Plant cell wall-mediated immunity: cell wall changes trigger disease resistance responses

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SUMMARY

Plants have evolved a repertoire of monitoring systems to sense plant morphogenesis and to face environmental changes and threats caused by different attackers. These systems integrate different signals into overreaching triggering pathways which coordinate developmental and defence-associated responses. The plant cell wall, a dynamic and complex structure surrounding every plant cell, has emerged recently as an essential component of plant monitoring systems, thus expanding its function as a passive defensive barrier. Plants have a dedicated mechanism for maintaining cell wall integrity (CWI) which comprises a diverse set of plasma membrane-resident sensors and pattern recognition receptors (PRRs). The PRRs perceive plant-derived ligands, such as peptides or wall glycans, known as damage-associated molecular patterns (DAMPs). These DAMPs function as 'danger' alert signals activating DAMP-triggered immunity (DTI), which shares signalling components and responses with the immune pathways triggered by non-self microbe-associated molecular patterns that mediate disease resistance. Alteration of CWI by impairment of the expression or activity of proteins involved in cell wall biosynthesis and/or remodelling, as occurs in some plant cell wall mutants, or by wall damage due to colonization by pathogens/pests, activates specific defensive and growth responses. Our current understanding of how these alterations of CWI are perceived by the wall monitoring systems is scarce and few plant sensors/PRRs and DAMPs have been characterized. The identification of these CWI sensors and PRR–DAMP pairs will help us to understand the immune functions of the wall monitoring system, and might allow the breeding of crop varieties and the design of agricultural strategies that would enhance crop disease resistance.

INTRODUCTION

In their natural environments, plants are under continuous biotic stresses caused by different attackers and are also exposed to abiotic stresses that compromise their survival and offspring. To cope with these environmental challenges, plants have evolved a variety of complex and efficient mechanisms of resistance, which include diverse molecular monitoring systems that perceive stress-derived signals triggering specific resistance responses (Atkinson and Atkinson and Urwin, 2012; Engelsdorf and Hamann, 2014). The general assumption is that resistance of plants to environmental stresses is costly, and that the constitutive expression of defensive mechanisms may not always be the best strategy for plants to cope with their potential colonization by pathogens/pests, most likely because allocation of defensive metabolites for resistance may constrain other physiological processes and have a negative impact on plant traits such as biomass and seed production (Manzaneda *et al.*, 2010; Viola *et al.*, 2010; Antonovics *et al.*, 2011; Kempel *et al.*, 2011; Denancé *et al.*, 2013; Lozano-Duran and Zipfel, 2015; de Vries *et al.*, 2017; Major

et al., 2017; Wasternack, 2017). These physiological constraints on plants have driven the evolution of cell-autonomous monitoring systems to perceive these stress-derived signals and to fine-tune defensive responses for environmental adaptation (Wolf *et al.*, 2012a; Lozano-Duran and Zipfel, 2015).

One of these plant monitoring systems is the plant cell wall, the physicochemical properties of which change upon exposure of the plant to different stresses/pathogens, and this strongly influence a plant's ability to grow and to cope with these adverse scenarios. Moreover, plants have developed an innate immunity system that is based on sets of plasma membrane-anchored pattern-recognition receptors (PRRs) that detect 'non-self' microbe-associated molecular patterns (MAMPs), activating pattern-triggered immunity (PTI). The plant immune system also recognizes microbial effectors (Avr proteins), via cytoplasmic proteins encoded by the resistance genes, and 'plant-self' derived damage-associated molecular patterns (DAMPs) (Boutrot and Zipfel, 2017). DAMPs comprise molecules released from the plant cell wall (e.g. wall-derived glycans) and peptides that upon exposure of the plant to different stresses are either *de novo* synthesized or processed to produce a mature active ligand.

THE PLANT CELL WALL: MORE THAN A PASSIVE DEFENSIVE BARRIER

The first obstacle encountered by pathogens attempting to colonize plant tissues is the plant cell wall, which is sometimes covered with a cuticle. Pathogens have evolved an arsenal of tools to penetrate and break down this barrier. Among these colonization tools are the secretion of cell wall-degrading enzymes (CWDEs) that modify wall glycans or hydrolyse the linkages between glycan moieties, and the formation of appressoria, penetration-specific structures that exert turgor pressure on the plant wall.

The plant cell wall is a dynamic and highly controlled structure that is essential for growth and development (Srivastava *et al.*, 2017). The molecular mechanisms behind synthesis and modifications of the plant cell wall, and how these modifications are communicated to the plant cell, are only partially understood. All plant cells in developmental expansion are surrounded by a primary cell wall mainly consisting of carbohydrate-based polymers (cellulose, pectins and hemicelluloses) that might harbour different types of biochemical modifications, such as acetylations, esterifications or methylations (Figure 1a; Carpita and McCann, 2000). In addition, those cells that have completed their cellular expansion and need to reinforce the wall structure for functional reasons (e.g. xylem formation) might deposit new layers of material in the inner face constituting the secondary cell wall that also contains cellulose, but is enriched in lignin and xylans (Figure 1a; Sarkar *et al.*, 2009).

Cellulose is the main load-bearing component in all plant cell walls, whereas different types of hemicelluloses and pectins are found in different plant phylogenetic groups (e.g. xyloglucan in dicots versus arabinoxylan in monocots; Carpita and Gibeaut, 1993). Moreover, there is a considerable variability in the fine structures of wall polymers (e.g. the degree of xylan/pectin acetylation or pectin methylation) among a given phylogenetic group of plants, and even between different tissues (e.g. leaves versus stems) of a given plant. All these chemical differences have an obvious impact on the three-dimensional architectures and physicochemical properties of plant walls. This wall heterogeneity is reflected in the diversity of mechanisms that pathogens have evolved to breach plant cell walls, including the secretion of numerous CWDEs, such as cellulases, polygalacturonases or xylanases (Annis and Goodwin, 1997). CWDEs represent a significant proportion of the encoded proteins of the genomes of plant-pathogenic fungi, further indicating their relevance for breaching the wall and suggesting that this fungal CWDE repertoire might determine plant-host specificity (Kubicek *et al.*, 2014). Similarly, it has been shown by genome-wide association studies (GWAS) that some plant loci related to plant cell wall integrity influence, together with defence-associated loci, the variation of leaf microbial communities (fungi and bacteria) of *Arabidopsis thaliana* (Horton *et al.*, 2014).

ALTERATIONS TO PLANT CELL WALL INTEGRITY AFFECT DISEASE RESISTANCE

Plant cell wall alterations, either by impairing or over-expressing cell wall-related genes, have been demonstrated to have a significant impact on disease resistance and/or on abiotic stresses (Bellincampi *et al.*, 2014; Malinovsky *et al.*, 2014; Miedes *et al.*, 2014; Kesten *et al.*, 2017). It was initially thought that these disease resistance phenotypes associated with alterations to cell wall integrity (CWI) were due to the inability of mis-adapted pathogens to overcome the genetically modified wall compositions/structures in the plant mutants or over-expressor lines. However, this view was an oversimplification of the disease resistance phenotypes of these plant genotypes since it considered the wall to be just a passive barrier, whereas it has been found that activation of defensive signalling pathways does take place in mutants/transgenic plants with wall alterations (Miedes *et al.*, 2014; Nafisi *et al.*, 2015; Houston *et al.*, 2016). In this section, we describe examples of the impact on disease resistance phenotypes of modifications of the main plant cell wall components, with a particular focus on published data for Arabidopsis genotypes (mutants and over-expressor lines) with altered wall composition (Table 1, Figure 1). We mainly focus on the modification of the wall's carbohydrate counterpart, since the role of cell wall phenolics (e.g. lignin) on the

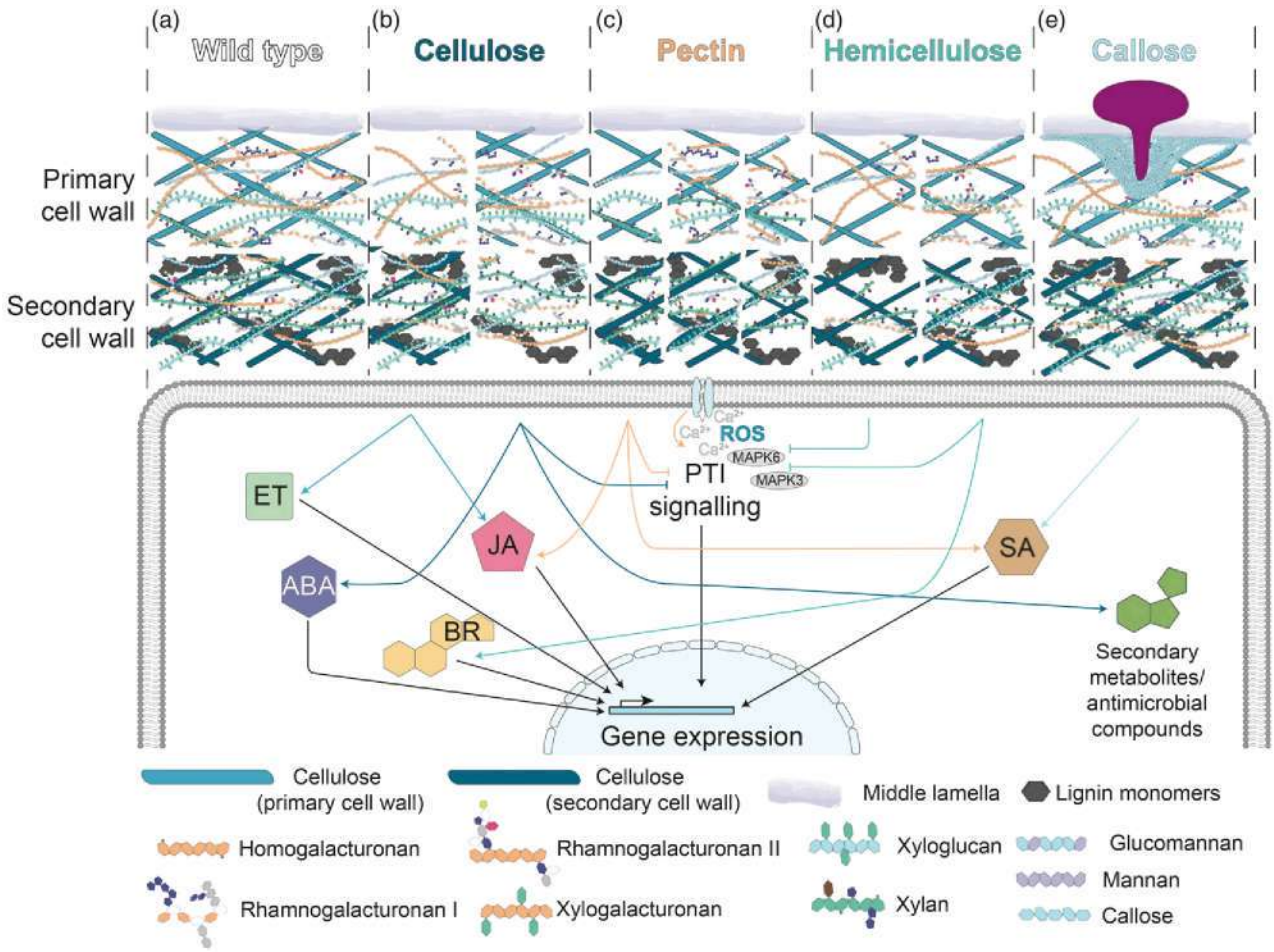

Figure 1. Alterations of *Arabidopsis thaliana* cell wall integrity triggers disease resistance responses. (a) The cell wall of Arabidopsis wild-type plants is composed of cellulose, pectin polysaccharides (homogalacturonan, rhamnogalacturonan I and rhamnogalacturonan II) and two main hemicelluloses, xyloglucan and xylan, together with minor proportions of mannan and glucomannan. In some plant tissues, cells also deposit a secondary cell wall that is mainly composed of cellulose, hemicelluloses (mostly xylans) and lignin. (b) Alterations in cellulose biosynthesis from primary (left) or secondary (right) cell wall trigger specific defensive responses, such as those mediated by the hormones jasmonic acid (JA), ethylene (ET) or abscisic acid (ABA), activate biosynthesis of antimicrobial compounds (like Trp-derived metabolites), but also might attenuate pattern triggered immunity (PTI) responses. (c) Alterations of wall pectins, either in their overall content (left), their degree of acetylation (middle) or methylation (right) activate specific defensive responses, such as those regulated by JA or SA, and trigger PTI responses, probably mediated by damage-associated molecular patterns like oligogalacturonides (OGs). (d) Alteration of wall hemicelluloses content (right) or the degree of acetylation (left) trigger defensive responses such as the activation of brassinosteroid (BR) and ABA signalling pathways, the biosynthesis of antimicrobial compounds, but also might attenuate PTI responses. (e) Callose deposition in the cell wall is a typical response that takes place at pathogen penetration sites. Callose deposition strengthens the plant cell wall and might activate the salycylic acid (SA) defensive pathway.

resistance of plants to pathogens has been recently reviewed in detail (Miedes *et al.*, 2014). Moreover, we also summarize the most significant disease resistance phenotypes that have been described in genotypes from other plant species – including crops – with modified wall composition due to the impairment or over-expression of cell wall-related genes (Table S1 in the online Supporting Information).

Impairment of cellulose biosynthesis activates differential defence responses

Cellulose plays a central role in determining the mechanical properties of plant cell walls, thus affecting many aspects of plant life and fitness (Somerville, 2006). Cellulose is synthesized at the plasma membrane by large protein complexes, known as cellulose synthase complexes (CSC); every CSA contains between 18 and 24 cellulose synthase (CESA) proteins that each synthesize an individual β-1,4-glucan chain (Guerriero *et al.*, 2010; Kumar *et al.*, 2017). Remodelling of primary and secondary cell walls by impairing the function of *CESA* genes has a specific impact on pathogen resistance and tolerance to abiotic stresses. Arabidopsis *irregular xylem* (*irx*) *5/3/1* cell wall mutants defective in CESA subunits (CESA4/7/8, respectively) required for secondary cell wall formation show enhanced resistance to different pathogens, including the necrotrophic fungi *Plectosphaerella cucumerina* and *Botrytis cinerea*, the vascular bacterium *Ralstonia solanacearum*

Table 1 Cell wall modifications in *Arabidopsis thaliana* associated with phenotypes of resistance/susceptibility to pathogens

Cell wall component	Gene name (mutant/ transgenic)	Pathogen tested	Phenotype[a]	Immune responses[b]	References
Cellulose	*CESA4/CESA7/CESA8* (*irx5/irx3/irx1*)	*P. cucumerina, R. solanacearum, B. cinerea, P. syringae*	R	ABA, antimicrobial, attenuated PTI	Hernández-Blanco *et al.* (2007)
	CESA3 (*cev1, rsw*)	*G. cichoracearum, G. orontii, Oidium lycopersicum*	R	JA, ET	Ellis and Turner (2001); Ellis *et al.* (2002)
	MYB46 (*myb46*)	*B. cinerea*	R	SA	Ramírez *et al.* (2011)
	ABA1 (*aba1-6*)	*P. cucumerina,*	R	ABA, JA, ET, SA	Sánchez-Vallet *et al.* (2012)
Pectin	*RAE* (*35S::AnRAE*)	*B. cinerea*	R	Callose deposition, ROS, transcriptional regulation	Pogorelko *et al.* (2013)
	PME (24 *pme* mutants)	*P. syringae*	S	JA	Bethke *et al.* (2014)
	PMEI (OE, *PMEI-1, PMEI-2*)	*B. cinerea*	R	nd	Lionetti *et al.* (2007)
	PMEI (OE; *AcPMEI*; PMEI-2)	*Tobacco mosaic virus, Turnip vein clearing virus*	S	nd	Lionetti *et al.* (2014)
	PLL (*pll18, pll19*)	*Heterodera. schachtii, Meloidogyne incognita*	R	nd	Wieczorek *et al.* (2014)
	GAE (*gae1 gae6*)	*P. syringae, B. cinerea*	S	JA, attenuated PTI	Bethke *et al.* (2016)
	ERF014 (RNAi)	*P. syringae, B. cinerea*	S	ROS production, SA,	Zhang *et al.* (2016)
	MUR8 (*mur8*)	*C. higginsianum*	S	nd	Engelsdorf *et al.* (2017a)
	PMR5 (*pmr5*) PMR6 (*pmr6-3*) (*pmr5 pmr6-3*)	*C. higginsianum*	R	nd	Engelsdorf *et al.* (2017a)
	PMEI (*pmei-10, pmei-12*)	*B. cinerea*	S	JA, ET	Lionetti *et al.* (2017)
	PMEI (*pmei-11*)			JA, ET, DAMPs	
	IDL6 (OE)	*P. syingae*	S	SA	Wang *et al.* (2017)
Hemicellulose	*RWA2* (*rwa2*)	*B. cinerea*	R	nd	Manabe *et al.* (2011)
		H. arabidopsidis	R	Transcriptional regulation	Pawar *et al.* (2016)
	AXE (*35S::AnAXE*)	*B. cinerea*	R	ROS, transcriptional regulation	Pogorelko *et al.* (2013)
		H. arabidopsidis	R	Transcriptional regulation	Pawar *et al.* (2016)
	ESK1 (*esk1-7*)	*P. cucumerina,*	R	AB, antimicrobials, attenuated PTI	Escudero *et al.* (2017)
	XYL1 (*xyl1-2*)	*P. cucumerina*	R	nd	Delgado-Cerezo *et al.* (2012)
	IRX6 (*irx6*)	*P. cucumerina*	R	nd	Delgado-Cerezo *et al.* (2012)
	DET3 (*det3*)	*P. cucumerina*	R	BR	Delgado-Cerezo *et al.* (2012)
	ER (*er*)	*P. cucumerina, R. solanacearum, Pythium irregulare*	S	Attenuated PTI	Llorente *et al.* (2005), Godiard *et al.* (2003). Adie *et al.* (2007), Sánchez-Rodríguez *et al.* (2009)
	AGB1 (agb1) AGG1/2 (*agg1 agg2*)	*P. cucumerina, A. brassicicola, B. cinerea, F. oxysporum*	S	Attenuated PTI	Trusov *et al.* (2010), Klopffleisch *et al.* (2011), Delgado-Cerezo *et al.*

(continued)

Table 1. (continued)

Cell wall component	Gene name (mutant/transgenic)	Pathogen tested	Phenotype[a]	Immune responses[b]	References
					(2012), Torres *et al.* (2013), Lorek *et al.* (2013), Liu *et al.* (2013)
Callose	*GSL5 (gsl5, pmr4)*	*G. cichoracearum, G. orontii, H. arabidopsidis*	R	SA, callose deposition	Jacobs *et al.* (2003), Nishimura *et al.* (2003)
		B. graminis	S		
	GSL5 (OE)	*G. cichoracearum*	R	SA, callose deposition	Ilinger *et al.* (2013)
	BG_papp (*bg_papp*)	*H. schachtii*	R	Callose deposition in plasmodesmata	ofmann *et al.* (2010)
Global modification of secondary cell walls	*WAT1* (*wat1*)	*R. solanacearum, P. cucumerina, X. campestris, V. dahlia, V. alboatrum*	R	SA, secondary metabolites	Denancé *et al.*, 2013
	PGM (*pgm*)	*C. higginsianum*	S	SA, secondary metabolites	Engelsdorf *et al.* (2017a)

[a]R, enhanced resistance compared with wild-type plants; S, enhanced susceptibility compared with wild-type plants.
[b]nd, not determined; PTI, pattern triggered immunity; ROS, reactive oxygen species; SA, salicylic acid; JA, jasmonic acid; ET, ethylene; ABA, absicic acid; BR, brassinosteroid.

and the vascular fungus *Fusarium oxysporum* (Table 1, Figure 1b; Hernández-Blanco *et al.*, 2007; Escudero *et al.*, 2017). In line with these results, an Arabidopsis mutant defective in the MYB46 transcription factor that directly regulates the expression of several secondary cell wall-related genes, including *CESA4/7/8*, also showed enhanced resistance to necrotrophic fungi (Table 1; Ramírez *et al.*, 2011).

The disease resistance phenotype of *irx5, irx3, irx1* and *myb46* plants is in part explained by the constitutive activation of plant immune responses, such as the abscisic acid (ABA) signalling pathway and the synthesis of antimicrobial compounds like peptides and tryptophan-derived metabolites (Figure 1b; Hernández-Blanco *et al.*, 2007; Escudero *et al.*, 2017). The constitutive activation of these defensive pathways in *irx1/3/5* plants probably explains their trade-off phenotypes (dwarf plants and reduced seed yield) (Hernández-Blanco *et al.*, 2007; Sánchez-Vallet *et al.*, 2010; Ramírez *et al.*, 2011). Despite the broad-spectrum resistance of *irx1-6* plants, some PTI responses, like production of reactive oxygen species (ROS) and phosphorylation of mitogen-activated protein kinases (MAPKs), are attenuated in the MAMP-treated *irx1-6* mutant compared with wild-type plants, indicating that CWI-mediated immunity might be sufficient to compensate partially defective PTI responses (Escudero *et al.*, 2017). Of note, *irx1-6* plants show enhanced resistance to several abiotic stresses like drought and salinity, which is in accordance with the constitutive activation of the ABA pathway and the accumulation in these plants of stress-associated metabolites, including some osmolites (Chen *et al.*, 2005; Hernández-Blanco *et al.*, 2007; Escudero *et al.*, 2017). Of note, impairment of ABA signalling, as occurs in the *P. cucumerina*-resistant *aba1-6* mutant, resulted in reduced cellulose and increased uronic acid in its cell wall (Table 1; Sánchez-Vallet *et al.*, 2012).

Plant resistance to pathogens is also altered in Arabidopsis mutants affected in CESA subunits required for cellulose biosynthesis of the primary cell wall, such as the CESA3-defective *isoxaben resistant* (*ixr1*)/*constitutive expression of VSP* (*cev1*) mutants. *ixr1/cev1* plants, which display constitutive activation of ethylene (ET) and jasmonic acid (JA) signalling, were more resistant than wild-type plants to *B. cinerea, Pseudomonas syringae* and different powdery mildew fungal isolates, whereas their resistance to *R. solanacearum* and *P. cucumerina* did not differ from that of wild-type plants (Table 1, Figure 1b; Ellis *et al.*, 2002; Hernández-Blanco *et al.*, 2007). These results with Arabidopsis CESA mutants illustrate that specific cell wall damage (CWD) of either the primary or secondary cell wall causes differential wall alterations, activating distinct immune responses. However, impairment of cellulose synthesis does not always lead to disease resistance since transient silencing of barley

Cellulose Synthase Like D2 (*CSLD2*) has been described to enhance susceptibility to powdery mildew (Table S1; Douchkov *et al.*, 2016).

Cellulose biosynthesis can be impaired genetically but also by chemical inhibition upon treatment of the plant with specific inhibitors (for a recent review see Tateno *et al.*, 2016). Such inhibitors can target specific CESAs (e.g. isoxaben) or affect biosynthesis indirectly by altering the movement of CSCs at the plasma membrane (e.g. dichlobenil). Chemical inhibition of cellulose biosynthesis in plants causes CWD and consequently the alteration of CWI, which leads to the ectopic production of JA, ROS, lignin and pectin, resulting in the activation of disease resistance responses and inhibition of plant growth (Ellis *et al.*, 2002; Caño-Delgado *et al.*, 2003; Manfield *et al.*, 2004; Hamann *et al.*, 2009; Mélida *et al.*, 2015; Largo-Gosens *et al.*, 2016).

The synthesis and degree of modification of pectins influence plant disease resistance

Pectins are a complex family of Golgi-synthesized plant cell wall polysaccharides and/or glycan domains that contain galacturonic acid (α-D-GalA) linked at both the 1 and 4 positions (Driouich *et al.*, 2012; Atmodjo *et al.*, 2013). Pectins have important functions, such as promoting cell–cell adhesion, providing structural support in primary walls and influencing secondary wall formation in fibres and woody tissues (Ogawa *et al.*, 2009; Singh *et al.*, 2009; Hongo *et al.*, 2012). Pectic polysaccharides are built using up to 12 different monosaccharides and can generally be grouped into three major types: homogalacturonan (HG), rhamnogalacturonan I (RG-I) and the substituted galacturonan rhamnogalacturonan II (RG-II). This structural diversity is reflected in a complex biosynthetic process which requires at least 67 different transferases, including glycosyltransferases, methyltransferases and acetyltransferases (Mohnen, 2008). Interestingly, a recent screening of mutants with alterations in content or modification of specific cell wall monosaccharides indicated an important function of pectic polymers for penetration resistance and hyphal growth of *Colletotrichum higginsianum* during its biotrophic phase (Engelsdorf *et al.*, 2017b). However, given the numerous functions of pectins and the number of players involved in their complex biosynthesis many examples of disease susceptibility/resistance phenotypes can be found in the literature. Here we will illustrate some of the most recent examples, since the complex contribution of pectin amount/structure to the regulation of plant innate immunity has been nicely reviewed in several recent publications that also describe the different virulence mechanisms used by pathogens to modify or degrade pectins in order to favour plant colonization (Ferrari *et al.*, 2012; Lionetti *et al.*, 2012; Bellincampi *et al.*, 2014).

Impairment of pectin biosynthesis, either directly though mutations on glucuronate 4-epimerases (GAEs) required to generate the nucleotide sugar used as donor in the biosynthesis, or indirectly through AtERF014, a nuclear-localized transcriptional activator, impaired disease resistance against *P. syringae* and *B. cinerea* (Table 1, Figure 1c; Bethke *et al.*, 2016; Zhang *et al.*, 2016). In particular, *gae1 gae6* double-mutant plants were more susceptible to both pathogens, whereas AtERF014-RNAi plants exhibited increased susceptibility to *P. syringae* but enhanced resistance to *B. cinerea*. On the other hand, examples of Arabidopsis mutants with pectin-enriched cell walls are *powdery mildew-resistant 5* and *6* (*pmr5* and *pmr6*), which were more susceptible to *P. syringae* than wild-type plants, while the penetration frequency of powdery mildew fungi and *C. higginsianum* was impaired in these mutants (Table 1; Vogel *et al.*, 2002, 2004; Engelsdorf *et al.*, 2017b). The synthesis of pectins and xylans has also been shown to be reduced in Arabidopsis starch-deficient mutants, such as *phosphoglucomutase* (*pgm*), and accordingly these mutants were found to be impaired in penetration resistance against *C. higginsianum* (Engelsdorf *et al.*, 2017b). Enhanced susceptibility to this fungus was also observed in Arabidopsis *mur8-1* mutants, which display reduced cell wall rhamnose and RG-I content compared with the wild type (Engelsdorf *et al.*, 2017b).

Pectic complexity is evenly increased by post-synthetic decorations such as acetylation and/or methylesterification (Atmodjo *et al.*, 2013). Pectin methylesterification status is strongly altered in response to necrotrophic fungal infections (Lionetti *et al.*, 2012). The methylesterification of pectin is controlled mainly by pectin methylesterases (PMEs), whose activity is post-transcriptionally regulated by endogenous protein inhibitors (PMEIs). As demonstrated recently in Arabidopsis, PME activity and pectin methylesterification are dynamically modulated by PMEIs during *B. cinerea* infection, pointing to AtPMEI10, AtPMEI11 and AtPMEI12 as mediators of maintenance of CWI in plant immunity (Table 1; Lionetti *et al.*, 2017). Notably, some of the Arabidopsis PMEI over-expressing lines were found to be more susceptible to viral infection, indicating the differential effect of the degree of pectin methylesterification on disease resistance (Lionetti *et al.*, 2014). Pectin complexity and the degree of methylesterification are also regulated by the activity of pectate lyases (PL), and it has been shown that impairment of some *PL-like* (*PLL*) genes in Arabidopsis affects the development and maintenance of syncytia during the colonization of roots by cyst nematodes (Table 1; Wieczorek *et al.*, 2014). Also, reduction of the degree of pectin acetylation in Arabidopsis by overexpression of *Aspergillus nidulans* (*AnRAE*) acetylase leads to specific defensive responses (e.g. callose deposition) and enhanced resistance to *B. cinerea* (Table 1; Pogorelko *et al.*, 2013).

Polygalacturonases (PG) are glycosyl hydrolases that depolymerize the pectic HG (D'Ovidio *et al.*, 2004). These enzymes are normally defined as pathogenicity factors produced at the earlier stages of a microbial infection and insect attack (Caprari *et al.*, 1993). However, they are also endogenously present in plants. Arabidopsis and wheat plants with increased PMEI activity or Arabidopsis plants with reduced PME activity exhibited increased resistance to some necrotrophic pathogens, suggesting that a high degree of methylesterification either reduces accessibility to pectin and its degradation by fungal PG or interferes with fungal penetration by altering cell wall stiffness (Table 1, Lionetti *et al.*, 2007; Raiola *et al.*, 2011). Moreover, a recent report shows that, in addition to pathogen-secreted PGs, *P. syringae* can promote pectin degradation by induction of the expression of the Arabidopsis *IDA-like 6* (*IDL6*) gene which, in turn, induces the expression of the plant PG gene *ADPG2* (Wang *et al.*, 2017). Arabidopsis *ADPG2* knockdown mutants and over-expression lines show decreased and increased resistance to *P. syringae,* respectively, further suggesting that such bacterially induced pectin degradation in plants is an efficient mechanism developed by microbes for colonizing plants (Table 1, Wang *et al.*, 2017). One of the defensive responses of plants to microbial PGs is the synthesis of polygalacturonase-inhibiting proteins (PGIPs; Spadoni *et al.*, 2006) to inhibit microbial and insect PGs. The over-expression of PGIPs in different plant species improves their resistance to necrotrophic fungi and bacteria (Tables 1 and Table S1; Aguero *et al.*, 2005; Ferrari *et al.*, 2012).

In summary, the complexity of pectins is such that we are still far from understanding the details of their contribution to CWI-mediated immunity. However, most of the resistance phenotypes found with altered pectic composition and structures could, at least partially, be explained by the enhanced accumulation of pectin-derived DAMP oligomers of α-galacturonosyl residues linked by α-1,4-glycosidic linkages, referred to as oligogalacturonides (OGs). OGs are perceived in Arabidopsis by wall-associated kinase 1 (WAK1), which functions as a PRR triggering plant immune responses (Figure 2, Table 2; He *et al.*, 1996; Wagner and Kohorn, 2001; Brutus *et al.*, 2010; Ferrari *et al.*, 2013).

The content and degree of acetylation of hemicellulose polysaccharides determine plant disease resistance

Hemicelluloses are plant cell wall polysaccharides that have β-1,4-linked backbones with an equatorial configuration. Diverse polysaccharides, very different from each other structurally and in physicochemical properties, such as xyloglucans, xylans, mannans, glucomannans and β-1,3-1,4-glucans, can be grouped under this definition (Scheller and Ulvskov, 2010).

The alteration of wall xylose content, the moiety that is present in xylans and xyloglucans, affects the resistance of Arabidopsis to pathogens. For example, plants with enhanced levels of wall-bound xylose, as occurs in the Arabidopsis *de-etiolated3* (*det3*) and *irx6* mutants (Brown *et al.*, 2005; Rogers *et al.*, 2005), or with modifications in the xyloglucan structure, as in the case of the Arabidopsis *xyl1-2* mutant (Sampedro *et al.*, 2010), show an enhanced resistance to the necrotrophic fungus *P. cucumerina* (Table 1, Figure 1d; Delgado-Cerezo *et al.*, 2012). Similarly, barley transgenic plants co-expressing glycosyltransferase-encoding genes responsible for xylan biosynthesis affected penetration resistance against *Blumeria graminis* (Table S1; Chowdhury *et al.*, 2017). In contrast, Arabidopsis *er* plants, impaired in ERECTA receptor-like kinase that harbours a leucine-rich repeat (LRR) ectodomain (ED), and *agb1* and *agg1 agg2* mutants, impaired in the Gß and Gγ subunits of heterotrimeric G proteins, have – besides other cell wall alterations – a reduced xylose content, and are hyper-susceptible to the necrotrophic fungus *P. cucumerina* (Table 1; Llorente *et al.*, 2005; Sánchez-Rodríguez *et al.*, 2009; Delgado-Cerezo *et al.*, 2012). Although the reasons for such resistance phenotypes are largely unknown, together these data suggest that shifts in the xylose content of the cell wall have a deep impact on CWI and susceptibility to pathogens.

Hemicelluloses can be acetylated. Four *Reduced Wall Acetylation* genes (*RWA1–RWA4*) are involved in the acetylation of xylan during secondary wall biosynthesis. Remarkably, the Arabidopsis *rwa2* mutant, with about 20% less polysaccharide *O*-acetylation, is more resistant than wild-type plants to the necrotrophic fungus *B. cinerea* and the biotroph *Hyaloperonospora arabidopsidis* whereas its resistance to *P. cucumerina* is not affected (Table 1; Manabe *et al.*, 2011; Pawar *et al.*, 2016). The relevance of the degree of xylan acetylation in plant resistance to pathogens is further supported by the enhanced resistance to necrotrophic fungi of transgenic plants with reduced xylan acetylation due to over-expression of a fungal xylan acetylesterase (Table 1; Pogorelko *et al.*, 2013). In addition to RWA proteins, members of the TRICHROME BIREFRINGENCE (TBR) and TBR-LIKE (TBL) protein families are involved in the *O*-acetylation of wall polysaccharides (Gille and Pauly, 2012). The Arabidopsis *powdery mildew resistant5* (*pmr5*) mutant, impaired in a TBL member, is more resistant than wild-type plants to powdery mildew fungi and *C. higginsianum*, whereas its resistance to *P. syringae* is similar to that of wild-type plants (Table 1; Vogel *et al.*, 2004; Gille and Pauly, 2012; Engelsdorf *et al.*, 2017b;. Another recent example of the activation of specific cell wall-triggered immune responses comes from the alteration of cell wall xylan acetylation caused by another TBL member, *Eskimo1* (*ESK1*), which encodes a plant-specific polysaccharide *O*-acetyltransferase involved in xylan acetylation (Xu *et al.*, 2014; Escudero *et al.*, 2017). In this case, the alterations

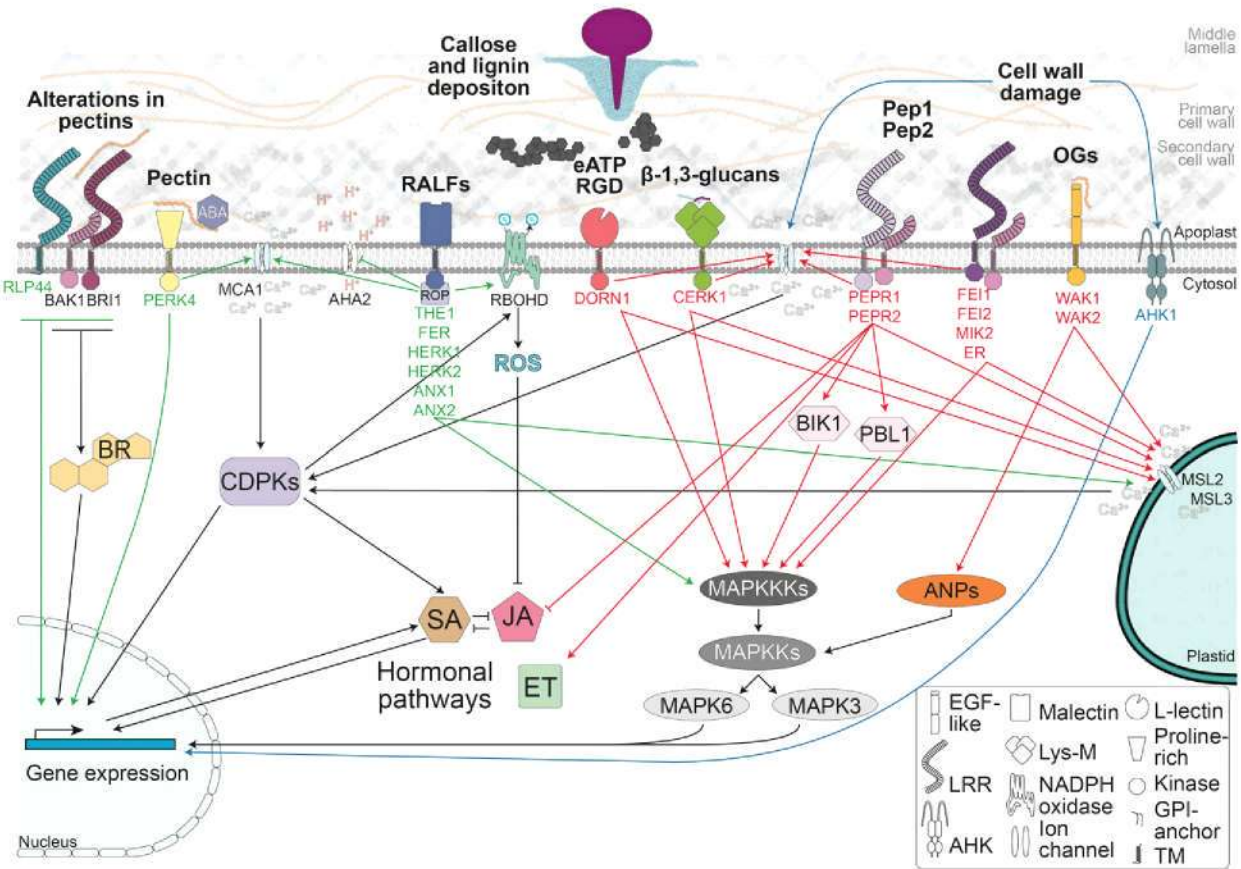

Figure 2. Plant cell wall sensing monitoring systems. Sensors and pattern recognition receptors (PRRs) that have been described to be involved in plant wall sensing mechanisms are shown. The different extracellular domains of these PRRs are indicated in the inset. The immune responses activated/repressed by the indicated PRR/sensor are indicated by arrows. Some key damage-associated molecular patterns and signalling components of the responses activated by these PRRs/sensors are also shown. For additional details and abbreviations see the main text.

in the degree of acetylation were minor, illustrating that subtle CWI impairments are sensed and trigger defence responses. Such impairment in xylan acetylation was accompanied by enhanced accumulation of ABA, the constitutive expression of genes encoding antimicrobial peptides and enzymes involved in the biosynthesis of tryptophan-derived metabolites, and the accumulation of disease resistance-related secondary metabolites and different osmolites, which might help explain the enhanced freezing, drought and salinity resistance phenotype of *esk1* plants (Xin *et al.*, 2007; Lugan *et al.*, 2009; Xu *et al.*, 2014; Escudero *et al.*, 2017). Some of the transcriptional and metabolomic changes observed in *esk1-7* overlapped with those observed in the *irx1-6* mutant and might contribute to explaining the restoration to wild-type levels of the defective PTI responses of *agb1-2* plants observed in *abg1-2 esk1-7* and *agb1-2 irx1-6* double mutants (Escudero *et al.*, 2017). Despite the similarities of the constitutively activated defence responses of *esk1-7* and *irx1-6*, the first showed enhanced resistance to *P. cucumerina* but not to *H. arabidopsidis*, which contrasted with the increased resistance of *irx1-6* to both pathogens (Table 1; Hernández-Blanco *et al.*, 2007; Escudero *et al.*, 2017).

A severe reduction in secondary wall thickness of fibre, but not that of xylem vessels, as occurs in the Arabidopsis *walls are thin 1* (*wat1*) mutant, also increased resistance to pathogens, such as the vascular bacteria *R. solanacearum* and *Xanthomonas campestris* pv. *campestris*, the soil-borne and vascular fungi *Verticillium dahliae* and *Verticillium alboatrum*, and the necrotrophic fungus *P. cucumerina* (Table 1; Denancé *et al.*, 2013). This resistance phenotype was explained by the higher salicylic acid (SA) content measured in *wat1* plants and by a general repression of indole metabolism (including Trp) in *wat1* roots. In support of this hypothesis, it was shown that introduction in *wat1* plants of *NahG*, the bacterial gene coding for a SA-degrading hydroxylase, restored full susceptibility to the bacterium *R. solanacearum*, and that crossing *wat1* with the *trp5* mutant, an over-accumulator of Trp, partially restored the susceptibility of *wat1* to the bacterium (Denancé *et al.*, 2013).

Table 2 Cell wall-related DAMPs and receptors identified in *Arabidopsis thaliana*

DAMP	Receptor	Ectodomain	Signalling hallmarks	Reference
Peptides				
PEP1	PEPR1/2	LRR	Ca^{2+}, MAPK3, MAPK6, ET, NO, ROS	Krol *et al.* (2010), Bartels, *et al.* (2013)
RALF1, RALF23	FER	Malectin	RAC/ROP GTPases, Ca^{2+}, ROS	Engelsdorf and Hamann (2014), Haruta *et al.* (2014), Stegmann *et al.* (2017)
Carbohydrates				
Pectins	PERK4	Proline-rich	Ca^{2+}	Bai *et al.* (2009a,b)
	WAK2	EGF-like repeat	MAPK3	Kohorn *et al.* (2009)
OGs DP10-16 (α-1,4-GalA)	WAK1	EGF-like repeat	Ca^{2+}, ROS, MAPK3, MAPK6, ANPs	Wagner and Kohorn (2001), Kohorn *et al.* (2009), Brutus *et al.* (2010)
Laminarioligos DP>6 (β-1,3 glucans)	CERK1	Lysin motif	Ca^{2+}, MAPK3, MAPK6	Mélida *et al.* (2018)
Laminarin (β-1,3-1,6 glucans)	nd	nd	SA	Menard *et al.* (2004); Boutrot and Zipfel (2017)
OGs DP3 (α-1,4-GalA)	nd	nd	MAPK3, MAPK6	Davidsson *et al.* (2017)
Cellobiose	nd	nd	Ca^{2+}, MAPK3, MAPK6	de Azevedo Sousa *et al.*, 2017

GalA, Galacturonic Acid; nd, Not determined; OGs, oligogalacturonides; LRR, Leucine-Rich Repeat; EGF, epidermal growth factor; MAPK, mitogen-activated protein kinase; ET, ethylene; ROS, reactive oxygen species; SA, salicylic acid.

Strengthening the wall door: CWI alterations triggered callose synthesis

Callose, a linear β-1,3-glucan polymer with hundreds of glucose units, can be accumulated in specialized plant cell walls, such as the cell plate that separates dividing cells and growing pollen tube walls, and also performs important functions during abiotic and biotic stress responses (Stone and Clarke, 1992). In particular, papillae, which are callose-enriched dome-shaped appositions between the epidermal wall and the plasma membrane synthesized as a plant cell wall reinforcement near the site of pathogen penetration, are structures of special interest in a CWI context (Figure 1e; Huckelhoven, 2007; Albersheim *et al.*, 2011; Chowdhury *et al.*, 2014). Formation of papillae is an early defence response which apparently slows down pathogen invasion in the attacked tissue, gaining time for the induction of additional defence responses (Schwessinger and Ronald, 2012). However, the extent to which papillae and the deposited callose would contribute to the plant's innate immunity and penetration resistance has been subject to an ongoing discussion (Voigt, 2014).

Callose, like cellulose, and in contrast to other wall polysaccharides which are synthesized in the Golgi, is synthesized at the plasma membrane by callose synthases (CalS or GSL for GLUCAN SYNTHASE-LIKE) (Gudesblat *et al.*, 2012). *GSL* gene family members comprise 12 members in Arabidopsis that fall into two major groups (Verma and Hong, 2001): (i) fertility and cell division (*GSL1, GSL2, GSL6, GSL8, GSL10*) and (ii) structural cell wall reinforcement (*GSL5, GSL7, GSL12*). Disruption mutants that lack the stress-induced callose synthase GSL5 (also known as Powdery Mildew Resistant 4, PMR4) do not deposit callose at sites of attempted fungal penetration (Table 1). However, the Arabidopsis *gsl5* mutant showed an increased resistance to *Golovinomyces cichoracearum* and *Golovinomyces orontii* which was contradictory to what would be expected (Jacobs *et al.*, 2003; Nishimura *et al.*, 2003). Additional analyses revealed that the over-expressed SA pathway caused the high resistance to adapted powdery mildews in *gsl5* mutants (Figure 1e; Nishimura *et al.*, 2003). Ellinger *et al.* generated *35S::GSL5* Arabidopsis over-expressor lines which in fact were able to accumulate enlarged callose deposits at sites of attempted fungal penetration (Ellinger *et al.*, 2013). Interestingly, in this case, Arabidopsis *35S::GSL5* showed a resistance phenotype to *G. cichoracearum* and *B. graminis* without triggering JA or SA pathways. More recently, double-stranded RNA interference (dsRNAi)-mediated silencing of *HvGSL6*, the functional orthologue of *AtGSL5* in barley, was shown to accumulate less callose and to be more susceptible to *B. graminis* penetration (Chowdhury *et al.*, 2016). In view of latest evidence, and considering the early contradictions, it could be reasoned that callose synthesized by GSL5 and its orthologues makes a positive contribution to immunity.

MOLECULAR MECHANISMS FOR THE PERCEPTION OF CWI ALTERATIONS AND THE ACTIVATION OF DEFENCE RESPONSES

Modification of plant CWI by external threats or internal developmental cues is monitored by the plant CWI maintenance system. Several reports implicate the plant CWI maintenance mechanism in the regulation of growth, immune responses and resource allocation between

development and immunity (Hernández-Blanco *et al.*, 2007; Hamann *et al.*, 2009; Wolf *et al.*, 2012a; Engelsdorf *et al.*, 2017a). The plant CWI monitoring system seems to share some similarities with that of yeasts, which is organized as a network of sensors and signalling pathways that play relevant functions during cell morphogenesis and adaptive responses to environmental stresses (Levin, 2011; Atkinson and Urwin, 2012; Nühse, 2012; Wolf *et al.*, 2012a; Hamann, 2015). It is unclear, with a few exceptions, what the signals are that alert the plant to CWI impairment. The most probable scenario comprises turgor pressure, which might cause displacement of the plasma membrane from the cell wall, and ligand–receptor interactions (Hamann, 2015).

The plant cell wall sensing network has been proposed to include monitoring of molecular systems for: (i) osmo-perception; (ii) mechano-perception; (iii) CWD perception; and (iv) wall-derived ligand–receptor (PRR) recognition (Figure 2). All these sensing systems activate signal relays via protein kinases (PKs) or/and calcium-based signalling cascades which induce the synthesis of some phytohormones (e.g. JA, SA and ABA), that in turn modulate downstream genes which regulate the adaptive changes in cell wall composition and structure and the activation of immune and defence responses (Figure 2; Engelsdorf and Hamann, 2014; Miedes *et al.*, 2014; Zipfel, 2014; Wolf, 2017). Only a few molecular components of these plant monitoring systems have been identified to date, and limited insights into their mode of action have been provided (Figure 2). Here, we describe the best-characterized components of the plant cell wall-associated monitoring system.

Osmo-perception and mechano-perception sensor systems in plants

Most of our current knowledge about the presence of osmo-receptors in Arabidopsis comes from their heterologous expression and functional study in yeast models (Engelsdorf and Hamann, 2014). Expression of several Arabidopsis histidine kinases (AHKs) in yeast complements osmosensing-deficient strains impaired in SYNTHETIC LETHAL OF N-END RULE 1 (SLN1) protein, a yeast turgor pressure sensor (Urao *et al.*, 1999; Engelsdorf and Hamann, 2014). The location of AHK1 in the Arabidopsis plasma membrane, as with SLN1 in yeast, is compatible with its role as an osmo-receptor (Tran *et al.*, 2007; Engelsdorf and Hamann, 2014). Furthermore, knocking out the *AHK1* gene affects Arabidopsis CWD responses upon manipulation of turgor pressure levels, similarly to what has been described in yeast (Figure 2; Hamann *et al.*, 2009; Wormit *et al.*, 2012). Conversely, AHK2 and AHK3 are located in Arabidopsis endoplasmic reticulum and they seem to function as cytokinin receptors negatively regulating stress responses (Engelsdorf and Hamann, 2014). AHK4/CYTOKININ RECEPTOR1 (CRE1) has been demonstrated to function as an endoplasmic reticulum-localized cytokinin receptor (Yamada *et al.*, 2001; Caesar *et al.*, 2011). However, is also involved in the transcriptional regulation triggered by osmotic changes caused by the inhibition of cellulose biosynthesis (Figure 2; Wormit *et al.*, 2012).

In Arabidopsis, two main groups of receptors seem to be involved in mechano-perception: mechanosensitive (MS) ion channels and receptor like kinases (RLKs) that perceive CWD (Hamant and Haswell, 2017). Expression in yeast of the Arabidopsis *MID1 Complementing Activity 1* (*MCA1*) and *MCA2* partially rescues the phenotype of yeast strains deficient in MID-1 Ca^{2+} channels (Nakagawa *et al.*, 2007; Yamanaka *et al.*, 2010; Engelsdorf and Hamann, 2014). In Arabidopsis, MCA1 and MCA2 are localized at the plasma membrane, mediating Ca^{2+} influx triggered by mechanical stimulus and hypo-osmotic pressure, and are necessary for CWD-induced responses, like synthesis and deposition of lignin, and JA and ROS production (Figure 2; Hamann, 2012; Kurusu *et al.*, 2013; Haswell and Verslues, 2015). Other MS ion channels are those belonging to the MscS-Like (MSL) family, which are homologues of the bacterial MS channels. Arabidopsis MSLs are plastid located and have been implicated in the regulation of Ca^{2+} influx (Figure 2; Kurusu *et al.*, 2013).

Arabidopsis CWD perception involves RLKs from different families

As stated before, one of the best-characterized plant CWDs is that caused by inhibition of CESA function. CWD caused by inhibition of primary cell wall CESAs leads to adaptive changes and ectopic responses (e.g. production of JA and ROS and lignin deposition), which together result in plant growth inhibition (Ellis *et al.*, 2002; Caño-Delgado *et al.*, 2003; Manfield *et al.*, 2004; Bischoff *et al.*, 2009; Hamann *et al.*, 2009; Denness *et al.*, 2011; Mélida *et al.*, 2015). A screening of suppressors of the *CESA6* mutant *procuste1-1* (*prc1-1*) identified the *the1-1* mutant, which partially recovered the constitutive growth inhibition and the ectopic lignin deposition observed in *prc1-1* plants (Hématy *et al.*, 2007). THESEUS1 (THE1) is a member of the *Catharanthus roseus* Receptor-Like Kinase 1-Like (CrRLK1L) family, that comprises 17 members in Arabidopsis (Lindner *et al.*, 2012). *the1-1* has been suggested to be impaired in the perception of unknown CWD-derived signals, and accordingly it would be unable to activate specific responses induced by inhibition of the activity of primary cell wall CESAs. The CrRLK1L members have an extracellular ED with a malectin-like domain that has been suggested to bind carbohydrates (Lindner *et al.*, 2012), but this hypothesis has not been experimentally demonstrated to date (Wolf, 2017). Instead, some CrRLK1L members have been demonstrated to bind peptides (Haruta *et al.*, 2014; Stegmann *et al.*, 2017). For example, FERONIA (FER), one of the best-characterized members of the CrRLK1L family, has

been shown to bind the peptides RAPID ALKALINIZATION FACTORS (RALF) 1 and 23 (Haruta *et al.*, 2014; Stegmann *et al.*, 2017). FER was initially characterized for its role in female gametophytic control of pollen tube reception (Huck *et al.*, 2003), but lately it has also been found to be involved in root hair formation, plant growth, mechanoperception, hormone-mediated signalling, ROS production and apoplast acidification, through molecular mechanisms that are dependent on Ca^{2+} influxes (Shih *et al.*, 2014; Li *et al.*, 2016; Voxeur and Höfte, 2016). Apoplast acidification, which occurs through RALF1 signalling and inhibition of H^+-ATPase2 (AHA2) (Haruta *et al.*, 2014), may be connected to cell wall modifications via the activation of pectin methyl esterases (PMEs) (Voxeur and Höfte, 2016). Active RALF peptides are generated upon cleavage of their propeptide by peptidases of the subtilisin family, such as SITE-1 PROTEASE (S1P), which cleaves RALF23 pro-peptide (Stegmann *et al.*, 2017). Interestingly, S1P also cleaves the pro-domain from PMEs (Srivastava *et al.*, 2009; Wolf *et al.*, 2009), suggesting coordinated secretion and processing of RALF23 and PMEs, and perhaps pointing to FER-mediated control of pH homeostasis in expanding cell walls (Voxeur and Höfte, 2016). Of note, the *fer-4* mutant is more resistant to the vascular fungus *F. oxysporum* than wild-type plants, probably due to its defect in the perception of a virulence RALF-like peptide secreted by the pathogen (Masachis *et al.*, 2016). Moreover, FER also participates in the modulation of immune responses acting as a RALF-regulated scaffold mediating the assembly of a receptor kinase complex including BRASSINOSTEROID INSENSITIVE 1-ASSOCIATED KINASE 1 (BAK1), the co-receptor of the immune receptor kinases EF-TU RECEPTOR (EFR) and FLAGELLIN-SENSING 2 (FLS2) (Stegmann *et al.*, 2017). These recent results represent a clear link between the sensing of CWI alterations and modulation of PTI, and suggest that some PRRs regulating CWD-associated defensive responses are targeted by pathogen virulence factors to suppress host immunity.

Domain swap experiments with FER, its closest CrRLK1L homologues, ANXUR1 (ANX1), ANX2 and HERCULES1 (HERK1), demonstrated that the kinase domain is exchangeable but not the extracellular domain, implying that the ED of each CrRLK1L might bind different ligands (Kessler *et al.*, 2015). However, these CrRLK1Ls might trigger similar downstream responses since all the CrRLK1L-mediated responses studied so far required similar signalling components like Rho of plants (ROP) guanidine nucleotide exchange factor (ROP-GEF1), ROP GTPases and NADPH oxidases (Figure 2; Kessler *et al.*, 2015; Li *et al.*, 2016; Wolf, 2017).

In addition to CrRLK1L family members, other RLKs belonging to different families have been implicated in CWD responses. For example, FEI1 and FEI2, two very similar LRR-RLKs of Arabidopsis (Figure 2), were identified on the basis of the sucrose-dependent swollen-root phenotype of *fei1 fei2* double-mutant seedlings that is similar to that observed in the *prc1-1* mutant and in isoxaben-treated seedlings (Fagard *et al.*, 2000; Xu *et al.*, 2008; Hamann *et al.*, 2009; Engelsdorf and Hamann, 2014). FEI1 and FEI2 control cellulose biosynthesis and anisotropic growth under high-sucrose and high-salinity conditions, acting together with SALT OVERLY SENSITIVE5 (SOS5), an extracellular glycosylphosphatidylinositol-anchored protein that has a similar conditional phenotype (Shi *et al.*, 2003; Xu *et al.*, 2008; Engelsdorf and Hamann, 2014; Wolf, 2017). FEIs seem to be implicated in a highly complex regulation network, that includes the biosynthesis of the ethylene-precursor 1-aminocyclopropane-1-carboxylic acid (ACC), which directly controls cellulose biosynthesis (Tsang *et al.*, 2011; Wolf, 2017). Interestingly, auxin biosynthesis also plays a role in FEI-regulated CWI signalling. This was revealed in a screening of suppressors of the *fei1 fei2* mutant phenotype that identified genes with a key role in auxin homeostasis as suppressors of *fei1 fei2* growth isotropy but also of isoxaben-induced phenotypes (Steinwand *et al.*, 2014; Wolf, 2017).

The LRR-RLK MALE DISCOVERER 1-INTERACTING RECEPTOR LIKE KINASE 2 (MIK2) (Figure 2) has been also proposed to have a role in sensing CWI caused by CWD, probably functioning in the same pathway as THE1 (Van der Does *et al.*, 2017). MIK2 was found in a screening of RLK mutants insensitive to cellulose biosynthesis inhibitors (Van der Does *et al.*, 2017). Although MIK2 does not present a completely overlapping pattern with THE1 in response to inhibition of cellulose biosynthesis, MIK2 requires THE1 for control of normal root growth direction and salt tolerance (Van der Does *et al.*, 2017). On the contrary, THE1 is not required for MIK2-mediated resistance to *F. oxysporum* (Van der Does *et al.*, 2017).

It has been experimentally demonstrated that both RLKs and MS ion channels work together to regulate CWD responses. For instance, FEI2 and the plasma membrane channel MCA1 function downstream of THE1, triggering Ca^{2+} influx, ROS production, JA and SA production and modulating immune-related gene expression (Figure 2; Engelsdorf *et al.*, 2017a).

Sensing cell-wall-derived, carbohydrate-based DAMPs to regulate disease resistance responses

Plant cell walls are a source of potential carbohydrate-based defence signalling molecules (DAMPs). These DAMPs can be released upon breakdown or modification of wall polymers by (i) CWDEs secreted by pathogens, (ii) the activity of plant enzymatic repertoires upon infection or abiotic stresses exposure, or (iii) the genetic manipulation (mutation or over-expression) of the levels of cell wall biosynthesis/remodelling associated proteins (Hahn *et al.*, 1981; Walton, 1994; Cosgrove, 2005). Such alterations of

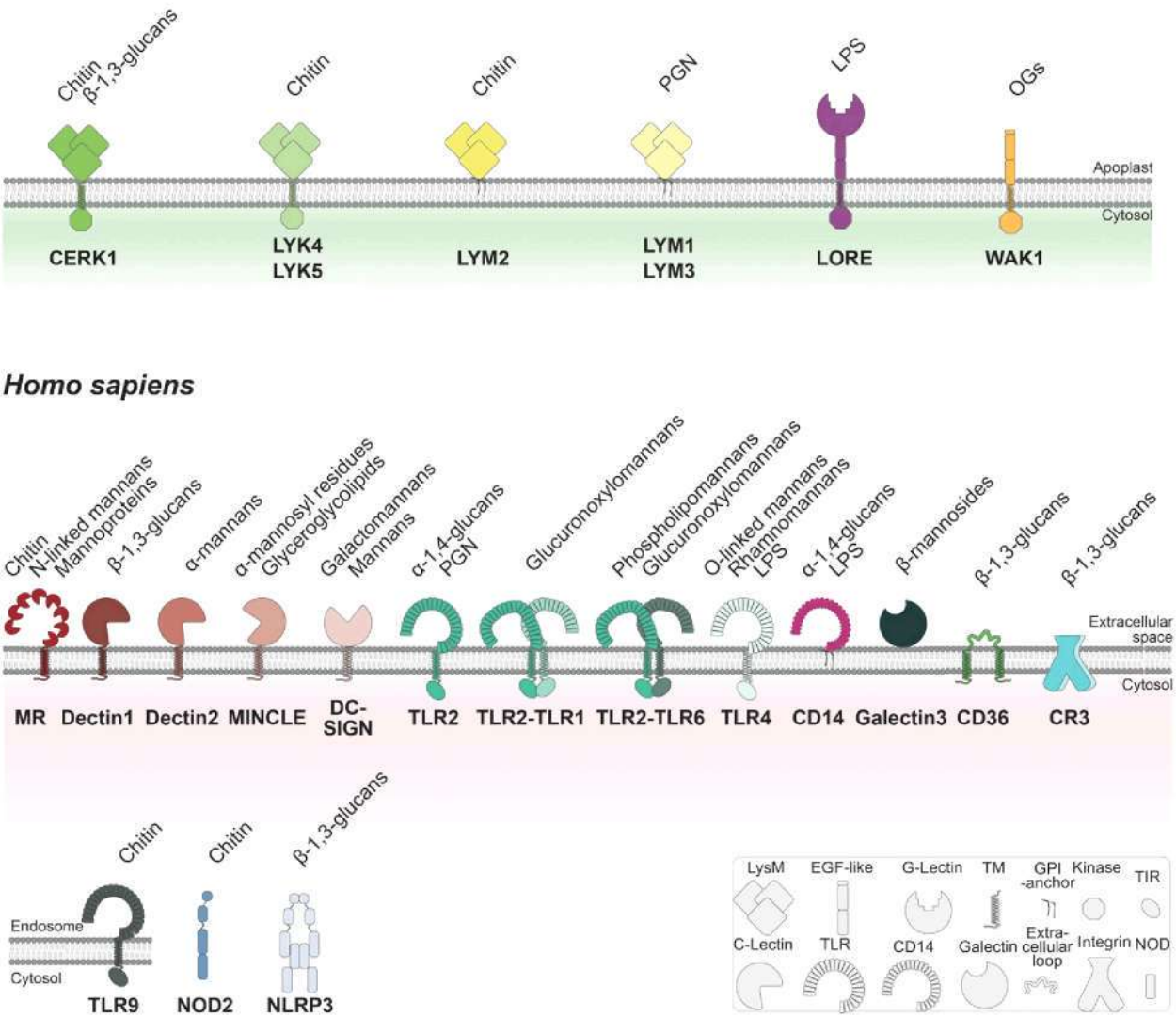

Figure 3. Receptor–carbohydrate ligand pairs in *Arabidopsis thaliana* and *Homo sapiens*. A few plant pattern recognition receptors (PRRs) have been described to bind microbe-associated molecular patterns (MAMPs) [chitin, lipopolysaccharide (LPS), peptidoglycan (PGN) or β-1,3-glucans] or damage-associated molecular patterns (DAMPs) [oligogalacturonides (OGs) or β-1,3-glucans] containing carbohydrate moieties. These plant receptors belong to three main PRR families: LysM (CERK1, LYM/LYK), WAKs and Lec-RLKs (LORE). The diverse set of carbohydrate-based patterns (MAMPs and DAMPs) perceived by the *Homo sapiens* monitoring system is also shown. The different domains of the human receptors that bind carbohydrate-based patterns are indicated.

CWI would trigger specific defensive responses (Figure 1, Table 1), but the mechanisms behind the activation of these adaptive responses and, in particular, the specific ligands (e.g. wall-derived DAMPs) and PRRs that regulate these responses, remain elusive (Figure 2). Until very recently, OGs were the only well-known and accepted cell wall-derived DAMPs in plants (Nothnagel *et al.*, 1983; de Azevedo Souza *et al.*, 2017). OGs, generally with a degree of polymerization (DP) of 10–15, act via many of the same signalling steps as MAMPs to elicit defence responses and to confer plant protection when applied exogenously (Ridley *et al.*, 2001; De Lorenzo *et al.*, 2011; Ferrari *et al.*, 2013; Benedetti *et al.*, 2015).

Despite pioneering work and recently regained momentum (Nothnagel *et al.*, 1983; Doares *et al.*, 1995; Willmann *et al.*, 2011; Liu *et al.*, 2012; Cao *et al.*, 2014; Mélida *et al.*, 2018), knowledge about the specific mechanisms of plant defence activation by carbohydrate-based patterns (MAMPs and DAMPs) clearly lags behind the animal field, where different types of receptors have been described to bind carbohydrate-based ligands (Figure 3; Erwig and Gow, 2016). Also, our current knowledge of carbohydrate-based pattern recognition by plants is clearly behind that of peptidic-based MAMP perception by the plant immune system (recently reviewed by Tang *et al.*, 2017). Despite the diversity of PRRs present in plants, only three families

of receptors have been described to be involved in the recognition of carbohydrate-based patterns (MAMPs or DAMPs) (Figures 2 and 3): (i) receptors of the lysin motif-(LysM)-PRR family, which are involved in the perception of fungal chitin [CHITIN ELICITOR RECEPTOR KINASE 1 (CERK1), LysM-CONTAINING RECEPTOR KINASE 4/5 (LYK4/5) and LysM DOMAIN-CONTAINING GPI-ANCHORED PROTEIN 2 (LYM2)], bacterial peptidoglycan (PGN) (CERK1, LYM1/LYM3) and β-1,3-glucans present in the walls of fungi/oomycetes/plants (CERK1); (ii) the PRR family with Epidermal Growth Factor (EGF)-Like ED, that includes the OGs receptor WALL ASSOCIATED KINASE1 (WAK1); and (iii) Lectin-like (Lec)-PRRs that include the LIPOOLIGOSACCHARIDE-SPECIFIC REDUCED ELICITATION (LORE) receptor for bacterial lipopolysaccharides (LPS) (Brutus *et al.*, 2010; Ranf *et al.*, 2011; Wolf *et al.*, 2012a; Zipfel, 2014).

Epidermal Growth Factor-Like receptors: WAK receptors and OGs perception

This PRR plant family contains an EGF ED (He *et al.*, 1996) with similarities to that present in mammalian EGF receptors (Figure 2). WAKs, first identified as RLKs physically linking the plasma membrane to the cell wall and later shown to bind pectins *in planta* and pectic polysaccharides and oligosaccharides *in vitro*, belong to this family (He *et al.*, 1996; Wagner and Kohorn, 2001; Decreux and Messiaen, 2005; Kohorn *et al.*, 2009). The WAK EDs interact preferably with de-esterified pectin cross-linked by Ca^{2+} ('egg-box' configuration) through a binding site formed by cationic amino acids (Decreux and Messiaen, 2005; Decreux *et al.*, 2006).

The WAK family is composed of 22 WAK-like genes identified on the basis of protein sequence homology (Verica and He, 2002). WAK1 and WAK2 both bind pectins (Decreux and Messiaen, 2005; Kohorn *et al.*, 2009), but so far only WAK1 has been experimentally characterized as an OGs receptor using a domain-swap approach (Brutus *et al.*, 2010). Furthermore, *WAK1* is the only WAK family gene to be upregulated in response to OGs (Denoux *et al.*, 2008), being also induced by wounding (Wagner and Kohorn, 2001). Otherwise, WAK2 is required for the activation of pectin-induced immune responses, like MAPK6 phosphorylation (Kohorn *et al.*, 2009).

OGs, usually with DP 10–15 as mentioned above, act via many of the signalling steps of MAMPs to elicit defences and provide protection against pathogens (Ridley *et al.*, 2001; De Lorenzo *et al.*, 2011; Ferrari *et al.*, 2013; Benedetti *et al.*, 2015). Downstream signalling regulated by OGs (Table 2) includes Ca^{2+} influx, calcium-dependent protein kinase (CDPK) activation and phosphorylation of MAPK3 and MAPK6, but only the latter is required for OGs-dependent regulation of defence genes and protection against *B. cinerea* (Figure 2; Galletti *et al.*, 2011; Gravino *et al.*, 2015). Moreover, the three members of the ARABIDOPSIS NPK1-RELATED PROTEIN KINASE (ANP) MAP kinase kinase kinases (MAP3Ks) family, ANP1, ANP2 and ANP3, are required for OGs-triggered signal transduction and ROS production (Figure 2; Asai *et al.*, 2002; Savatin *et al.*, 2014a). In addition, OGs-triggered signalling has been shown to inhibit the expression of auxin-induced genes (Savatin *et al.*, 2011, 2014a), thus reflecting its diverging roles in controlling plant immunity and development. More recently, it has been described that shorter OGs (DP = 3) also trigger immune responses (e.g. Ca^{2+} influx, MAPK phosphorylation and gene expression), which partially overlap with the response activated by OGs with a higher DP, but, remarkably, the shorter OGs do not trigger ROS production (Davidsson *et al.*, 2017). In this sense, treatment with DP = 3 OGs provided protection against *Pectobacterium carotovorum* (Davidsson *et al.*, 2017).

The role of WAKs in plant immunity is further supported by the demonstration that Arabidopsis plants over-expressing WAK1 are more resistant to *B. cinerea* (Brutus *et al.*, 2010) and that another member of the family, WAKL22, is required for resistance against *F. oxysporum* (Diener and Ausubel, 2005). Remarkably, *WAK-like* genes have been identified as major regulators of crop resistance to different diseases, like rice blast (Li *et al.*, 2009) and corn head smut and leaf blight (Hurni *et al.*, 2015; Zuo *et al.*, 2015).

Another PRR that does not belong to the WAK family, the PROLINE-RICH EXTENSIN-LIKE RECEPTOR KINASE 4 (PERK4), has been also shown to bind pectin. PERK4 is required for ABA-mediated inhibition of root growth through perturbation of Ca^{2+} homeostasis and regulates the expression of *PGs* genes that are related to cell wall loosening (Figure 2; Bai *et al.*, 2009a,b).

LysM-PRRs: a few receptors perceiving a diverse set of carbohydrate-based structures

LysM domains are widespread in eukaryotic and prokaryotic proteins, and are typically implicated in the recognition of *N*-acetylglucosamine (GlcNAc)-containing glycans (Buist *et al.*, 2008; Bellande *et al.*, 2017). LysM-PRRs have been described in various plant species as receptors for MAMPs such as chitin and PGN, both in symbiotic and pathogenic interactions (Zipfel and Oldroyd, 2017). In Arabidopsis, eight LysM-PRRs have been identified so far: five LYKs that are RLKs, and three LYMs that are receptor-like proteins (RLPs) lacking the cytoplasmic kinase domain (Tanaka *et al.*, 2013). LYK1, known as CERK1, has a crucial role in glycan-based-MAMP perception. CERK1 is implicated in chitin recognition and binding, although at low affinity (Miya *et al.*, 2007; Wan *et al.*, 2008). However, for effective chitin-triggered immune signalling, LYK4 and LYK5, two additional members of the family, are also necessary (Cao *et al.*, 2014). These proteins bind chitin with higher affinity than CERK1, but their kinase domain is not active, and thus the formation of LYK4/5–CERK1

heterodimers has been suggested to be required for the activation of immune responses (Cao *et al.*, 2014).

CERK1 is also implicated in PGN perception (probably as a co-receptor), forming heteromeric complexes with LYM1 and LYM3 RLPs (Willmann *et al.*, 2011). Moreover, CERK1 has recently been shown to be necessary for 1,3-β-D-glucan-triggered immune responses (Mélida *et al.*, 2018). The 1,3-β-D-glucans are an important component of fungal and oomycete cell walls but are also present in plant cells, although in minute amounts, thus suggesting that CERK1 might also be a DAMP receptor (Mélida *et al.*, 2018). The central role of CERK1 in Arabidopsis immunity is supported by its role in resistance against fungi such as *Alternaria brassicicola*, *G. cichoracearum* and *P. cucumerina* (Miya *et al.*, 2007; Wan *et al.*, 2008; Mélida *et al.*, 2018), the bacterium *P. syringae* (Gimenez-Ibanez *et al.*, 2009) and the oomycete *H. arabidopsidis* (Mélida *et al.*, 2018). In contrast, LYK3 seems to negatively regulate resistance to *B. cinerea* and *P. carotovorum* by controlling the balance between ABA-dependent responses and pathogen resistance (Paparella *et al.*, 2014). Interestingly, *LYK3* expression was repressed upon plant infection with these pathogens and upon treatment with OGs, chitin or flg22 (Paparella *et al.*, 2014). In contrast, *LYK3* is the only LYK member whose expression is induced in response to *A. brassicicola* that is consistent with its positive function in the resistance of Arabidopsis to this fungus (Paparella *et al.*, 2014).

Lectin-like (Lec)-PRRs: a diverse set of plant receptors sharing EDs with mammalian glycan receptors

Lectins are widespread carbohydrate-binding proteins that can bind mono- and oligosaccharides (Bellande *et al.*, 2017). Plant Lec-RLKs comprise PRRs with lectin-type extracellular domains that have been divided into three subclasses: (i) G- (GNA-related or S-locus) lectins; (ii) C- (calcium-dependent) lectins; and (iii) L- (legume) lectins (Wang and Bouwmeester, 2017). In animals, C-lectin receptors are key players in innate immune responses. It is worth highlighting Dectin-1, the mammalian β-glucan receptor, which was the first mammalian non-Toll-like receptor discovered that is capable of coupling microbial recognition with transcriptional regulation of genes (Figure 3; Brown and Gordon, 2001; Dambuza and Brown, 2015; Wang and Bouwmeester, 2017). In plants, Lec-RLKs have a prominent role in biotic stress responses (Bouwmeester *et al.*, 2011; Desclos-Theveniau *et al.*, 2012; Singh *et al.*, 2012; Wang *et al.*, 2012, 2014; Gouhier-Darimont *et al.*, 2013; Huang *et al.*, 2013; Balague *et al.*, 2017), in contrast to their minor role in developmental processes (Bellande *et al.*, 2017). However, only two ligand–LecRLK pairs have been identified (Bellande *et al.*, 2017; Wang and Bouwmeester, 2017): lipopolysaccharides (LPSs)–LORE and extracellular ATP (eATP)–DORN1.

LORE is a G-type Lec-RLK that has been identified as a plant receptor for LPSs, carbohydrate-containing MAMPs (Ranf *et al.*, 2015). Intriguingly, BAK1 is not implicated in LPS perception (Ranf *et al.*, 2015), which contrasts with its involvement in the perception of other well-known bacterial MAMPs such as elf18 or flg22 (Chinchilla *et al.*, 2007). DORN1/LecRK-I.9 was first found in a screening performed for the identification of plant interactors of the IPI-O effector from *Phytophthora infestans*, a protein containing a RGD (arginine–glycine–aspartic acid) sequence (Gouget *et al.*, 2006). In Arabidopsis, RGD is able to interfere with cell wall–plasma membrane contacts that are required for resistance of plants to fungi (Canut *et al.*, 1998; Mellersh and Heath, 2001; Gouget *et al.*, 2006; Bouwmeester *et al.*, 2011). Later on, in an independent screening, DORN1 was also identified as displaying altered responses to ATP (Choi *et al.*, 2014a). *In vitro* binding assays revealed a high affinity of the DORN1/LecRKI.9 ectodomain for eATP (Choi *et al.*, 2014b), which might act as a DAMP released after physical damage of cells as it occurs during fungal infections (Kim *et al.*, 2006, 2008; Tanaka *et al.*, 2014). The downstream signalling events triggered by eATP, such as Ca^{2+} influx and MAPK activation, are similar to those triggered by other DAMPs or MAMPs (Choi *et al.*, 2014b). Nevertheless, neither RGD peptides nor eATP are the expected ligands for this L-LecRLK. Instead, cell wall-derived molecules or glycoproteins have been suggested to be the bona fide ligands based on *in silico* modelling of LecRKI.9 ED (Bellande *et al.*, 2017). These data suggest that LecRKI.9 and/or other L-Lec-RLKs might be implicated in the perception of as-yet-unknown cell wall-derived DAMPs.

Orphan carbohydrate-based DAMPs: looking for PRR pairs

Given both the complexity of the plant cell wall and the fact that many pathogens secrete a wide range of cell wall-degrading enzymes, it could be reasoned that the breakdown products of other cell wall polymers could act as DAMPs. In line with this hypothesis, de Azevedo Souza *et al.* (2017) recently demonstrated that cellobiose, a disaccharide consisting of two glucose units in a β-1,4-glycosidic linkage, was perceived as a DAMP by Arabidopsis, triggering a signalling cascade that shares some similarities with the responses modulated by well-known glycan elicitors such as chitooligosaccharides and OGs (Table 2). Thus, with around 20 different monosaccharide moieties building the polysaccharides of plant cell walls, it can be hypothesized that other carbohydrate-based cell wall molecules, in addition to OGs and cellobiose, could act as DAMPs in plants, modulating DAMP-triggered immunity (DTI; Escudero *et al.*, 2017).

Sensing peptidic-based DAMPs to regulate disease resistance responses

The LRR extracellular domain is the most frequent ED in RLKs and RLPs (Wolf, 2017). All known LRR-containing PRRs recognize peptidic ligands (Tang *et al.*, 2017), and

many of them play important roles in plant immunity (Gómez-Gómez and Boller, 2000; Bauer *et al.*, 2001; Huffaker *et al.*, 2006; Zipfel *et al.*, 2006; Krol *et al.*, 2010; Yamaguchi *et al.*, 2010; Zhang *et al.*, 2010, 2014; Jehle *et al.*, 2013b; Wolf *et al.*, 2014; Albert *et al.*, 2015; Yamada *et al.*, 2016). LRR-RLKs act by forming heterodimers with RLKs from the SOMATIC EMBRYOGENESIS RECEPTOR KINASEs (SERKs) family, like BAK1/SERK1, in a ligand-dependent manner (Figure 2; Chinchilla *et al.*, 2007; Schulze *et al.*, 2010; Roux *et al.*, 2011; Sun *et al.*, 2013; Tang *et al.*, 2015; Wolf, 2017). Equivalently, RLPs, need to interact with proteins that contain cytoplasmic kinase domains such as SUPPRESSOR OF BIR1 (SOBIR1) and BAK1 (Figure 2; Jehle *et al.*, 2013a; Liebrand *et al.*, 2013; Zhang *et al.*, 2014; Albert *et al.*, 2015; Couto and Zipfel, 2016; Wolf, 2017).

Besides the already described LRR-RLKs FEI1, FEI2 and MIK2 that have been implicated in CWI sensing (see above) upon CWD, some LRR-RLKs and LRR/RLPs have been related to cell wall-mediated immune responses or the perception of peptides that are considered DAMPs released upon CWD. For example, Arabidopsis Pep1, has been described as a peptide ligand synthesized in response to CWD, since the *PROPEP1* gene, encoding a Pep1 precursor, is upregulated after isoxaben treatment (Engelsdorf *et al.*, 2017a). Moreover, *PROPEP1* is also induced upon pathogen infection and wounding, which cause CWI alterations (Huffaker *et al.*, 2006). Pep1 is sensed by the LRR-RLKs PEPR1 and PEPR2 (Figure 2; Bartels and Boller, 2015) and has been shown to function as a PTI response enhancer. Pep1–PEPR1/2 interaction represses phytohormone accumulation induced by CWD, thus suggesting that PTI signalling controls the magnitude of the CWD response (Engelsdorf *et al.*, 2017a). Moreover, PEPR1 and PEPR2 are also implicated in some OGs-triggered responses like upregulation of the *PATHOGENESIS RELATED 1* (*PR1*) gene and enhanced resistance to *B. cinerea* (Gravino *et al.*, 2017). PEPR1 and PEPR2 seem to be required for full activation of the immune response, but if these PRRs are not functional, as occurs in *pepr1 pepr2* mutants, the CWI monitoring system seems to function as backup activating basal broad-spectrum defences (e.g. phytohormone, lignin and callose accumulation) that can compensate for impaired PTI and defective regular defence responses (Engelsdorf *et al.*, 2017a; Gravino *et al.*, 2017).

Some PRRs mediate important developmental processes that may need to include CWI input to fine-tune the balance between growth and defence. This might be the case for the signalling pathways regulated by brassinosteroids (BRs), since BRs are hormones that play a key role in balancing growth–immunity trade-offs (Lozano-Duran and Zipfel, 2015). In support of this hypothesis, cell wall-related genes are an important group of BR targets (Sun *et al.*, 2010 2011), and BRs have been shown to promote pectin biosynthesis and PME activity, and to mediate the response to pectin demethylesterification (Wolf *et al.*, 2012b). The BR signalling module is a deeply characterized signalling pathway involving the LRR-RLK BRASSINOSTEROID INSENSITIVE 1 (BRI1), that upon BR-binding creates a docking platform for SERK co-receptors such as BAK1 (Santiago *et al.*, 2013; Sun *et al.*, 2013; Belkhadir and Jaillais, 2015; Singh and Savaldi-Goldstein, 2015; Wolf, 2017). It has been found that RLP44 interacts with this complex via BAK1 (Figure 2) and mediates the BR-mediated response to pectin demethylesterification (Wolf *et al.*, 2014). RLP44 is required for normal growth and stress responses, but does not affect the response to altered levels of BR (Wolf *et al.*, 2014; Wolf, 2017). Taking these results together, it has been proposed that RLP44 mediates the integration of cell wall status and BR signalling (Wolf, 2017). Important members of the CrRLK1L family, like THE1 and HERK1/2, are also implicated in BR-controlled growth (Guo *et al.*, 2009), and FER mediates the antagonistic effect of BR and ET on hypocotyl growth of etiolated seedlings (Deslauriers and Larsen, 2010). Together these data suggest that some CrRLK1L and RLPs play a role in sensing cell wall status.

Similarly, LRR-RLK ERECTA (ER) might also regulate CWI since *er* mutants are highly susceptible to different pathogens and show alterations in cell wall composition, both phenotypes being restored to wild-type levels in the *ser* (*suppressors of er*) *er* double mutants (Llorente *et al.*, 2005; Sánchez-Rodríguez *et al.*, 2009; Jordá *et al.*, 2016). Remarkably, it was recently found that BAK1 and ER interact, and that they cooperate with other members of the ER pathway [ER-like1 (ERL1) and ERL2, and the TOO MANY MOUTHS (TMM) RLP] in the regulation of innate immune response (Meng *et al.*, 2015; Jordá *et al.*, 2016). Moreover, the YODA MAP3K functioning downstream of the ER–ERLs–TMM–BAK1 complex in the regulation of developmental processes like stomata patterning has also been found to be required for Arabidopsis broad-spectrum disease resistance (Sopeña-Torres *et al.*, 2018). Remarkably, *yda11* and *er-105* mutants show similar cell wall modifications compared with wild-type plants, as revealed by glycomics profiling (Sopeña-Torres *et al.*, 2018). The overexpression of a constitutively activated YODA protein (*CA-YDA* plants) results in broad-spectrum disease resistance that is independent of canonical immune pathways (e.g. defensive phytohormones and PTI). *CA-YDA* plants also show constitutive expression of genes encoding putative small secreted peptides (SSPs) and PRRs and altered cell wall composition. These SSPs and some carbohydrate-derived ligands present in wall fractions of *CA-YDA* plants have been suggested to function as novel DAMPs regulating the constitutive immune responses of *CA-YDA* plants (Sopeña-Torres *et al.*, 2018).

METHODOLOGICAL CHALLENGES AND FUTURE PERSPECTIVE OF CELL-WALL-MEDIATED IMMUNITY

As stated above, the main Achilles' heel hampering carbohydrate CWI research is the limited number of identified DAMPs. The high supramolecular complexity of the plant cell wall and the difficulties in obtaining pure carbohydrate structures resembling the *in vivo* released DAMPs are limiting progress in this area. These putative DAMPs could either be synthesized or purified. However, carbohydrate synthesis by organic chemistry is challenging – connection of the anomeric hydroxyl of the glycosyl donors to the alcoholic hydroxyl groups of the glycosyl acceptors has been achieved so far. Protection of the hydroxyl groups of the acceptor with the target alcoholic hydroxyl group being unprotected ensures regiochemical control but hinders the process. Moreover, the yields are usually quite low, and these technologies are usually difficult to scale up (Levy and Fügedi, 2005).

Therefore, nowadays, the best strategy to isolate as-yet uncharacterized cell wall-derived DAMPs is to take advantage of those that are naturally produced by plants and purify them. This is not a straight-forward task either, because it requires harsh chemical extractions combined with enzymatic digestions and chromatographic purifications. By using such extraction methods, several immune active plant cell wall fractions enriched in specific components (i.e. pectins or hemicelluloses) can be obtained. These wall fractions trigger immunity hallmarks such as early Ca^{2+} influx and ROS production, and the subsequent activation of downstream events such as MAPK and CDPK phosphorylation (Couto and Zipfel, 2016; Bacete L. *et al.*, 2017). These fractions can then be further purified and the identity of the DAMP structures characterized using classical carbohydrate analytical procedures (Pettolino *et al.*, 2012). However, since glycan sequencing is far from being achievable, a combination of methodologies might be required. In this regard, new tools for cell wall characterization have emerged, such as glycomics profiling or carbohydrate microarrays that are based on the use of extensive collections of monoclonal antibodies (mAbs) developed against specific cell wall polymers/epitopes (Pattathil *et al.*, 2012; Cornuault *et al.*, 2014; Wood *et al.*, 2017). These technologies are being used to screen Arabidopsis cell wall fractions from selected mutants with altered resistance to pathogens (Bacete L. *et al.*, 2017; Engelsdorf *et al.*, 2017b) and to identify molecular markers for plant cell wall composition in breeding programs (Wood *et al.*, 2017).

Recent results also indicate that insoluble polymers are not able to induce immune responses in plants, and that these polymers should be shortened to smaller entities (oligosaccharides) to get active DAMPs recognized by their corresponding PRRs (Mélida *et al.*, 2018). Thus, oligosaccharide production by the enzymatic machinery from either the host or the invader is necessary for DAMP release. However, all the above-mentioned technologies require the use of strong chemical solvents, which normally alter the *in vivo* conformations of carbohydrates. Moreover, not all plant cell wall-derived oligosaccharides obtained by fractionations triggering PTI responses may function as bona fide DAMPs *in vivo*. Therefore, chemical information should be complemented *in vivo* with spatial information through either imaging coupled with antibody labelling (Lee *et al.*, 2011), FT-IR microspectroscopy (Philippe *et al.*, 2006) or Raman microspectroscopy analyses (Robert *et al.*, 2011). More recently, matrix-assisted laser desorption/ionization (MALDI) mass spectrometry imaging (MSI) has emerged as a powerful method for resolving both the spatial distribution and the structures of many kinds of molecules in intact tissue sections. Following tissue mounting on a conductive glass plate and application of the MALDI matrix, the MS instrument captures a series of mass spectra, each of which represents the mass profile of a laser beam-irradiated region of the sample (Velickovic *et al.*, 2014). The major advantage of MSI over other imaging techniques is that it would allow the tracing of potential purified DAMPs during a plant–pathogen interaction without the requirement to develop specific probes or antibodies. Once these novel cell-wall-derived DAMPs are purified *in vitro*, biomolecular interaction assays can be performed with ED of putative receptors to allow quantification of potential DAMP–ED binding (Reinhard and Nurnberger, 2017). These assays will also require the production of EDs from different PRRs, but progress during the last decade in the field of peptidic ligand perception has allowed the development of successful pipelines for such a task (Sahdev *et al.*, 2008; Young *et al.*, 2012; Hohmann *et al.*, 2017).

CONCLUDING REMARKS

The plant cell wall has been considered for a long time as 'just' a passive barrier which pathogens had to surpass to enter host cells, colonize them and complete their life cycle. More recently it has been demonstrated that plants have developed sophisticated stress monitoring systems through CWI maintenance mechanisms. Part of such CWI monitoring systems relies on the recognition of 'danger' alert signals activating DTI, which shares signalling components and responses with the immune pathways triggered by non-self MAMPs. Only a reduced number of molecular partners involved in carbohydrate-based DAMP/MAMP perception and signal triggering have been characterized so far, and consequently our current knowledge about the specificity of PTI and DTI responses, and the cross-regulation between these immune mechanisms, is limited (Brutus *et al.*, 2010; Willmann *et al.*, 2011; Liu *et al.*, 2012; Mélida *et al.*, 2018). The plant model Arabidopsis offers an important reservoir of receptors with specific and

diverse recognition specificities awaiting their functional characterization. Once novel carbohydrate-based potential DAMPs become available, natural genetic variation between different Arabidopsis ecotypes may allow us to identify ecotypes that are partially or fully insensitive to DAMP-containing wall fractions and thus enable the identification of, for instance, the corresponding receptors or DTI signalling components. Progress in the understanding of DTI and the identification of DAMP–PRR pairs might allow us to breed crop varieties harbouring specific PRRs and to design agricultural strategies (e.g. use of DAMPs as agrobiologicals that modulate crop immunity) that would enhance crop disease resistance.

ACKNOWLEDGEMENTS

This work was supported by Spanish Ministry of Economy and Competitiveness (MINECO) grants BIO2015-64077-R to AM. HM was funded by an IEF grant (SignWALLINg-624721) from the European Union and LB was recipient of an FPI fellowship (BES-2013-065010) from MINECO.

CONFLICT OF INTEREST

All authors declare that there are no conflicts of interest.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article.

Table S1. Cell wall modifications in crops associated with phenotypes of resistance/susceptibility to pathogens.

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