gene for which expression data is available in both of the datasets compared. **G)** Density plots displaying the distribution of the logarithm of the root/shoot expression ratios of all genes and NLR genes, for each *Lotus* expression data set indicated. **H)** Root/shoot expression correlations for *Lotus* NLR genes between two datasets. Each circle represents one NLR gene for which expression data is available in both datasets. **I)** Phylogenetic tree of species for which both shoot and root expression data is available, along with their average NLR gene root/shoot expression values (black dots). Error bars indicate SEM. The symbiotic status of each species is indicated on the right; M: Mycorrhiza. R: Rhizobia. +: engages in endosymbiosis. -: does not engage in endosymbiosis. Significance of each species group is indicated on the far right; ****: Significant difference with $p \leq 0.0001$. n.s.: No significance. ANOVA and Tukey's multiple comparison test was used for calculation of *P*-values. See **Supplemental table 5** for *P*-values for inter-group and inter-species differences.

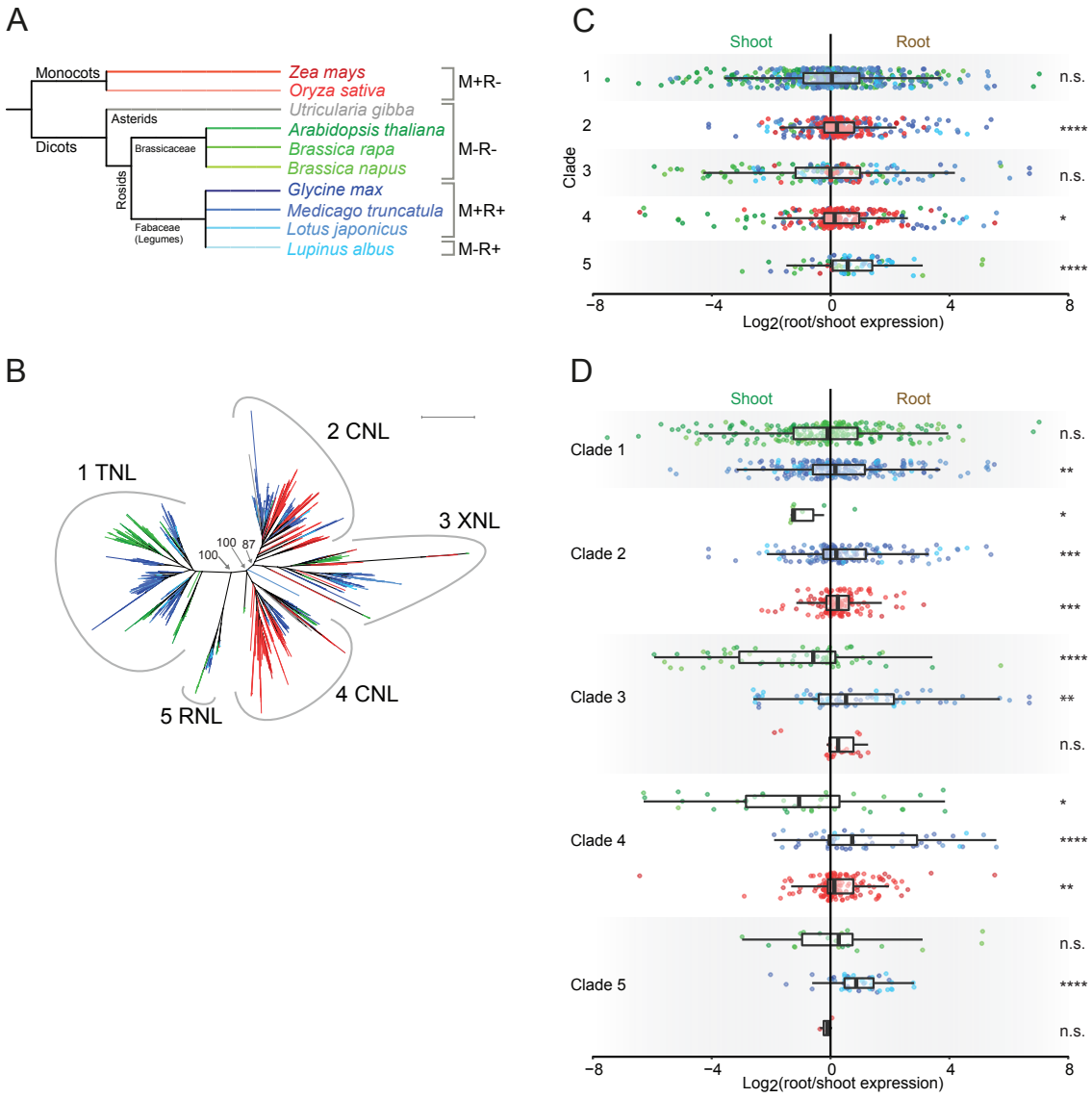
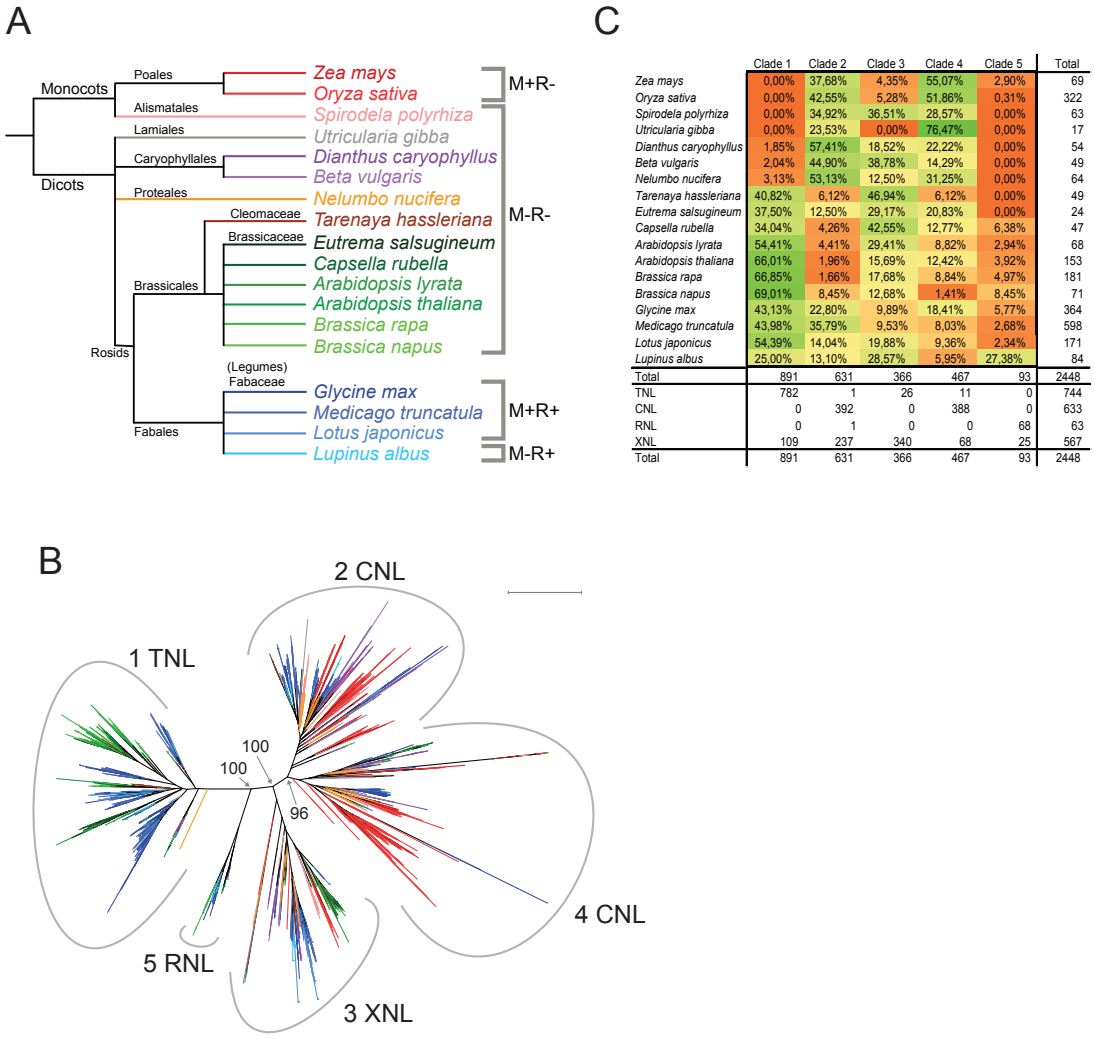


Figure 2. NBD protein expression patterns and sequence phylogeny. Colors indicate the plant species from which the NLR originates, with reference to **Figure 2A**. **A)** Species-level phylogenetic tree. The symbiotic status of each species is indicated on the right; M: Mycorrhiza. R: Rhizobia. +: engages in endosymbiosis. -: does not engage in endosymbiosis. **B)** Phylogenetic tree based on the NBD protein sequence of identified NLR genes in the species indicated in **Figure 2A**. Numbers at branches indicate bootstrap values for the branching of the 5 major clades. Peripheral numbers indicate clade designation, and

NLR designation indicate enrichment of the corresponding NLR type in the given clade. Scale bar indicate 1.0 average amino acid substitutions per site. See **Supplemental File 2** for full bootstrap analysis of the tree. See **Supplemental table 7** for NLR distribution at the clade and species level. **C)** Per clade log2 root/shoot expression ratios of the NLR genes shown in B) for which expression data is available. Each colored dot represents one NLR gene. Box plot bars show median with boxes indicating 25th and 75th percentiles and whiskers indicating 1.5 times the interquartile range. **D)** Same as C) but with expression data separated into groups depending on the species evolutionary descent colored according to **Figure 2A**. See **Supplemental table 8** for *P*-values for inter-clade and inter-species differences.



	Clade 1	Clade 2	Clade 3	Clade 4	Clade 5	Total
Zea mays	0,00%	37,68%	4,35%	55,07%	2,90%	69
Oryza sativa	0,00%	42,55%	5,28%	51,86%	0,31%	322
Spirodela polyrhiza	0,00%	34,92%	36,51%	28,57%	0,00%	63
Utricularia gibba	0,00%	23,53%	0,00%	76,47%	0,00%	17
Dianthus caryophyllus	1,85%	57,41%	18,52%	22,22%	0,00%	54
Beta vulgaris	2,04%	44,90%	38,78%	14,29%	0,00%	49
Nelumbo nucifera	3,13%	53,13%	12,50%	31,25%	0,00%	64
Tarenaya hassleriana	40,82%	6,12%	46,94%	6,12%	0,00%	49
Eutrema salsugineum	37,50%	12,50%	29,17%	20,83%	0,00%	24
Capsella rubella	34,04%	4,26%	42,55%	12,77%	6,38%	47
Arabidopsis lyrata	54,41%	4,41%	29,41%	8,82%	2,94%	68
Arabidopsis thaliana	66,01%	1,96%	15,69%	12,42%	3,92%	153
Brassica rapa	66,85%	1,66%	17,68%	8,84%	4,97%	181
Brassica napus	69,01%	8,45%	12,68%	1,41%	8,45%	71
Glycine max	43,13%	22,80%	9,89%	18,41%	5,77%	364
Medicago truncatula	43,98%	35,79%	9,53%	8,03%	2,68%	598
Lotus japonicus	54,39%	14,04%	19,88%	9,36%	2,34%	171
Lupinus albus	25,00%	13,10%	28,57%	5,95%	27,38%	84
Total	891	631	366	467	93	2448
TNL	782	1	26	11	0	744
CNL	0	392	0	388	0	633
RNL	0	1	0	0	68	63
XNL	109	237	340	68	25	567
Total	891	631	366	467	93	2448

Figure 3. NBD phylogeny including additional non-mycorrhizal species.

A) Species-level phylogenetic tree. The symbiotic status of each species is indicated on the right. M: Mycorrhiza. R: Rhizobia. +: engages in endosymbiosis. -: does not engage in endosymbiosis. **B**) Phylogenetic tree based on the NBD protein sequence of identified NLR genes in the species indicated in **Figure 3A**. Numbers at branches indicate bootstrap values for the branching of the 5 major clades. Peripheral numbers indicate clade designation. Scale bar indicate 1.0 average amino acid substitutions per site. Colors indicate the plant species from which the NBD originates, with reference to **Figure 3A**. See Supplemental File 3 for full bootstrap analysis of the tree. See **Supplemental table 9** for NLR distribution at the clade and species level. **C**) Table showing the percentage of NLRs in each species, with respect to each clade shown in **Figure 3B**.

Supplemental Information

Supplemental figures

Supplemental figure 1. Density plots displaying the distribution of the normalized logarithm of the root/shoot expression ratios of all genes and NLR genes in *Lotus*, from two different data sets, excluding or including inoculated samples.

Supplemental figure 2. Density plots displaying the distribution of the normalized logarithm of the root/shoot expression ratios of all genes and NLR genes for the species and experiments indicated.

Supplemental figure 3. Log root/shoot expression ratios split by NLR domain type.

Supplemental files

Supplemental file 1. Complete set of expression data for all species.

Supplemental file 2. NB-ARC alignment of sequences used to construct the phylogenetic tree in Figure 2B, along with bootstrap analysis and the resulting phylogenetic tree.

Supplemental file 3. NB-ARC alignment of sequences used to construct the phylogenetic tree in Figure 3B, along with bootstrap analysis and the resulting phylogenetic tree.

Supplemental file 4. Full length sequences for all NLRs identified and used in this study.

Supplemental file 5. Full list of NLR genes identified, with domains, designations and normalized $\log_2$ root/shoot expression ratios.

Supplemental tables

Supplemental table 1. Sources of the protein sequences used in the NLR analysis.

Supplemental table 2. Expression data sources used in the NLR analysis.

Supplemental table 3. Number of NLR genes identified through computational analyses for all species where expression analysis was carried out.

Supplemental table 4. Mean root/shoot gene expression ratios for all genes and NLR genes.

Supplemental table 5. Cross-species comparison of normalized $\log_2$ root/shoot NLR expression ratios supporting Figure 1I. ANOVA and Tukey's multiple comparison test was used for calculation of *P*-values.

Supplemental table 6. Cross-species domain comparison of normalized $\log_2$ root/shoot NLR gene expression ratios for Supplemental figure 3. ANOVA and Tukey's multiple comparison test was used for calculation of *P*-values.

Supplemental table 7. Clade distribution of NLR genes for the phylogenetic tree used in Figure 2B, including number of identified NLRs identified with at least one TIR (TNL), CC (CNL) or CC_R (RNL) domain, or none of the former three (XNL), and their clade distribution patterns, with reference to Figure 2B.

Supplemental table 8. Cross-species clade comparison of normalized $\log_2$ root/shoot NLR gene expression ratios for Figure 2C and 2D. ANOVA and Tukey's multiple comparison test was used for calculation of *P*-values.

Supplemental table 9. Clade distribution of NLR genes for the phylogenetic trees used in Figure 3B, including number of identified NLRs identified with at least one TIR (TNL), CC (CNL) or CC_R (RNL) domain, or none of the former three (XNL), and their clade distribution patterns, with reference to Figure 3B.

Supplemental references

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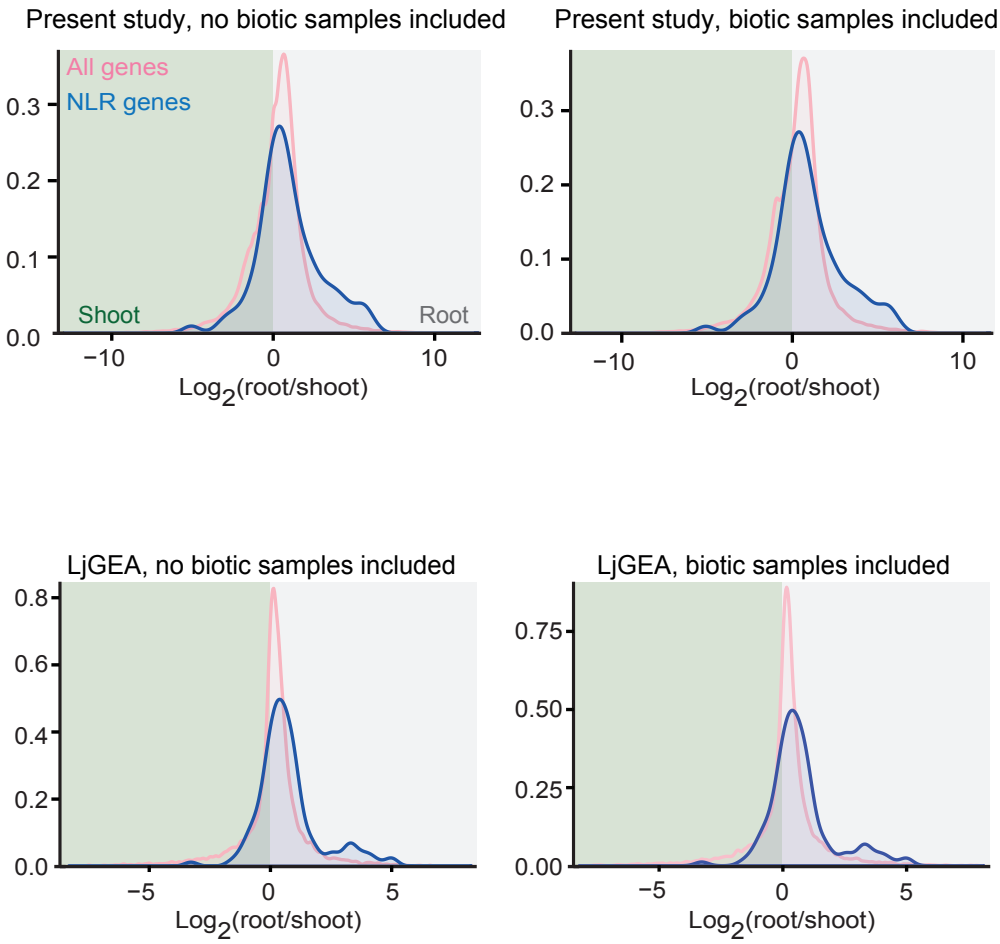
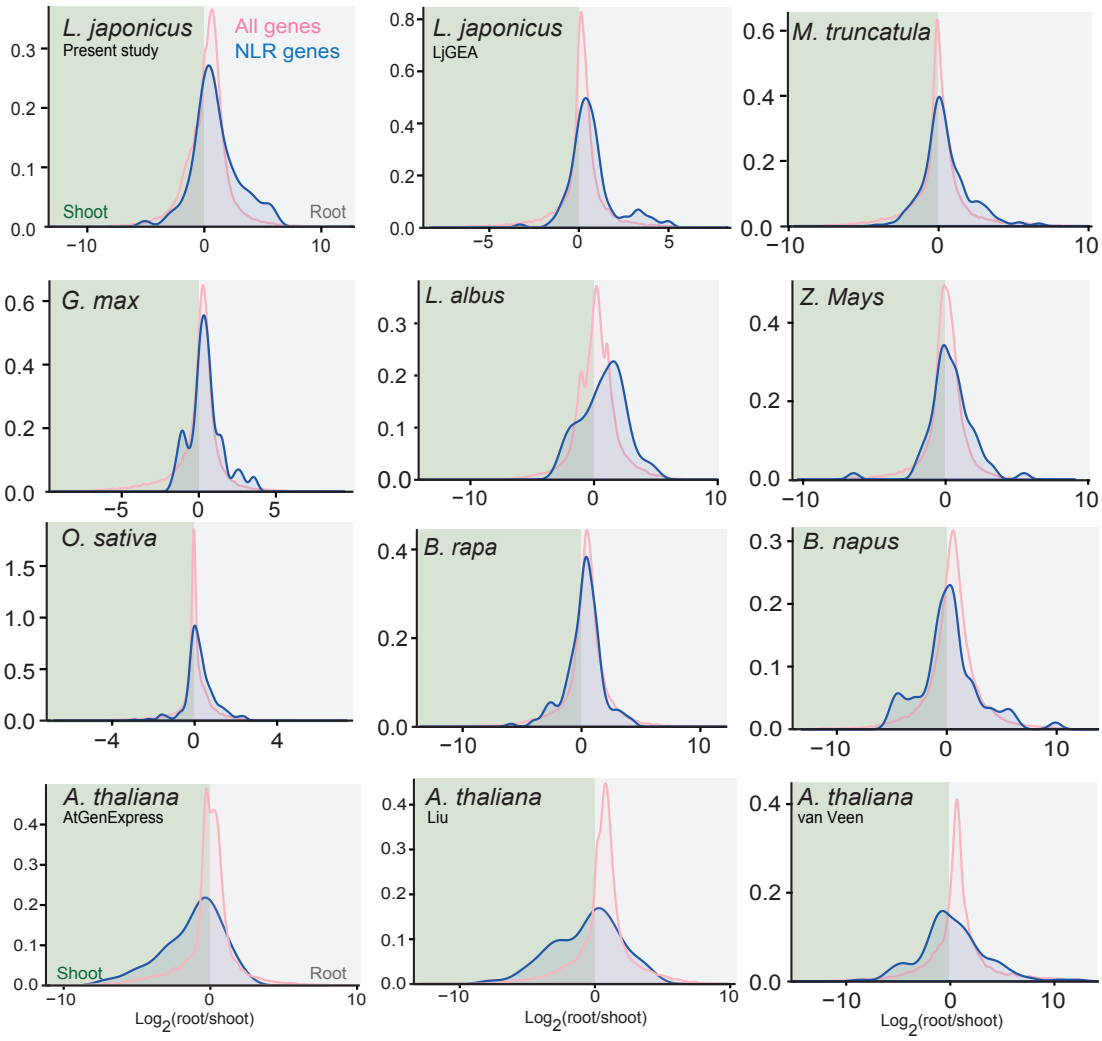
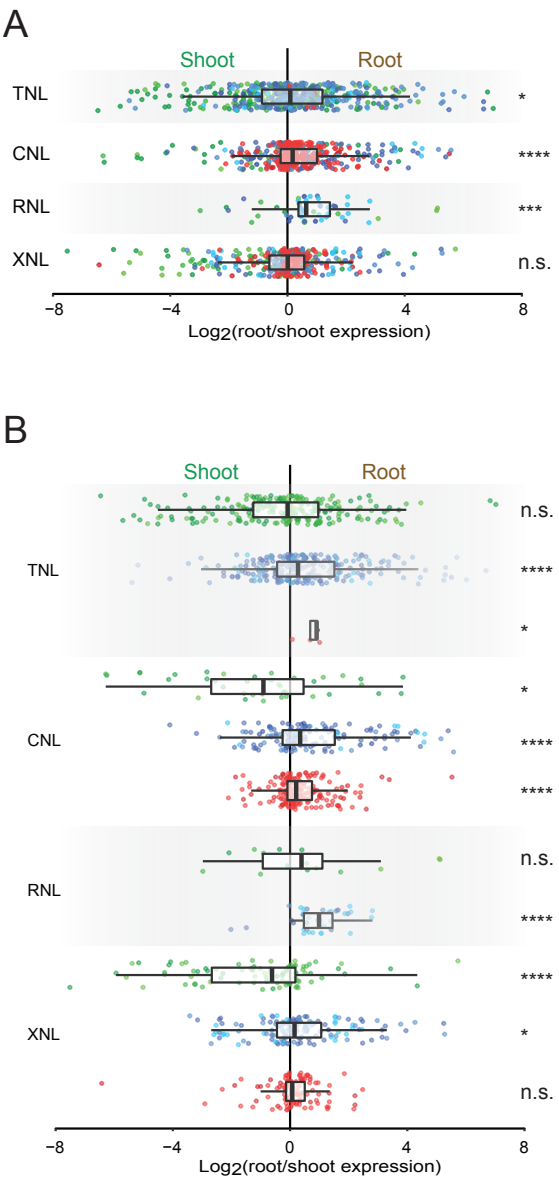
Supplemental figures



Supplemental Figure 1. Density plots displaying the distribution of the logarithm of the root/shoot expression ratios of all genes and NLR genes in Lotus, from two different data sets, excluding or including inoculated samples.



Supplemental figure 2. Density plots displaying the distribution of the logarithm of the root/shoot expression ratios of all genes and NLR genes for the species indicated.



Supplemental figure 3. Colors indicate the plant species from which the NLR originates, with reference to Figure 2A. **A)** Log2 root/shoot expression ratios. Each colored dot represents one NLR gene. Box plot bars show median, with boxes indicating 25th and 75th percentiles and whiskers indicating 1.5 times the interquartile range. **C)** Same as B) but with expression data separated into groups depending on the species evolutionary descent colored according to Figure 2A.

Supplemental tables

Species	Version	Source
A. thaliana	10	ftp://ftp.arabidopsis.org/home/tair/Sequences/blast_datasets/TAIR10_blastsets/TAIR10_pep_20101214_updated
B. napus	-	http://www.ncbi.nlm.nih.gov/sra/SRP028575
B. rapa	1.2	http://www.plantgdb.org/download/Download/xGDB/BrGDB/Brapa_197_peptide.fa.gz
G. max	1.09	http://www.plantgdb.org/download/Download/xGDB/GmGDB/Gmax_109_peptide.fa.gz
L. japonicus	3.0	http://www.kazusa.or.jp/lotus/
L. albus	*	http://lupal.comparative-legumes.org/data/2013/797f6de2ea8102be4b875c165ab5e994/LAGI01_express_annotate.txt
M. truncatula	3.5	ftp://ftp.jcvi.org/pub/data/m_truncatula/Mt3.5/Annotation/Mt3.5v5/Mt3.5v5_GenesProteinSeq_20111014.fa
O. sativa	7.0	ftp://ftp.plantbiology.msu.edu/pub/data/Eukaryotic_Projects/o_sativa/annotation_dbs/pseudomolecules/version_7.0/all.dir/all.pep
Z. mays	3	http://plantgdb.org/download/download.php?dir=/PublicPlantSeq/Dump/Z/Zea_mays
U. gibba	4.1	http://de.iplantcollaborative.org/dl/d/4E6FBB33-2FE7-4404-8548-D3BC14E2CEFC/Utricularia_gibba-ft-CDS-gid-19475-prot.fasta

Supplemental table 1. Sources of the protein sequences used in the NLR analyses. *: The *L. albus* analysis was based on *de novo* assembled transcripts and not on annotated protein coding genes.

Species	Reference	Data type	Source
A. thaliana	Schmid et al., 2005	Microarray	http://www.weigelworld.org/resources/microarray/AtGenExpressand
	Liu et al., 2016	RNA-seq	https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE74856
	van Veen et al., 2016	RNA-seq	http://www.plantphysiol.org/content/172/2/668/suppl/DC1
B. napus	Yong et al., 2014	RNA-seq	http://www.ncbi.nlm.nih.gov/sra/SRP028575
B. rapa	Tong et al., 2013	RNA-seq	http://www.biomedcentral.com/content/Supplemental/1471-2164-14-689-s2.zip
G. max	Dash et al., 2012	Microarray (log)	http://www.plexdb.org/plex.php?database=Soybean
L. japonicus	Hogslund et al., 2009; Verdier et al., 2013	Microarray,	http://ljgea.noble.org/v2/
	Current study	RNA-seq	
L. albus	O'Rourke et al., 2013	RNA-seq, FPKM	http://lupal.comparative-legumes.org/data/2013/797f6de2ea8102be4b875c165ab5e994/LAGI01_express_annotate.txt
M. truncatula	He et al., 2009	Microarray	http://mtgea.noble.org/v3/experiments.php
O. sativa	Dash et al., 2012	Microarray (log)	http://www.plexdb.org/plex.php?database=Rice
Z. mays	Dash et al., 2012	Microarray (log)	http://www.plexdb.org/plex.php?database=Corn

Supplemental table 2. Expression data sources used in the NLR gene analysis. Please refer to the data sources listed for a detailed description of the samples.

	Total number of NLRs	Root and shoot gene expression data available?	NLRs with gene expression data available
Arabidopsis lyrata	70	No	0
Arabidopsis thaliana	160	Yes	141
Beta vulgaris	51	No	0
Brassica napus	75	Yes	70
Brassica rapa	194	Yes	158
Capsella rubella	48	No	0
Dianthus caryophyllus	60	No	0
Eutrema salsugineum	24	No	0
Glycine max	393	Yes	33
Lotus japonicus	198	Yes	162
Lupinus albus	87	Yes	78
Medicago truncatula	628	Yes	292
Nelumbo nucifera	65	No	0
Oryza sativa	340	Yes	241
Spirodela polyrhiza	63	No	0
Tarenaya hassleriana	49	No	0
Utricularia gibba	17	No	0
Zea mays	75	Yes	60
Total	2597	9 out of 18	1235

Supplemental table 3. Number of NLR genes identified through computational analyses for all species. Expression analysis was carried out where root and shoot expression data was available for the indicated number of NLRs with expression data available. The numbers in this table include sequences that were subsequently filtered out as explained in the Materials and Methods section.

Species	Source	Gene category	Mean	STD	SEM	Number of transcripts	Protein coding genes	t-test p-value (All vs. NLR)
A. thaliana	Schmid et al., 2005	NLR	-1,215	2,088	0,202	107	153	< 0,0001
		All	-0,105	1,804	0,013	19203	22591	
	Liu et al., 2016	NLR	-0,578	2,531	0,212	142	153	< 0,0001
		All	0,588	2,048	0,014	22495	28496	
	van Veen et al., 2016	NLR	0,216	3,184	0,258	152	153	0,0484
		All	0,681	2,893	0,017	27776	32679	
B. napus	Yong et al., 2014	NLR	0,206	2,739	0,327	70	71	0,2667
		All	0,520	2,364	0,006	180676	258551	
B. rapa	Love et al., 2010; Tong et al., 2013	NLR	0,205	1,534	0,122	158	180	0,3559
		All	0,348	1,949	0,012	28237	37649	
G. max	Dash et al., 2012	NLR	0,446	1,107	0,193	33	366	0,0862
		All	-0,028	1,586	0,011	20006	21058	
L. japonicus	Hogslund et al., 2009; Verdier et al., 2013	NLR	0,731	1,321	0,119	123	174	< 0,0001
		All	0,106	1,402	0,008	29027	34149	
	Present study	NLR	0,963	2,054	0,161	162	174	< 0,0001
		All	0,209	1,871	0,008	49890	83153	
L. albus*	O'Rourke et al., 2013	NLR	0,767	1,775	0,201	78	83	0,0015
		All	0,136	1,753	0,005	106676	125821	
M. truncatula	He et al., 2009	NLR	-0,011	1,556	0,091	292	592	< 0,0001
		All	0,436	1,768	0,012	20225	23793	
O. sativa	Dash et al., 2012	NLR	0,239	0,750	0,048	241	317	< 0,0001
		All	-0,008	0,975	0,005	32960	38781	
Z. mays	Dash et al., 2012	NLR	0,352	1,613	0,208	60	74	0,0341
		All	-0,033	1,406	0,006	59555	70064	

Supplemental Table 4. Root/shoot mean gene expression ratios for NLR and all genes. Mean: average $\log_2$(root/shoot expression). STD: standard deviation. SEM: Standard error of the mean. N: Number of genes with expression values included in the analysis. *: The *L. albus* analysis was based on *de novo* assembled transcripts and not on annotated protein coding genes.

Tukey's multiple comparisons test	Mean Diff.	95% CI of diff.	Significant	Summary	Adjusted P Value
Lotus, present study vs. Lotus, LjGEA	0,1203	-0,6249 to 0,8656	No	ns	> 0,9999
Lotus, present study vs. Soybean	0,2713	-0,9188 to 1,461	No	ns	0,9999
Lotus, present study vs. Medicago	0,2834	-0,3271 to 0,8939	No	ns	0,9355
Lotus, present study vs. Lupin	0,1142	-0,7446 to 0,9731	No	ns	> 0,9999
Lotus, present study vs. Arabidopsis, van Veen	1,21	0,5062 to 1,914	Yes	****	< 0,0001
Lotus, present study vs. Arabidopsis, Liu	1,911	1,195 to 2,628	Yes	****	< 0,0001
Lotus, present study vs. Arabidopsis, AtGenExpress	1,855	1,079 to 2,632	Yes	****	< 0,0001
Lotus, present study vs. B. rapa	0,8887	0,1919 to 1,585	Yes	**	0,0019
Lotus, present study vs. B. napus	1,059	0,1679 to 1,951	Yes	**	0,0059
Lotus, present study vs. Rice	0,4983	-0,1348 to 1,131	No	ns	0,2942
Lotus, present study vs. Maize	0,3604	-0,5814 to 1,302	No	ns	0,9845
Lotus, LjGEA vs. Soybean	0,151	-1,071 to 1,373	No	ns	> 0,9999
Lotus, LjGEA vs. Medicago	0,163	-0,5068 to 0,8329	No	ns	0,9997
Lotus, LjGEA vs. Lupin	-0,006107	-0,9081 to 0,8959	No	ns	> 0,9999
Lotus, LjGEA vs. Arabidopsis, van Veen	1,09	0,3338 to 1,845	Yes	***	0,0002
Lotus, LjGEA vs. Arabidopsis, Liu	1,791	1,023 to 2,559	Yes	****	< 0,0001
Lotus, LjGEA vs. Arabidopsis, AtGenExpress	1,735	0,9112 to 2,559	Yes	****	< 0,0001
Lotus, LjGEA vs. B. rapa	0,7683	0,01901 to 1,518	Yes	*	0,0386
Lotus, LjGEA vs. B. napus	0,9389	0,005914 to 1,872	Yes	*	0,0469
Lotus, LjGEA vs. Rice	0,378	-0,3126 to 1,069	No	ns	0,8228
Lotus, LjGEA vs. Maize	0,2401	-0,7412 to 1,221	No	ns	0,9997
Soybean vs. Medicago	0,01204	-1,132 to 1,156	No	ns	> 0,9999
Soybean vs. Lupin	-0,1571	-1,451 to 1,137	No	ns	> 0,9999
Soybean vs. Arabidopsis, van Veen	0,9386	-0,2582 to 2,135	No	ns	0,2996
Soybean vs. Arabidopsis, Liu	1,64	0,4357 to 2,844	Yes	***	0,0005
Soybean vs. Arabidopsis, AtGenExpress	1,584	0,3432 to 2,825	Yes	**	0,0018
Soybean vs. B. rapa	0,6174	-0,5754 to 1,810	No	ns	0,8711
Soybean vs. B. napus	0,7879	-0,5280 to 2,104	No	ns	0,7208
Soybean vs. Rice	0,227	-0,9297 to 1,384	No	ns	> 0,9999
Soybean vs. Maize	0,08906	-1,262 to 1,440	No	ns	> 0,9999
Medicago vs. Lupin	-0,1691	-0,9634 to 0,6251	No	ns	> 0,9999
Medicago vs. Arabidopsis, van Veen	0,9265	0,3033 to 1,550	Yes	****	< 0,0001
Medicago vs. Arabidopsis, Liu	1,628	0,9904 to 2,266	Yes	****	< 0,0001
Medicago vs. Arabidopsis, AtGenExpress	1,572	0,8677 to 2,276	Yes	****	< 0,0001

Medicago vs. B. rapa	0,6053	-0,01014 to 1,221	No	ns	0,0589
Medicago vs. B. napus	0,7759	-0,05344 to 1,605	No	ns	0,0924
Medicago vs. Rice	0,215	-0,3274 to 0,7573	No	ns	0,9795
Medicago vs. Maize	0,07702	-0,8063 to 0,9603	No	ns	> 0,9999
Lupin vs. Arabidopsis, van Veen	1,096	0,2277 to 1,964	Yes	**	0,0022
Lupin vs. Arabidopsis, Liu	1,797	0,9188 to 2,675	Yes	****	< 0,0001
Lupin vs. Arabidopsis, AtGenExpress	1,741	0,8133 to 2,669	Yes	****	< 0,0001
Lupin vs. B. rapa	0,7745	-0,08790 to 1,637	No	ns	0,1282
Lupin vs. B. napus	0,945	-0,08096 to 1,971	No	ns	0,1052
Lupin vs. Rice	0,3841	-0,4277 to 1,196	No	ns	0,9266
Lupin vs. Maize	0,2462	-0,8239 to 1,316	No	ns	0,9998
Arabidopsis, van Veen vs. Arabidopsis, Liu	0,7014	-0,02587 to 1,429	No	ns	0,0708
Arabidopsis, van Veen vs. Arabidopsis, AtGenExpress	0,6454	-0,1410 to 1,432	No	ns	0,234
Arabidopsis, van Veen vs. B. rapa	-0,3212	-1,029 to 0,3868	No	ns	0,9448
Arabidopsis, van Veen vs. B. napus	-0,1507	-1,051 to 0,7495	No	ns	> 0,9999
Arabidopsis, van Veen vs. Rice	-0,7116	-1,357 to -0,06612	Yes	*	0,0166
Arabidopsis, van Veen vs. Maize	-0,8495	-1,800 to 0,1006	No	ns	0,1326
Arabidopsis, Liu vs. Arabidopsis, AtGenExpress	-0,056	-0,8538 to 0,7418	No	ns	> 0,9999
Arabidopsis, Liu vs. B. rapa	-1,023	-1,743 to -0,3021	Yes	***	0,0002
Arabidopsis, Liu vs. B. napus	-0,8521	-1,762 to 0,05800	No	ns	0,0918
Arabidopsis, Liu vs. Rice	-1,413	-2,072 to -0,7538	Yes	****	< 0,0001
Arabidopsis, Liu vs. Maize	-1,551	-2,510 to -0,5914	Yes	****	< 0,0001
Arabidopsis, AtGenExpress vs. B. rapa	-0,9667	-1,747 to -0,1864	Yes	**	0,0031
Arabidopsis, AtGenExpress vs. B. napus	-0,7961	-1,754 to 0,1619	No	ns	0,2173
Arabidopsis, AtGenExpress vs. Rice	-1,357	-2,081 to -0,6331	Yes	****	< 0,0001
Arabidopsis, AtGenExpress vs. Maize	-1,495	-2,500 to -0,4899	Yes	****	< 0,0001
B. rapa vs. B. napus	0,1706	-0,7242 to 1,065	No	ns	> 0,9999
B. rapa vs. Rice	-0,3904	-1,028 to 0,2476	No	ns	0,6917
B. rapa vs. Maize	-0,5283	-1,473 to 0,4167	No	ns	0,8016
B. napus vs. Rice	-0,5609	-1,407 to 0,2852	No	ns	0,5721
B. napus vs. Maize	-0,6989	-1,795 to 0,3975	No	ns	0,6328
Rice vs. Maize	-0,1379	-1,037 to 0,7612	No	ns	> 0,9999

Tukey's multiple comparisons test	**Mean Diff,**	**95% CI of diff,**	**Significant**	**Summary**
Brassicaceae vs. Legumes	-1,207	-1,458 to -0,9556	Yes	****
Brassicaceae vs. Monocots	-0,9098	-1,229 to -0,5905	Yes	****
Legumes vs. Monocots	0,2969	-0,01776 to 0,6115	No	ns

Supplemental Table 5. Cross-species comparison of root/shoot NLR expression ratios for Figure 1I. ANOVA and Tukey's multiple comparison test was used for calculation of *P*-values. ns: $P > 0.05$; *: $P \leq 0.05$; **: $P \leq 0.01$; ***: $P \leq 0.001$; ****: $P \leq 0.0001$.

Deviation from a mean of zero (significant root or shoot skewed NLR expression):

	P value (two tailed)	Significant (p=0.05)
All NLRs	0,0004	**
All TNL	0,0343	*
All CNL	< 0,0001	****
All RNL	0,0002	***
All XNL	0,1902	ns
Brassicaceae TNL	0,1951	ns
Brassicaceae CNL	0,0139	*
Brassicaceae RNL	0,3267	ns
Brassicaceae XNL	< 0,0001	****
Legume TNL	< 0,0001	****
Legume CNL	< 0,0001	****
Legume RNL	< 0,0001	****
Legume XNL	0,0286	*
Monocot TNL	0,0131	*
Monocot CNL	< 0,0001	****
Monocot XNL	0,3712	ns

Tukey's multiple comparisons test	Mean Diff.	95% CI of diff.	Summary
Brassicaceae TNL vs. Legumes TNL	-0,7424	-1.181 to -0.3038	***
Brassicaceae TNL vs. Monocots TNL	-0,9144	-3.076 to 1.247	ns
Legumes TNL vs. Monocots TNL	-0,1719	-2.331 to 1.987	ns

Tukey's multiple comparisons test	Mean Diff.	95% CI of diff.	Summary
Brassicaceae CNL vs. Legumes CNL	-1,798	-2.447 to -1.148	****
Brassicaceae CNL vs. Monocots CNL	-1,442	-2.075 to -0.8088	****
Legumes CNL vs. Monocots CNL	0,3556	-0.04291 to 0.7541	ns

Unpaired t-test	t, df	One- or two-tailed?	P value
Legumes RNL vs. Brassicaceae RNL	t=0.8081 df=50	Two-tailed	ns

Tukey's multiple comparisons test	Mean Diff.	95% CI of diff.	Summary
Brassicaceae XNL vs. Legumes XNL	-1,39	-1.921 to -0.8596	****
Brassicaceae XNL vs. Monocots XNL	-1,18	-1.740 to -0.6198	****
Legumes XNL vs. Monocots XNL	0,2107	-0.2855 to 0.7070	ns

Supplemental table 6. Cross-species domain comparison of normalized log_2 root/shoot NLR gene expression ratios, for Supplemental figure 3. ANOVA and Tukey's multiple comparison test was used for calculation of *P*-values.

	Clade 1	Clade 2	Clade 3	Clade 4	Clade 5	Total
Arabidopsis thaliana	101	2	25	19	6	153
	66,01%	1,31%	16,34%	12,42%	3,92%	
Brassica napus	49	3	9	4	6	71
	69,01%	4,23%	12,68%	5,63%	8,45%	
Brassica rapa	120	2	33	16	9	180
	66,67%	1,11%	18,33%	8,89%	5,00%	
Glycine max	159	83	37	66	20	365
	43,56%	22,74%	10,14%	18,08%	5,48%	
Lotus japonicus	94	26	34	15	4	173
	54,34%	15,03%	19,65%	8,67%	2,31%	
Lupinus albus	21	11	24	4	23	83
	25,30%	13,25%	28,92%	4,82%	27,71%	
Medicago truncatula	262	214	57	48	16	597
	43,89%	35,85%	9,55%	8,04%	2,68%	
Oryza sativa	0	139	20	165	1	325
	0,00%	42,77%	6,15%	50,77%	0,31%	
Utricularia gibba	0	4	0	13	0	17
	0,00%	23,53%	0,00%	76,47%	0,00%	
Zea mays	0	26	6	35	2	69
	0,00%	37,68%	8,70%	50,72%	2,90%	
Total	806	510	245	385	87	2033
TNL	708	2	34			744
CNL		307		326		633
RNL		1			62	63
XNL	97	200	211	59	25	567
Total	805	510	245	385	87	2032

Supplemental Table 7. Clade distribution of NLR genes for the phylogenetic tree used in Figure 2B, including number of identified NLRs identified with at least one TIR (TNL), CC (CNL) or CC_R (RNL) domain, or none of the former three (XNL).

Deviation from a mean of zero (significant root or shoot skewed NLR expression)

	P value (two tailed)	Significant (p=0.05)
All NLRs	0,0004	***
Clade 1	0,6038	ns
Clade 2	< 0,0001	****
Clade 3	0,7291	ns
Clade 4	0,0179	*
Clade 5	< 0,0001	****
Brassicaceae Clade 1	0,1119	ns
Brassicaceae Clade 2	0,0425	*
Brassicaceae Clade 3	0,0001	****
Brassicaceae Clade 4	0,0117	*
Brassicaceae Clade 5	0,3697	ns
Legume clade 1	0,003	**
Legume clade 2	0,0003	***
Legume clade 3	0,0018	**
Legume clade 4	< 0,0001	****
Legume clade 5	< 0,0001	****
Monocot clade 2	0,0007	***
Monocot clade 3	0,2093	ns
Monocot clade 4	0,0011	**
Monocot clade 5	0,3632	ns

Unpaired t-test	**t, df**	**One- or two-tailed?**	**P value**
Legumes clade 1 vs. Brassicaceae clade 1	t=3.068 df=493	Two-tailed	0,0023

Tukey's multiple comparisons test	**Mean Diff.**	**95% CI of diff.**	**Summary**
Brassicaceae clade 2 vs. Legumes clade 2	-1,263	-2.443 to -0.08233	*
Brassicaceae clade 2 vs. Monocots clade 2	-1,042	-2.228 to 0.1439	ns
Legumes clade 2 vs. Monocots clade 2	0,2205	-0.1446 to 0.5856	ns

Tukey's multiple comparisons test	**Mean Diff.**	**95% CI of diff.**	**Summary**
Brassicaceae clade 3 vs. Legumes clade 3	-2,063	-2.943 to -1.184	****
Brassicaceae clade 3 vs. Monocots clade 3	-1,398	-2.661 to -0.1357	*
Legumes clade 3 vs. Monocots clade 3	0,6650	-0.5884 to 1.918	ns

Tukey's multiple comparisons test	**Mean Diff.**	**95% CI of diff.**	**Summary**
Brassicaceae clade 4 vs. Legumes clade 4	-2,479	-3.329 to -1.629	****
Brassicaceae clade 4 vs. Monocots clade 4	-1,505	-2.223 to -0.7860	****
Legumes clade 4 vs. Monocots clade 4	0,9745	0.3373 to 1.612	**

Tukey's multiple comparisons test	**Mean Diff.**	**95% CI of diff.**	**Summary**
Brassicaceae clade 5 vs. Legumes clade 5	-0,4421	-1.359 to 0.4747	ns
Brassicaceae clade 5 vs. Monocots clade 5	0,5864	-1.488 to 2.661	ns
Legumes clade 5 vs. Monocots clade 5	1,029	-0.9643 to 3.021	ns

Supplemental table 8. Cross-species clade comparison of normalized $\log_2$ root/shoot NLR gene expression ratios. ANOVA and Tukey's multiple comparison test was used for calculation of *P*-values.

	Clade 1	Clade 2	Clade 3	Clade 4	Clade 5	*Total*
Arabidopsis lyrata	37	3	20	6	2	68
	54,41%	4,41%	29,41%	8,82%	2,94%	
Arabidopsis thaliana	101	3	24	19	6	153
	66,01%	1,96%	15,69%	12,42%	3,92%	
Beta vulgaris	1	22	19	7	0	49
	2,04%	44,90%	38,78%	14,29%	0,00%	
Brassica napus	49	6	9	1	6	71
	69,01%	8,45%	12,68%	1,41%	8,45%	
Brassica rapa	121	3	32	16	9	181
	66,85%	1,66%	17,68%	8,84%	4,97%	
Capsella rubella	16	2	20	6	3	47
	34,04%	4,26%	42,55%	12,77%	6,38%	
Dianthus caryophyllus	1	31	10	12	0	54
	1,85%	57,41%	18,52%	22,22%	0,00%	
Eutrema salsugineum	9	3	7	5	0	24
	37,50%	12,50%	29,17%	20,83%	0,00%	
Glycine max	157	83	36	67	21	364
	43,13%	22,80%	9,89%	18,41%	5,77%	
Lotus japonicus	93	24	34	16	4	171
	54,39%	14,04%	19,88%	9,36%	2,34%	
Lupinus albus	21	11	24	5	23	84
	25,00%	13,10%	28,57%	5,95%	27,38%	
Medicago truncatula	263	214	57	48	16	598
	43,98%	35,79%	9,53%	8,03%	2,68%	
Nelumbo nucifera	2	34	8	20	0	64
	3,13%	53,13%	12,50%	31,25%	0,00%	
Oryza sativa	0	137	17	167	1	322
	0,00%	42,55%	5,28%	51,86%	0,31%	
Spirodela polyrhiza	0	22	23	18	0	63
	0,00%	34,92%	36,51%	28,57%	0,00%	
Tarenaya hassleriana	20	3	23	3	0	49
	40,82%	6,12%	46,94%	6,12%	0,00%	
Utricularia gibba	0	4	0	13	0	17
	0,00%	23,53%	0,00%	76,47%	0,00%	
Zea mays	0	26	3	38	2	69
	0,00%	37,68%	4,35%	55,07%	2,90%	
Total	891	631	366	467	93	2448
TNL	782	1	26	11		744
CNL		392		388		633
RNL		1			68	63
XNL	109	237	340	68	25	567
Total	891	631	366	467	93	2448

Supplemental Table 9. Clade distribution of NLR genes for the phylogenetic tree used in Figure 3B, including number of identified NLRs identified with at least one TIR (TNL), CC (CNL) or CC_R (RNL) domain, or none of the former three (XNL).