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Apoplastic and vascular defences

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Abstract (250 words)

The apoplast comprises the intercellular space between cell membranes, includes the xylem, and extends to the rhizoplane and the outer surfaces of the plant. The apoplast plays roles in different biological processes including plant immunity. This highly specialised space is often the first place where pathogen recognition occurs, and this then triggers the immune response. The immune response in the apoplast involves different mechanisms that restrict pathogen infection. Among these responses, secretion of different molecules like proteases, proteins related to immunity, small RNAs and secondary metabolites play important and often additive or synergistic roles. In addition, production of reactive oxygen species occurs to cause direct deleterious effects on the pathogen as well as reinforce the plant's immune response by triggering modifications to cell wall composition and providing additional defence signalling capabilities. The pool of available sugar in the apoplast also plays a role in immunity. These sugars can be manipulated by both interactors, pathogens gaining access to nutrients whilst the plant's responses restrict the pathogen's access to nutrients. In this review, we describe the latest findings in the field to highlight the importance of the apoplast in plant – pathogen interactions and plant immunity. We also indicate where new discoveries are needed.

Introduction

The apoplast encompasses the intercellular spaces between cell membranes including the interfibrillar and intermicellar space of the cell walls, the xylem, and extends to the rhizoplane and cuticle of the outer plant surfaces [1]. The apoplast plays roles in signalling, water, ion, and nutrient transport as well as in plant – pathogen interactions. Often the apoplast is the first location where pathogen detection occurs. Pathogen recognition leads to activation of a wide range of different immune responses in the apoplast (**Figure 1**).

Apoplastic defences triggered by microbe associated molecular patterns (MAMPs) and damage associated molecular patterns (DAMPs)

Different plant plasma membrane localised receptors recognise a plethora of MAMPs from bacteria (e.g. flagellin, elongation factor Tu, lipopolysaccharide and peptidoglycans) [2], fungi (e.g. β-glucan and chitin) [3], insects (e.g. inceptin) [4], and oomycetes (e.g. elicitins, oligopeptide and phospholipid elicitors) [5, 6]. Perception of MAMPs by immune receptors leads to the activation of Pattern-triggered immunity (PTI) which includes massive transcriptional reprogramming and the production of different molecules that strengthen cell walls, induce stomata closure and/or impose adverse effects on pathogens. The transient and highly localised production of reactive oxygen species (ROS) in the apoplast, known as the

ROS burst, is one of the earlier responses detected [7]. The different types of ROS molecules produced by plants include singlet oxygen ($^1O_2$), superoxide anion ($O_2^{\cdot-}$), hydroxyl radicals ($^{\cdot}OH$), and hydrogen peroxide (H_2O_2). These not only cause direct deleterious effects to pathogens associated with oxidative damage to DNA, lipids and proteins [8], but also serve as additional signals that amplify and trigger different immune responses locally [7, 9]. In addition, apoplastic H_2O_2 can move across the plasma membrane, mediated by aquaporins [10], where other proteins associated with the activation of plant immunity can be targeted because of alterations in redox activity. The proteins responsible for the ROS burst are plasma membrane localised nicotinamide adenine dinucleotide phosphate (NADPH) oxidases, also known as respiratory burst oxidases homologs (RBOHs), superoxide dismutases (SODs), and apoplastic class III peroxidases such as PRX33 and PRX34 [11, 12]. Increased accumulation of ROS in the apoplast leads to the activation of several critical defence mechanisms. Direct interaction of H_2O_2 with peroxidases lead to cross-linking of different cell wall components and lignin formation which strengthens the cell wall against pathogen penetration [13]. Callose is a cell wall component that accumulates upon pathogen infection strengthening the cell wall upon PTI [14]. An Arabidopsis mutant deficient in the production of RBOHD, a key player in apoplastic ROS production, accumulates lower level of callose upon treatment with flagellin, indicating that ROS is involved in callose deposition [15]. PTI also leads to stomata closure. Activation of the Arabidopsis Ca^{2+} permeable channel OSCA1.3 upon flagellin perception by the cell-surface receptor Flagellin-Sensing-2 (FLS2) is mediated by phosphorylation. Activated OSCA1.3 produces a rapid influx of cytosolic calcium that leads to stomata closure and thus restricts further pathogen entry into the apoplast [16]. Ca^{2+} influx into the cell is also required for RBOHD activation and increased ROS production [17].

Adapted pathogens often secrete proteinaceous effectors with multiple functions including suppression of different aspects of plant immunity to promote infection. Some effectors localise to and exert their function in the apoplast, whereas others act in the cytoplasm of the host plant cell following translocation from the apoplast. Moreover, some pathogens deliver effectors directly into plant cells. Plants have evolved intracellularly located receptors that recognise a subset of pathogen effectors and activate a second tier of defence called effector-triggered immunity (ETI) [18]. This intracellular ETI amplifies the PTI response by inducing a biphasic ROS burst that is stronger and longer lasting as well as a long-lasting calcium influx which further strengths apoplastic defences. On the other hand, PTI boosts ETI by amplifying the hypersensitive response which leads to rapid death of the responding plant cell [19-21].

During infection some pathogens secrete different cell wall degrading enzymes (CWDEs) to deconstruct host cell walls and obtain access to nutrients or penetrate the cell wall to reach the nutrient richer host cell's interior [22]. Due to the action of CWDEs, degradation products such as cutin monomers are released from the cuticle and are derived from different cell wall polysaccharides such as oligogalacturonides (OGs), cello-oligomers, xyloglucan, and the recently described Poaceae specific oligosaccharides containing a β-1,3-1,4-glucan backbone [23, 24]. These degradation products can act as DAMPs which are perceived by plasma membrane receptors and trigger a range of immune responses that can include ROS production, callose deposition, activation of defence-related genes, Ca^{2+} influx and/or the production of antimicrobial substances called phytoalexins. Polysaccharide derived DAMPs are not the only products arising from pathogen incited degradation. DAMPs can also be proteins, peptides, nucleotides, sugars and amino acids released into the apoplast as the plant cells and tissues progressively lose integrity following pathogen attack. DAMP-triggered immunity shares similarities and signalling components with PTI. In addition, DAMPs can function as an amplifier of PTI and on some occasions DAMPs can negatively regulate PTI. The roles of DAMPs in plant immunity have been recently comprehensively reviewed [24].

Secreted secondary metabolites with a role in plant defence

Different types of secondary metabolites are known to be formally linked with resistance to pathogens from different kingdoms [25]. Some are secreted to the apoplast and in recent years progress has been made in understanding their mode of action. Camalexin is an important tryptophan (Trp)-derived phytoalexin that provides resistant against bacterial, fungal and oomycete pathogens within the Brassicaceae family [26]. Camalexin synthesis is induced after pathogen challenge and is then secreted to the apoplast by the pleiotropic drug resistance (PDR) transporter PENETRATION3 (PEN3)/ PDR8 [26]. In addition, these two transporters can export a set of unidentified metabolites derived from Trp. Glucosinolates are also derived from the Trp-metabolites and are mainly present in plant species within the Brassicaceae family [27]. For example, sulforaphane (SFN) is constitutively secreted into the apoplast and provides resistance to *Pseudomonas syringae* by inhibiting type III secretion system (T3SS), a method used by many phytopathogenic bacteria species to inject effector proteins directly into the host cells [28]. Interestingly, these SFN concentration in the apoplast do not affect the normal leaf microbiota. Polyphenols are another group of secondary metabolites present in the apoplast. Polyphenols affect different processes connected with bacterial pathogenicity including swarming, biofilm formation and quorum sensing [29]. Three polyphenols, namely tannic acid (TA), 1,2,3,4,6-pentagalloylglucose (PGG) and epigallocatechin gallate (EGCG) present in the apoplast, were shown to interact with and inhibit the phosphatase activity of RhpS, a master bacteria regulator, that also controls T3SS gene expression [29]. However, in this study suppression of bacterial virulence was achieved by exogenously applying the different polyphenols at concentrations above those normally found in the apoplast. Noteworthy, is the hypothesis that adapted pathogenic bacteria could have evolved specific detoxifying system(s) to overcome the inhibitory effects of the polyphenols and SFN *in planta*.

The role of apoplastic sugars in anti-pathogen host defence

The apoplastic free simple sugars, glucose, hexose, and fructose provide a nutrient rich carbon niche frequently used by diverse pathogens. Once a pathogen infects a tissue, the changes induced frequently lead to the localised downregulation of photosynthetic genes, upregulation of respiratory gene and accumulation of monosaccharides thereby transforming the colonised tissue into an alien sink tissue [30, 31]. Pathogen induced activation of members of the transporter family Sugar Will Eventually Be Exported Transporter (SWEET), which release monosaccharides to the apoplast, is a common strategy deployed by bacterial and fungal pathogens to increase nutrient availability [32, 33]. A well-documented case is the compatible interaction between rice and *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) whereby upon infection, *Xoo* secretes a transcriptional activator-like (TAL) effector PthXo1 that binds to the regulatory region in the promoter of the rice OsSWEET11 gene to induce its expression and promote sugar efflux to feed the bacteria in the xylem and/or apoplast [34]. But to protect themselves, plants also differentially express other sugar transporters during pathogen infection to keep the apoplast free of sugar and therefore, reduce or stop infection. For example, the Sugar Transport Protein (STP) family of high-affinity proton/sugar symporters localise mainly in the plasma membrane of sink cells and are more active at higher pH [35]. Often an increase in apoplastic pH occurs during pathogen infections. The *Arabidopsis* STP13 and STP11 are major contributors to monosaccharides uptake from the apoplast [36]. Arabidopsis double mutant lines for these two transporters are more susceptible to *Pseudomonas syringae* (*P. syringae*) infection [37]. Interestingly, STP13 is phosphorylated by the complex FLS2 and Brassinosteroid insensitive 1-associated receptor kinase 1 (BAK1)

upon flagellin recognition. This phosphorylation enhances STP13 activity leading to a reduction in apoplastic sugars thereby depriving the bacteria not only from an energy source but also restricting the delivery of virulence factors [37].

Recently, an apoplastic glycosidase, an enzyme that hydrolises sugar bonds, was shown to play a role in monitoring for infection by bacteria. Bacteria use flagella for motility, and plants can perceive bacteria by detecting fragments of flagellin protein, the primary component of flagella. Plant extracellular proteases and hydrolases were hypothesised to be responsible for releasing short flagellin-derived peptides. The released peptides, such as flg22, could then be recognised by the immune receptor FLS2 which triggers the immune response. In *Nicotiana benthamiana,* the enzyme β-galactosidase 1 (BGAL1) was shown to be responsible for the deglycosylation of flagellin, but only when flagellin carries a terminal modified unusual sugar viosamine. Following this critical deglycosylation step, the flagellin can be digested by unknown extracellular protease(s) into immunogenic fragments detectable by FLS2 [38]. Bacteria can avoid plant detection either by producing a BGAL1 inhibitor or by producing BGAL1-insensitive glycans to shield the flagellin protein.

Invertases cleave sucrose into glucose and fructose increasing the pool of hexose available in the apoplast. Activation of plant cell wall invertases during plant pathogen interactions has been reported [39]. Changes in the pool of free hexoses can activate the immune response. However, depending on the interaction, elevated invertase activity could also favour the pathogen [40]. Understanding how changes in the sucrose/hexose ratio are sensed and thus trigger the immune response have not yet been elucidated.

Defence related proteins secreted into the apoplast

Pathogen recognition mediated by plant immune receptors triggers defence-related gene expression and the secretion into the apoplast of many different pathogenesis-related (PR) proteins and proteases [41]. These well characterised groups of PR-proteins include PR-1, β-1,3-glucanases (PR-2), chitinases (PR-3) and thaumatin-like (PR-5) which provide resistance against fungal pathogens and peroxidases (PR-9) that are effective against both bacteria and fungi. In the past years PR-proteins have been extensively reviewed in [42] and [43].

As described above, to penetrate the plant cell wall, pathogens release different CWDEs such as cellulases, pectinases, xylanases which degrade cellulose, pectins and hemicellulose, respectively. On the other hand, plants secrete proteins that inhibit the activity of CWDEs, for example polygalacturonase-inhibiting proteins (PGIPs) which minimise the function of pathogen produced polygalacturonases (PGs) [44, 45]. PGs secreted by pathogens release oligogalacturonides (OGs) by hydrolysis of homogalacturonan, the most abundant type of pectin [22]. OGs have been formally proven to be DAMPs because transgenic Arabidopsis plants engineered to generate OGs, through partial inhibition of PGs by PGIPs, activate various immune responses, including the expression of defence-related genes, ROS production and accumulation of phytoalexins, which are effective against different pathogens [44]. Release of xylanase inhibitors (XIs) as a component of plant immunity has also been reported [22].

Plant proteases are a group of proteins that catalyse the cleavage of other proteins. Members of the aspartic protease (AP) family have been reported to be induced by different pathogens. In soybean, root apoplastic GmAP1 confers resistance to *Phytophthora sojae* and *P. capsici* in tomato and *N. benthamiana*, respectively [46]. Interestingly, this protease is targeted by the *P. sojae* effector protein PsAvh240 thereby supressing its secretion into the apoplast [46]. Another example in the same pathosystem is GmAP5, a secreted protease that degrades the effector PsXEG1, an apoplastic xyloglucan endoglucanase, required for *P. sojae* virulence [47]. APs also play a role in defence against phytopathogenic bacteria. Secreted aspartic proteases 1 and 2 (SAP1 and SAP2) in *Arabidopsis* can cleave the highly conserved

bacterial protein MucD which is essential for *P. syringae* growth [48]. Subtilases are serine peptidases associated with plant defence. The tomato subtilase P69B localises to the leaf apoplast [49] and following cleavage of the *P. infestans* secreted effector PC2 activates immunity in different *Solanum* species [50]. Proteolytic cascades involve a set of proteases that activate each other by cleavage. This mechanism has been described in animals but there was no direct evidence of its existence in plants [51] until a recent study revealed a connection between a proteolytic cascade occurring in the apoplast and activation of plant defence. Tomato Rcr3 is an apoplastic papain-like Cys protease (PLCP) that confers resistance against *P. infestans* [52], the mold fungus *Cladosporium fulvum* and the root nematode *Globodera rostochiensis* [53]. Rcr3 is secreted as an inactive precursor proRcr3, which is then proteolytically cleaved by P69B and other subtilases [54]. The mature version of Rcr3 is able to bind to Avr2, an apoplastic effector secreted by *Cladosporum fulvum*. The complex Rcr3-Avr2 can be perceived by the plasma membrane receptor Cf-2 to trigger cell death [54]. Rcr3 [55], GmAP5 [47] and SAP1/2 [48] are all induced during pathogen infection, but how protease gene expression and protein accumulation is triggered by pathogens remains to be elucidated.

Targeting the integrity and function of pathogen cell walls, through the secretion of enzymes other than chitinases and glucanases has recently been reported. In maize two secreted proteins AFP1 and AFP2 with mannose binding capacity were found to confer resistance against *Ustilago maydis* [56]. These two DUF26-domain proteins are induced upon pathogen infection and mutations in the mannose binding sites reduce their antifungal activity. The mode of action of these proteins remains unknown. One hypothesis could be that binding of AFP1 and AFP2 to mannose in the hyphae may affect the integrity of the fungal cell wall. In addition, degradation of fungal cell wall would lead to the release of molecules that could trigger the immune response [56]. Interestingly, *U. maydis* secretes an effector Rsp3 able to block the functions of maize AFP1 and AFP2.

Another way to inhibit the deconstruction of plant cell walls by pathogens has recently been identified. Soybean plants secrete a protein GmGIP1 that has homology with the secreted aspartic protease GmAP5 [47], but instead possesses a mutation in a critical catalytic amino acid. GmGIP1 binds to the *P. sojae* xyloglucan endoglucanase effector PsXEG1 but this does not lead to its degradation as reported for the interaction between GmAP5 and PsXEG1. GmGIP1 reduces the glucanase activity of PsXEG1 thereby reducing pathogen virulence. However, *P. sojae* is able to protect PsXEG1 by N-glycosylation of PsXEG1 that shields the effector from degradation by GmAP5 and attenuates binding to GmGIP1. In addition, the pathogen secretes a paralogous PsXEG1-Like Protein (PsXLP1) that does not possess glucanase activity but binds strongly to GmGIP1 and therefore also protects PsXEG1 [57]. This example together with the one described above for *U. maydis* show that pathogens can also counteract apoplastic defences.

Role of extracellular RNAs in plant immunity

In addition to the many defence-related mechanisms discussed above, the plant apoplast is also a reservoir for numerous species of small RNA (sRNA) differing in their biogenesis, including micro-RNA (miRNA) and small interfering RNA (siRNA) ranging in size from 21- to 24-nt, which regulate various cellular processes including immune responses during the plant-pathogen interactions via an RNA interference (RNAi) pathway [58]. A brief description of the different sRNA species is provided in **Box1**. Plant sRNAs respond to attack by pathogens by promoting disease resistance either directly or indirectly, via regulation of hormone signalling pathways comprehensively reviewed in [59]. A very exciting discovery made during the past decade is that both miRNAs and siRNAs could traffic from the plants to the invading pathogens where these molecules direct silencing of genes required for virulence. This phenomenon, coined ‘cross-kingdom RNAi’, has been reported for several pathosystems

including cotton – *Verticillium dahlia* [60], *Arabidopsis thaliana – Botrytis cinerea* [61], wheat – *Fusarium graminearum* [62], and *A. thaliana – Phytophthora capsici* [63]. The mechanism by which the plant sRNAs are secreted and transported from the plant to the pathogen cells remains unresolved, with the most popular hypothesis being that this process somehow or other involves membrane-derived extracellular vehicles originating from the Golgi apparatus [64]. However, the latest study by Karimi and collaborators [65] demonstrated that apoplastic RNAs are largely located outside of extracellular vesicles, as complexes with various RNA-binding proteins. It appears that numerous pathogen species also produce sRNAs that act as effectors, which when delivered to plant cells during invasion suppress host immunity and promote susceptibility by silencing defence-related genes [66-68]. Interestingly, the plant apoplast also contains thousands of circular RNAs (circRNAs), long noncoding RNA species with a role in plant immunity [69]. In animals, circRNAs are involved in regulation of gene expression including by acting as sponges for miRNAs [70]. This discovery has led to the proposition of a fascinating hypothesis that plant apoplastic circRNAs could act as a counter-defence measure by sequestering pathogens' sRNA effectors, and therefore preventing them suppressing host immunity [65].

Box 1 Biogenesis and mode of action of mRNAs

miRNA and siRNA originate from different RNA precursors. miRNA are processed from the MIR gene whilst siRNA precursors are long double stranded RNAs or long hairpin RNA. siRNA associate to proteins from the Argonaute family to form RNA-induced silencing complexes. Then, post-transcriptional gene silencing (PTGS) is achieved by different mechanisms like transcript cleavage, translation repression or transcriptional gene silencing [71]. The circRNAs are non-coding single-stranded RNAs, in which the 5' and 3' termini are covalently linked, that are derived from back-splicing of pre-mRNA. Similar to other non-coding RNAs, circRNAs have been implicated in regulation of a plethora of processes in plants [69].

Xylem defences against pathogens

Xylem plays an important role in water and mineral transport and is a direct extension of the apoplast. Although nutrient poor compared with the sugar rich phloem, the xylem can be colonised by several fungal, bacteria and oomycete pathogens causing vascular wilt diseases [72]. One potential explanation proposed for this taxonomically widespread pathogen species colonisation is that the xylem is composed of dead treachery elements with lower osmotic pressure compared to the living phloem cells which together facilitate pathogen colonisation [72]. The xylem also contains living parenchyma cells with roles in storage and transport [73]. The parenchyma cells may play role(s) in pathogen recognition and triggering the immune response [72]. Resistance to vascular pathogens involves the production of different physical barriers which prevent the spread of the pathogen in the xylem vessels. These include the formation of tyloses, balloon-like structures produced by the parenchyma cells that protrude into the xylem vessels [74], the secretion of gels and gums around the tyloses and the localised deposition of lignin, suberin and callose reviewed in [75]. Although these inducible barriers were first described over 50 years ago, the signalling events behind their activation remain largely unknown. Similarly, xylem localised elemental sulphur (S^0) is known to be produced by cocoa, tomato, cotton and French bean plants in response to different xylem invading bacterial and fungal pathogens [76]. In each pathosystem, S^0 production is higher and faster in resistant cultivars. The signalling mechanisms leading to S^0 synthesis remain to be elucidated.

The main issue when studying xylem defences is the distinction between the immune responses observed in non-xylem and xylem-specific tissues. In general, xylem colonising pathogens must first enter the host tissue through wounds, mechanical penetration, or natural openings such as stomata and hydathodes. Next, pathogen colonisation is somehow

preferentially orientated toward the vascular tissue [72]. During this initial colonisation process, pathogens can be recognised and trigger PTI and ETI responses in the non-xylem tissues. However, some pathogens are delivered directly into the vascular system by insect vectors and in these pathosystems dissection of the xylem immunity can be achieved. *Xylella fastidiosa* (*Xf*), the causal agent of Pierce's disease in grapevines exclusively occupies the xylem tissue and represents a suitable tool to dissect the xylem immune responses. In this context, a mutant *Xf* strain defective in lipopolysaccharide O-antigen synthesis which compromises the bacterial virulence [77] has been used to study xylem immunity responses. Grapevine xylem tissue displayed a faster and more intense immune response against the mutant strain compared to the wild-type strain. This immune response involves production of ROS, phytoalexins and PR gene induction [78] clearly indicating that PTI-like responses can also occur in xylem tissues. Metabolomic and proteomics analyses of xylem sap from infected plants are two useful approaches to elucidate the immune response in xylem tissues. Xylem sap from *Xf* infected grapevines was found to contain an increased amount of defence-associated phenolic compounds [79]. The interaction between the fungus *Fusarium oxysporum* f.sp. *lycopersici* (*Fol*) with tomato is a model pathosystem used to explore root and xylem specific responses. Proteins present in xylem sap from *Fol* infected tomato plants include two PR-1 isoforms, PR-2, PR-3, PR-4, PR-5, peroxidases, a xyloglucan-specific endoglucanase inhibitor protein, and xyloglucan endotransglycosylase which might be involved in cell wall remodelling in response to the pathogen [80, 81].

Regarding a role for ETI in the xylem, several dominant disease resistance (*R*) loci have been described that provide effective defence to different vascular pathogens. The *Ve1* tomato gene and the *Xa21* rice gene both code for extracellular receptor proteins that mediate resistance against the fungus *Verticillium dahlia* [82] and the bacteria *Xoo* [83], respectively. Co-expression of Ve1 and the interacting *Verticillium* effector Ave1 confers a hypersensitive response in tomato leaves [84], whereas recognition of the *Xoo* secreted RaxX peptide by Xa21 in rice leaves leads to ROS production and defence-related gene induction [85]. However, whether these receptor proteins reside solely in xylem tissues or are also present in the tissues surrounding the vasculature still needs to be elucidated. To date only a few studies have reported on *R* gene expression in xylem tissues. The tomato intracellular receptor *I-2* which provides resistance to *Fol* is mainly expressed in tissues surrounding the xylem vessels [86]. In addition to *I-2*, two transmembrane R proteins *I* and *I-3* provide *Fol* resistance once the fungal hypha reaches the xylem, which might indicate that *I* and *I-3* are also expressed in vascular tissues [87]. Overall, the immune responses evident in xylem tissues possess some similarities to those described for non-xylem tissues. However, the connection(s) between the induction of physical barriers such as tyloses and gels that block pathogen dispersal in xylem vessels and PTI and ETI has still to be elucidated.

Summary

- Apoplastic defences encompass a wide array of immune responses including reactive oxygen species, secondary metabolites, changes in the apoplastic sugar contents, secreted small RNAs, proteases and defence-related proteins associated with immunity (**Figure 1**).

- Different types of secondary metabolites accumulate in the apoplast and are connected to or required for immunity, but for most their mode of action remains obscure.

- Sugar depravation in the apoplast restricts the growth of apoplast dwelling pathogenic bacteria, but the molecular immune mechanism sensing changes in the pool of free sugar in the apoplast has still to be elucidated.

- Secreted small RNAs have recently been connected with immunity, but how plant sRNAs are secreted and transported from the plant to the pathogen cells is still poorly understood.

- Pattern and effector-triggered immunity responses can occur in xylem tissues, but the signalling cascades and networks leading to activation of specific xylem defences are unresolved.

Abbreviations

$^1O_2$	singlet oxygen
AP	aspartic protease
BAK1	Brassinosteroid insensitive 1-associated receptor kinase 1
BGAL1	β-galactosidase 1
CWDEs	cell wall-degrading enzymes
circRNAs	circular RNAs
DAMPs	damage associated molecular patterns
EGCG	epigallocatechin gallate
ETI	effector-triggered immunity
FLS2	Flagellin-Sensing-2
Fol	*Fusarium oxysporum* f. sp*. lycopersici*
H_2O_2	hydrogen peroxide
HR	hypersensitive response
MAMPs	microbe-associated molecular patterns
miRNA	micro-RNA
NADPH	nicotinamide adenine dinucleotide phosphate
$O_2^{\cdot-}$	superoxide anion
OGs	oligogalacturonides
·OH	hydroxyl radicals
PDR	pleiotropic drug resistance
PEN3	PENETRATION3
PGG	1,2,3,4,6-pentagalloylglucose
PGIPs	polygalacturonase-inhibiting proteins
PGs	polygalacturonases
PLCP	papain-like Cys protease
PR	pathogenesis-related
PRRs	pattern-recognition receptors
PsXLP1	PsXEG1-Like Protein
P. syringae	*Pseudomonas syringae*
PTI	pattern-triggered immunity
PTGS	post-transcriptional gene silencing
PGs	polygalacturonases
RBOHs	respiratory burst oxidases homologs
RNAi	RNA interference
ROS	reactive oxygen species
S^0	elemental sulphur
SAP	secreted aspartic proteases

SFN	sulforaphane
siRNA	small interfering RNA
SOD	superoxide dismutase
sRNA	small RNA
STP	Sugar Transport Protein
SWEET	Sugar Will Eventually Be Exported Transporter
T3SS	type III secretion system
TA	tannic acid
TAL	transcriptional activator-like
XIs	xylanase inhibitors
Xf	*Xylella fastidiosa*
Xoo	*Xanthomonas oryzae* pv. *oryzae*

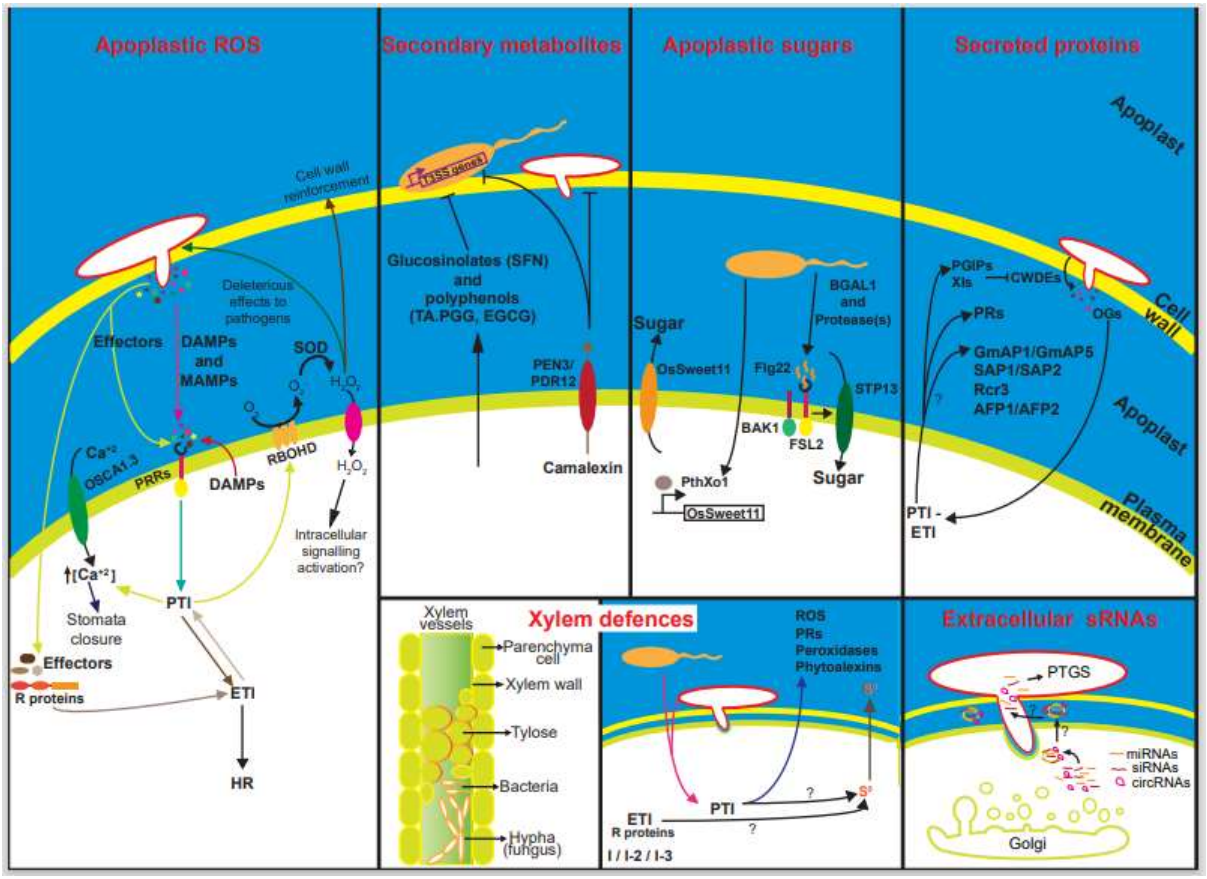

Figure 1. Apoplastic and vascular defences involve multiple responses. Secreted effectors proteins and/or MAMPs from pathogens can be recognised by different membrane localised pattern-recognition receptors (PRRs) and cytoplasmic receptors (R proteins). In addition, release of degradation products by the action of CWDEs such as oligogalacturonides (OGs) and other molecules released from the infected plant cells can act as DAMPs perceived by PRRs. Receptor recognition leads to activation of PTI and ETI, both responses may boost one another's effectiveness. Activation of the immune response leads to production of ROS which not only has a direct deleterious effect on pathogen fitness but also leads to the reinforcement of plant cell walls and the activation of intracellular defences. PTI also increases cytoplasmic Ca^{2+} concentration and thus promote stomata closure and ROS production. ETI leads to activation of the hypersensitive response (HR), i.e. the rapid death of the responding cell, and some of these cell contents may also accumulate in the apoplast to further boost immunity. Pathogen recognition leads to the activation of defence signalling mechanisms that results in the secretion of proteins connected with immunity (PRs, PGIPs, XIs and AFP1/AFP2), proteases (Rcr3, SAP1/SAP2, GmAP5 and GmAP1), sRNAs (miRNA, siRNA, and circRNA) and secondary metabolites (camalexin, EGCG, PGG, SFN and TA). However, for most of these molecules the molecular signalling events that leads to the temporal and spatial co-ordination of their production and apoplastic accumulation as well as how these molecules are secreted are only partially elucidated. In the case of sRNA and circRNAs the role of the extracellular vesicles in RNA secretion and the mechanisms controlling movement within the apoplast are not known. In bacterial infection, the available pool of hexoses in the apoplast plays an important role in the outcome of the infection. Bacteria promote expression of SWEET11, a transporter that releases sugars in the apoplast. BGAL1, an apoplastic glycosidase, together with unknown protease(s) digests bacterial flagellin into fragments (for example, flg22) to permit detection by FSL2. Flagellin recognition leads to activation of the immune response including activation of another transporter STP13 that reduces the amount of free sugars in the apoplast. Xylem defences include the production of tyloses covered by gels and gums that physically blocks the xylem vessels and thus restrict the spread of pathogens. However, the molecular signalling that triggers these defences is still unknown. PTI-like responses like ROS, phytoalexin production, and PRs and peroxidases gene expression have been reported in xylem tissues. Secretion into xylem vessels of S^0 which has direct anti-fungal activity is long known but its connection with PTI or ETI has still to be investigated. Some canonical *R* genes are known to be expressed in xylem tissues and suggest that some components of ETI are active in xylem vessels.

Conflict of interest

The authors declare that there are no competing interests associated with this article.

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Authors contributions

MD, KK and KHK wrote the manuscript. MD prepared the figure. KK prepared the box.

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