International Journal of Medical Microbiology 309 (2019) 252–257

Contents lists available at ScienceDirect

International Journal of Medical Microbiology

journal homepage: www.elsevier.com/locate/ijmm

Interplay of plant glycan hydrolases and LysM proteins in plant—Bacteria interactions

Maria A. Schlöffel, Christoph Käsbauer, Andrea A. Gust*

Plant Biochemistry, Center for Plant Molecular Biology (ZMBP), University of Tübingen, 72076 Tübingen, Germany

ARTICLE INFO

Keywords:
Peptidoglycan
Nodulation factor
Lysin motif
Plant receptor
Chitinase

ABSTRACT

Plants are always found together with bacteria and other microbes. Although plants can be attacked by phytopathogenic bacteria, they are more often engaged in neutral or mutualistic bacterial interactions. In the soil, plants associate with rhizobia or other plant growth promoting rhizosphere bacteria; above ground, bacteria colonise plants as epi- and endophytes. For mounting appropriate responses, such as permitting colonisation by beneficial symbionts while at the same time fending off pathogenic invaders, plants need to distinguish between the "good" and the "bad". Plants make use of proteins containing the lysin motif (LysM) for perception of *N*-acetylglucosamine containing carbohydrate structures, such as chitooligosaccharides functioning as symbiotic nodulation factors or bacterial peptidoglycan. Moreover, plant hydrolytic enzymes of the chitinase family, which are able to cleave bacterial peptidoglycan or chitooligosaccharides, are essential for cellular signalling induced by rhizobial nodulation factors during symbiosis as well as bacterial peptidoglycan during pathogenesis. Hence, LysM receptors seem to work in concert with hydrolytic enzymes that fine-tune ligand availability to either allow symbiotic interactions or trigger plant immunity.

1. Introduction

In their natural environment, plants are exposed to a plethora of microbes, both in the phyllosphere and the resource-rich rhizosphere (Fig. 1). Whereas many microbes are only associated with the plant surface as epiphytes, several organisms also reside in plants as endophytes. Endophytes are regarded as non-phytopathogenic microbes living within the plant tissue at least part of their lifetime (Mercado-Blanco, 2015) and, intriguingly, almost all plants analysed so far contained endophytes. Therefore, an endophyte-free plant would be considered a rare exception (Partida-Martinez and Heil, 2011).

Some bacteria are simply associated with plant surfaces as commensals without having any impact on the plant health. Others, such as plant growth promoting rhizobacteria (PGPRs), benefit the plant by increasing the availability of nutrients or by improving plant root growth without invading plant tissue (Verbon and Liberman, 2016; Weyens et al., 2009). However, some mutualistic and pathogenic bacteria enter their host and establish a chronic infection, which is only possible by evading or suppressing the plant immune system (Zipfel and Oldroyd, 2017).

Classically, endophytic bacteria that have a negative impact on plant fitness are termed pathogenic (Fig. 1). These bacteria, of which *Pseudomonas syringae* and *Agrobacterium tumefaciens* pathovars were nominated the most devastating (Mansfield et al., 2012), can cause severe agronomical losses due to disease symptoms such as galls, wilts, leaf spots, specks, blights, soft rots, scabs and cankers (van der Wolf and De Boer, 2015). Notably, plant pathogenic bacteria generally do not enter the host cells but reside and grow in the apoplastic space between plant cells. Here, they cause disease, for instance by colonizing the water-conducting xylem vessels, producing toxins or cell wall degrading enzymes or by injecting special effector proteins into the plant cell to interfere with the plant innate immune response (Alfano and Collmer, 1996; van der Wolf and De Boer, 2015). *Agrobacterium* species even inject part of their Tumour-inducing (Ti) plasmid into the plant cell to force the host to alter its metabolism to the advantage of the bacterium, which ultimately leads to the formation of cancer-like galls (Hooykaas, 2015).

On the other hand, endophytes with rather neutral or positive effects on the plant host are considered the classical endophytes. One prominent example of beneficial endophytes are bacteria of the genus

Abbreviations: GlcNAc, *N*-acetylglucosamine; LysM, lysin motif; MurNAc, *N*-acetylmuramic acid; NF, nodulation factor; PAMP, pathogen-associated molecular pattern; PGN, peptidoglycan; PGPRs, plant growth promoting rhizobacteria; PRR, pattern recognition receptor; RP, receptor-like protein; RK, receptor kinase

* Corresponding author at: Plant Biochemistry, Center for Plant Molecular Biology (ZMBP), Universität Tübingen, Auf der Morgenstelle 32, 72076 Tübingen, Germany.

E-mail address: andrea.gust@zmbp.uni-tuebingen.de (A.A. Gust).

https://doi.org/10.1016/j.ijmm.2019.04.004
Received 27 December 2018; Received in revised form 10 April 2019; Accepted 25 April 2019
1438-4221/

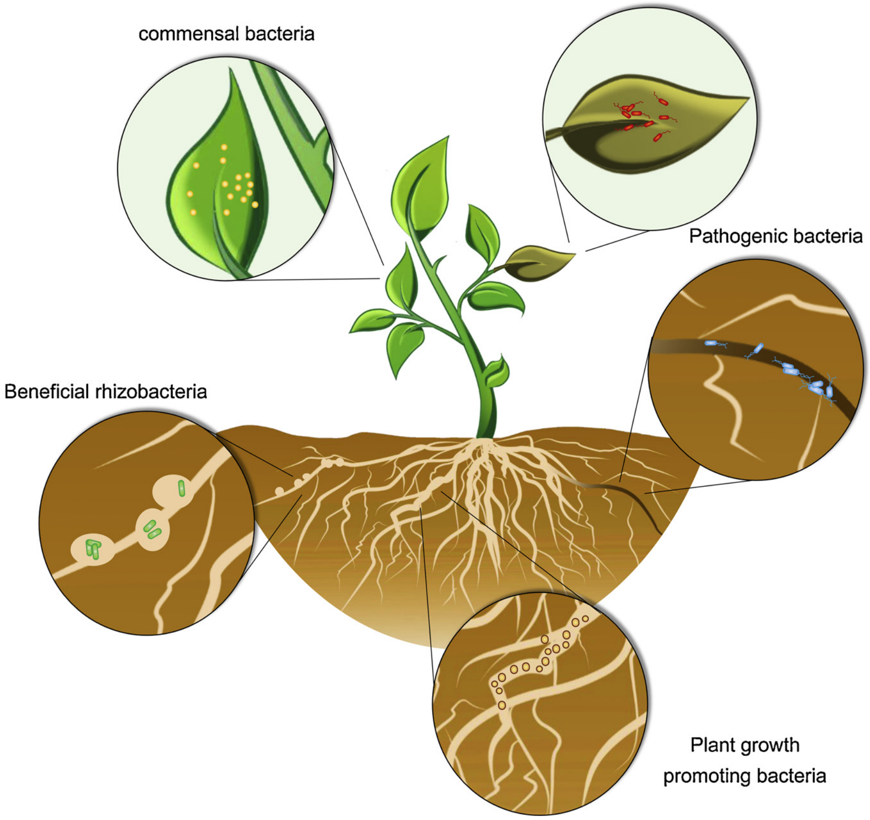

Fig. 1. Plants are associated with a multitude of different bacteria in the phyllosphere and the rhizosphere. Epiphytic, commensal bacteria can use the leaf surface as habitat; however, pathogenic bacteria enter the apoplastic space via stomata, other natural openings or wounds to cause disease. Many different microbes are attracted to the nutrient-rich soil near the plant roots (i.e. the rhizosphere). Plant roots can take up nutrients made available by plant growth promoting rhizobacteria (PGPRs) or can establish root nodule symbiosis with rhizobia. Phytopathogenic strains are also among the rhizosphere bacteria.

Rhizobium, which colonise the internal root tissue of legume plants to form root nodules for nitrogen fixation (Oldroyd, 2013). The prerequisite for being granted access by the plant tissue without inducing plant defense responses is the molecular dialog between rhizobia and their specific host. Plants attract symbiotic bacteria by secreting flavonoids, which are recognised by compatible rhizobia (Oldroyd, 2013; Perret et al., 2000). Flavonoid perception, in turn, triggers the production of strain-specific bacterial nodulation factors (NFs). NFs are lipochitooligosaccharides composed of four to five chitin monomers modified with fatty acid moieties and other substitutions in a strain-specific manner (D'Haeze and Holsters, 2002; Oldroyd, 2013; Perret et al., 2000). NFs can be regarded as entry tickets permitting colonisation of the plant tissue, which ultimately results in root nodule formation and nitrogen fixation.

This review will focus on rhizobia as beneficial endophytes versus plant-pathogenic bacteria, their communication with the host plant and response stimulus modulation by plant hydrolytic activities.

2. LysM receptors in plant – bacteria communication

For a plant to mount the appropriate cellular responses upon a bacterial encounter, it is crucial to recognise and to distinguish between harmful and beneficial bacteria. Bacterial signals, which can either be immunity-triggering pathogen-associated molecular patterns (PAMPs) or symbiotic signals, are perceived by cell surface-located plant receptor complexes (Böhm et al., 2014; Boutrot and Zipfel, 2017; Ranf, 2017; Saijo et al., 2018; Zipfel and Oldroyd, 2017). Bacterial PAMPs that are perceived in plants include flagellin, elongation factor Tu (EF-Tu), lipopolysaccharides and peptidoglycans (Boller and Felix, 2009; Zipfel and Oldroyd, 2017). The corresponding plant receptors constitute either receptor kinases (RKs), which contain an extracellular domain for ligand binding and an intracellular kinase domain linked by a single-pass transmembrane domain, or receptor-like proteins (RPs) lacking the kinase domain (Boutrot and Zipfel, 2017; Zipfel and Oldroyd, 2017).

Whereas plants typically use receptors with extracellular leucine-rich repeats for perception of proteinaceous ligands, the detection of *N*-acetylglucosamine (GlcNAc)-containing glycan patterns, such as chitin, peptidoglycan or NFs, is mediated by Lysin motif (LysM) domain-containing proteins (Böhm et al., 2014; Boutrot and Zipfel, 2017; Desaki et al., 2018b; Gust et al., 2012). The LysM is an ancient and ubiquitous protein motif found in virtually all living organisms except in Archaea (Bateman and Bycroft, 2000; Buist et al., 2008; Zhang et al., 2009). LysMs are approximately 40 amino acids in length with a three-dimensional βααβ structure containing two α-helices sandwiching a two-stranded antiparallel β-sheet (Bateman and Bycroft, 2000; Bielnicki et al., 2006; Mulder et al., 2006). A LysM domain is built up of several lysin motifs separated by short peptide spacers (Buist et al., 1995; Ohnuma et al., 2008). Initially, LysM domains were reported from secretory bacterial hydrolases including lysozyme, autolysins, and transglycosylases (Buist et al., 2008; Ponting et al., 1999). These enzymes are implicated in bacterial cell wall biogenesis, modification and degradation. Mechanistically, bacterial LysM domains were proposed to mediate physical contact between LysM domain-containing proteins and complex bacterial carbohydrate surface structures, such as PGN. Intriguingly, although LysM domains occur in many bacterial and eukaryotic proteins, LysM receptor-like kinases are unique to plants.

2.1. LysM proteins are involved in plant – rhizobia interactions

The first plant LysM proteins shown to be required for the perception of GlcNAc-containing carbohydrates were *Lotus japonicus* NFR1 (NODULATION FACTOR RECEPTOR 1) and NFR5 (Fig. 2), two plasma-membrane located receptor kinases which mediate responses to rhizobacterial NFs (Madsen et al., 2003; Radutoiu et al., 2003). Nodulation factors are key signalling molecules during the establishment of legume–rhizobium symbiosis. The bacterium-specific decorations on the chitin oligosaccharide backbone impact on the binding of NFs to the

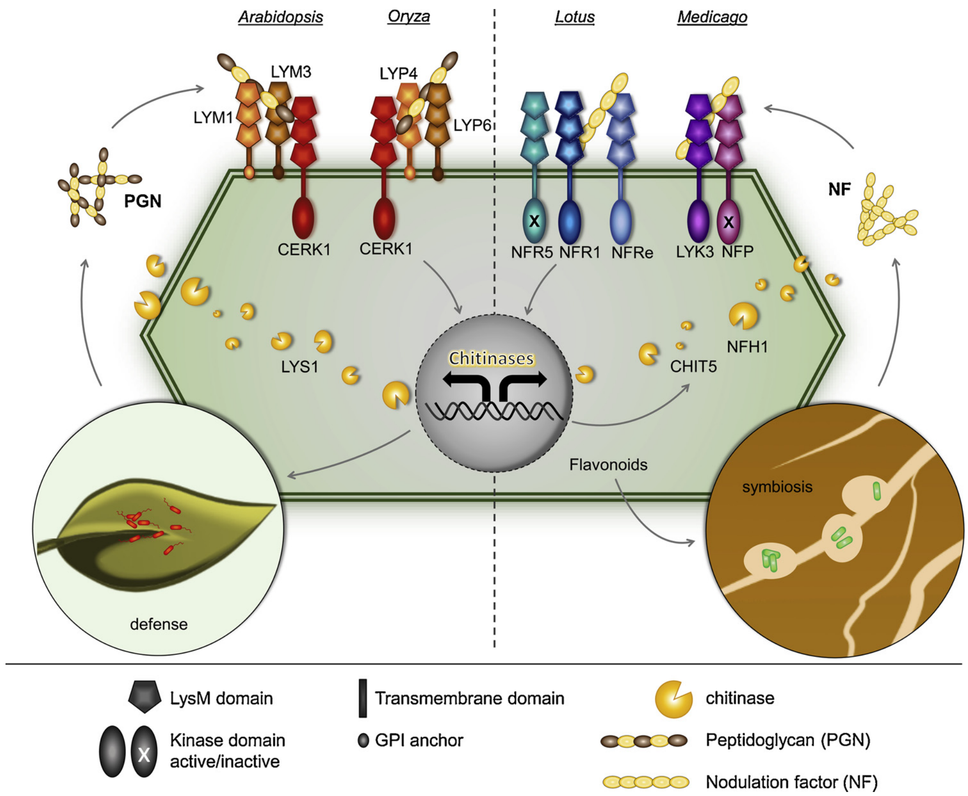

Fig. 2. Concerted action of LysM receptors and chitinases in PGN and NF perception. Plants such as *Arabidopsis thaliana* (the model dicot) and rice (*Oryza sativa*, the model monocot) perceive PGN via muropeptide-binding to the plasma-membrane tethered LysM protein complexes AtLYM1/AtLYM3 and OsLYP4/OsLYP6, respectively. Upon ligand-binding, complex formation with signalling competent RKs such as AtCERK1/OsCERK1 is likely. In Arabidopsis, PGN-solubilisation by the chitinase/lysozyme-like protein LYS1 is required for effective immunity and chitinase genes are transcriptionally up-regulated upon PGN perception. Moreover, chitinases might have a direct bacteriocidal activity. Ultimately, PGN-perception leads to a defense response. In legume plants such as *Lotus japonicus* and *Medicago truncatula*, protein complexes of the LysM-RKs LjNFR1/LjNFR5 (and LjNFRe) and MtNFP/MtLYK3 mediate the perception of NFs, which are produced upon bacterial detection of plant flavonoids. Notably, LjNFR5 and MtNFP have non-functional kinase domains. The action of NF-cleaving chitinases LjCHIT5 and MtNFH1 maintain balanced NF-levels to obtain the desired cellular output during symbiosis.

LysM receptor kinases, thus only allowing root nodule establishment when NF – LysM-RK pairs are matching (Antolin-Llovera et al., 2014; Perret et al., 2000). Indeed, both NFR1 and NFR5 directly bind to NFs and are able to discriminate between un-modified chitin-pentamers and decorated NFs (Broghammer et al., 2012; Murakami et al., 2018). Recently, an additional *Lotus* LysM-RK, NFRe (NF SIGNALLING IN EPIDERMAL LAYER), was also shown to participate in NF perception. Like NFR1 and NFR5, NFRe directly binds to NFs and forms receptor complexes with NFR1 and NFR5 (Murakami et al., 2018). Similarly, the two NFR1/5 homologs LYK3 and NFP were also shown to be required for NF perception in *Medicago truncatula* (Amor et al., 2003; Limpens et al., 2003) (Fig. 2). Notably, both *Lotus* NFR5 and *Medicago* NFP possess non-functional kinase domains, suggesting complex formation with kinase-active NFR1 and LYK3, respectively, to form functional signalling complexes (Limpens and Bisseling, 2003). In addition to NF-perception, legume plants are also equipped with receptors for bacterial exopolysaccharides, which are secreted into the rhizosphere and can contribute to host specificity during rhizobial symbiosis (Downie, 2010). One example is the exopolysaccharide receptor 3 (EPR3), a LysM-RK that recognises *Rhizobium leguminosarum* exopolysaccharides (Kawaharada et al., 2015).

2.2. LysM proteins as pattern recognition receptors in plant immunity

Consistent with its structural similarity to NFs, chitin is also perceived by LysM receptors in plants (Desaki et al., 2018b; Gust et al., 2012; Tanaka et al., 2013). Chitin, one of the first microbial structures with proven PAMP activity (Felix et al., 1993), is the major structural component of fungal cell walls, crustacean shells and the exoskeleton of insects and nematodes. The first known chitin receptor was isolated from rice plasma membranes and identified as the LysM domain-containing chitin elicitor binding protein (CEBiP) (Kaku et al., 2006). Upon chitin binding, CEBiP forms homodimers where a chitin octamer links two receptor monomers. The LysM-RK OsCERK1 (*Oryza sativa* chitin elicitor receptor kinase 1) is subsequently recruited to finally form an activated signalling complex (Hayafune et al., 2014; Shimizu et al., 2010). Likewise, the LysM-RKs AtCERK1 and AtLYK5 (and possibly AtLYK4) were identified as the key elements for chitin perception in *Arabidopsis thaliana* (Cao et al., 2014; Miya et al., 2007; Wan et al., 2012, 2008). Elucidation of the AtCERK1 crystal structure after soaking the crystal with chitin provided a mechanistic explanation for AtCERK1 dimer formation. Chitin, of a minimal length of eight GlcNAc units, bridges the two AtCERK1 monomers, thereby stabilizing the AtCERK1 dimer (Liu et al., 2012b). Notably, although AtLYK5 has the higher chitin binding affinity compared to AtCERK1, it possesses a non-functional kinase domain (Cao et al., 2014). In contrast, AtCERK1 does not bind chitin as efficiently, but its kinase activity is essential for chitin signalling. Thus, AtCERK1 acts as co-receptor together with LYK5, which is the *bona-fide* chitin receptor.

Another ubiquitously found carbohydrate structurally similar to chitin is bacterial PGN, which was demonstrated to trigger typical plant immune responses in model plants such as *Arabidopsis thaliana* (Erbs et al., 2008; Gust et al., 2007; Millet et al., 2010) and rice (Liu et al., 2012a). In Arabidopsis a plasma membrane localised, tripartite PGN recognition system with shared functions in PGN sensing and transmembrane signalling was described (Willmann et al., 2011). This system comprises PGN-binding LysM-domain proteins AtLYM1 and AtLYM3, which are membrane-tethered receptor-like proteins (RPs) structurally similar to rice CEBiP, and the transmembrane LysM-RK AtCERK1 (Fig. 2). Although evidences for direct physical interactions among AtCERK1, AtLYM1 and AtLYM3 is still lacking, AtCERK1 is likely required for conveying the extracellular signal across the plasma membrane and for initiating intracellular signal transduction. Similar to chitin-perception, AtCERK1 poorly binds to PGN and functions as co-receptor, whereas AtLYM1 and AtLYM3 act as ligand-binding moieties. Importantly, all three proteins were shown to be indispensable for PGN sensitivity and immunity to bacterial infection (Willmann et al., 2011), which is in agreement with their proposed role as the PGN sensor system. In rice, a similar PGN perception system comprising LysM domain proteins OsLYP4 and OsLYP6, and the LysM-RK OsCERK1 has been reported (Ao et al., 2014; Liu et al., 2012a) (Fig. 2). Interestingly, OsLYP4 and OsLYP6 proteins are dual specificity receptors, binding both PGN and chitin. Upon ligand binding, the constitutive OsLYP4/OsLYP6 complex dissociates to form a signalling competent complex with the co-receptor OsCERK1 (Ao et al., 2014). Interestingly, OsCERK1 was also demonstrated to be involved in the perception of yet another bacterial MAMP, namely lipopolysaccharides (Desaki et al., 2018a), indicating that CERK1 in different plant species might act as a multi-functional co-receptor for ligand-binding LysM-proteins.

Notably, symbiotic LysM receptors are not only closely related to chitin innate immune receptors, but some of these receptors function in immunity as well as symbiosis. For instance, both OsCERK1 from rice and the CERK1-homolog from *Pisum sativum*, PsLYK9, have a bifunctional role in defense and arbuscular mycorrhizal symbiosis (Leppyanen et al., 2017; Miyata et al., 2014). However, for the rhizobia-legume interaction two distinct perception systems for chitin and NFs were postulated. This hypothesis is supported by the identification of *Lotus* and *Medicago* LysM-RKs, which are uniquely required for chitin perception but not for NF-detection (Bozsoki et al., 2017).

Based on the close analogy between pathogenic and symbiotic perception systems in plants it might be speculated that during evolution symbiotic LysM receptors might have arisen from LysM receptors involved in pathogenic plant – bacteria interactions.

3. Plant chitinases as important signalling determinants

When considering plant – bacteria interactions, plant chitinases might not be the first enzymes that come to mind. Neither plants nor bacteria contain chitin. Still, plants express a multitude of chitinases which have been implicated not only in plant growth and development but also in plant defense and plant-bacterial symbiosis (Grover, 2012; Kasprzewska, 2003). Although bacteria do not possess chitin, they produce chitin-like structures, such as the cell wall component PGN and NFs as symbiosis signalling molecules. Whereas chitin is a homomer of β-1,4 linked GlcNAc, PGN is composed of alternating β-1,4 linked GlcNAc and *N*-acetylmuramic acid (MurNAc), and these glucan strands are cross-linked by short peptides bridges (Bertsche et al., 2015; Dworkin, 2014). NFs, in contrast, are in fact short chitin fragments with various bacterium-specific decorations (D'Haeze and Holsters, 2002; Oldroyd, 2013; Perret et al., 2000).

3.1. Chitinases are required for balanced NF levels during symbiosis

Chitinases are defined as enzymes that can cleave the β-1,4 glycosidic linkage between GlcNAc units in chitooligosaccharides (Collinge et al., 1993), including NFs. During nodule formation, chitinase genes are transcriptionally up-regulated in plant roots such as soybean (Salzer et al., 2004), and purified NFs can induce an increase in chitinase activities in this legume (Xie et al., 1999). Moreover, NFs can act as substrate for various plant chitinases (reviewed in (Perret et al., 2000)). NF cleavage products were shown to be considerably less active than the undigested NFs (Staehelin et al., 1994), suggesting that plant chitinases secreted from plant roots control the rhizobial infection process by inactivating NFs in the rhizosphere (Fig. 2). The importance of fine-tuning NF-levels during rhizobial root-infection was revealed in two recent reports in *Medicago* and *Lotus* (Cai et al., 2018; Malolepszy et al., 2018). The *Medicago* class V chitinase/Nod factor hydrolase MtNFH1, although belonging to the plant chitinase family, specifically hydrolyses NFs but shows no activity towards chitin (Tian et al., 2013). Interestingly, *MtNFH1* deficiency resulted in a delayed rhizobial root hair infection, suggesting that an excess of NFs has a negative impact on the initial stages of this symbiosis (Cai et al., 2018). Moreover, *MtNFH1*-mutant plants only allowed the formation of abnormal nodules. In *L. japonicus*, mutants of the class V chitinase *LjChit5* displayed nitrogen deficiency symptoms upon infection with nodule-inducing *Mesorhizobium loti* bacteria, due to the formation of a large number of nodules that were arrested in development (Malolepszy et al., 2018). Whereas NF signalling was initially unaffected in *LjChit5* mutants, LjCHIT5 enzyme activity was essential for the complete establishment of symbiotic nitrogen fixation. In their model, the authors propose that low NF levels within infected plant tissue are required to maintain the balance between symbiotic and defense signalling and that LjCHIT5 is the key factor for keeping this balance by hydrolysing excess NF. Hence, only balanced NF levels lead to the formation of a functional symbiotic interaction and chitinases like MtNFH1 or LjCHIT5 might help in fine-tuning these NF levels within the plant tissue and in the rhizosphere.

3.2. Chitinases can produce ligands for plant immune receptors

Whereas NFs are synthesised with the appropriate size for receptor binding, bacterial PAMPs are mostly part of supramolecular structures. PAMPs are often even buried within these structures, making direct receptor binding rather unlikely if not impossible. It was thus a long-standing question whether and how PAMPs in general are released from complex molecules such as bacterial flagella or cell walls. PGN fragments are generated by bacteria as turnover products during bacterial cell wall growth, and these fragments might be sensed by plants and animals to assess a potential bacterial threat (Bertsche et al., 2015). Indeed, soluble oligomeric PGN fragments have immune-stimulatory activity in plants such as *Arabidopsis* (Gust et al., 2007). However, the concentration of immune-stimulatory PGN fragments might be increased by host hydrolytic enzymes such as lysozyme to amplify the immune response. In mice, an effective immune response towards infection with *Streptococcus pneumoniae* requires digestion of PGN by lysozyme followed by perception of soluble PGN-fragments by the PGN sensor Nod2 (Davis et al., 2011). Likewise, *Drosophila* Gram-negative bacteria-derived binding protein 1 (GNBP1) possesses PGN-hydrolysing activity and delivers fragmented PGN to the PGN-sensor, PGRP-SA (Filipe et al., 2005; Wang et al., 2006). As some plant chitinases also have lysozyme-like activity (Beintema and Terwisscha van Scheltinga, 1996; Bokma et al., 1997), they might also function in PGN digestion to facilitate perception in plants. Indeed, upon bacterial infection or exposure to microbial patterns *Arabidopsis* produces the metazoan lysozyme-like hydrolase AtLYS1, which is able to hydrolyse β-1,4-linkages between *N*-acetylmuramic acid and *N*-acetylglucosamine residues in bacterial PGN (Liu et al., 2014). Importantly, soluble PGN fragments generated by AtLYS1 are immunogenic in plants and can trigger typical immunity-related responses in an AtLYM1, AtLYM3 and AtCERK1-dependent manner. Moreover, *Arabidopsis lys1* mutants are impaired in PGN-triggered immune responses and display a decreased resistance towards bacterial infections similar to that observed in PGN receptor mutants (Liu et al., 2014; Willmann et al., 2011). Thus, AtLYS1 lysozyme/chitinase activity contributes to the generation of immunogenic PGN ligands for receptor activation and ultimately to bacterial resistance.

The examples given above, for both plants and animals, suggest that eukaryotic hosts make concerted use of PGN-hydrolytic activities and of pattern recognition receptors in order to cope with bacterial infections (Gust, 2015).

4. Conclusions

As higher eukaryotes, plants have evolved mechanisms to detect and discriminate between a wide range of beneficial and harmful microorganisms. LysM domain proteins are key components in the perception machineries for GlcNAc-containing bacterial structures including PGN and NFs. Intriguingly, although structurally quite similar, most LysM-receptors are able to discriminate between specific GlcNAc-containing ligands. The mechanism for specific ligand-recognition is currently unknown, but it was proposed that the cooperation of multiple LysM-domain containing proteins within one receptor complex determines specificity (Sanchez-Vallet et al., 2013; Wong et al., 2015). Indeed, all known plant LysM receptor complexes characterised so far are composed of homo- and/or heteromers of several LysM proteins, irrespective whether they mediate PGN, NF or chitin perception. The receptor affinities and the amount of available ligand might also contribute to specificity and in this respect, enzymes that adjust or generally alter ligand levels might be crucial for the induction of the desired cellular responses. For instance, levels of immunogenic PGN-fragments solubilised from supramolecular bacterial cell wall structures is increased by the action of plant chitinases/lysozyme-like activities, resulting in a boosted immune response. Likewise, NF-levels are fine-tuned by plant chitinases to allow for a balanced cellular response during symbiotic interactions. Hence, during the interaction of plants (or generally eukaryotic hosts) with bacteria (or generally microbes) functional perception machineries are essential, but mechanisms to "optimise" ligand levels or availability might also be critical for mounting efficient cellular responses.

Acknowledgements

Our research on peptidoglycan perception in plants was supported by the Deutsche Forschungsgemeinschaft (DFG) Sonderforschungsbereich (SFB: Collaborative Research Consortium) 766, project B07. We thank Thorsten Nürnberger and Rory Pruitt for helpful comments on the manuscript.

References

Alfano, J.R., Collmer, A., 1996. Bacterial pathogens in plants: life up against the wall. Plant Cell 8, 1683–1698.
Amor, B.B., Shaw, S.L., Oldroyd, G.E., Maillet, F., Penmetsa, R.V., Cook, D., Long, S.R., Denarie, J., Gough, C., 2003. The NFP locus of *Medicago truncatula* controls an early step of Nod factor signal transduction upstream of a rapid calcium flux and root hair deformation. Plant J. 34, 495–506.
Antolin-Llovera, M., Petutsching, E.K., Ried, M.K., Lipka, V., Nürnberger, T., Robatzek, S., Parniske, M., 2014. Knowing your friends and foes - plant receptor-like kinases as initiators of symbiosis or defence. New Phytol. 204, 791–802.
Ao, Y., Li, Z., Feng, D., Xiong, F., Liu, J., Li, J.F., Wang, M., Wang, J., Liu, B., Wang, H.B., 2014. OsCERK1 and OsRLCK176 play important roles in peptidoglycan and chitin signaling in rice innate immunity. Plant J. 80, 1072–1084.
Bateman, A., Bycroft, M., 2000. The structure of a LysM domain from *E-coli* membrane-bound lytic murein transglycosylase D (MltD). J. Mol. Biol. 299, 1113–1119.
Beintema, J.J., Terwisscha van Scheltinga, A.C., 1996. Plant lysozymes. EXS 75, 75–86.
Bertsche, U., Mayer, C., Götz, F., Gust, A.A., 2015. Peptidoglycan perception - sensing bacteria by their common envelope structure. Int. J. Med. Microbiol. 305, 217–223.
Bielnicki, J., Devedjiev, Y., Derewenda, U., Dauter, Z., Joachimiak, A., Derewenda, Z.S., 2006. *B. subtilis*ykuD protein at 2.0 A resolution: insights into the structure and function of a novel, ubiquitous family of bacterial enzymes. Proteins 62, 144–151.
Böhm, H., Albert, I., Fan, L., Reinhard, A., Nürnberger, T., 2014. Immune receptor complexes at the plant cell surface. Curr. Opin. Plant Biol. 20, 47–54.
Bokma, E., van Koningsveld, G.A., Jeronimus-Stratingh, M., Beintema, J.J., 1997. Hevamine, a chitinase from the rubber tree *Hevea brasiliensis*, cleaves peptidoglycan between the C-1 of N-acetylglucosamine and C-4 of N-acetylmuramic acid and therefore is not a lysozyme. FEBS Lett. 411, 161–163.
Boller, T., Felix, G., 2009. A renaissance of elicitors: perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors. Annu. Rev. Plant Biol. 60, 379–406.
Boutrot, F., Zipfel, C., 2017. Function, discovery, and exploitation of plant pattern recognition receptors for broad-spectrum disease resistance. Annu. Rev. Phytopathol. 55, 257–286.
Bozsoki, Z., Cheng, J., Feng, F., Gysel, K., Vinther, M., Andersen, K.R., Oldroyd, G., Blaise, M., Radutoiu, S., Stougaard, J., 2017. Receptor-mediated chitin perception in legume roots is functionally separable from Nod factor perception. Proc. Natl. Acad. Sci. U. S. A. 114, E8118–E8127.
Broghammer, A., Krusell, L., Blaise, M., Sauer, J., Sullivan, J.T., Maolanon, N., Vinther, M., Lorentzen, A., Madsen, E.B., Jensen, K.J., Roepstorff, P., Thirup, S., Ronson, C.W., Thygesen, M.B., Stougaard, J., 2012. Legume receptors perceive the rhizobial lipochitin oligosaccharide signal molecules by direct binding. Proc. Natl. Acad. Sci. U. S. A. 109, 13859–13864.
Buist, G., Kok, J., Leenhouts, K.J., Dabrowska, M., Venema, G., Haandrikman, A.J., 1995. Molecular cloning and nucleotide sequence of the gene encoding the major peptidoglycan hydrolase of *Lactococcus lactis*, a muramidase needed for cell separation. J. Bacteriol. 177, 1554–1563.
Buist, G., Steen, A., Kok, J., Kuipers, O.R., 2008. LysM, a widely distributed protein motif for binding to (peptido)glycans. Mol. Microbiol. 68, 838–847.
Cai, J., Zhang, L.Y., Liu, W., Tian, Y., Xiong, J.S., Wang, Y.H., Li, R.J., Li, H.M., Wen, J., Mysore, K.S., Boller, T., Xie, Z.P., Staehelin, C., 2018. Role of the Nod factor hydrolase mtnfh1 in regulating nod factor levels during rhizobial infection and in mature nodules of Medicago truncatula. Plant Cell 30, 397–414.
Cao, Y., Liang, Y., Tanaka, K., Nguyen, C.T., Jedrzejczak, R.P., Joachimiak, A., Stacey, G., 2014. The kinase LYK5 is a major chitin receptor in *Arabidopsis* and forms a chitin-induced complex with related kinase CERK1. Elife 3.
Collinge, D.B., Kragh, K.M., Mikkelsen, J.D., Nielsen, K.K., Rasmussen, U., Vad, K., 1993. Plant chitinases. Plant J. 3, 31–40.
D'Haeze, W., Holsters, M., 2002. Nod factor structures, responses, and perception during initiation of nodule development. Glycobiology 12, 79R–105R.
Davis, K.M., Nakamura, S., Weiser, J.N., 2011. Nod2 sensing of lysozyme-digested peptidoglycan promotes macrophage recruitment and clearance of *S. pneumoniae* colonization in mice. J. Clin. Invest. 121, 3666–3676.
Desaki, Y., Kouzai, Y., Ninomiya, Y., Iwase, R., Shimizu, Y., Seko, K., Molinaro, A., Minami, E., Shibuya, N., Kaku, H., Nishizawa, Y., 2018a. OsCERK1 plays a crucial role in the lipopolysaccharide-induced immune response of rice. New Phytol. 217, 1042–1049.
Desaki, Y., Miyata, K., Suzuki, M., Shibuya, N., Kaku, H., 2018b. Plant immunity and symbiosis signaling mediated by LysM receptors. Innate Immun. 24, 92–100.
Downie, J.A., 2010. The roles of extracellular proteins, polysaccharides and signals in the interactions of rhizobia with legume roots. FEMS Microbiol. Rev. 34, 150–170.
Dworkin, J., 2014. The medium is the message: interspecies and interkingdom signaling by peptidoglycan and related bacterial glycans. Annu. Rev. Microbiol. 68, 137–154.
Erbs, G., Silipo, A., Aslam, S., De Castro, C., Liparoti, V., Flagiello, A., Pucci, P., Lanzetta, R., Parrilli, M., Molinaro, A., Newman, M.A., Cooper, R.M., 2008. Peptidoglycan and muropeptides from pathogens *Agrobacterium* and *Xanthomonas* elicit plant innate immunity: structure and activity. Chem. Biol. 15, 438–448.
Felix, G., Regenass, M., Boller, T., 1993. Specific perception of subnanomolar concentrations of chitin fragments by tomato cells: induction of extracellular alkalinization, changes in protein phosphorylation, and establishment of a refractory state. Plant J. 4, 307–316.
Filipe, S.R., Tomasz, A., Ligoxygakis, P., 2005. Requirements of peptidoglycan structure that allow detection by the *Drosophila* toll pathway. EMBO Rep. 6, 327–333.
Grover, A., 2012. Plant chitinases: genetic diversity and physiological roles. Crit. Rev. Plant Sci. 31, 57–73.
Gust, A.A., 2015. Peptidoglycan perception in plants. PLoS Pathog. 11 e1005275.
Gust, A.A., Biswas, R., Lenz, H.D., Rauhut, T., Ranf, S., Kemmerling, B., Götz, F., Glawischnig, E., Lee, J., Felix, G., Nürnberger, T., 2007. Bacteria-derived peptidoglycans constitute pathogen-associated molecular patterns triggering innate immunity in Arabidopsis. J. Biol. Chem. 282, 32338–32348.
Gust, A.A., Willmann, R., Desaki, Y., Grabherr, H.M., Nürnberger, T., 2012. Plant LysM proteins: modules mediating symbiosis and immunity. Trends Plant Sci. 17, 495–502.
Hayafune, M., Berisio, R., Marchetti, R., Silipo, A., Kayama, M., Desaki, Y., Arima, S., Squeglia, F., Ruggiero, A., Tokuyasu, K., Molinaro, A., Kaku, H., Shibuya, N., 2014. Chitin-induced activation of immune signaling by the rice receptor CEBiP relies on a unique sandwich-type dimerization. Proc. Natl. Acad. Sci. U. S. A. 111, E404–413.
Hooykaas, P.J.J., 2015. *Agrobacterium*, the genetic engineer. In: Lugtenberg, B. (Ed.), Principles of Plant-Microbe Interactions: Microbes for Sustainable Agriculture. Springer International Publishing, Cham, pp. 355–361.
Kaku, H., Nishizawa, Y., Ishii-Minami, N., Akimoto-Tomiyama, C., Dohmae, N., Takio, K., Minami, E., Shibuya, N., 2006. Plant cells recognize chitin fragments for defense signaling through a plasma membrane receptor. Proc. Natl. Acad. Sci. U. S. A. 103, 11086–11091.
Kasprzewska, A., 2003. Plant chitinases—Regulation and function. Cell. Mol. Biol. Lett. 8, 809–824.
Kawaharada, Y., Kelly, S., Nielsen, M.W., Hjuler, C.T., Gysel, K., Muszynski, A., Carlson, R.W., Thygesen, M.B., Sandal, N., Asmussen, M.H., Vinther, M., Andersen, S.U., Krusell, L., Thirup, S., Jensen, K.J., Ronson, C.W., Blaise, M., Radutoiu, S., Stougaard, J., 2015. Receptor-mediated exopolysaccharide perception controls bacterial infection. Nature 523, 308–312.
Leppyanen, I.V., Shakhnazarova, V.Y., Shtark, O.Y., Vishnevskaya, N.A., Tikhonovich, I.A., Dolgikh, E.A., 2017. Receptor-like kinase LYK9 in *Pisum sativum* L. is the CERK1-like receptor that controls both plant immunity and AM symbiosis development. Int. J. Mol. Sci. 19, 8.
Limpens, E., Bisseling, T., 2003. Signaling in symbiosis. Curr. Opin. Plant Biol. 6, 343–350.
Limpens, E., Franken, C., Smit, P., Willemse, J., Bisseling, T., Geurts, R., 2003. LysM domain receptor kinases regulating rhizobial Nod factor-induced infection. Science 302, 630–633.
Liu, B., Li, J.F., Ao, Y., Qu, J., Li, Z., Su, J., Zhang, Y., Liu, J., Feng, D., Qi, K., He, Y., Wang, J., Wang, H.B., 2012a. Lysin motif-containing proteins LYP4 and LYP6 play dual roles in peptidoglycan and chitin perception in rice innate immunity. Plant Cell 24, 3406–3419.
Liu, T., Liu, Z., Song, C., Hu, Y., Han, Z., She, J., Fan, F., Wang, J., Jin, C., Chang, J., Zhou, J.M., Chai, J., 2012b. Chitin-induced dimerization activates a plant immune receptor. Science 336, 1160–1164.
Liu, X., Grabherr, H.M., Willmann, R., Kolb, D., Brunner, F., Bertsche, U., Kühner, D., Franz-Wachtel, M., Amin, B., Felix, G., Ongena, M., Nürnberger, T., Gust, A.A., 2014. Host-induced bacterial cell wall decomposition mediates pattern-triggered immunity in Arabidopsis. Elife e01990.
Madsen, E.B., Madsen, L.H., Radutoiu, S., Olbryt, M., Rakwalska, M., Szczyglowski, K., Sato, S., Kaneko, T., Tabata, S., Sandal, N., Stougaard, J., 2003. A receptor kinase gene of the LysM type is involved in legume perception of rhizobial signals. Nature 425, 637–640.
Malolepszy, A., Kelly, S., Sorensen, K.K., James, E.K., Kalisch, C., Bozsoki, Z., Panting, M., Andersen, S.U., Sato, S., Tao, K., Jensen, D.B., Vinther, M., Jong, N., Madsen, L.H., Umehara, Y., Gysel, K., Berentsen, M.U., Blaise, M., Jensen, K.J., Thygesen, M.B., Sandal, N., Andersen, K.R., Radutoiu, S., 2018. A plant chitinase controls cortical infection thread progression and nitrogen-fixing symbiosis. Elife 7 e38874.
Mansfield, J., Genin, S., Magori, S., Citovsky, V., Sriariyanum, M., Ronald, P., Dow, M., Verdier, V., Beer, S.V., Machado, M.A., Toth, I., Salmond, G., Foster, G.D., 2012. Top

10 plant pathogenic bacteria in molecular plant pathology. Mol. Plant Pathol. 13, 614–629.
Mercado-Blanco, J., 2015. Life of microbes inside the plant. In: Lugtenberg, B. (Ed.), Principles of Plant-Microbe Interactions: Microbes for Sustainable Agriculture. Springer International Publishing, Cham, pp. 25–32.
Millet, Y.A., Danna, C.H., Clay, N.K., Songnuan, W., Simon, M.D., Werck-Reichhart, D., Ausubel, F.M., 2010. Innate immune responses activated in *Arabidopsis* roots by microbe-associated molecular patterns. Plant Cell 22, 973–990.
Miya, A., Albert, P., Shinya, T., Desaki, Y., Ichimura, K., Shirasu, K., Narusaka, Y., Kawakami, N., Kaku, H., Shibuya, N., 2007. CERK1, a LysM receptor kinase, is essential for chitin elicitor signaling in *Arabidopsis*. Proc. Natl. Acad. Sci. U. S. A. 104, 19613–19618.
Miyata, K., Kozaki, T., Kouzai, Y., Ozawa, K., Ishii, K., Asamizu, E., Okabe, Y., Umehara, Y., Miyamoto, A., Kobae, Y., Akiyama, K., Kaku, H., Nishizawa, Y., Shibuya, N., Nakagawa, T., 2014. The bifunctional plant receptor, OsCERK1, regulates both chitin-triggered immunity and arbuscular mycorrhizal symbiosis in rice. Plant Cell Physiol. 55, 1864–1872.
Mulder, L., Lefebvre, B., Cullimore, J., Imberty, A., 2006. LysM domains of *Medicago truncatula* NFP protein involved in Nod factor perception. Glycosylation state, molecular modeling and docking of chitooligosaccharides and Nod factors. Glycobiology 16, 801–809.
Murakami, E., Cheng, J., Gysel, K., Bozsoki, Z., Kawaharada, Y., Hjuler, C.T., Sorensen, K.K., Tao, K., Kelly, S., Venice, F., Genre, A., Thygesen, M.B., Jong, N., Vinther, M., Jensen, D.B., Jensen, K.J., Blaise, M., Madsen, L.H., Andersen, K.R., Stougaard, J., Radutoiu, S., 2018. Epidermal LysM receptor ensures robust symbiotic signalling in Lotus japonicus. Elife 7 e33506.
Ohnuma, T., Onaga, S., Murata, K., Taira, T., Katoh, E., 2008. LysM domains from *Pteris ryukyuensis* chitinase-A: a stability study and characterization of the chitin-binding site. J. Biol. Chem. 283, 5178–5187.
Oldroyd, G.E., 2013. Speak, friend, and enter: signalling systems that promote beneficial symbiotic associations in plants. Nat. Rev. Microbiol. 11, 252–263.
Partida-Martinez, L.P., Heil, M., 2011. The microbe-free plant: fact or artifact? Front. Plant Sci. 2, 100.
Perret, X., Staehelin, C., Broughton, W.J., 2000. Molecular basis of symbiotic promiscuity. Microbiol. Mol. Biol. Rev. 64, 180–201.
Ponting, C.P., Aravind, L., Schultz, J., Bork, P., Koonin, E.V., 1999. Eukaryotic signalling domain homologues in archaea and bacteria. Ancient ancestry and horizontal gene transfer. J. Mol. Biol. 289, 729–745.
Radutoiu, S., Madsen, L.H., Madsen, E.B., Felle, H.H., Umehara, Y., Gronlund, M., Sato, S., Nakamura, Y., Tabata, S., Sandal, N., Stougaard, J., 2003. Plant recognition of symbiotic bacteria requires two LysM receptor-like kinases. Nature 425, 585–592.
Ranf, S., 2017. Sensing of molecular patterns through cell surface immune receptors. Curr. Opin. Plant Biol. 38, 68–77.
Saijo, Y., Loo, E.P., Yasuda, S., 2018. Pattern recognition receptors and signaling in plant-microbe interactions. Plant J. 93, 592–613.
Salzer, P., Feddermann, N., Wiemken, A., Boller, T., Staehelin, C., 2004. *Sinorhizobium meliloti*-induced chitinase gene expression in *Medicago truncatula* ecotype R108-1: a comparison between symbiosis-specific class V and defence-related class IV chitinases. Planta 219, 626–638.
Sanchez-Vallet, A., Saleem-Batcha, R., Kombrink, A., Hansen, G., Valkenburg, D.J., Thomma, B.P., Mesters, J.R., 2013. Fungal effector Ecp6 outcompetes host immune receptor for chitin binding through intrachain LysM dimerization. eLife 2 e00790.
Shimizu, T., Nakano, T., Takamizawa, D., Desaki, Y., Ishii-Minami, N., Nishizawa, Y., Minami, E., Okada, K., Yamane, H., Kaku, H., Shibuya, N., 2010. Two LysM receptor molecules, CEBiP and OsCERK1, cooperatively regulate chitin elicitor signaling in rice. Plant J. 64, 204–214.
Staehelin, C., Granado, J., Muller, J., Wiemken, A., Mellor, R.B., Felix, G., Regenass, M., Broughton, W.J., Boller, T., 1994. Perception of *Rhizobium* nodulation factors by tomato cells and inactivation by root chitinases. Proc. Natl. Acad. Sci. U. S. A. 91, 2196–2200.
Tanaka, K., Nguyen, C.T., Liang, Y., Cao, Y., Stacey, G., 2013. Role of LysM receptors in chitin-triggered plant innate immunity. Plant Signal. Behav. 8 e22598.
Tian, Y., Liu, W., Cai, J., Zhang, L.Y., Wong, K.B., Feddermann, N., Boller, T., Xie, Z.P., Staehelin, C., 2013. The nodulation factor hydrolase of *Medicago truncatula*: characterization of an enzyme specifically cleaving rhizobial nodulation signals. Plant Physiol. 163, 1179–1190.
van der Wolf, J., De Boer, S.H., 2015. Phytopathogenic bacteria. In: Lugtenberg, B. (Ed.), Principles of Plant-Microbe Interactions: Microbes for Sustainable Agriculture. Springer International Publishing, Cham, pp. 65–77.
Verbon, E.H., Liberman, L.M., 2016. Beneficial microbes affect endogenous mechanisms controlling root development. Trends Plant Sci. 21, 218–229.
Wan, J., Zhang, X.C., Neece, D., Ramonell, K.M., Clough, S., Kim, S.Y., Stacey, M.G., Stacey, G., 2008. A LysM receptor-like kinase plays a critical role in chitin signaling and fungal resistance in Arabidopsis. Plant Cell 20, 471–481.
Wan, J., Tanaka, K., Zhang, X.C., Son, G.H., Brechenmacher, L., Nguyen, T.H., Stacey, G., 2012. LYK4, a lysin motif receptor-like kinase, is important for chitin signaling and plant innate immunity in Arabidopsis. Plant Physiol. 160, 396–406.
Wang, L., Weber, A.N., Atilano, M.L., Filipe, S.R., Gay, N.J., Ligoxygakis, P., 2006. Sensing of Gram-positive bacteria in *Drosophila*: GNBP1 is needed to process and present peptidoglycan to PGRP-SA. EMBO J. 25, 5005–5014.
Weyens, N., van der Lelie, D., Taghavi, S., Newman, L., Vangronsveld, J., 2009. Exploiting plant-microbe partnerships to improve biomass production and remediation. Trends Biotechnol. 27, 591–598.
Willmann, R., Lajunen, H.M., Erbs, G., Newman, M.A., Kolb, D., Tsuda, K., Katagiri, F., Fliegmann, J., Bono, J.J., Cullimore, J.V., Jehle, A.K., Gotz, F., Kulik, A., Molinaro, A., Lipka, V., Gust, A.A., Nürnberger, T., 2011. *Arabidopsis* lysin-motif proteins LYM1 LYM3 CERK1 mediate bacterial peptidoglycan sensing and immunity to bacterial infection. Proc. Natl. Acad. Sci. U. S. A. 108, 19824–19829.
Wong, J.E., Midtgaard, S.R., Gysel, K., Thygesen, M.B., Sorensen, K.K., Jensen, K.J., Stougaard, J., Thirup, S., Blaise, M., 2015. An intermolecular binding mechanism involving multiple LysM domains mediates carbohydrate recognition by an endopeptidase. Acta Crystallogr. D Biol. Crystallogr. 71, 592–605.
Xie, Z.-P., Staehelin, C., Wiemken, A., Broughton, W.J., Müller, J., Boller, T., 1999. Symbiosis-stimulated chitinase isoenzymes of soybean (Glycine max (L.) Merr.). J. Exp. Bot. 50, 327–333.
Zhang, X.C., Cannon, S.B., Stacey, G., 2009. Evolutionary genomics of LysM genes in land plants. BMC Evol. Biol. 9, 183.
Zipfel, C., Oldroyd, G.E., 2017. Plant signalling in symbiosis and immunity. Nature 543, 328–336.