Rhizobium-legume symbioses: the crucial role of plant immunity

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New research results have significantly revised our understanding of the rhizobium-legume infection process. For example, Nod factors (NF), previously thought absolutely essential for the symbiosis, were shown to be dispensable under certain circumstances. Similarly, the NF receptor, previously considered solely symbiotic, was shown to function during plant pathogen infections. Indeed, there is a growing realization that plant innate immunity is a critical component in the establishment and maintenance of the symbiosis. Here, we review the factors involved in the suppression of plant immunity during the rhizobium-legume symbiosis and attempt to place this information into context with the most recent and sometimes surprising research results.

Signaling during rhizobium-legume symbioses: a field in motion

Symbiotic nitrogen fixation is a major contributor to soil fertility. In legumes, this process depends on the compatible interaction between the plant and soil bacteria referred to as rhizobia. During these interactions, a facultative organ, the nodule, is developed by the plant, generally on the root system. Nodules house spectacular densities of rhizobia that literally fill the symbiotic cells after invasion [1]. The infection processes and the massive colonization of nodule tissues raise the question as to how the plant host tolerates the rhizobia without eliciting a defense response. Indeed, there are clear commonalities between aspects of rhizobial and pathogen infection. For example, the bacterial type III secretion system (TTSS), commonly associated with virulence functions in plant pathogenic bacteria, can function in rhizobia to modify host range (for review, [2]). Furthermore, during the infection process of the semi-aquatic plant *Sesbania rostrata* and plants of the genus *Aeschynomene*, massive cell death, reminiscent of the hypersensitive response to pathogens, was observed [3, 4]. These few examples illustrate a possible role of plant immunity during the symbiotic process.

39 However, in general, the role of the plant innate immunity response has been largely ignored
40 by mainline, nodulation research and usually given only minor attention even when noted.

41 The development of nodules is a direct consequence of plant recognition of the
42 bacterial lipochitooligosaccharide (LCO) signals, called Nod factors (NFs), that are secreted
43 in the rhizosphere. NFs were, until recently, believed to be absolutely required for nodule
44 development [1, 5]. However, recent publications challenge this vision (see below) and place
45 the previous data in a new perspective with NF signaling seemingly part of a larger concept of
46 "control of immunity" during symbiotic establishment. One of the most striking results
47 illustrating this role is that *Bradyrhizobium elkanii* strains lacking the *Nod* genes (responsible
48 for the synthesis of NF) can nodulate soybean mutant lines lacking the NF receptor [6]. In this
49 case, the crucial function was supplied by the TTSS [6].

50 In this review, we highlight examples of plant defenses elicited during compatible and
51 incompatible rhizobium-legume symbiotic interactions to document that such immune
52 responses do occur. Plant and symbiont determinants involved in immunity suppression will
53 be presented (Figures 1 and 2) and the potential role of immunity suppression as a host
54 specificity determinant during the interaction will be discussed. Due to the nature of this
55 review, only the most recent literature is cited and, therefore, we apologize to those authors
56 whose work we were unable to highlight.

58 **Activation of legume defenses during the rhizobium-legume symbiosis**

59 Legume defenses can be elicited by rhizobial inoculation. This is illustrated by a
60 transcriptomic study in soybean that showed strong induction of plant defense related genes
61 12 hours after *Bradyrhizobium japonicum* inoculation but with expression returning to
62 baseline within 24 hours, perhaps due to active suppression of the defense response [7].
63 Similar transient induction of defense related genes were observed in *Medicago truncatula*

where many defense response genes were up-regulated one hour after *Sinorhizobium meliloti* inoculation but down-regulated as early as 6 hours after inoculation [8]. Similarly, in *Lotus japonicus*, transient induction of defense related genes [9] was observed upon *Mesorhizobium loti* inoculation. In agreement with these observation, phosphorylation of MAP kinases 3 and 6 (a typical plant defense response) was shown in *L. japonicus* roots in response to a *M. loti* cell suspension [10].

Increased production of reactive oxygen species (ROS) is also a common plant defense response. H₂O₂ was proposed to be required for optimal infection of *Medicago sativa* (alfalfa). Indeed, a mutant strain of the *Medicago* symbiont *S. meliloti*, degrading H₂O₂ very efficiently (thanks to the overexpression of a catalase gene), is altered for the infection process and induces the formation of a reduced number of nodules [11]. In agreement, more than 300 genes induced during the initial steps of the symbiotic process are likely regulated by H₂O₂ in *M. truncatula* [12]. Beyond infection, ROS production by the plant seems to be required for optimal symbiosis during the later stages of the symbiotic process. Indeed, in *M. truncatula*, the gene *MtRbohA* which encodes an NADPH oxidase that produces ROS, is required for optimal nodule functioning [13]. Such a positive role of ROS was also observed in *Phaseolus vulgaris*, in which the *RbohB* gene (encoding a ROS producing enzyme) was found to stimulate the symbiosis with rhizobia [14, 15]. Together, these results indicate that plant innate immunity functions are transiently activated during the earliest stages of rhizobial infection and symbiotic establishment.

Suppression of legume defenses: a prerequisite for the rhizobium-legume symbiosis

Plant immunity mechanisms rely on the recognition of MAMPs (sometimes termed pathogen-associated molecular patterns = PAMPs) or on the detection of microbial secreted proteins called effectors [16]. To our knowledge, no rhizobial MAMPs have been identified as active

on legumes. However, as discussed in the previous section, defense reactions are activated upon rhizobium inoculation in the legume roots and appear to be rapidly suppressed.

Succinoglycan (an exopolysaccharide, EPS) is produced by *S. meliloti* and was suggested to suppress plant defenses during the early stages of the symbiosis. Transcriptomic analyses of inoculated *M. truncatula* roots showed that inoculation with a *S. meliloti* *exoY* mutant, deficient for the synthesis of succinoglycan, resulted in a much stronger induction of defense related genes relative to control plants infected with wild-type *S. meliloti* [17]. This observation is in agreement with the described role of EPS from various bacteria, including *S. meliloti*, in suppressing MAMP triggered immunity (MTI) [18]. EPS were proposed to suppress MTI by chelating calcium [18], which is an important cellular signal involved in MTI. In agreement with the idea that MTI has to be suppressed during the symbiosis, MAMP elicitation is deleterious for the symbiotic process. For example, treatment with flg22, the active MAMP epitope of bacterial flagellin, reduced the *L. japonicus* response to NF. In addition, nodulation of *L. japonicus* roots treated with flg22 was significantly delayed relative to controls [10]. This result might have been interpreted as a decreased fitness of rhizobia caused by the flg22 triggered immunity. However, this negative effect of flg22 on nodulation kinetics was also observed with a *Lotus snfl* mutant, which produces spontaneous nodules in the absence of rhizobia, indicating that the flg22 negative effect on nodulation is not solely a consequence of reduced rhizobial fitness [10]. Moreover, these findings indicate that flg22 affects not only infection but also nodule organogenesis. Similar to the flg22 effect, the defense phytohormone salicylic acid (SA) has a negative impact on nodulation as demonstrated in *M. truncatula* and *L. japonicus* [19]. Indeed, strong, ectopic expression of NPR, a key regulatory protein in the SA response pathway, was also shown to suppress NF-induced root hair curling, as well as expression of nodulation-related genes (i.e., nodulins; [20]). Jasmonic acid and ethylene are also considered defense phytohormones and both inhibit

development of the rhizobial-legume symbiosis [21, 22]. Together these results clearly indicate that immunity has to be suppressed in legumes from the very first stages of the symbiotic process.

A role for Nod Factors in plant immunity suppression

The very first rhizobial response to legume perception is the production and secretion of NF that initiates the infection process and induces development of the nodule. Rhizobial genes responsible for NF production are not only actively transcribed before the infection but also remain active in the nitrogen fixation zone of *M. truncatula* nodules [23]. This observation suggests that NF might have a role beyond organogenesis because, at this stage, the nodule is already formed. Furthermore, rhizobial mutants overproducing NF are not only more competitive for nodulation but also display enhanced nodule development [24] suggesting again a possible role for NF after nodule organogenesis. Recently, NF were shown to partially suppress MTI [25]. This suppression is independent of the nodulation signaling pathway as it was observed in two soybean mutants unable to express the NF receptors (NFR1 and NFR5). In agreement with a NF action independent of the nodulation genes, MTI suppression by NF was observed with both legumes and non-legumes (i.e., tomato and corn). Furthermore, this NF suppressive effect on MTI was also observed on the model plant *Arabidopsis thaliana* (*Arabidopsis*) that is unable to develop a mycorrhizal symbiosis [25]. Interestingly, mycorrhizal and rhizobial symbioses, in part, rely on a set of common plant genes. It is now known that both of these symbioses involve recognition of LCO molecules, NF for rhizobia and so-called Myc factors for the mycorrhizal symbiosis [26]. Given the ancient nature and widespread occurrence of mycorrhizal symbiosis in plants, it is assumed that this ability to recognize LCOs and the common symbiotic pathway genes necessary for this recognition evolved first to support this fungal symbiosis. Later, the ability to nodulate evolved in

legumes, in part, by co-opting this symbiotic machinery. NF suppression of MTI suggests that LCO recognition may be even more ancient, perhaps evolving first to support a pathogenic role and only later being adapted to support symbiotic infection [25, 27]. However, although NF treatment suppressed MTI in both *Arabidopsis* leaves and roots, the ability of NF to suppress a MTI involved in rhizobial-legume root infection was not directly tested.

In contrast to the study indicating that NF suppresses MTI [25], other research suggests that NF, or at least NF receptors, may also play a role in induction of plant defense. For example, the *M. truncatula nfp* (encoding a NF receptor) mutant was shown to be significantly more susceptible to the oomycete *Aphanomyces euteiches* and to the fungus *Colletotrichum trifolii* [28]. The expression of active NF receptors from *M. truncatula*, or *L. japonicus* in *Nicotiana benthamiana* leaves triggered cell death [29, 30]. Consistent with these findings, strong, ectopic expression of *NFP* in *M. truncatula* nodules appeared to trigger defense-like reactions [31]. Although this area clearly requires further study, it is important to note that NF and chitin elicitor (MAMP) have structural similarities and that recognition of both molecules is mediated by lysin motif receptor-like kinases (LysM RLKs). Indeed, the construction of chimeric proteins; for example, in which the ectodomain of the chitin receptor CERK1 was replaced with the ectodomains of the NF receptors NFR1 or NFR5 conferred, when expressed together so presumably a heterodimer could form, the ability to induce defense related responses upon the addition of NF [32]. Conversely, replacement of the ectodomains of NFR1 and NFR5 with those derived from the rice CERK1 and CeBiP chitin co-receptors conferred, when expressed together, the ability to induce nodulin gene expression upon addition of chitin elicitor. It seems that only minor changes in the LysM RLK amino acid sequence confers specificity for either the NF or chitin MAMP [33]. With this in mind, for example, it is not surprising that a strong expression of the NF receptors in tobacco confers cell death since CERK1 can also induce cell death [34]. It is also clear that

both the NF and chitin receptors function as heterodimers. For example, NFP was shown to interact *in vivo* in *M. truncatula* nodules with the Lyk3 LysM RLK [31]. In both rice and Arabidopsis, CERK1 is not the major chitin binding protein with this function provided by a co-receptor with higher chitin binding affinity; CeBiP in rice [35] and LYK5 in Arabidopsis [36]. These results are not consistent with the interpretation of some published results to mean that specific binding of CERK1 to various ligands explains the apparent symbiotic and pathogenic roles of this protein [37]. An alternative and more consistent suggestion would be these different roles are determined, not by CERK1, but by the specific co-receptors that are recruited for the differing functions. It is now clear that both LCO and chitin receptors are multiprotein complexes that respond depending on the chemistry and concentration of the ligand and composition of the receptor complex formed [27]. Likewise, the specifics of treatment and the way in which responses are measured can also affect the interpretation of results [27].

What is the role of effector-triggered immunity (ETI) in the rhizobial-legume symbiosis?

As discussed above, plants defend against invading pathogens by activation of MTI. This form of immunity is generally broad but also relatively weak. However, plants also have the ability to recognize pathogen-derived effector proteins through the direct or indirect action of resistance (R) genes. This form of immunity is termed effector triggered immunity (ETI) and is generally very specific (e.g., limited to only specific pathogen strains) but also much stronger than MTI [38]. In the case of bacteria, the best studied effectors are those that are directly injected into the plant cytoplasm by action of the type III protein secretion system (TTSS) [39]. In the case of pathogens, effectors are thought to promote virulence, sometimes by inhibiting MTI [referred to as effector triggered susceptibility (ETS)]. Effectors can also trigger defense when, and only when, the cognate R gene is expressed in the host; giving rise

to the gene-for-gene model of plant immunity. There is now a growing body of literature that suggests that ETS and ETI are important, perhaps even central, to the rhizobial-legume symbiosis.

A very interesting experiment demonstrated the conversion of a pathogenic bacterium, *Ralstonia solanacearum*, into a bacterium that could nodulate mimosa, which has a relatively wide host range [40]. The authors first transferred the *Cupriavidus taiwanensis* symbiotic plasmid into *R. solanacearum* and then selected mutants able to infect and nodulate. Isolation of bacteria from the nodules that formed on mimosa roots showed that only strains mutated in the TTSS system (or in a master regulator controlling the expression of the TTSS genes) were able to nodulate [40]. Subsequently, these authors showed that the loss of TTSS, as well as other virulence factors, are also required for intracellular maintenance of the chimeric *R. solanacearum* [41]. Consistent with these findings, a functional TTSS restricts the host range of *Cupriavidus taiwanensis* [42]. These results are fully consistent with many other published reports showing a role for the TTSS in modulating rhizobial host range. For example, extensive work with the very broad host range rhizobium, NGR234, showed that specific TTSS mutations could affect to varying extents, both positively and negatively, the host range of this bacterium [2, 43-48]. Amongst the few NGR234 effectors described was NopM that was shown to be an E3 ubiquitin ligase. When expressed in *N. benthamiana*, NopM inhibits the plant's defense response involved in MAMP induction of ROS. In NGR234, NopM was shown to specifically promote nodulation on *Lablab purpureus* roots [49]. Similarly, ectopic expression of the NGR234 effector NopL in *N. benthamiana* was found to suppress defense responses when plants were challenged by infection with tobacco mosaic virus [50]. As mentioned above, it was shown that the *B. elkanii* TTSS was crucial for the ability to nodulate soybean mutants defective in the NF receptor [6]. Because both TTSS effectors and NF can

suppress innate immunity, in this specific case, the *Bradyrhizobium* effectors may have provided this essential function in the absence of NF signaling.

Not all rhizobial strains have a TTSS system. In these cases, effectors delivered via a type IV secretion system (T4SS) may be involved in symbiosis. For example, both *M. loti* and *S. meliloti* secrete proteins via a type IV system that both positively and negatively affect nodulation [51, 52]. Interestingly, in some rhizobia, the genes responsible for NF synthesis and for TTSS and T4SS depend on a common regulator that is activated by legume emitted flavonoids (for review [2]). Remarkably, and in contrast to pathogens, rhizobial effector proteins appear to display a high degree of conservation in content and sequence [53]. This is particularly striking in the context of the otherwise high genomic diversity amongst rhizobia and suggests that fitness conflicts between rhizobia and their hosts have been a key driver for the evolution of symbiotic-related genes [53].

In order to counter the virulence functions of pathogen effector proteins, the plant host evolved resistance genes encoding nucleotide-binding site/leucine-rich repeat proteins (NBS-LRR) that activate defenses during ETI. A number of legume mutants were isolated that either blocked nodulation or affected nodulation by specific rhizobial strains. Very recently, the soybean *Rj2* and *Rfg1* were found to be allelic genes encoding NBS-LRRs [54]. Although the cognate effector for this protein was not identified, *Rj2/RFG1* restricts nodulation with specific strains of *B. japonicum* and *S. fredii* [54]. In addition, and in agreement with an R gene function, TTSS mutants of *Bradyrhizobium* gain the ability to efficiently nodulate *Rj2* soybean plants [55]. The involvement of R genes in the restriction of rhizobial host range may be a widespread, although little studied, phenomenon. For example, a dominant gene, *Mt-NS1*, restricting nodulation by *S. meliloti* strain RM41 but not by strains NGR247 and NGR34, was mapped in *M. truncatula* [56]. Although still not cloned and identified, such host restriction conferred by a dominant gene is very reminiscent of an R gene effect.

The above discussion illustrates that the data clearly support a role for ETI in legume nodulation but, compared to our understanding in plant-pathogen interactions, much remains to be elucidated. Perhaps the most important question is whether ETI plays strictly a minor role (e.g., to modulate strain-specific nodulation) or a more fundamental role.

Beyond the effectors, rhizobial factors preventing a legume host defense response

Aside from the effector proteins, most of the bacterial factors required to prevent defense-like reactions during the symbiotic process are not specifically required for symbiosis and likely have a broader role for the bacteria. For example, nodules induced by *B. japonicum* mutants altered in the general stress response display necrotic lesions reminiscent of defense reactions [57]. Perhaps the best examples relevant to the rhizobial symbiosis are those dealing with defects in cell surface polysaccharides, lipopolysaccharide (LPS), EPS and cyclic glucans (reviewed in [58]). The literature in this area is a bit confusing, because the phenotypes seen very much depend on which rhizobium, host or polysaccharide is being tested. However, overall the data are clear that these polysaccharides play an important role in the symbiosis and, at least in part, this can be explained by their effect on plant host immunity. Much of this work is older than 5 years and, therefore, we will not review it in detail here (see Table 1). Given the increased interest in the role of the immune response in legume nodulation, however, the ability of rhizobial polysaccharides to control the immune response is likely an area deserving of renewed interest.

Role of defense response during intracellular colonization

Once internalized within the plant cells, the rhizobia are separated from the plant cytoplasm by a plant-host derived membrane, the symbiosome membrane [59] (Figure 2). In these infected cells immunity is modulated. Recent work showed that the expression of defense

genes is reduced in the infected cells of *M. truncatula* nodules relative to expression in the non-infected cells [60], suggesting a modification of the genetic program to accommodate the rhizobia within the cells. This tolerance program, preventing expression of defense related genes, does not seem to be dependent on active nitrogen fixation. Indeed, in *M. truncatula*, nodules induced by the *S. meliloti bacA* mutant, that does not fix nitrogen, defense-like reactions are not induced [61].

A few plant genes were proposed to play a role in the control of plant immunity during intracellular colonization. For example, the *M. truncatula RSD* gene, standing for regulator of symbiosome differentiation encodes a C2H2 transcriptional factor, which represses expression of the vesicle associated membrane protein 721a (Vamp721a). In *Arabidopsis*, a homolog of this gene is involved in plant immunity. Interestingly, bacteroids rapidly senesce in nodules formed on *rsd* mutant plants and a brown pigment accumulates suggesting activation of plant defenses [62]. Two other *M. truncatula* genes, *DNF2* and *SymCRK*, are also required for rhizobial persistence within the nodule [61, 63, 64]. Nodules formed on roots of these mutants are necrotic, express defense-related genes and accumulate phenolic compounds [61, 64]. *DNF2* encodes a phospholipase-like protein, whereas *SymCRK* encodes a non-arginine-aspartate motif, receptor-like kinase, typical of those involved in innate immunity [65, 66]. Although the biochemical functions of these genes are not yet fully described, like *RSD*, they are required for bacteroid differentiation (in nodules with a persistent meristem such as those formed on *M. truncatula*, bacteroids elongate but do not divide). However, in *rsd*, *dnf2* and *symCRK* mutants, the lack of bacteroid differentiation is likely not the inducer of defense reactions because infection with a bacterial mutant that is unable to differentiate does not trigger defenses on WT plants [61].

Interestingly, even in “healthy” *M. truncatula* symbiotic cells, elements reminiscent of plant defenses are present. For example, *M. truncatula* bacteroid differentiation is governed

by a large family of antimicrobial peptides rich in cysteine [so-called nodule cysteine-rich (NCR) peptides], which are specifically produced in nodules [67]. NCR activity is similar to defensins produced by plants in response to pathogen invasion. NCR have a toxic effect *in vitro* on *S. meliloti* and on other bacteria [67]. *In planta*, the rhizobial BacA protein, which is believed to act as a peptide transporter, partially protects bacteroids against NCR peptide toxicity which, in this situation are only bacteriostatic [68].

Nodule senescence and plant immunity

Beyond defense-like reactions, *symCRK*, *dnf2* and *rsd* mutants also display typical features of early senescence [62-64]. It was proposed that suppression of plant innate immunity is reversed as the nodules senesce [69]. However, this appears to be independent of nitrogen fixation because early senescing nodules elicited by the *bacA* mutant, which lacks the ability to fix nitrogen, do not accumulate phenolics, are not necrotic and do not express defense markers [61]. Nevertheless, there are some parallels between defenses and the senescence process. For example, nitric oxide (NO), which was initially reported to be a plant signal involved in the response to pathogens [70, 71], also promotes nodule senescence [72]. Recently, it was shown that *S. meliloti* degrades NO in *Medicago* nodules that leads to a delay in nodule senescence [72]. Cysteine proteinases (CP) constitute another example of convergence between defense and senescence. CPs are routinely used as senescence markers [73]. Nevertheless, some members of this gene family are not specifically involved in senescence but are also required for optimal defense responses against pathogenic organisms [74, 75]. Thus, nodule senescence and defenses, which both result in bacterial death, appear to share some common signaling elements (e.g., NO) and effectors (CP). More work is now required to precisely determine to what extent the genetic program of the two processes overlap.

Environmental influences on plant innate immunity during nodulation

A number of publications attest to the significant impact that the plant growth environment can have on plant immunity (e.g., [76, 77]). Therefore, it would not be surprising to find that environmental conditions can also interfere with immunity during the symbiotic process. As mentioned earlier, the presence of flg22 in a plant growth substrate delayed the symbiotic process. Abiotic environmental conditions also seem to modulate nodule immune status. For example, in *Pisum sativum* and *Phaseolus vulgaris*, boron deficiency triggered defense-like reactions as illustrated by nodule necrosis, as well as PR10 gene expression [78]. The role of the environment is also illustrated by the *M. truncatula dnf2* mutant that produces necrotic nodules when grown on perlite/sand substrate or in axenic condition using agar as a solidifying agent. In contrast, when cultivated on medium solidified with Phytigel or agarose, the *dnf2* mutants form non-necrotic nodules that can fix nitrogen suggesting the presence of an unidentified factor(s) present in sand/perlite and agar that trigger(s) defense-like reactions. The inability to fix nitrogen is restored when ulvan, a defense priming agent, is added to the agarose solidified medium [61]. Plant growth conditions also determine the requirement for a wild-type EPS during interaction of *L. japonicus* with *M. loti* [79]. Indeed, when the root system is exposed to light, nodulation by the rhizobial EPS mutant is drastically reduced [79]. Temperature also acts on nodule immune status; during the interaction between *Bradyrhizobium* sp. ORS285 and *Glycine max*, defense-like reactions are observed when plants are cultivated at high temperature but not under low-temperature [80].

Symbiotic suppression of legume innate immunity as a result of coevolution

It seems that rhizobia and legumes co-evolved to modify critical elicitors/MAMPs and their corresponding receptors to avoid the development of defense reactions. For instance, the flg22

epitope is exceptionally divergent in *S. meliloti* and is completely inactive as an elicitor [81]. Similarly, purified *M. loti* flagellin does not elicit MAP kinase phosphorylation in *L. japonicus* roots [10]. In addition, the expression of the plant flagellin sensing receptor *FLS2* is drastically reduced in *L. japonicus* nodules [10], suggesting an adaptation to reduce defense induction during nodulation.

NFs were believed for a long time to be absolutely required for rhizobium-legume symbiosis. However, not all rhizobia are able to produce NFs. Indeed, the genome sequencing of two *Aeschynomene* rhizobial symbionts revealed that these rhizobial strains completely lack the canonical Nod genes [82]. Although not ignoring this report, the community appeared to absorb it as an exotic exception and perhaps not really relevant to mainline research. However, this view became untenable with the demonstration that soybean and a mutant of *Lotus japonicus* could be nodulated and/or infected, respectively, in the absence of either NF or the NF receptors [6, 83]. Thus, some rhizobia do not require NF. Similarly, some rhizobia use TTSS (or T4SS) and some do not while some rhizobia appear to use both the NF and the TT/4SS. These data are perhaps best explained by co-evolution of symbiont and host, perhaps originating from a more pathogenic interaction, to one in which pathogenic features were lost while the host response became more benign.

Concluding remarks

Rhizobium-legume interactions are beneficial for plants in spite of the massive and chronic infection of nodule tissues. A growing body of literature indicates that successful symbiotic development depends on the ability to actively suppress plant innate immunity. However, suppression of immunity during symbiosis may carry a cost for the plant as this suppression could constitute an opportunity for infection by pathogenic organisms. Indeed, the ability to allow active suppression of plant immunity while allowing infection by the compatible

363 symbiont may have been a driving selective force that led to mechanisms of rhizobium-host
364 specificity. Now that transferring a nitrogen fixation symbiosis to non-legume crop plants
365 appears as a reasonable challenge [84, 85], understanding how legumes tolerate the massive
366 rhizobial colonization is of prime interest. Hence, there is a clear need to better define the role
367 of the plant innate immunity response during rhizobial nodulation.

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Table 1: Non exhaustive summary of determinants acting on plant immunity during rhizobia/legumes symbioses

Rhizobium species	Bacterial/Legume Factors	Host species	Phenotypes	Refs.
<i>Rhizobium</i> sp. NGR234	NopM	<i>Nicotiana benthamiana</i>	Reduces flg22 triggered oxidative burst Stimulates flg22 triggered gene expression	[49]
<i>Sinorhizobium meliloti</i>	LPS	<i>Nicotiana. Tabacum</i> <i>Medicago sativa</i> and <i>Medicago truncatula</i>	Elicit oxidative burst on cell cultures Supress oxidative burst on cell cultures	
<i>Sinorhizobium fredii</i>	LPS	<i>Glycine max</i>	Lytical clearance of the bacteroids Accumulation of phenolics-like compounds	
<i>Bradyrhizobium japonicum</i>	Nod factors	<i>G. max</i> <i>Arabidopsis thaliana</i> <i>Zea maidis</i> <i>Solanum lycopersicum</i>	PTI inhibition on leave disks	[25]
<i>S. meliloti</i> <i>S. medicae</i>	DNF2	<i>M. truncatula</i>	Conditional phenotype Expression of defense related genes Accumulation of phenolic compounds Bacteroid death	[61, 64]
<i>S. meliloti</i> <i>S. medicae</i>	SymCRK	<i>M. truncatula</i>	Expression of defense related genes Accumulation of phenolic compounds Bacteroid death	
<i>S. meliloti</i>	RSD	<i>M. truncatula</i>	Accumulation of brown compounds	
<i>B. japonicum</i>	PhyR EcfG	<i>G. max</i>	Nodule necrosis	[57]
<i>Bradyrhizobium elkani</i>	TTSS	<i>G. max</i>	Compensates absence of Nod factor	[6]
<i>Ralstonia solanacearum</i> chimeric strain	TTSS	<i>Mimosa pudica</i>	Inhibits nodulation and intracellular invasion	[40, 41]
<i>Cupriavidus taiwanensis</i>	TTSS	<i>Leucaena leucocephala</i>	Compromise symbiosis	[42]
<i>Rhizobium leguminosarum</i>		<i>Phaseolus vulgaris</i> <i>Pisum sativum</i>	PR10 genes induction and nodules necrosis upon boron deficiency	[78]
<i>S. meliloti</i>	NCR peptides	<i>M. truncatula</i>	Trigger <i>in vivo</i> terminal differentiation of bacteroids	[67]
<i>S. meliloti</i>	BacA	<i>M. truncatula</i>	BacA partially protects bacteroids against NCR toxicity	[68]
<i>B. japonicum</i>	<i>Rj2</i>	<i>G. max</i>	Restrict nodulation with specific strains of <i>B. japonicum</i>	[54]
<i>S. fredii</i>	<i>Rfg1</i>	<i>G. max</i>	Restrict nodulation with specific strains of <i>S. fredii</i>	
<i>B. japonicum</i>	TTSS	<i>G. max</i>	Restrict nodulation on <i>Rj2</i>	[55]
<i>S. meliloti</i>	exoY/succinoglycan	<i>M. truncatula</i>	Reduce defense related gene induction	[17]
<i>S. meliloti</i>	NFP	<i>M. truncatula</i>	Nodulation deficient mutant Mutants are more suceptible to <i>Aphanomyces euteiches</i> and <i>Colletotrichum trifolii</i>	[86] [28]
<i>M. loti</i>	TTSS	<i>Lotus corniculatus</i> subsp. frondosus and <i>L. filicaulis</i> <i>Lotus halophilus</i> and two other species	Affects positively nodulation Affects negatively nodulation	[87]

1

2 **Legends to figures :**

3

4 **Figure 1: Defense suppression during early symbiotic interactions.** Plants can perceive

5 bacterial MAMP using Pattern Recognition Receptors (PRR) that trigger MTI characterized

6 by calcium influx, ROS production and immune signaling. Rhizobia can escape MTI by

7 producing modified MAMP (for example rhizobial flg22 peptide is exceptionally divergent in

8 *S. meliloti*), through NF suppression of MTI, by inhibiting the defense reactions through

extracellular calcium chelation using bacterial exopolysaccharides and/or through ROS production inhibition using bacterial lipopolysaccharides. LysM receptor-like kinases can recognize Nod factors and inhibit MTI signaling by an unknown mechanism. Nod factors perception by symbiotic LysM RLK receptors (NFR/NFP) induces the symbiotic process. In addition, some recent data suggest that these symbiotic receptors activate defense reactions. Certain rhizobia use T3SS or T4SS to inject symbiotic effectors (for example Nop proteins) into the host cytoplasm to short circuit immune signaling, directly or via the proteasome pathway. Plants can recognize Nop effectors via NBS-LRR R genes that will turn on ETI (Effector Triggered Immunity). It is important to note that the figure presents an ensemble of responses induced by rhizobia in different plant species and different plant cells.

Fig 2: Defense suppression after intracellular bacterial invasion: Host PRR can interact with MAMP and induce defense reactions characterized by accumulation of phenolics and defense gene expression. Rhizobia can block host signaling using effector proteins secreted by T3SS/T4SS. LysM RLKs, for example homologous to AtLyk3, may mediate Nod factor-dependent suppression of immunity. However, NFP and NFR5, the characterized nod factors, are not expressed in the infected nodule cells. *DNF2* and *SymCRK* plant genes, encoding a PI PLC-like protein and a cysteine-rich, non-RD receptor-like kinase, can suppress defense reactions by an unknown mechanism. The C2H2 transcription factor RSD represses *VAMP721a* expression, which may play a role in defense compounds secretion in response to bacterial invasion. Immunity can be regulated at the transcriptional level by repressing defense genes or PRR expression. For example a *L. japonicus* the *FLS2* homolog is down regulated during symbiosis. Nitric oxide (NO) plays a role in the senescence process by inducing expression of *cysteine proteinases (CP)*. Rhizobia suppress NO production to delay early senescence. Different studies have shown that *CPs* can also participate in defense

1 reactions. Finally, NCRs are antimicrobial peptides produced by plants that are internalized
2 via a bacterial ABC transporter called BacA. The *bacA* mutants are unable to survive
3 intracellularly.

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5