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β-Glucan-binding proteins are key modulators of immunity and symbiosis in mutualistic plant–microbe interactions

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Abstract

In order to discriminate between detrimental, commensal, and beneficial microbes, plants rely on polysaccharides such as β-glucans, which are integral components of microbial and plant cell walls. The conversion of cell wall-associated β-glucan polymers into a specific outcome that affects plant-microbe interactions is mediated by hydrolytic and non-hydrolytic β-glucan-binding proteins. These proteins play crucial roles during microbial colonization: they influence the composition and resilience of host and microbial cell walls, regulate the homeostasis of apoplastic concentrations of β-glucan oligomers, and mediate β-glucan perception and signaling. This review outlines the dual roles of β-glucans and their binding proteins in plant immunity and symbiosis, highlighting recent discoveries on the role of β-glucan-binding proteins as modulators of immunity and as symbiosis receptors involved in the fine-tuning of microbial accommodation.

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Introduction

The ability for plants to differentiate between beneficial and detrimental microbes in their environment is important for their survival. β-Glucans comprise a structurally highly diverse group of β-linked D-glucose polysaccharides, varying in linkage, chain length and branching patterns. Among other polysaccharides, β-glucans are conserved components of cell walls of both microbes and plants. For example, β-glucans contribute to a large proportion of the polysaccharide content of fungal cell walls [1,2]. In contrast to chitin, which often forms a rigid inner layer of the cell wall, β-glucans are mainly present in the outer, flexible layer, predominantly in the form of β-1,3-linked glucans with β-1,6-linked side branches or as β-1,3- and β-1,4- mixed-linked glucans (MLGs) in varying ratios [3,4]. Additionally, high molecular weight β-1,3/β-1,6-glucans are present in the mobile gel-like extracellular polysaccharide (EPS) matrix which is loosely attached to the outer cell wall layer [4–6]. In bacteria, β-glucans are found in the EPS matrix [7,8]. In plants, the β-1,4-linked glucan cellulose is the major β-glucan of the cell wall, while the β-1,3-linked glucan callose, though a minor component, plays a functionally important role. MLGs are found in several plant species, especially abundant in the cell walls of grasses like barley, oats, and wheat, where they contribute to cell wall flexibility and growth [9–12]. As building blocks of cell walls, β-glucans are present at the first contact sites of plants and microbes, the apoplast. Here, fragments/oligomers can be released by plant- or microbe-derived hydrolytic enzymes (glucanases) [11]. These fragments, derived from microbes or plants, act as microbe-associated molecular patterns (MAMPs) or damage-associated molecular patterns (DAMPs), respectively. They are detected by pattern recognition receptors (PRRs) on the plant plasma membrane. Receptor binding can activate various hallmarks of pattern-triggered immunity (PTI), such as apoplastic reactive oxygen species (ROS) accumulation, cytosolic calcium influx, or phosphorylation of mitogen-associated protein kinases (MAPK) [13–16]. Recent studies have shown that certain β-glucans can bind to symbiosis receptors that do not induce plant immunity but are involved in the accommodation of beneficial microbes. In mutant lines lacking those symbiosis receptors, establishment of arbuscular mycorrhizal symbiosis (AMS) and bacterial root nodule symbiosis (RNS) is restricted [5]. Here, we discuss established and emerging roles of β-glucan-binding

proteins in plant—microbe interactions and highlight several recent studies that identified novel β-glucan-binding proteins which, rather than promoting resistance, act as compatibility factors by enhancing microbial accommodation.

β-Glucan-binding proteins as immunity receptors

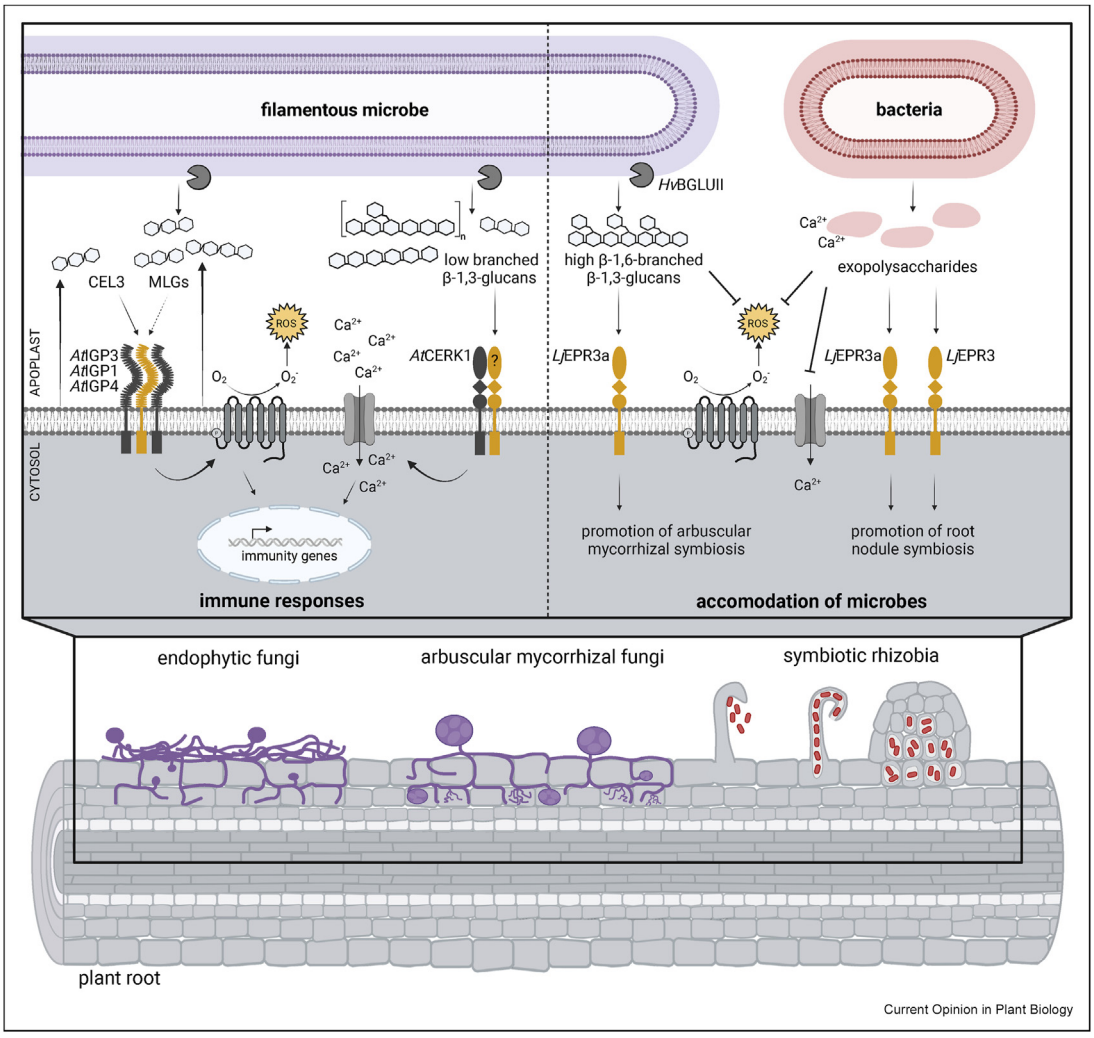
In the past years, β-glucans have been identified as major microbial cell wall MAMPs, providing a gateway for studying the mechanisms of β-glucan perception in plants and the receptors involved. Recent studies demonstrated that recognition of linear β-1,3-glucans varies between plant species and depends largely on the degree of polymerization (DP) [14,15]. While both longer (laminarin) and shorter (laminarihexaose [hereafter lam6]) β-1,3-glucans can be perceived in leaves of the monocotyledons *Hordeum vulgare* (hereafter barley) and *Brachypodium distachyon*, leaves of the dicotyledon *Nicotiana benthamiana* perceive only the long β-1,3-glucan laminarin. In barley, *Nicotiana tabacum* and *N. benthamiana* leaves, perception of laminarin was independent of the presence of β-1,6-branches, emphasizing that the DP is important for laminarin perception in these plant species. Branch length and DP can also influence supramolecular structure formation (i.e. aggregation into helical bundles of individual β-glucan strands) which might be relevant for β-glucan protein binding [6,15,17,18]. In *N. tabacum*, sulfation was additionally found to impact laminarin perception and activate a different, SA-dependent signaling pathway [19]. In leaves of the dicotyledons *Arabidopsis thaliana* (hereafter Arabidopsis) and *Capsella rubella*, short lam6 but not the long laminarin was perceived [15]. In Arabidopsis, this perception depends on the co-receptor kinase CHITIN ELICITOR RECEPTOR KINASE 1 (*At*CERK1) [14,20] (Figure 1), while perception of laminarin in rice and *N. benthamiana* is independent of *Os*CERK1 and *Nb*CERK1, respectively [15]. However, *in silico* analysis and isothermal titration calorimetry binding assays did not predict a direct interaction of *At*CERK1 and lam6 [20], suggesting that *At*CERK1 might act as a co-receptor. Therefore, other immune receptors must be present which can directly bind to β-1,3-glucans. One example of a soluble β-glucan receptor is the β-glucan-binding protein (*Gm*GBP) from *Glycine max* L. (soybean), which binds to a β-1,6-linked, β-1,3-branched heptaglucoside (HG) of the oomycete *Phytophthora sojae*. Treatment of soybean tissue with the HG induces phytoalexin production [21]. Additionally, *Gm*GBP has been shown to possess bifunctional properties, exhibiting both binding and endoglycosidic activity on β-1,3-oligoglucosides. This dual functionality allows *Gm*GBP to potentially release fragments from microbial cell walls that it can recognize, thereby initiating defense responses [21,22]. Besides linear β-1,3-glucans, MLGs can trigger PTI in both monocots and dicots [23,24] (Figure 1). MLGs, consisting of linear, unsubstituted chains of β-1,3- and β-1,4-linked glucan subunits, are widely distributed in the cell walls of monocot plants (e.g., Poaceae), fungi, and oomycetes. These MLGs can be recognized as either DAMPs or MAMPs [23,24]. The trisaccharide β-D-cellobiosyl-1,3-β-D-glucose is currently the smallest known MLG structure capable of triggering immunity in Arabidopsis, resulting in enhanced disease resistance to the oomycete pathogen *Hyaloperonospora arabidopsidis* [24]. During *Magnaporthe oryzae* infection, two hemicellulose-derived oligosaccharides are released from rice, 3^1-β-D-cellobiosyl-glucose and 3^1-β-D-cellotriosyl-glucose, and are perceived as DAMPs by an immune complex containing *Os*CERK1 and *Os*CEBiP [25].

Besides MLGs, the β-1,4-linked cellulose derivatives cellobiose and cellotriose are perceived as DAMPs in Arabidopsis [26,27]. Recently, it was found that activation of plant immunity by MLG43 and cellotriose depends on the LRR-MAL-RKs *At*IGP1/CORK1, *At*IGP3 and *At*IGP4 in Arabidopsis [28,29]. Cross-elicitation experiments of cellotriose with the xylo-oligosaccharide DAMPs β-1,4-D-xylotetraose (XYL4) and the pentasaccharide 3^3-alpha-l-arabinofuranosyl-xylotetraose (XA3XX) showed refractory responses in the respective combinations, suggesting shared perception mechanisms [30]. Therefore, the LRR-MAL-RKs *At*IGP1/CORK1, *At*IGP3 and *At*IGP4 might function as common receptors for DAMPs with β-1,4-linked sugar backbones. A more detailed description of plant, fungal or oomycete glycan structures inducing PTI responses in plants and the respective glucan-binding proteins involved in DAMP/MAMP perception, is provided in recent reviews [4,31].

Microbial evasion of β-glucan-triggered immunity

To successfully colonize the host, microbes have evolved various mechanisms to suppress perception and activation of plant immunity [37] (Figure 2). One strategy is downregulating MAMP biosynthesis to avoid MAMP exposure. For example, the hemibiotroph *Colletotrichum graminicola* downregulates the β-1,6-glucan biosynthesis pathway genes, *KRE5* and *KRE6*, in biotrophic hyphae [38] (Figure 2a). Overexpression of *KRE5* and *KRE6* in biotrophic hyphae led to activation of broad-spectrum plant defense responses, including papilla and H_2O_2 formation, as well as transcriptional activation of several defense-related genes [38]. Another strategy to avoid MAMP release is through secretion of inhibitors of plant hydrolytic enzymes. The oomycete pathogen *P. sojae* secretes the Glucanase Inhibiting Protein1 (GIP1), which inhibits the activity of the plant-secreted endoglucanase EGaseA to avoid the release of β-1,3/1,6-glucan MAMPs from the fungal cell wall [39] (Figure 2b).

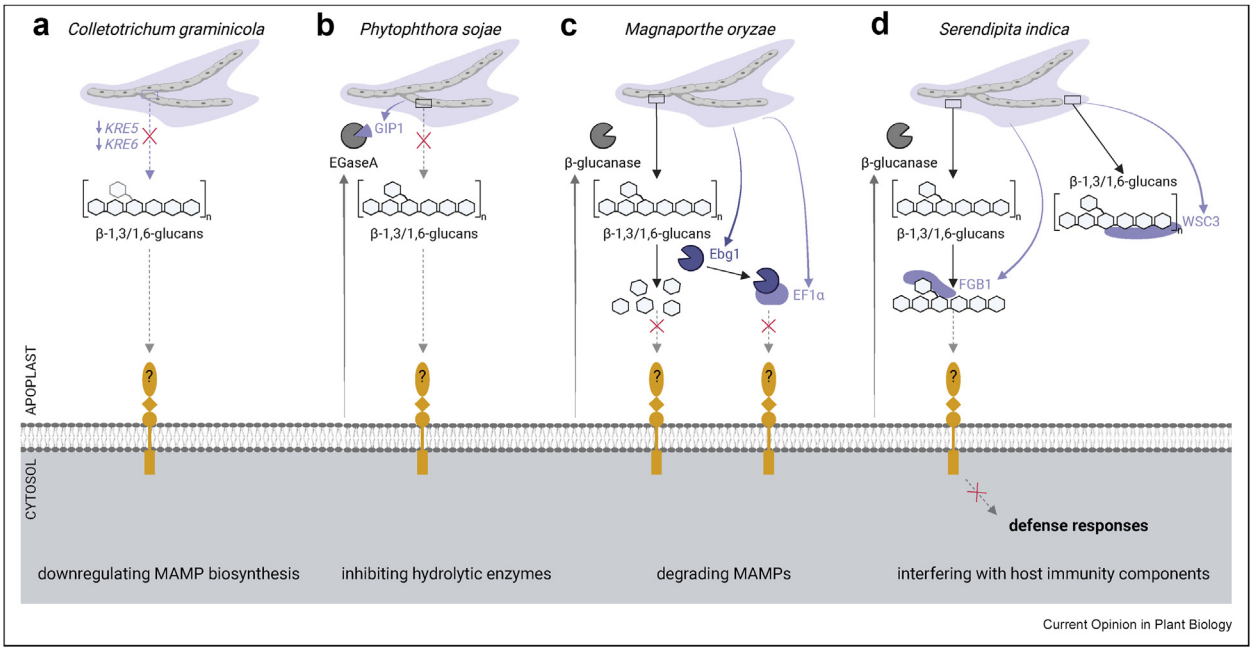
Figure 1



β-Glucan-binding proteins are involved in activation of plant innate immunity or accommodation of microbes as symbiosis receptors. Activation of plant immune responses: Cellotriose (CEL3) and MLGs can be released from plant and fungal cell walls through hydrolytic enzymes (gray circles) and are perceived in Arabidopsis by the LRR-MAL-RKs *At*IGP1/CORK1, *At*IGP3 and *At*IGP4 [28]. CEL3 directly binds to *At*IGP1, while no direct binding of MLG43 could be shown. However, plant immunity activation in response to MLG43 was reduced in *igp1*, *igp3* and *igp4* mutants [28]. Perception leads to cytosolic calcium influx, apoplastic ROS accumulation and subsequently transcriptional activation of plant immunity genes. In addition, low branched β-1,3-glucans can be released from fungal or oomycete cell walls or EPS matrices and bind to an unknown receptor. In Arabidopsis, *At*CERK1 is involved in laminarihexaose perception as a co-receptor (black) [14,15]. Accommodation of microbes: The plant-derived endoglucanase *Hv*BGLUII releases a β-1,6-branched β-1,3-glucan decasaccharide (β-GD) from fungal EPS matrix which scavenges ROS [32]. β-GD was also shown to bind to the *Lotus japonicus* symbiosis receptor *Lj*EPR3a which promotes arbuscular mycorrhizal symbiosis (AMS) [5]. Similarly, bacterial EPS can chelate calcium ions to prevent cytosolic calcium influx and scavenge apoplastic ROS to suppress plant immunity [33,34]. Furthermore, binding of bacterial EPS to the symbiosis receptors *Lj*EPR3 and *Lj*EPR3a in *L. japonicus* promotes root nodule symbiosis (RNS) [5,35,36]. Receptors that directly bind to ligands are indicated in gold, co-receptors in black. Lower box: Beneficial fungi and bacteria colonizing the plant root epidermis and cortex layer (light gray outermost layers). Endodermis (white) and central cylinder (dark gray innermost layers) are not colonized.

A third strategy, acting further downstream from MAMP release, is the degradation of the released MAMPs to avoid their perception. The rice blast fungus *M. oryzae* secretes a glycoside hydrolase family 17 (GH17) exo-β-1,3-glucanase (Ebg1), which hydrolyzes the β-1,3-glucans released from the fungal cell wall and/or EPS matrix into monomeric glucose to prevent PTI responses [40]. Ebg1 itself can be perceived as a MAMP. Therefore, *M. oryzae* secretes another protein, elongation factor 1 alpha (EF1α), which binds to Ebg1, thereby preventing its recognition [40] (Figure 2c). Similar to Ebg1, the xylo- and β-glucanase XEG1 of *P. sojae* can be recognized as a MAMP in *N. benthamiana* and induce immunity [41]. This response can be suppressed by *P. sojae* RXLR effectors, indicating the use of similar strategies to avoid

Figure 2



Microbial strategies to evade β-glucan-triggered immunity. a) Downregulating MAMP biosynthesis: To avoid MAMP exposure during infection, the hemibiotroph *Colletotrichum graminicola* downregulates the β-1,6-glucan biosynthesis pathway genes, *KRE5* and *KRE6*, in biotrophic hyphae [38]. **b)** Inhibiting hydrolytic enzymes: To avoid the release of β-glucan MAMPs from the cell wall, the oomycete pathogen *Phytophthora sojae* secretes the Glucanase Inhibitor Protein1 (GIP1), which inhibits the activity of the plant-secreted endoglucanase EGaseA [39]. **c)** Degrading MAMPs: The rice blast fungus *Magnaporthe oryzae* secretes a GH17 exo-β-1,3-glucanase (Ebg1), which hydrolyzes the β-1,3-glucans released from the fungal cell wall and/or EPS matrix (light purple) into single glucose units to prevent PTI responses [40]. Ebg1 itself can be perceived as a MAMP, therefore *M. oryzae* secretes a second protein, elongation factor 1 alpha (EF1α), which sequesters Ebg1. **d)** Interfering with host immunity components: The beneficial root endophytic fungus *Serendipita indica* releases the lectin Fungal Glucan-Binding 1 (FGB1) which binds to β-1,3/1,6-glucans [42]. This lectin localizes on the fungal cell wall and is also extensively secreted into the environment. Substoichiometric amounts of FGB1 were sufficient to significantly reduce glucan perception, indicating that FGB1 might interfere with host immunity components to prevent perception. In addition, *S. indica* secretes the lectin WSC3, which is highly induced during both plant- and microbe-interactions [6]. WSC3 specifically binds to β-1,3-linked glucans of the fungal EPS matrix where it accumulates [6], and potentially functions in adhesion between hyphal cells and strengthening of the cell wall against external stresses. Adapted from Buscaill et al., 2021 [37].

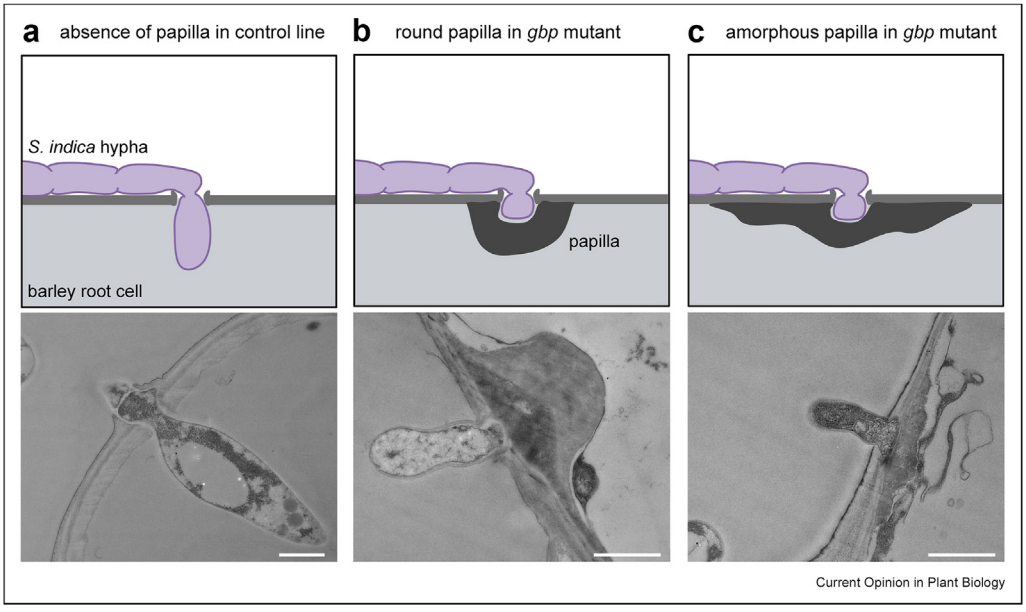
perception of β-glucanases as MAMPs. Alternatively, a fourth strategy is interfering with downstream host immunity components, that activate defense responses upon β-glucan perception (Figure 2d). The beneficial root endophytic fungus *Serendipita indica* secretes the lectin Fungal Glucan-Binding 1 (FGB1) which can bind to the β-1,6-linkages of various β-glucans including laminarin and the disaccharide gentiobiose (D-glucose subunits with β-1,6-linkage) (Figure 2d). Substoichiometric amounts of FGB1 were sufficient to significantly reduce laminarin-induced ROS production in both barley and Arabidopsis, indicating that FGB1 might interfere with host immunity components e.g. through competing for receptor binding and/or blocking of receptor assembly [42]. Moreover, *S. indica* colonization was significantly higher in barley when FGB1 was added, suggesting that this effector promotes fungal colonization in the host [6,42]. *S. indica* additionally expresses the lectin WSC3, which is highly induced *in planta* and in confrontation with other fungi [6,43]. WSC3 is associated with the EPS matrix of *S. indica* [32] and contains three Wall Stress Component (WSC) domains which can each bind to long β-1,3-linked glucans (Figure 2d). While WSC domains were initially described as cell surface sensors for cell wall integrity in yeast [44,45], *S. indica* WSC3 promotes adhesion between hyphal cells and potentially strengthens the cell wall against external stresses. This is likely achieved through the formation of helical bundles involving three β-1,3-linked glucan polymers [6]. WSC-domain-containing proteins are prominent among carbohydrate binding proteins of fungi of various lifestyles [46] and are found to be induced in other plant-fungal systems during colonization [47,48], suggesting a function beyond cell wall stress sensing. These findings suggest that fungal lectins such as FGB1 and WSC3 can bind β-glucans present in the fungal cell wall, EPS matrices and released fragments in the apoplast and might interfere with host immunity signaling components. Further research is needed to investigate the potential role of these lectins in intermicrobial interactions.

Plant-derived β-glucanases modulate immune responses

Downstream of the release and perception of microbial MAMPs during host colonization, several mechanisms evolved that can fine-tune the degree in which plant immunity affects microbial accommodation. Previous reports indicated that some microbial β-glucans (e.g. bacterial EPS) can suppress plant immune responses due to their physicochemical properties, for instance, by sequestration of calcium ions or ROS scavenging [33,34]. A more recent study demonstrated how the interplay between the microbial EPS matrix and plant hydrolases has led to the emergence of a similar principle in fungi [32]. During colonization by *S. indica*, barley secretes an endoglucanase, *Hv*BGLUII, of the family GH17 into the apoplastic space. This enzyme acts on the EPS matrix of the fungus, and releases a specific fragment, a β-1,3/1,6-glucan decasaccharide (β-GD) which does not activate plant immunity (Figure 1). Moreover, β-GD was resistant to further degradation by host glucanases due to the presence of its β-1,6-branches and found to actively scavenge ROS in the apoplast (Figure 1). *S. indica* colonization of barley was enhanced by exogenous application of β-GD, confirming the role of this β-glucan fragment and *Hv*BGLUII in accommodation of beneficial fungi [32]. In addition, *Hv*BGLUII releases β-GD from the cell wall of the pathogenic fungus *Bipolaris sorokiniana* which also possesses immunity-suppressive function [32]. This confirms that the outer microbial EPS layer of fungi and bacteria function as a protective shield against oxidative stress.

An additional response in plant immunity is the production of cell wall appositions (CWA) to restrict fungal and oomycete colonization [49–51]. A recent study identified a previously undescribed barley β-glucanase via a pulldown approach using laminarin as bait. This GH81 member, named β-glucan-binding protein (*Hv*GBP1), hydrolyzes the β-1,3-linkages of laminarin. Barley double mutant lines of the only two GBP paralogs (*gbp1 gbp2*) were significantly less colonized by fungi of various lifestyles compared to the control line (Golden Promise Fast) and relative expression of *HvPR10*, a barley defense marker gene, was significantly higher in the *gbp* mutant lines compared to the control line, implying that *Hv*GBP acts as a compatibility factor in barley by attenuating immune signaling. *Hv*GBP1 was not active on the EPS of *S. indica*, indicating a degree of specificity. Further investigation revealed that when colonized by *S. indica*, the *gbp* mutant lines displayed significantly more CWA compared to the control lines [52]. Since CWA are composed of callose (a β-1,3-glucan), it was inferred that *Hv*GBP likely operates on

Figure 3



Barley β-glucan-binding protein (GBP) affects the barley cell wall responses at *Serendipita indica* hyphae penetration sites. a) Example of the absence of papilla formation during hyphal penetration in the barley control line Golden Promise Fast [53]. **b)** During hyphal penetration, round, compact papilla structures can be deposited at the barley cell wall such as in the roots of the barley *gbp* mutant line [52]. **c)** In addition to round papilla structures, also spatially unrestricted, amorphous papilla were detected in barley *gbp* roots lacking GBP [52]. These amorphous papillae were not observed in the control line Golden Promise Fast. Top panel: graphical representations of the images below. Bottom panel: transmission electron micrographs of cross-sections of chemically fixed barley roots colonized by *S. indica* at 6 dpi. Scale bars = 1 μm.

the plant cell wall, hydrolyzing callose, thereby enabling fungal penetration of the plant cells [52]. Transmission electron microscopy analysis suggests that both round-shaped, as well as spatially unrestricted, amorphous papillae can be found in *gbp* mutant barley roots at fungal hyphae penetration sites (Figure 3). Amorphous papillae were never found in the control line Golden Promise Fast (Figure 3). This implies that apoplastic β-glucanases can not only target microbial substrates but also modify the plant cell wall to enhance microbial accommodation. Taken together, these findings suggest that β-glucanases can facilitate the colonization of both beneficial and pathogenic fungi by modulating/attenuating the effect of activated defense responses. This indicates a potential homeostatic mechanism to prevent hyperactivation of immunity, which is particularly important in the context of beneficial plant−microbe associations, where an overly aggressive immune response could be detrimental.

β-Glucan symbiosis receptors promote accommodation of beneficial microbes

In contrast to the extensive knowledge of β-glucan receptors in animals, plant β-glucan receptors are relatively understudied, with only a few linked to microbial accommodation. Recent reports have identified two β-glucan receptors from *Lotus japonicus*. One of these is EXOPOLYSACCHARIDE RECEPTOR 3 (*Lj*EPR3), a LysM receptor kinase that plays a crucial role in promoting root nodule symbiosis during rhizobia accommodation in *L. japonicus* [35,36,54] (Figure 1). In a more recent study, *Lj*EPR3a, another LysM receptor kinase, was shown to promote arbuscular mycorrhizal fungus accommodation in *L. japonicus* [5] (Figure 1). *epr3a* mutants exhibited significantly reduced intracellular arbuscule formation compared to the wild-type. Characterization by microscale thermophoresis revealed that both *Lj*EPR3 and *Lj*EPR3a bind the immunosuppressive β-GD released from EPS of endophytic fungi. ROS accumulation upon β-glucan treatment was not affected in *epr3* and *epr3a* mutant plants, indicating β-glucan perception by *Lj*EPR3 and *Lj*EPR3a is not involved in immunity signaling. Instead, *Lj*EPR3 and *Lj*EPR3a act as symbiosis receptors [5,36] (Figure 1). Symbiosis receptor kinases are known from legume-rhizobia symbiosis, where they are important in symbiosis initiation through perception of lipochitooligosaccharides (LCOs) from bacteria (Nodulation-factors) [55,56]. Similarly, perception of LCOs as well as other chitin oligomers from arbuscular mycorrhizal fungi (Myc-factors) by symbiosis receptor kinases plays a key role in AM symbiosis establishment in the host [55−57]. These recent reports about EPR3/EPR3a show for the first time that symbiosis receptors also exist for β-glucan-type ligands, opening up an exciting field of research.

Conclusions

β-Glucans are a diverse group of polysaccharides that can be released by plant- or microbe-derived glucanases and perceived as MAMPs or DAMPs, triggering plant immunity. However, recent studies identified β-glucan-binding proteins which prevent activation of host immunity. Host-derived β-glucanases were also shown to modulate plant immune responses after MAMP perception, for instance by releasing β-glucan fragments which scavenge ROS or by reducing callose deposition along the plant cell wall. A growing amount of evidence suggests that the β-glucan-rich EPS matrix surrounding the cell wall of microbes acts as a protective shield against oxidative stress and that components of the EPS matrix are recognized by symbiosis receptors, which are important in the establishment of mutualistic plant−microbe interactions including AMS and RNS. Similar to the well-studied contrasting roles of chitin-oligosaccharides in immunity and symbiosis establishment [58−61], these findings collectively show that β-glucans play important roles beyond immunity activation in microbial accommodation. The β-glucans themselves, as well as their interacting β-glucan-binding proteins, can be considered as compatibility factors operating at different stages of colonization. These recent advances highlight the unexplored potential for β-glucans of different lengths and branching patterns as symbiosis messengers, shifting our attention to the search for β-glucan ligand/receptor pairs to unravel their mechanisms during beneficial plant−microbe interactions.

Author contributions

SvB: Conceptualization, Investigation, Writing-original draft preparation. PS: Conceptualization, Writing-original draft preparation. AW: Writing-reviewing and editing. UN: Investigation, Supervision, Writing-reviewing and editing. AZ: Conceptualization, Supervision, Writing-reviewing and editing, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

Data availability

Data will be made available on request.

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