

Not All Allies Are Welcome: Partner Discrimination in Legume–Rhizobium Symbiosis

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The nitrogen-fixing symbiosis between leguminous plants and soil bacteria, collectively termed rhizobia, is a major contributor of fixed nitrogen to the biosphere. The ability of legumes to secure nitrogen from the atmosphere underlies their ecological success and has made them important crops in both traditional and modern sustainable agriculture. Many genes directing the establishment and functioning of this beneficial interaction have been identified under laboratory conditions using a limited number of bacterial strains and plant species in pairwise combinations. Under natural and field conditions, however, plants encounter numerous potential partners, as soil microbiomes contain diverse bacteria equipped with the necessary toolkit for symbiosis. Consequently, legumes must possess mechanisms to select for or against specific partners. This review highlights how legumes employ elements of their immune system for the negative selection of rhizobia via processes resembling the gene-for-gene model of effector-triggered immunity in plant–pathogen interactions.

Keywords: bacteroid development, infection, symbiotic incompatibility, symbiotic nodule

The intensive use of chemical fertilizers has been a crucial factor in the increased agricultural production that could be achieved by new crop varieties that have high yield potential and require far more fertilizer than traditional cultivars. A recent study envisages that fertilizer use would need to increase fourfold over current use to satisfy the increasing global food

demand while adapting to climate change (Martre et al. 2024). This increase would unavoidably lead to higher ecological impacts, as the production and use of chemicals, especially nitrogen (N) fertilizers, are not environmentally friendly because (i) their synthesis and transport to fields require a lot of fossil energy and (ii) close to 50% of the applied nitrogen is not used by the targeted crops; it is lost through volatilization of ammonia and greenhouse gases, leaching and runoff that pollute ground and surface waters, and the (de)nitrification activities of microbes (Bhatt et al. 2025).

Legumes (Fabaceae), which constitute the third largest family of flowering plants and can be found in climates ranging from cold tundra to warm deserts across the globe, except in Antarctica (Doyle and Luckow 2003), require much less nitrogen fertilizer because they can establish a symbiotic association with nitrogen-fixing soil bacteria, collectively termed rhizobia. This association ensures legumes' nitrogen supply from the unlimited source of nitrogen gas (N₂) in the atmosphere, which is not accessible to most organisms. Thus, these plants constitute a major input of combined nitrogen in the biosphere, and this unique ability contributes to their success both in nature and as crops. In nature, early stages of primary succession—which begin in barren environments such as volcanic rocks, sand dunes, or glacial tills, where soil is essentially absent or very low in nutrients, particularly nitrogen—are frequently dominated by leguminous (and actinorhizal) plants (Walker 1993). The nitrogen and carbon inputs provided by these nitrogen-fixing pioneer plants are very important for the development of soil organic matter and nitrogen pools, the establishment of the microbial communities responsible for nutrient retention and cycling, which are essential for subsequent colonization by other plant species (Halvorson et al. 2005). At later stages of the succession, the non-fixer plants benefit from the neighborhood of nitrogen-fixing legumes by accumulating more nitrogen than the individuals growing alone on the biological soil crusts (Chapin et al. 1994; Wang et al. 2021). Similarly, in established ecosystems, the presence of legumes positively affects soil nitrogen pools, as well as the biomass and nitrogen content of non-legume plants (Soudzilovskaia et al. 2012; Spehn et al. 2002). Because of the ease with which legumes can acquire nitrogen, their seeds and forage tend to be highly proteinaceous, and therefore, these plants are an invaluable source of protein in both human and animal nutrition. Thus, in anthropogenic landscapes, legume crops have played an important role in both classical and modern sustainable agricultural practices, serving as elements in crop rotations or being used for intercropping and cover cropping purposes (Kokkini et al. 2025; Stagnari et al. 2017) or during restoration of, for example, abandoned fields and burned forest/meadow ecosystems (Li et al. 2007;

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Naydenova et al. 2022; Yuan et al. 2016). In both natural and anthropogenic ecosystems, the key benefits of legumes are their contribution to soil health in general and the resistance of soils to ecosystem disturbances: Through carbon sequestration and nitrogen fixation, these plants incorporate atmospheric carbon and nitrogen into soil and thus increase its organic carbon and biologically available nitrogen content, which supports the growth of other organisms. Their presence also improves the soil structure by enhancing its porosity and aggregation, which leads to reduced erosion, as well as a reduced salinity impact in saline soils (Gao et al. 2017; Srivastava et al. 2025).

Because of the ecological and economic importance of legume–rhizobium symbioses—as well as the “Holy Grail” goal of extending this symbiosis to major non-legume crops—a significant amount of research has been devoted to legume–rhizobium interactions. These studies aim to unravel the steps of the symbiotic process, particularly the formation of the functional nodule, a novel plant organ that hosts nitrogen-fixing bacteria known as bacteroids. Researchers have sought to identify the genetic determinants required for nodule formation, the infection of nodule cells by rhizobia, the differentiation of eukaryotic and prokaryotic cells, the reduction of atmospheric nitrogen, and the assimilation and transport of combined nitrogen in both the plant and bacterial partners. These discovery investigations to reveal what makes a symbiotic partner eligible have been performed under laboratory conditions using one-on-one experimental setups (i.e., a single host infected with a single partner). For this purpose, a limited number of eukaryotic model organisms, such as *Medicago truncatula*, *Lotus japonicus*, and, more

rarely, pea and soybean, but more rhizobial species and strains have been investigated.

In natural and agricultural soils, however, microbiomes may contain hundreds of rhizobial strains capable of establishing effective nitrogen-fixing symbioses with compatible legumes. Thus, plants must employ mechanisms beyond the basic symbiotic toolkit to select their preferred bacterial partners. Moreover, plants monitor whether the considerable amounts of resources invested in the rhizobial partners are reciprocated with a comparable symbiotic benefit from the bacteria to impose sanctions on “cheaters” that would only exploit the interaction and would cause a fitness deficit for the host (Chen et al. 2025). Unfortunately, very little is known about the genetic determinants and mechanisms resulting in preference for or discrimination against certain eligible bacterial strains. In this review, we summarize current knowledge on the mechanisms plants use to discriminate among rhizobia and the bacterial signatures that are recognized and negatively discriminated by host plants.

Development and Functioning of a Nitrogen-Fixing Nodule

The establishment of nodules is a complex process involving several interconnected developmental events, including bacterial infection, nodule organogenesis, bacteroid and host cell differentiation, and maturation (Fig. 1). It requires multiple signal exchanges, as well as the activity and coordinated regulation of the expression of numerous genes.

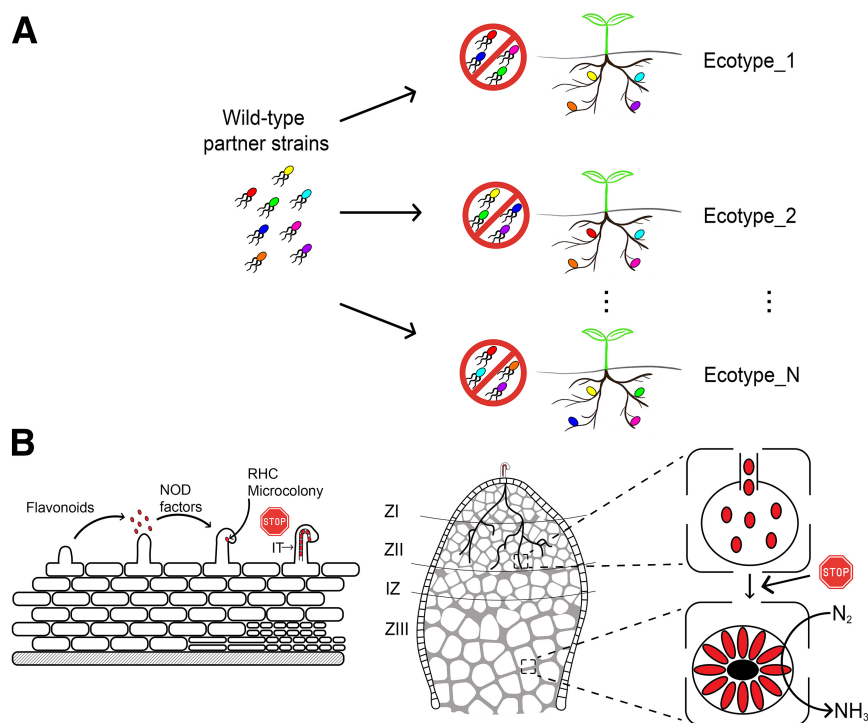


Fig. 1. Concept of partner selection in symbiosis. **A**, Soils contain diverse rhizobial populations (colored bacterial cells), all of which are generally capable of forming nitrogen-fixing symbioses with a given plant species. Natural variants (ecotypes) of the plant species, however, may discriminate among rhizobial groups, excluding incompatible strains from forming nitrogen-fixing nodules. **B**, The development of an indeterminate nodule (for example, on alfalfa or pea roots) is initiated by Nod factors (NFs) of the appropriate chemical structure, produced by rhizobia in response to specific compounds within the diverse flavonoid cocktails released by nitrogen-starved plants. NF-producing rhizobia induce root hair curling (RHC), in which the entrapped bacteria form a microcolony. In parallel, cortical cells re-enter the cell cycle, divide, and form a persistent meristem (Zone I) that continually produces new cells. These cells undergo several endoreduplication cycles to reach their final DNA content and size. A transcellular infection thread forms and delivers rhizobia into the developing nodule tissues (Zone II, the infection zone), where they are released into the cytoplasm of plant cells enclosed within a plant-derived membrane. In the interzone (IZ), both bacterial and plant cells differentiate into their symbiotic forms. In the nitrogen-fixation zone (Zone III), bacteroids—the nitrogen-fixing forms of rhizobia—reduce atmospheric N_2 to ammonia, which, after assimilation, supports plant growth. Plants can interrupt the symbiotic interaction (STOP sign) either at early stages (infection thread initiation or growth) or at later stages by eliminating bacteroids.

Bacterial signal molecules and their perception in the early steps of symbiosis

Usually, plant species establish symbiosis with a limited number of bacterial partners, and rhizobia are able to induce nodules only on certain plant species (together, they are termed a cross-inoculation group); however, there are bacteria with a broad host range (Perret et al. 2000; Pueppke and Broughton 1999). For example, *Sinorhizobium meliloti* is the partner of plants in the *Medicago*, *Melilotus*, and *Trigonella* genera, whereas *Rhizobium leguminosarum* biovar *viciaea* forms nodules on *Pisum*, *Lens*, *Lathyrus*, and *Vicia* species. The legume–rhizobial interaction begins at the developing root hairs with the mutual recognition of partners, where host-derived flavonoids and rhizobial lipo-chitooligosaccharide Nod factors (NFs) play essential roles and determine the specificity of the partnership. In nitrogen-deficient environments, legumes release diverse cocktails of flavonoids, of which only specific compounds are recognized by the individual NodD transcriptional regulator proteins of different rhizobial species, providing the first layer of specificity in partner selection. As a result, each rhizobial strain responds to particular legumes and activates its nodulation (*nod*, *nol*, *noe*) genes (Györgypal et al. 1991). Activation of these genes leads to the production and export of NFs, which are composed of a chitin oligomer backbone, typically containing four to five β -1,4-linked N-acetyl-D-glucosamine residues, with an acyl chain attached to the C2 position of the non-reducing terminal sugar. The common *nod* genes encode proteins responsible for chitin oligomer synthesis, acyl chain attachment, and NF export. Meanwhile, species- and strain-specific nodulation genes determine the structure of the acyl chain and the unique substitutions on the reducing and non-reducing terminal glucosamine residues, which vary depending on the rhizobial species (Perret et al. 2000).

NFs, and thus rhizobia, are recognized by a membrane-anchored receptor complex centered on a heterodimer of two LysM domain-containing receptor kinases, which bind NFs with high affinity (Broghammer et al. 2012; Limpens et al. 2003; Madsen et al. 2003; Radutoiu et al. 2003). In *L. japonicus*, the NF receptor pair NFR1 and NFR5 binds the fucosylated NFs produced by *Mesorhizobium loti*, whereas in *M. truncatula*, the LYK3 and NFP receptors orthologous to NFR1 and NFR5, respectively, recognize the sulfated NFs from *S. meliloti*. The ability of different NF receptor variants to recognize chemically distinct NFs has been demonstrated through their ectopic expression in heterologous hosts, as well as through domain and amino acid swaps between the proteins (Radutoiu et al. 2007). Furthermore, variation in the number, sequence, and expression levels of NFR1/LYK3-like receptors (Wang et al. 2022) may modulate the stringency of NF recognition: For example, wild pea accessions, commonly referred to as Afghan peas, carrying the *Sym2^A* allele can be nodulated only by rhizobia expressing the *nodX* gene (Firmin et al. 1993), which confers O-acetylation at the C6 position of the N-acetylglucosamine residue at the reducing end of the NF. In contrast, European cultivated pea lines carrying the *Sym2^C* allele can establish effective symbiosis with rhizobia lacking *nodX* (Geurts et al. 1997; Kozik et al. 1995). The *Sym2* gene has been mapped to a genomic region (Gualtieri et al. 2002) containing four LYK3-like receptor kinase genes (Sulima et al. 2025), including *Sym37* (*PsK10*), which controls infection thread (IT) initiation and nodule development, at least in European pea varieties (Zhukov et al. 2008). Based on nodulation phenotypes, we suppose that *Sym37*, which can recognize both acetylated and non-acetylated NFs, is inactive in Afghan peas. In contrast, the Afghan pea variant of *Sym2* proposed to be *LykX* (Solovev et al. 2021) recognizes only the acetylated NF and is also present in some European lines, such as cultivar Finale, where *sym37* mutants can still be nodulated by *nodX*-

expressing rhizobia (Zhukov et al. 2008). In *M. truncatula* cultivar Jemalong, two LysM receptor kinases, LYK2 and LYK3, are orthologous to *Lotus* NFR1. In *M. littoralis* R108, three such receptors (LYK2, LYK2bis, and LYK3) have been identified. Interestingly, only the *hcl* mutant of Jemalong, defective in LYK3, and the triple and double mutants (*lyk2bis/lyk2*, *lyk2bis/lyk3*) of R108 fail to form nodules after inoculation with *S. meliloti* (Li et al. 2024; Smit et al. 2007). However, overexpression of any of the three R108 receptor kinase genes in the triple mutant restores nodulation ability (Li et al. 2024). Moreover, the R108-specific *LYK2bis* gene—a chimera of *LYK2* and *LYK3*—uniquely enables nodulation with *nodF/nodL* mutant *S. meliloti*, which produces NFs lacking O-acetylation at the non-reducing end and contains an alternative lipid at the same end (Luu et al. 2023).

Signal transduction from the cell membrane to the nucleus

The perceived signal is transmitted through the so-called common symbiosis signaling pathway (Kouchi et al. 2010), which is also shared by the beneficial symbiosis established with arbuscular mycorrhizal fungi. The maleatin-like domain leucine-rich repeat receptor kinase (MLD-LRR-RLK) SYMRK (Endre et al. 2002; Stracke et al. 2002) is part of the NF receptor complex in the plasma membrane (Ried et al. 2014), whereas other components—such as the nucleoporins NUP85, NUP133, and NENA (Groth et al. 2010; Kanamori et al. 2006; Saito et al. 2007); the potassium channel formed by one (MtDMI1) protein or two (LjCASTOR-LjPOLLUX) proteins (Ané et al. 2004; Imaizumi-Anraku et al. 2005); the CNGC15 cyclic nucleotide-gated channels; and the MCA8 Ca^{2+} -dependent ATPase (Capoen et al. 2011; Charpentier et al. 2016; Cook et al. 2025)—are located in the nuclear envelope or the nucleus. Nuclear-localized elements include the calcium- and calmodulin-dependent serine/threonine protein kinase DMI3 (Lévy et al. 2004) and its target, the IPD3 (CYCLOPS in *Lotus*) transcription factor (Messinese et al. 2007; Yano et al. 2008).

Elongating root hairs respond to NFs within 1 to 5 min by a Ca^{2+} influx, followed by an anion efflux that leads to membrane depolarization, which in turn triggers a potassium ion efflux initiating repolarization (Felle et al. 1998). A few minutes later, calcium spiking (periodic, nucleus-associated oscillations in Ca^{2+} levels) begins (Ehrhardt et al. 1996). The signaling pathway components located in the plasma membrane and nuclear envelope are essential for calcium spiking, and the DMI3 protein plays a key role in decoding these oscillations (Oldroyd and Downie 2006). It remains to be elucidated how receptor activation at the plasma membrane is communicated to the nuclear envelope and nucleus to initiate downstream signaling events, including the identity of the second messenger(s) involved. Activated DMI3 associates with and phosphorylates IPD3 (Yano et al. 2008), leading to the activation of the symbiotic transcriptional network through the NSP1–NSP2 transcription complex (Fonouni-Farde et al. 2016; Jin et al. 2016). This complex binds to the promoters of NF-inducible genes, including *NIN* and *ERN1*, which encode additional transcription factors with nodule-specific functions (Hirsch et al. 2009). It must be noted that some *Aeschynomene* species can form root and stem nodules with *Bradyrhizobium* bacteria even though the bacteria lack the canonical nodulation genes and do not produce NFs (Giraud et al. 2007). Surprisingly, a photosynthetic *Bradyrhizobium* isolated from a marine diatom and possessing no nodulation genes induces nitrogen-fixing nodules on *A. indica* (Chandola et al. 2025). It was shown that NF-independent nodule formation required the activity of the common symbiosis signaling pathway (Fabre et al. 2015).

Nodule formation and bacterial infection

NF signal perception and transduction initiate two processes: (i) the mitotic activation of root cortical and the underlying en-

dodermis/pericycle cells radial to the xylem pole to produce the nodule meristem and the uninfected cells at the base of the mature nodule, respectively, and (ii) infection of the newly formed meristem-derived nodule cells by rhizobia. Based on the maintenance of meristematic activity, two major morphological types of nodules can be distinguished. Determinate nodules lack a persistent meristem and are usually spherical in shape. In contrast, the typically cylindrical indeterminate nodules contain an apical meristem that continuously produces new cells capable of being infected by bacteria, resulting in an age and developmental gradient along the nodule axis (Ferguson et al. 2010). In indeterminate nodules, distinct zones corresponding to the developmental stages of symbiotic cells can be identified: the apical meristem (Zone I), the infection zone (Zone II), the interzone (Zones II to III), the nitrogen-fixing zone (Zone III), and, in older nodules, the senescence zone (Zone IV). In both nodule types, differentiation of the newly formed cells into mature, bacterium-filled symbiotic cells requires successive rounds of endoreduplication (DNA replication without cell division). As a consequence, nuclear DNA content increases from 2C up to 64C (C corresponds to the haploid DNA content), the cells enlarge drastically (approximately 80-fold), and each can host up to 50,000 nitrogen-fixing bacteria (Maroti and Kondorosi 2014).

Parallel to the formation of the nodule primordium, rhizobia invade roots either intercellularly (Ibáñez et al. 2017; Quilbé et al. 2022)—a mode considered the ancestral route—or through transcellular ITs, which are tubular structures that guide bacteria into the inner tissues of the nodule (Tsyganova et al. 2021). In the latter route, NF production by rhizobia near the tips of actively growing root hairs induces continuous reorientation of their growth, resulting in a characteristic shepherd's crook-like curl. This curled root hair forms an infection pocket that encloses the bacteria, which then establish a microcolony. Within the infection pocket, the IT is initiated by localized degradation of the cell wall and invagination of the root hair membrane. The thread then extends via inverted polar growth toward the base of the root hair cell; it is bordered by a membrane and cell wall of plant origin and contains a matrix produced by both partners. At the base of the root hair, the IT fuses with the distal cell wall, thereby bridging the IT into the adjacent cell (Su et al. 2023). In the cortical cells ahead of the advancing IT, cytoplasmic bridges aligned in radial rows traverse the central vacuole. These structures, termed pre-infection threads, help determine the future path of the IT (van Brussel et al. 1992). The polar growth of the IT requires the coordinated and dynamic action of various proteins involved in membrane domain specification, cell polarity, cytoskeletal rearrangement, vesicle trafficking, and regulation of NF levels (Gao et al. 2024). Among these, a protein complex termed the infectosome plays a crucial role in maintaining the normal polar growth of ITs (Liu et al. 2019). The infectosome localizes to the tip of the growing IT, as well as to cytoplasmic puncta near the nucleus. It is believed to mediate exocyst-dependent vesicle trafficking toward the IT tip. The exact composition of the vesicle cargo remains unclear; it may consist of lipid vesicles to support plasma membrane expansion or enzymes and other materials required for cell wall modification and extension within the IT.

The population of bacteria infecting an IT remains localized in the extracellular space and typically descends usually from a single or—if the rhizobium density at the root hair is sufficiently high—very few founder cells. Bacterial cell division occurs near the tip of the IT; consequently, only the descendants of the tip-most bacteria populate the nodule (Gage 2002, 2004). Beyond producing NFs with the correct structure and quantity, rhizobia must adapt to the specific environment of the IT and present surface polysaccharides with appropriate composition and mod-

ifications. These polysaccharides may participate in adaptation and/or act as additional signaling molecules. Rhizobia that have lost the ability to produce periplasmic cyclic β -(1 $\rightarrow$ 2)-glucan and to adapt to hypoosmotic conditions are unable to successfully carry out the infection process (Dylan et al. 1990). Similarly, a K^+/H^+ antiporter mutant of *S. meliloti*—which is defective in K^+ efflux at alkaline pH and, therefore, sensitive to potassium—fails to invade nodule tissues due to early abortion of ITs (Putnoky et al. 1998). Rhizobia produce a wide variety of species-specific (EPS, extracellular polysaccharide) and strain-specific (lipopolysaccharide; K-antigen or capsular polysaccharide) surface polysaccharides, all of which are implicated in the infection process. However, their essentiality depends on the host plant (Acosta-Jurado et al. 2021; Frayse et al. 2003). In general, lipopolysaccharide appears to be essential for infection of determinate nodules, as shown for soybean, bean, and *Lotus* species (Margaret et al. 2013; Noel et al. 2000; Turska-Szewczuk et al. 2009). In contrast, EPS production was found to be dispensable for these hosts, because EPS-defective rhizobial mutants still formed effective symbioses with them (Borthakur et al. 1988; Hotter and Scott 1991; Kelly et al. 2013; Rodríguez-Navarro et al. 2014). Conversely, IT development in legumes that form indeterminate nodules typically requires EPS production (Borthakur et al. 1988; Chakravorty et al. 1982; Glazebrook et al. 1990; Hotter and Scott 1991). In the absence of EPS, either no ITs form or they abort prematurely. Interestingly, the lack of EPS production can be compensated for by the production of the symbiotically active K5-antigen in *S. meliloti* strain Rm41 on *M. sativa* and certain *M. truncatula* ecotypes (Putnoky et al. 1990). *M. loti* mutants affected in the early biosynthetic steps of EPS synthesis can still establish effective symbiosis with *Lotus* species. However, mutants defective in mid- or late biosynthetic steps induce uninfected nodule primordia (Kelly et al. 2013). It was later shown that the secretion of monomeric and oligomeric truncated EPS oligosaccharides disrupts the infection process (Wightman et al. 2024). These incompatible EPS fragments are perceived by the plant through a LysM-type receptor, which is expressed only after NF perception within the nodulation-susceptible zone (Kawaharada et al. 2015).

Both nodule organogenesis and infection programs are regulated and coordinated by all the major plant hormones and by their cross-talk (Lin et al. 2020; Velandia et al. 2022); however, because of the complexity of the topic and space limitation, this review does not provide details on the hormonal regulation of nodule development.

Invasion of nodule cells and bacteroid development

When the ramifying ITs reach the young nodule cells (Zone II in the indeterminate nodules), bacteria—enclosed by a host-derived membrane that becomes the peribacteroid membrane (PBM)—are released from the unwalled tip regions of the IT into the plant cytoplasm as infection droplets (Catalano et al. 2007), and organelle-like structures, termed symbiosomes, are formed. The PBM not only serves as a barrier between rhizobia and the plant cell, preventing a pathogenic interaction, but also performs the crucial function of mediating nutrient exchange between the symbionts (Udvardi and Poole 2013). Within nodule cells undergoing the second to fifth endoreduplication cycles (resulting in 8C to 64C nuclear DNA content), typically in Zone II and the interzone of indeterminate nodules, the bacteria and the surrounding PBM divide and develop into mature symbiosomes containing nitrogen-fixing bacteroids (Vasse et al. 1990). During this process, the nodule interior becomes microaerobic, and bacterial nitrogen fixation genes (*nif*, *fix*) are strongly induced. The fully mature symbiotic cells (in Zone III of indeterminate nodules) reduce atmospheric nitrogen (N_2) to ammonia, which is then assimilated and transferred to non-infected cells and other

parts of the plant (Prell and Poole 2006). The symbiosome membrane, depending on the host plant, may enclose either a single or multiple bacteroids. For example, in the determinate nodules of soybean and *Lotus*, as well as in the indeterminate nodules of *Leucaena*, symbiosomes typically contain multiple bacteroids that resemble free-living bacteria. These types of symbiosomes may form via PBM fusion or bacterial division within the symbiosome. In contrast, symbiosomes in the indeterminate nodules of inverted repeat-lacking clade (IRLC) legumes (e.g., *Medicago*, *Pisum*, *Trifolium*, and *Cicer*) and in the determinate nodules of Dalbergoid legumes (e.g., peanut and *Aeschynomene*) contain a single, enlarged, and often elongated, branched, or spherical bacteroid (Kereszt et al. 2011). These bacteroids exhibit increased DNA content (up to 24C), greater membrane permeability, and loss of cell division capacity—features characteristic of terminally differentiated bacteria (Mergaert et al. 2006). Terminal bacteroid differentiation is directed by nodule-specific cysteine-rich (NCR) peptides in IRLC legumes and by NCR-like peptides in *Aeschynomene* species (Czernic et al. 2015; Van de Velde et al. 2010). Among different IRLC legume species, terminal differentiation is associated with varied bacteroid morphologies, which correlate with the number (ranging from 7 to approximately 700) and type of NCR peptides (Montiel et al. 2016, 2017). Despite the presence of hundreds of NCR genes in *M. truncatula*, a few NCR peptides have unique and essential functions in nodule and bacteroid development and function (Yang et al. 2025). An evolutionary analysis (Oono et al. 2010) concluded that the reversible bacteroid state is ancestral, whereas terminal differentiation of bacteria evolved independently at least five times in Papilionoideae legumes, suggesting convergent evolution and a selective advantage. Indeed, studies revealed that terminally differentiated bacteroids outperformed their reversible counterparts (Oono and Denison 2010; Sen and Weaver 1980). Furthermore, it was shown that *Aeschynomene* species inducing spherical bacteroids gained more benefit than those inducing elongated bacteroids, even when inoculated with the same bacterial strain (Lamouche et al. 2019). Nitrogen fixation in nodules is maintained for several weeks. Then, the symbiotic cells begin to senesce; both bacterial and plant cells degrade, and their components are recycled by the plant. In indeterminate nodules, the senescing cells form the senescence zone (Zone IV). However, nitrogen-fixing cells are continually replaced by younger cells due to the persistent activity of the apical meristem. In contrast, when determinate nodules senesce, new nodules are formed on recently developed portions of the root (Rolfe and Gresshoff 1988; Van de Velde et al. 2006).

Choosing the Few from the Many: At the End, Plants Decide

Partner preference in natural environments

We may assume that most of the genes involved in the establishment and functioning of nitrogen-fixing nodules in the majority of legume–rhizobium interactions (collectively, we termed them the “essential symbiotic toolkit”), along with their expression patterns, have been identified. Several components of this essential symbiotic toolkit have also been validated in crop plants and their microbial partners. However, the functions, interactions, and modes of action of the encoded proteins and RNAs are still being elucidated (Mergaert et al. 2020; Roy et al. 2020). These studies were performed using a limited number of eukaryotic and prokaryotic model organisms under controlled laboratory or glasshouse conditions, typically testing one plant line and a single bacterial strain. Occasionally, the competitiveness of laboratory strains has been investigated (Sanchez-Cañizares and Palacios 2013).

In natural and anthropogenic soil environments, in which different strains of rhizobial genospecies coexist and compete, the success of a bacterial strain (whether indigenous or introduced as an inoculant)—that is, its ability to induce, infect, and occupy nodules to establish a niche where it can multiply and achieve a higher cell density than in the surrounding soil—depends on multiple variables (Mendoza-Suárez et al. 2021). Often, the frequency of rhizobial genotypes in soil differs markedly from that recovered from nodules on plants grown in the same soil (Bromfield et al. 1995; Laguerre et al. 2003; Li et al. 2023). A higher abundance of a strain—due to better adaptation to soil characteristics such as texture, pH, nutrient and water availability, and the composition of the microbiome—may predetermine its dominance in nodules. However, some rhizobial genotypes were found to occupy nodules according to their soil abundance at one location but were under- or overrepresented at another site, suggesting that environmental factors influence their success (Bromfield et al. 1989; Laguerre et al. 2003). In addition, competitiveness (the ability to form nodules in the presence of other strains) is also a key adaptive feature of rhizobia; however, its genetic basis is not yet fully understood. Moreover, it is often difficult to distinguish between rhizobial competitiveness and plant preference (or rejection) for a strain, as they may represent two sides of the same coin. The dependence of bacterial success on the plant host(s) present has been observed both in field studies and in experiments inoculating soil-derived or synthetic bacterial communities onto their respective cross-inoculation group plants. For example, the partner preference of *M. sativa* and *Melilotus albus* for *S. meliloti* genotypes differed when they were inoculated with the same soil bacterial community (Bromfield et al. 1995). Inoculation of a core collection of 104 pea lines with five different *R. leguminosarum* bv. *viciae* strains (Bourion et al. 2017), as well as inoculation of a core collection of 32 *R. leguminosarum* bv. *viciae* strains onto nine genetically diverse *Pisum sativum*, *Vicia faba*, and *Lens culinaris* genotypes (Boivin et al. 2021), revealed clear evidence of partner selection or host filtering (shaping associated microbial communities) by the plants. Similarly, host filtering—measured as deviation from the initial *Rhizobium* inoculant composition—was detected under both field and controlled greenhouse conditions, in which different clover varieties and even genotypes within the same variety selected for significantly different *R. leguminosarum* bv. *trifolii* nodule communities (Fields et al. 2023). It has also been shown that when multiple bacterial taxa can form symbiosis with a host plant, such as *Bradyrhizobium* and *Sinorhizobium* spp. with soybeans, one plant genotype may favor bradyrhizobia, which are more common in acidic soils, whereas another genotype may associate equally with both taxa (Li et al. 2023). Furthermore, a geographic distribution analysis of 1,821 soybean accessions (wild isolates and landraces) revealed that plants with the first genotype were enriched in acidic soils dominated by bradyrhizobia, whereas soybeans with the second genotype were most prevalent in alkaline soils dominated by sinorhizobia. In neutral soils, no clear preference for either genotype was observed. Similarly, marked differences were observed in the ability of *S. medicae* and *S. meliloti* strains to establish symbiosis with different *Medicago* species (Garau et al. 2005). Both bacteria were effective partners of *M. sativa*, but *S. meliloti* induced either no nodules or only ineffective nodules on the roots of *M. murex* and *M. polymorpha*, respectively. The authors concluded, from the observed symbiotic relationships between different *Medicago* species and the two *Sinorhizobium* species, that these bacteria have co-evolved with their host plants, adapting to different soil pH conditions. *S. medicae* is typically associated with medics adapted to moderately acidic soils (*M. murex*, *M. arabica*, *M. sphaerocarpos*, and *M. polymorpha*), whereas species that appear preferentially associated with *S. meliloti* (e.g., *M. littoralis*

and *M. tornata*) are more common in alkaline or neutral and sandy soils.

The art of saying NO: Are there gene-for-gene interactions in symbiosis?

Plants are continuously challenged by microbial pathogens; therefore, they have evolved a multilayered defense system (Dodds et al. 2024). As a first line of active defense, the plant surface is monitored by membrane-spanning receptors that recognize microbe- or pathogen-associated molecular patterns (MAMPs/PAMPs), which are, in many cases, conserved motifs on the surface of or within microbial cells. The binding of extracellular elicitors such as MAMPs/PAMPs by elicitor-recognition (pattern-recognition) receptors triggers downstream defense responses, leading to MAMP-/PAMP-triggered immunity (MTI/PTI). The extracellular, ligand-binding region of receptor proteins may contain various domain types, such as lectin, epidermal growth factor, cysteine-rich, and MLDs or LRRs (Dodds et al. 2024), as well as combinations of these, as exemplified by the MLD-LRR-RLK SYMRK required for different symbioses (Endre et al. 2002). One other class of elicitor-recognition receptors contains LysM motifs in their extracellular domains, which are implicated in oligo-/polysaccharide binding and are found in NF receptors (NFR1/NFR5), as well as in the EPR3 receptors discussed earlier. The response of the latter to truncated EPS fragments may represent a specific MTI/PTI reaction, although the associated cellular responses—such as the signaling pathways, gene expression changes, reactive oxygen species production, and changes in ion fluxes—remain unknown. Legumes can distinguish beneficial from harmful microbes by employing different LysM-receptor kinases to differentiate between the perception of chitin oligomeric MAMPs and the perception of NFs by the NFR1/NFR5 receptor complex (Bozsoki et al. 2017).

In the constant arms race between plants and pathogens, microbes have evolved virulence factors and specialized apparatus for delivering them into plant cells, thereby overcoming the otherwise effective MTI/PTI by modifying or attenuating the original immune responses. In resistant plants, effector-triggered immunity (ETI) prevents the action of the effectors and the spread of pathogens. The effectors or the products of their biochemical activity are recognized by intracellular nucleotide-binding LRR (NLR) receptors, triggering a cascade of signaling events—including changes in ion fluxes, reactive oxygen species production, and hormonal signaling—that culminate in the hypersensitive response (HR), a rapid and localized form of programmed cell death (Dodds et al. 2024). A central player at the intersection of PTI, effector-mediated immune suppression, and ETI is the RPM1-interacting protein 4 (RIN4), which negatively regulates PTI and is modified by several pathogen effectors to inhibit immune defense, and its modification by the effectors triggers ETI by NLRs (Toruño et al. 2019). RIN4 of soybean was shown to be rapidly phosphorylated upon rhizobial inoculation in the root hairs by SYMRK and to be required for proper symbiosis formation, indicating its role in distinguishing friends and foes (Tóth et al. 2025).

Certain rhizobia, similar to other gram-negative (pathogenic) bacteria, possess one or more apparatuses of the type III, IV, or VI secretion systems (T3SS, T4SS, or T6SS) used to deliver effector proteins (T3, T4, or T6 effectors) with diverse biochemical functions into plant cells. Among these, the T3SS and its associated effectors are the most numerous and best studied in rhizobia (Jiménez-Guerrero et al. 2022; Teulet et al. 2022). The components of the T3SS and the secreted T3 effectors are termed nodulations outer proteins (Nops) in rhizobia and are produced upon NF induction, indicating their role in symbiosis. Their effects on symbiosis vary with the host: their production can be neutral in certain interactions, essential for nodule formation in

others, or can inhibit nodulation/infection in specific species or ecotypes (Teulet et al. 2022; Wang et al. 2022). Notably, T3 effectors from some rhizobia can bypass the requirement for NFs to trigger nodulation (Ratu et al. 2023). For example, a *nodC* mutant of *Bradyrhizobium elkanii* strain USDA61, which cannot produce NFs, forms nodules on *Aeschynomene indica* provided its T3SS is functional.

Beyond the specific local defense reactions in PTI and ETI, both processes can trigger broad-spectrum immune responses to secondary infections via the production of the defense hormones salicylic acid and jasmonic acid, leading to systemic acquired resistance and local acquired resistance, respectively (Kumar et al. 2025). A common feature of both induced resistance types is the production of pathogenesis-related (PR) proteins, although different PRs from the approximately 20 known classes are expressed in the two processes (Ali et al. 2018). Many PRs possess antimicrobial activity, such as PR1, PR2 (β -1,3-glucanases), and PR5 (thaumatin-like proteins), which are characteristic of systemic acquired resistance, or PR3 and PR4 chitinases and PR12 defensins, which are molecular markers of local acquired resistance. The NCR peptides, which are essential for terminal bacteroid differentiation, are most similar to defensins, and the cationic members of this nodule-specific peptide family have antimicrobial activity (Maróti et al. 2011).

Large collections of symbiotic partners, including plant ecotypes/accessions and rhizobium strains isolated as effective symbionts, have enabled investigations of natural variation in symbiotic interactions. These studies revealed, for example, that certain soybean genotypes restrict nodulation with specific rhizobial strains (Hayashi et al. 2012) and that there is substantial variation in nodulation capacity and nitrogen fixation specificity among different *Medicago* species/ecotypes and *S. meliloti*/*S. medicae* host-strain pairs (Crook et al. 2012; Liu et al. 2014). These symbiotic incompatibilities (Fig. 1) are highly specific between the interacting partners and are determined by dominant alleles in the host plants, a striking similarity to the resistance specificity observed in plant-pathogen interactions that led to the “gene-for-gene” concept later replaced with the term ETI discussed above (Flor 1971; Gassmann and Bhattacharjee 2012). It must be emphasized that in cases of “gene-for-gene” incompatibilities, including those in which T3 effectors negatively affect symbiosis and are recognized by classical intracellular ETI receptors (see later), no HR has been observed. This indicates that the nitrogen-fixing legume-rhizobium symbiosis might have taken advantage of the existing plant defense inventory but modified some elements to affect the symbiotic program to discriminate between potential symbionts.

Gene-for-gene incompatibilities mediated by membrane-bound receptor kinases

Several studies have reported that *S. meliloti* and *S. medicae* strains differ in their symbiotic efficiency not only with different *Medicago* species but also with *M. truncatula* ecotypes (Crook et al. 2012; Liu et al. 2014; Snyman and Strijdom 1980; Tirichine et al. 2000). A detailed analysis of the incompatible interaction between *S. meliloti* strain Rm41 and *M. truncatula* ecotype F83005.5 revealed that the bacterium could induce root hair curling and nodule primordium formation, but IT initiation and elongation—and thus the invasion process—were blocked (Liu et al. 2014). A very similar phenotype was observed with all but one *S. medicae* strain, which failed to induce infected nodules on the roots of ecotype DZA220H (Yu et al. 2024). This ecotype may belong to *M. littoralis* or may be an introgressant derived from it, according to a recent classification based on plastid genome sequences (Choi et al. 2022). In both plants, the trait was determined by a single dominant genetic locus, termed *NS1* (*Nodulation Specificity 1*) in F83005.5 and *NS2* in DZA220H.

The *NS1* locus in *M. truncatula* ecotype F83005.5 comprises two tandem genes, *NS1a* and *NS1b*, both of which are required for the strain-specific nodulation blockade. In other ecotypes that are normally infected, the locus varies in the sequence and copy number of these genes (Liu et al. 2022). Expression of the *NS1* genes is induced by NF-producing rhizobia and depends on NF perception by the host. *NS1a* and *NS1b* encode MLD-LRR-RLKs that are homologous to MtSYMRK and share its domain structure. The two proteins interact at the plasma membrane; *NS1a*, but not *NS1b*, self-associates to form homomers, but neither interacts with SYMRK. On the bacterial side, a gene present in approximately half of the sequenced *S. meliloti* isolates (and causing incompatibility in all tested bacteria) was identified through loss-of-function mutations in multiple strains and heterologous expression in compatible rhizobia. Notably, this gene, termed *rns1* (*required for activation of the NS1 locus*), is located in an approximately 55-kb strain-specific gene cluster on the pSymB megaplasmid. *Rns1* encodes a protein that is secreted via the type I secretion system (T1SS) and contains right-handed parallel β -helix repeats, a motif often found in polysaccharide lyases such as pectate lyases and glycanases. However, its biochemical function remains to be elucidated.

The *NS2* gene is part of a complex gene cluster encoding MLD-LRR-RLK-type receptor-like kinases, with homologous clusters located in syntenic genomic regions of both legumes and non-legumes (Yu et al. 2024). Similar to *NS1*, the expression of *NS2* is induced by NFs produced by both compatible and incompatible bacteria. Most sequenced *Medicago* lines lack allelic copies of *NS2*, for which multiple possible explanations exist: (i) there are selective pressures excluding this gene from natural populations; (ii) *NS2* may have arisen from a recent gene duplication followed by mutations; (iii) in the absence of systematic compatibility tests between *M. littoralis* accessions and *S. medicae* strains, and without long-read genome sequencing of *M. littoralis* ecotypes, *NS2* alleles may simply not have been detected yet. Another question regarding *NS2* is whether an allelic variant of a second gene from the MLD-LRR-RLK cluster is required for incompatibility, similar to *NS1*. The genetic determinant in *S. medicae* termed *rns2* was identified in the same way as *rns1* in *S. meliloti*. The *rns2* genes in different *S. medicae* strains code for putative secreted or structural cell wall proteins containing an N-terminal hydrophobic amino acid sequence that may serve as a secretory signal or as a transmembrane domain, as well as a variable number of perfect tandem β -repeats of a glycine-rich octapeptide. In contrast, the *rns2*-like genes in *S. meliloti* genomes code for proteins with imperfect tandem repeats consisting of octa- and hexapeptides in variable numbers and arrangements. These differences suggest that it is the regular structure, rather than the size, of the *Rns2* proteins that determines incompatibility. Similar to *Rns1*, no biochemical or structural function has been assigned to *Rns2* yet.

No physical interaction between the NS and Rns proteins could be detected in vitro, suggesting that either two receptor proteins are required for efficient Rns binding or the bacterial proteins are not the direct ligands perceived by the receptor kinases. In the latter case, the Rns proteins are likely involved in biochemical reactions that generate products recognized by the receptor kinases. In support of this latter scenario, there are multiple examples for plant immune responses when microbial effectors do not interact with the resistance proteins but rather the result of their biochemical activity is sensed by the receptors. For example, the apoplastic Avr2 protease inhibitor effector of *Cladosporium fulvum* binds and inhibits the Rcr3 cysteine protease of tomato, and, most probably, the resulting conformational change of Rcr3 triggers the HR mediated by the Cf-2 receptor-like resistance protein (Rooney et al. 2005). The intracellular RIN4 is post-translationally modified by multiple *Pseudomonas*

effector proteins (AvrRpm2, AvrB, and HopZ5) with diverse biochemical activities, such as ADP ribosylation, rhamnosylation, or acetylation, respectively, all activating RPM1 resistance protein-dependent immunity in *Arabidopsis* (Choi et al. 2021; Peng et al. 2024; Yoon et al. 2022). Based on the high levels of structural and sequence similarity, as well as their expression patterns, the recognition mechanisms and downstream targets of the NS receptor systems might be similar.

Intracellular receptors and PR proteins restrict infection in soybeans

Similar to the *Medicago*–*Sinorhizobium* incompatibilities described in the previous section, several soybean genotypes were identified that restrict nodulation with specific rhizobial strains after the initial root hair curling and cortical cell divisions. Each of the genetic determinants, termed *Rj2* (Caldwell 1966), *Rj3* (Vest 1970), *Rj4* (Vest and Caldwell 1972), and *Rfg1* (Trese 1985), behaves as a single dominant gene. Bacterial mutants defective in the T3SS or in the production of certain T3 effectors could form nitrogen-fixing nodules on the roots of their incompatible hosts (Faruque et al. 2015; Meinhardt et al. 1993; Shobudani et al. 2020; Tsukui et al. 2013; Tsurumaru et al. 2015), indicating that the four incompatibilities are specific manifestations of ETI without an HR. In this sense, these incompatibilities are reminiscent of the extreme resistance (Ross et al. 2021) characterized by absence of symptoms and limited or lack of virus replication and spread, often mediated by resistance proteins, that may cause HR. Indeed, allelic variants of a single classical resistance (R) gene, *Glyma16g33780*, encoding a Toll/interleukin-1 receptor–nucleotide-binding site–LRR (TIR–NBS–LRR) protein, are responsible for both the *Rj2* and *Rfg1* incompatibilities (Yang et al. 2010). A single amino acid difference (I490R) after the NBS domain distinguishes the restrictive *Rj2* and permissive *rj2* alleles, whereas the *Rfg1* and *rfg1* alleles differ at five positions in and after the sixth LRR domain (Sugawara et al. 2019; Yang et al. 2010). All four possible allelic combinations (*rj2/rfg1*, *Rj2/rfg1*, *rj2/Rfg1*, and *Rj2/Rfg1*) of the gene were constructed and shown to determine the expected compatibility profile when transformed into a non-restrictive (*rj2/rfg1*) soybean (Fan et al. 2017). A genome-wide association study identified a gene termed *G. max Nodule Number Locus 1* (*GmNNL1*) encoding another TIR–NBS–LRR receptor protein that restricts nodulation with *B. diazoefficiens* strain USDA110 (Zhang et al. 2021). Surprisingly, a thaumatin-like protein, which belongs to the PR-5 family of PR proteins, is the determinant of incompatibility in *Rj4* soybeans (Tang et al. 2016); however, it cannot be excluded that another NBS–LRR receptor, which has not yet been identified, also contributes to the nodulation arrest.

In light of these discoveries, the previously mentioned results regarding the connections between soybean genotypes with different bacterial preferences and soil pH (Li et al. 2023) can be explained. In their investigation, the SC1 genotype, which favors bradyrhizobia and was more prevalent in acidic soils where these bacteria are common, carried the *rj2/Rfg1* allele combination, whereas HH1 soybeans carried the non-restrictive *rj2/rfg1* allele combination, as can be deduced from the sequence data presented in the cited article. Soybeans with the *Rfg1* genotype were shown to be incompatible with *S. fredii* strains USDA193, USDA205, and USDA257. Thus, it is possible that *Rfg1* confers broad-spectrum resistance to *S. fredii*, similarly to *NS2* in *M. littoralis*, which prevents nodulation and infection by *S. medicae* strains. Accordingly, the preference for bradyrhizobia could be a direct consequence of this incompatibility. In addition, the lack of such strain restriction in the *rj2/rfg1* genotypes can be favorable in environments in which sinorhizobia are more common. It would be worth reexamining the large dataset obtained by the

authors to look for polymorphisms at amino acid position 490 and to identify *Rj2* alleles and then reevaluating the bacterial and soil preferences, as well as the geographical distribution of the *Rj2* genotypes.

All incompatibilities between soybeans and different bacterial strains depend on bacterial T3 effectors, as well as genes affecting T3SS function or potentially modulating plant innate immunity (Wang et al. 2022). Incompatibilities mediated by GmNNL1 and *Rj2*—restricting nodulation by *B. diazoefficiens* strains USDA110 and USDA122, respectively—are caused by bacterial NopP proteins (Sugawara et al. 2019; Zhang et al. 2021). The two NopP variants differ at only four amino acid positions, which renders USDA110 compatible with *Rj2* soybeans (Sugawara et al. 2019). Unfortunately, neither USDA122 nor a USDA110 derivative carrying the USDA122 NopP has been tested on GmNNL1 soybeans to determine whether these four residues are responsible for the specificity of the physical interaction between the receptor and effector proteins.

Post-infection collapse of the interaction: NCR peptides in action and in the crosshairs of peptidases

Legume plants safeguard nodule cells from bacteria not only at the (root hair) infection step but also during and after the release of bacteria from ITs; however, these defense reactions are under strict control and are usually suppressed (Gourion et al. 2015). Termination of the interaction due to incompatibility is not restricted to the infection stage, as late-stage arrest of symbiosis (Fig. 1) has been observed between *M. truncatula* ecotype Jemalong and *S. meliloti* strains A145 and Rm41 (Liu et al. 2014; Tirichine et al. 2000), as well as between ecotype A20 and strain USDA1963 (Crook et al. 2012).

S. meliloti strains A145 and Rm41 invade the nodules of Jemalong plants and develop into elongated bacteroids that begin expressing nitrogen-fixation genes. These bacteroids are subsequently eliminated from nodule cells, triggering early nodule senescence, and saprophytic bacteria then occupy the nodule space. The dominant alleles of two genes, *NFS1* (*Nitrogen Fixation Specificity 1*) and *NFS2*, were identified as negative regulators of symbiotic persistence (Wang et al. 2017, 2018; Yang et al. 2025). Both genes code for NCR peptides; however, their in vitro antimicrobial activity was similar against both compatible and incompatible bacteria, indicating that incompatibility is not caused by higher sensitivity of incompatible strains. The bacterial determinant responsible for incompatibility in strains A145 and Rm41—or compatibility in other strains—has not yet been identified. One possibility is that in these two strains, these NCR peptide versions target strain-specific (versions of) proteins that are essential for bacterial maintenance/survival in the nodule cells and prevent this activity. On the other hand, the genome of compatible bacteria may code for proteins that counteract the negative action of the peptide variants.

Conversely, the accessory genome of strain USDA1963 harbors a gene encoding an M16A family zinc-metallopeptidase, designated “host range restriction peptidase” (HrrP), which causes incompatibility with ecotype A20 (Price et al. 2015). HrrP degrades a range of NCR peptides, and this activity is most likely responsible for the gradual degeneration of bacteroids, activation of senescence genes, and subsequent invasion of saprophytic bacteria, as reflected by high levels of cultivable bacteria in the nodule. The symbiotic effect of HrrP depends on both host and bacterial genetic background: expression of *hrrP* in diverse *Sinorhizobium* strains caused variable effects—from complete elimination of symbiosis to unimpeded nitrogenase activity—with different hosts. The genetic determinants behind the differences in both partners have yet to be discovered.

These observations highlight that plant hosts may differ in the composition and biological activity of their NCR arsenal, and

bacterial partners may vary in intrinsic sensitivity to nodule-specific peptides. Thus, the symbiotic performance of a given interaction depends on the combined effects of the NCR peptide complement and the bacterial capacity to resist or degrade these peptides.

The NCR-related phenotypes during the incompatible interactions are reminiscent of the premature senescence observed in a number of *M. truncatula* Fix[−] mutants, including three lines defective in the production of NCR peptides essential for bacteroid maturation or persistence (Pál et al. 2025). It seems that failure to support plant growth through the production of fixed nitrogen—be it due to mutation, incompatibility, or microbial cheating—typically triggers early nodule senescence. This response prevents further allocation of resources to ineffective nodules and enables the plant to recycle materials from the non-functional organ.

Conclusions, Open Questions, and Future Directions

Currently, very little is known about how legume plants choose their partners from the soil microbiome, in which many different strains, equipped with all the necessary tools to establish effective symbiosis, are present. Existing evidence indicates that the plant’s defense machinery (including elicitor/microbial-associated molecular pattern receptors, intracellular receptors, and PR proteins) is involved in discriminating among potential rhizobial partners. However, the outcome of this “fight”—whether mediated by PTI or ETI elements or PR proteins—differs from classical defense reactions: no eradication of bacteria or HR takes place.

One open question is whether legumes employ additional mechanisms to discriminate against incompatible (“incompatible” or “undesirable”) bacteria. Another is whether the choice of defense strategy (e.g., ETI-type receptors in soybeans versus PTI-type receptors in *Medicago* spp.) depends on the mode of nodule development (i.e., whether the plant forms determinate or indeterminate nodules). Further, what renders a rhizobium *persona non grata*, and why are genes causing incompatibility maintained? Do the T3SSs and T3 effectors of soybean-nodulating bacteria, or the extracellular proteins of sinorhizobia interacting with medics, confer advantages in certain ecological niches or with certain hosts that justify sacrificing compatibility with others? Answering these questions will require the identification and characterization of additional cases of symbiotic incompatibility, ideally supported by high-throughput investigations.

The more specific questions concern the already known players in the discrimination processes. What are the targets of the plasma membrane and cytoplasmic receptors that block the infection process? Does recognition of their ligands trigger a signal transduction cascade (such as via the MAP kinase pathway) that leads to transcriptional changes attenuating or completely arresting NF-induced gene expression? Alternatively, are components of the NF signal transduction pathway and/or the infectiousome (infection machinery) directly targeted, for instance by inhibitory phosphorylation? Two MLD-LRR-RLKs, SIF2 and IOS1, encoded in the *Arabidopsis* MLD-LRR-RLK cluster syntenic to the *Medicago* NS2 region, have been shown to play important roles in (stomatal) immunity. IOS1 facilitates the formation of complexes involving the BAK1-dependent receptors FLS2 and EFR, as well as the BAK1-independent receptor CERK1, in response to MAMP treatment (Yeh et al. 2016), whereas SIF2 functions in FLS2-, EFR-, and CERK1-mediated signaling pathways that prime PTI activation (Chan et al. 2020). SIF2 phosphorylates the guard cell SLOW ANION CHANNEL1 (SLAC1) and acts between the FLS2–BAK1 immunity recep-

tor complex and SLAC1, which is required for abscisic acid-mediated stomatal closure.

One can speculate that the NS1 and NS2 receptors in legumes may alter the composition of NF receptor complexes, thereby inhibiting their function, or directly phosphorylate key proteins to block NF signaling, infectosome assembly, or vesicular transport required for IT growth.

The roles of the extracellular proteins Rns1 and Rns2 also remain unclear. Are they direct ligands of the receptor complexes? Do they produce or process the ligand? Or do they compete with or degrade the ligand, thereby preventing receptor activation? It is equally important to determine which bacterial proteins are targeted by the NFS-type NCRs and which bacterial proteins confer resistance to these peptides in compatible strains. Conversely, in the host plant, what genetic determinants prevent “cheating” strategies, such as those involving the HrrP protease?

To address these questions, it will be necessary to identify the proteins involved in partner selection, map their interaction networks, and determine their biochemical functions. Comparative transcriptome analyses of infected root hairs should also be performed.

To achieve the long-held goal of transferring nitrogen-fixing symbiosis to non-legume crops, many steps must be engineered in the future host. In addition to enabling non-legume plants to recognize NFs and form nodule structures, it will be necessary to ensure effective colonization of nodule tissues by rhizobia. These goals require research into how plants recognize and select their partners from the soil microbiome and how bacteria invade nodule tissues while simultaneously evading or suppressing plant defense responses and avoiding host discrimination.

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