Title: PTI-ETI crosstalk: an integrative view of plant immunity

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Abstract

Plants resist attacks by pathogens via innate immune responses, which are initiated by cell surface-localized pattern-recognition receptors (PRRs) and intracellular nucleotide-binding domain leucine-rich repeat containing receptors (NLRs) leading to pattern-triggered immunity (PTI) and effector-triggered immunity (ETI), respectively. Although the two classes of immune receptors involve different activation mechanisms and appear to require different early signalling components, PTI and ETI eventually converge into many similar downstream responses, albeit with distinct amplitudes and dynamics. Increasing evidence suggests the existence of intricate interactions between PRR- and NLR-mediated signalling cascades as well as common signalling components shared by both. Future investigation of the mechanisms underlying signal collaboration between PRR- and NLR-initiated immunity will enable a more complete understanding of the plant immune system. This review discusses recent advances in our understanding of the relationship between the two layers of plant innate immunity.

Keywords: Plant pathogen; plant immunity; ROS burst; MAPK; Ca^{2+} influx, resistosome

Introduction

Plants have evolved a two-layered innate immune system to detect and cope with diverse biotic attacks [1-3]. The first layer of the immune system is triggered upon recognition of the pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs) via cell surface localized PRRs, leading to PTI [1]. PTI plays a prominent role in curtailing pathogen invasion [4,5] and maintaining the homeostasis of endophytic leaf

microbiota in the plant leaf [6]. To facilitate invasion and proliferation, many pathogens, including bacteria, fungi, oomycete and nematodes, deliver virulence-associated molecules, such as effectors secreted via bacterial type III secretion system (T3SS) into plant cells or the apoplast to suppress host immunity [7,8]. To combat pathogen virulence, plants activate a second and generally stronger immune signalling known as ETI, upon direct or indirect recognitions of effectors by NLRs [1]. An influential "zig-zag" model was proposed by Jones and Dangl in 2006 to describe physiological outputs of the two-layered plant immune system in response to different pathogens [1], yet how PTI and ETI contribute to the quantitative or/and qualitative output of immunity and how they work together when both being activated were both being activated were not known

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Of note, PTI and ETI involve activation of two distinct classes of receptors (i.e., PRRs and NLRs, respectively) and different steps in early signalling [9-11]. Yet, they lead to a number of overlapping downstream outputs, such as mitogen-activated protein kinase (MAPK) cascades, calcium flux, reactive oxygen species (ROS) burst, transcriptional reprograming and phytohormone signalling [12-14], suggesting converging and intersectional points of these two signalling cascades. Recent years witnessed significant progress in understanding how PTI and ETI crosstalk to ensure a robust immunity. Given the blurred distinction between PAMPs and effectors, discussions in this review are confined to PRR-mediated PTI and NLR-mediated ETI.

Distinct activation mechanisms and early signalling events in PTI versus ETI

PTI signalling is activated upon direct recognition of PAMPs or DAMPs by PRRs, which so far include two types of cell surface proteins, receptor-like kinases (RLKs) and receptor-like proteins (RLPs) [15]. The extracellular portion of these proteins often contain a leucine-rich repeats (LRR) (e.g., FLS2, EFR, PEPRs and RLP23), LysM (e.g., LYK4/5) or S-lectin domain (e.g., LORE) [9], which perceive ligands derived from microbes or plants. RLKs contain an intracellular kinase domain, while RLPs lack a kinase domain, have a short or no intracellular tail, and usually complex with the adaptor protein SOBIR1 for ligand recognition [16-18]. Upon binding of ligands, RLKs or RLP-SOBIR1 receptors recruit co-receptors such as BAK1 or CERK1 to form a receptor complex, in which trans-phosphorylation occurs (Figure 1A) [16,19-21]. The activated heteromeric receptor complex further phosphorylates receptor-like cytoplasmic kinases (RLCKs) [22-24], which subsequently activate a variety of substrate proteins, leading to diverse physiological outputs including ROS production, stomatal closure, MAPK activation and production of defence hormones (summarized in Table 1). For example, in Arabidopsis, one of the best-studied members of the RLCK family, BIK1, directly activates the cyclic nucleotide-gated ion channel CNGC2/4 for calcium (Ca2+) influx, when plants are grown under sufficient Ca2+ condition [25], and Ca2+-permeable channel OSCA1.3 in the guard cell for stomatal closure upon PAMP treatment [26]. Whether there are additional calcium channels (e.g., in mesophyll cells and/or under different calcium concentrations) in PTI will be an interesting topic for future investigation. Similarly, rice OsRLCK185 plays an essential role in activating OsCNGC9 for calcium influx and MAPK signalling cascade in response to PAMPs [27-29].

Table 1. The role of RLCK family in PTI and ETI responses in plants

Species	RLCK genes	Substrates	Immune outputs	Involvement in PTI or ETI	References
Arabidopsis	BIK1/PBLs[a]	RBOHD	ROS production	Both	[30-33]
	BIK1[a]	CNGC2/4	Ca^{2+} influx	PTI	[25]
	BIK1[a]	OSCA1.3	Ca^{2+} influx and stomatal closure	PTI	[26]
	PBL27[a]	SLAH3	Stomatal closure	PTI	[34]
	RLCK VII-4 subfamily[a], BSK1[b]	MAPKKK3/5	MAPK activation	PTI	[33,35,36]
	BIK1[a]	WRKY33	Salicylic acid (SA)	PTI	[37]
	BIK1[a]	WRKY50/57	Jasmonate	PTI	[37]
	PBL19/20[a], PCRK1/2[a]	Unknown	Salicylic acid (SA)	PTI	[38,39]
	PCRK1	Unknown	Callose deposition	PTI	[40]
	PBL31[a]	Unknown	Ethylene	PTI	[41]
Rice	OsRLCK185[a]	OsMAPKKKs	MAPK activation	PTI	[28,29]
	OsRLCK185[a]	OsCNGC9	Ca^{2+} influx	PTI	[27]
	OsRLCK 57[a], OsRLCK107[a], OsRLCK118[a], OsRLCK176[a]	Unknown	ROS production and defense gene expression.	PTI	[42,43]

This table summarizes direct substrate proteins of different members of the RLCK family in Arabidopsis and rice and their mediated immune responses in PTI (in response to different PAMPs such as flagellin, elongation factor Tu, chitin and necrosis and ethylene-inducing peptide 1-like proteins [nlps]) and/or ETI.

[a]These genes belong to the RLCK-VII subfamily.

[b]These genes belong to the RLCK-XII subfamily.

ETI signalling is initiated following direct or indirect recognition of pathogen effectors by NLRs (Figure 1A) and activation of ETI results in enhanced resistance and hypersensitive cell death response (HR) [10]. Most NLRs in plants contain three domains, an N-terminal variable domain, a middle nucleotide binding domain and a C-terminal LRR domain [11]. NLRs can be classified into three major groups based on their N-terminal domain, including the coiled-coil (CC)-type NLRs (CNLs), Toll/interleukin-1 receptor/Resistance protein (TIR)-type NLRs (TNLs) and the Resistance to powdery mildew 8-like domain (RPW8)-type NLR (RNLs) [11,44]. They may function as a "sensors" or "helpers" during recognition [44]. Emerging studies on helper NLRs demonstrated their essential roles in mediating ETI resistance or the

hypersensitive cell death response (HR) initiated by sensor NLRs [45-48]. Recent breakthroughs include determination of the three-dimensional structure of the Arabidopsis CNL ZAR1 "resistosome", which adopts a putative "pore" structure in an oligomeric state, and TNLs Roq1 from tobacco and RPP1 from Arabidopsis, which form tetrameric resistosomes, as well as demonstration of the enzymatic activity of TNLs in cleaving NAD^+ molecule. These advances represent exciting opportunities toward a mechanistic understanding of ETI signal transduction [49-54]. Compared to a large body of knowledge in early signalling of PTI, how NLR activation leads to various ETI downstream events remains largely elusive. Interestingly, the RLCK protein BIK1 mediates ETI-associated ROS production in Arabidopsis [32]. However, while the RLCK family function as a core "hub" to evoke downstream responses in PTI (as described above), whether RLCKs (other than BIK1) are broadly involved in ETI responses are still largely unknown.

Role of PRR signalling in ETI

Despite distinct ligands perceived and activation modes in PTI and ETI, increasing evidence suggests that the two signalling branches are functionally linked. For example, the PTI co-receptors BAK1 and BKK1 in Arabidopsis are required for ETI-associated pathogen restriction mediated by TNLs RPP2 and RPP4 against *Hyaloperonospora arabidopsidis* (*Hpa*) races *Emoy2* and *Cala2* [55]. Consistently, recent studies showed that ETI-associated resistance against *Pseudomonas syringae* pv *tomato* (*Pst*) DC3000 carrying AvrRpt2 (recognized by a CNL, RPS2), AvrPphB (recognized by a CNL, RPS5) or AvrRps4 (recognized by a TNL, RPS4) is compromised in different PRR or co-receptor mutants including *fls2/efr*, *fls2/efr/cerk1* and *bak1-5/bkk1-1/cerk1* [32,56]. One could argue that ETI-associated restriction of pathogen growth measured in these studies actually represented "PTI+ETI", since avirulent pathogens carry both PAMPs and effectors. To clearly dissect the relationship between PTI and ETI, a careful examination using treatment of PAMP alone, transgenic expression of effectors alone or both demonstrated that PRR signalling is indeed important for ETI-associated responses[32,56].

HR is a hallmark response of ETI. Studies showed that HR development in response to RPS2 activation by AvrRpt2 was compromised in PRR/co-receptor mutants including *fls2*, *pepr1/2*, *fls2/efr/cerk1* and *bak1-5/bkk1-1/cerk1* [32,57]. Consistently, activation of PRR signalling by PAMP or non-pathogenic bacterial strains (*P. fluorescence* and *Pst* DC3000 *hrcC*) can promote HR mediated by inducible expression of effectors (i.e., AvrRps4, ATR4, AvrRpt2, AvrRpm1 and AvrPphB) recognized by cognate NLRs [32,56]. Notably, TNL-mediated HR seems to particularly rely on PRR signalling, as transgenic expression of AvrRps4 and AvrRpp4 alone (without PRR signalling), to activate RPS4 and RPP4 NLRs, respectively, does not lead to macroscopic HR [56]. Interestingly, Hatsugai and colleagues identified an ETI signalling sector, named ETI-Mediating and PTI-Inhibited Sector (EMPIS) in Arabidopsis, which is inhibited by PRR signalling [58]. This type of PTI-ETI crosstalk was uncovered in an Arabidopsis quadruple mutant *dde2/ein2/pad4/sid2* (*deps*), which lacks multiple signalling sectors including jasmonate, ethylene, PAD4 and salicylate, and, in this mutant, AvrRpt2- and AvrRpm1-triggered HR is inhibited by PAMP treatment [58]. There are likely complex interactions between PTI, defense hormones and ETI underlying the different modes of PTI-ETI crosstalk discovered in different plant backgrounds. In addition to HR, other ETI responses

such as ROS production and activation of MAPK cascade are also modulated by PRR signalling (see sections below), supporting a general notion that PTI co-regulates multiple ETI responses, but to different degrees and in a NLR type-specific manner.

Regulation of PTI by ETI

The influence between PTI and ETI appears to be mutual. Recent studies showed that an upregulation of PTI components is an important feature of ETI. Activation of multiple NLRs (i.e., RPM1, RPS2, RPS5, RPS4 and RPP4) triggers transcript and protein accumulation of multiple PRR signaling components, including BAK1, SOBIR1, BIK1/PBLs, RBOHD and MPK3 in a PTI-independent manner [32,56]. Similarly, activation of the N protein (a TNL that confers resistance to tobacco mosaic virus in *Nicotiana tabacum*) leads to *de novo* synthesis of WIPK (an ortholog of Arabidopsis MPK3) [59]. In addition, the ETI activation by RRS1/RPS4, which mediates resistance against the fungal pathogen *Colletotrichum higginsianum* in Arabidopsis [60], potentiates ROS production and cell death triggered by the fungal PAMP chitin [56].

The detailed mechanisms by which ETI potentiates PRR signaling components are still unclear. While exogenous application of SA can lead to accumulation of PRRs, MPK3 and RBOHD in both Arabidopsis and tobacco [61-64], upregulation of PRR signaling components during ETI is independent of ICS1 (SID2), a key enzyme involved in SA biosynthesis [32]. Therefore, SA alone does not seem to be responsible for ETI upregulation of PTI components. Curiously, transcription and translation are poorly correlated during PTI [65], but well-correlated during ETI [66,67]. Shortly following activation, PTI is negatively regulated, through protein turnover or de-activation, to prevent prolonged immune responses [9]. It is therefore possible that ETI potentiation of PTI components involve both transcriptional and translational mechanisms, which remains to be further explored.

Overlapping immune responses in PTI and ETI

ROS production

ROS function as key defence and signaling molecules and are induced in both PTI and ETI. While PTI induces a fast and transient ROS burst, ETI is associated with a biphasic ROS burst with the second peak usually much stronger and more sustained than the first (Figure 1B) [68-71]. The mechanisms of ROS production in PTI have been extensively studied. Multiple PTI-associated protein kinases, including BIK1/PBLs, CPKs, SIK1 and CRK2, directly phosphorylate RBOHD to trigger extracellular ROS production in Arabidopsis [30,31,72-75]. RBOHD also mediates the production of ROS during RPS2- and RPM1-initiated ETI, and phosphorylation of RBOHD at S343 and S347 residues is important for ROS production in both PTI and ETI [32,76,77]. Two recent studies found that the second ROS burst (during ETI) requires, surprisingly, plant exposure to PAMP treatment [32,56]. This suggests that the second phase of ETI-associated ROS is dependent on PRR signalling. Furthermore, PRR signalling is required for maximal phosphorylation of RBOHD during ETI, whereas NLR signaling upregulates the levels of RBOHD [32,56], highlighting the dual requirement of PRR and NLR signaling to ensure robust ROS production during ETI. How RBOHD transcripts and proteins

are upregulated in ETI is not clear and could involve MAPK-WRKY module as shown in a related study in *Nicotiana benthamiana* [78]. In addition to RBOH-mediated ROS, ROS can also be generated extracellularly by peroxidases on the membrane or in the chloroplast during infection [79-81]. It will be interesting to investigate whether PTI and ETI display a similar coordination in these processes.

Ca^{2+} influx

Activation of PRR signalling leads to a fast and transient Ca^{2+} influx into the plant cell and Ca^{2+} influx is important for many subsequent immune responses, including ROS production and stomatal immunity [26,82]. NLR signalling, on the other hand, induces a slower but longer-lasting Ca^{2+} influx (Figure 1B) [83]. Previous studies showed that two independent Arabidopsis mutants, *dnd1* and *dnd2* (*defense, no death*), in which two calcium channels, CNGC2 and CNGC4 [84-86], were mutated, show constitutively elevated SA, enhanced bacterial resistance and, interestingly, largely compromised AvrRpt2/RPS2-mediated HR. Introduction of SA-metabolizing *NahG* gene into the *dnd2* mutant abolished the constitutively elevated SA and enhanced resistance but only weakly impacted the HR phenotype, further suggesting an involvement of CNGC2 and CNGC4 in ETI-associated HR [87]. In addition, CNGC11 and CNGC12 from Arabidopsis are important for ETI resistance against an avirulent pathogen *Hyaloperonospora parasitica Emwa1* [88]. However, how Ca^{2+} influx is regulated in ETI remains unclear. Recent analysis of the CNL-type ZAR1 resistosome revealed a funnel-shaped structure [50] and suggests a possible channel activity of the ZAR1 complex on the membrane [3]. Given the role of CNGC2/4 and CNGC11/12 in HR and ETI resistance, whether these proteins are involved in CNL-mediated Ca2+ influx warrants future investigation. Recently determined structure of TNL resistosomes suggest both similarity and difference in CNLs and TNLs activation mechanisms [51,52]. Moreover, some CNLs and TNLs are not localized on membranes and thus less likely to function as a channel to direct calcium flux. Whether these NLRs trigger Ca2+ influx through downstream "pore-forming" components (e.g., "CNL-type" helper NLRs, given the similarity between helper NLRs and ZAR1 [51]) or by other calcium-releasing mechanisms [89] (e.g., NAD+/NADP+ degradation products by TNLs) is an exciting area to explore in the future. We anticipate that future efforts will uncover the basis of ETI-associated Ca^{2+} features and its relationship to Ca^{2+} channels implicated in PTI.

MAPK activation

Rapid activation of MAPK cascade is a well-known feature of PRR signalling [90] Activation of NLR signalling triggers a slower but longer-lasting MAPK activation (Figure 1B) [81,91]. While the RLCK-family kinases directly phosphorylate MAPKKKs following PAMP perception during PTI [35,36,92], how NLR signalling activates MAPK cascade remains to be elucidated. Interestingly, RRS1/RPS4 and RPP4, which are TNLs, cannot trigger MAPK activation in transgenic Arabidopsis expressing AvrRps4 or AvrRpp4 in the absence of PRR signaling [56,93], suggesting that TNL-associated MAPK phosphorylation signals through PTI pathway. Similarly, induced overexpression of EDS1/PAD4 cannot activate MAPKs [94]. However, the activation of MAPK cascade by CNLs such as RPS2, RPS5 and RPM1 seems to be independent of PRR signalling ([32]; Figure 1B), indicating that PRRs and CNLs might

activate the MAPK cascade by different mechanisms [32,56]. Whether PRR- and CNL-signalling pathways converge to activate MAPKs remains to be determined.

Transcriptional reprogramming

Various studies have used different pathosystems (e.g., Arabidopsis-*Pseudomonas syringae*, tobacco-*Pseudomonas syringae*, rice-*Magnaporthe oryzae*, wheat-*Puccinia striiformis f. sp. tritici* and poplar-*Melampsora larici-populina*) to comparatively examine the expression profiles in plants inoculated with virulent or avirulent pathogens [95-100]. Results support a prevailing notion that compatible and incompatible interactions trigger largely overlapping changes in host gene expression and that incompatible interactions are usually associated with a faster and more robust response [99-102]. Although powerful, these transcriptome studies cannot easily disentangle PTI-ETI relationships because wild-type pathogens contain numerous effectors that interfere with PTI and ETI branches [7]. To circumvent this complication, recent studies utilized natural or engineered *Pseudomonas* strains *(P. fluorescence* strain Pf0-1 or *Pst* DC3000 D36E, which contains no endogenous effector genes), to deliver a single avirulent effector (e.g., AvrRpt2 or AvrRps4) to examine immune gene transcription during PTI and ETI [32,103]. It was found that inoculation of ETI-eliciting bacteria induces a globally similar, but stronger expression pattern in Arabidopsis Col-0 plant, compared to that induced by PTI-eliciting bacteria, which is consistent with previous studies. Interestingly, RPS2 signalling induced by D36E-delivered AvrRpt2 also globally rescues the expression defects of PTI-associated genes in the Arabidopsis PRR/co-receptor *bak1-5/bkk1-1/cerk1* triple mutant [32]. Nonetheless, the ETI resistance and HR are largely compromised in the same mutant, suggesting that the transcriptional activation of PTI-associated gene is not sufficient to trigger normal ETI responses.

Consistently, transcriptomic analysis of transgenic plants that conditionally express ETI-eliciting effectors [58,93] or the N-terminal CC domain of a barley NLR, MLA (Mildew resistance locus A) [104], also showed highly similar gene expression patterns for PTI and ETI. The same study has identified calmodulin-binding transcription activator 3 (CAMTA3) in Arabidopsis as an important player in both PTI- and ETI-mediated transcriptional regulation, as CAMTA3-binding sites are enriched in the promoters of upregulated PTI and ETI genes [104].

Other possible converging points of PTI and ETI

In addition to immune regulators mentioned above, other plant components also play dual roles in PTI and ETI, suggesting additional converging points of the two pathways. For instance, two Arabidopsis receptor-like kinases, ANXUR1 (ANX1) and ANX2, interact with BAK1 and BIK1 to interfere with the ligand-induced PRR complex formation and interact with RPS2 to promote RPS2 degradation, thereby negatively regulating both PTI and ETI [105]. Similarly, rice OsRac1 interacts with both the PRR co-receptor OsCERK1 and NLR Pit to form different complexes and positively transduce PTI and ETI signals [106]. Whether OsRac1 is similarly or differentially regulated during PTI and ETI and the temporal and spatial coordination of OsRac1 in two different complexes will be interesting topics of future research. The *miR472-*

RDR6 (RNA-dependent RNA polymerase 6) gene silencing pathway negatively regulates both PTI and ETI in Arabidopsis, via post-transcriptional control of a subset of mRNAs encoding CNL proteins, although the effect on PTI is likely indirect [107,108]. Interestingly, recent studies demonstrated that helper NLRs ADR1/NRG1, EDS1, PAD4 and SAG101, which were previously identified as key ETI components, are indispensable for fully activating PTI responses upon treatment of microbial PAMPs in Arabidopsis [38,41]. Therefore, helper NLRs and EDS1/PAD4/SAG101 seem to be additional intersectional points of PTI and ETI. Details of how these components are cross-regulated by PRRs and NLRs remain to be determined. In addition, key components of PTI pathway, such as BAK1 and MPK4, are guarded by NLRs [109-111], suggesting crosstalk between PTI and ETI at different contexts.

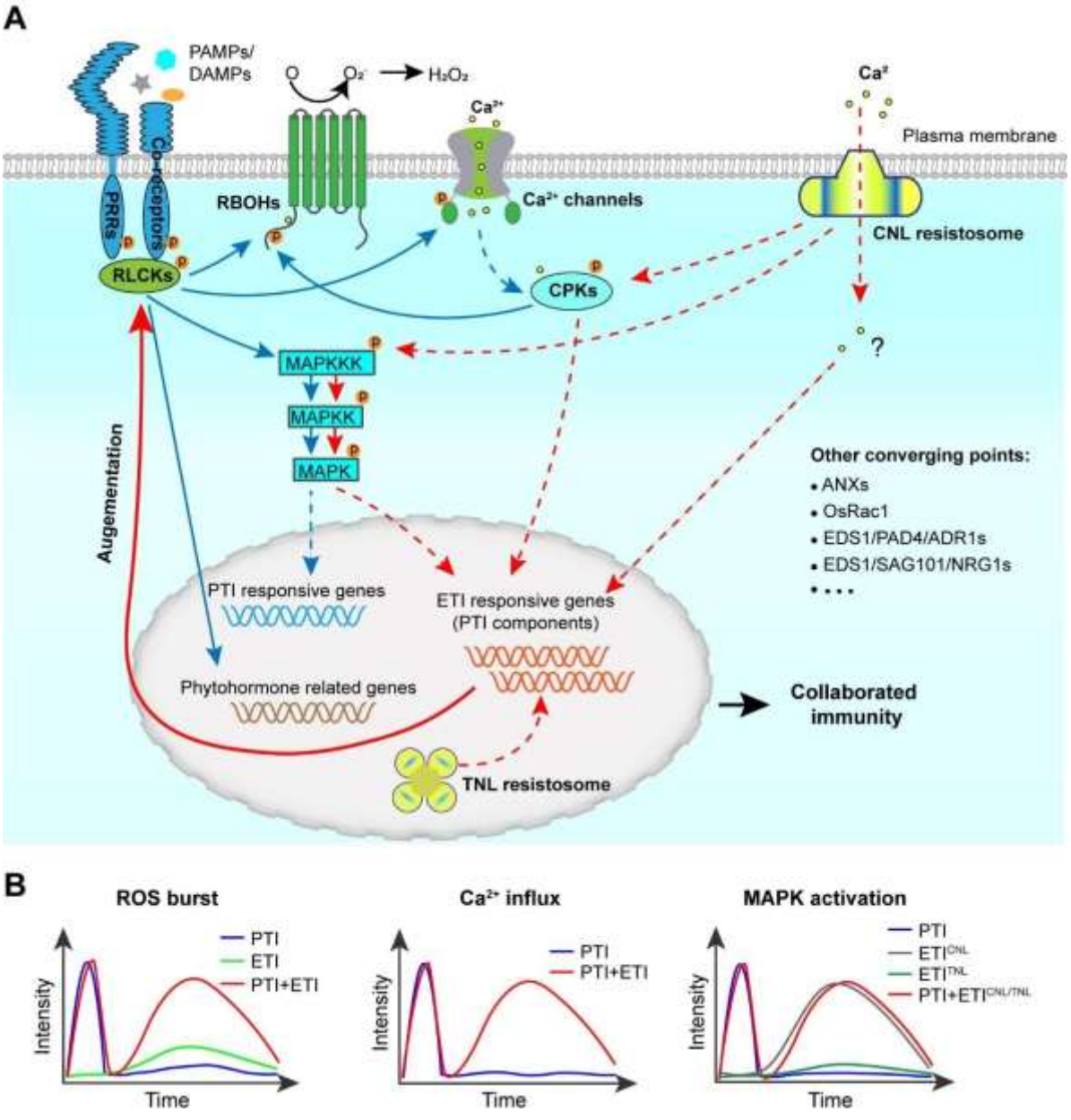
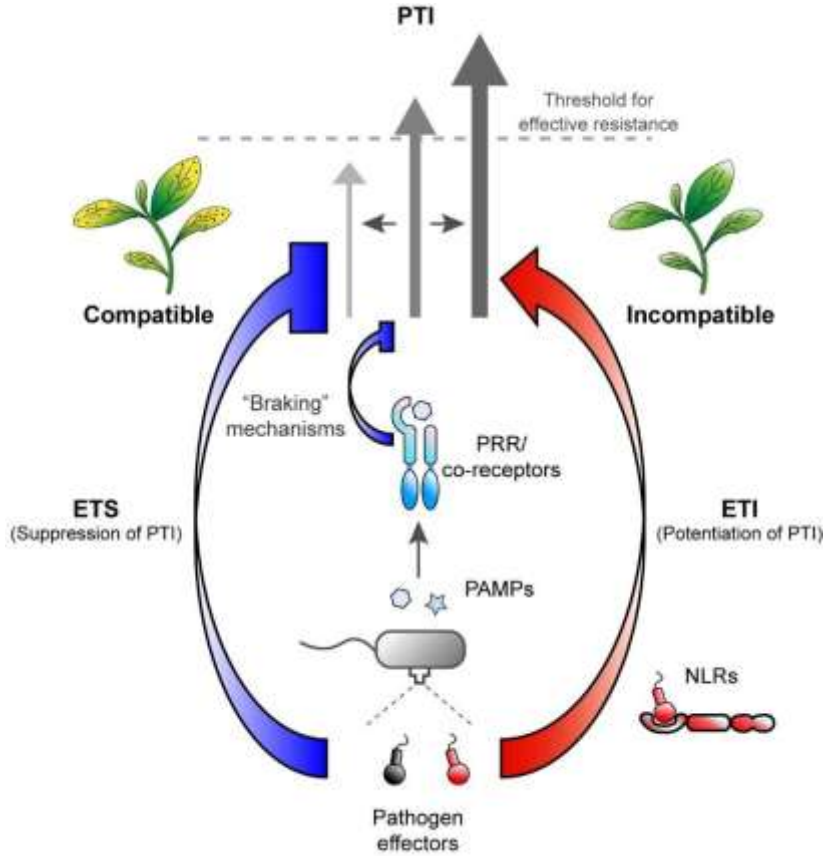


Figure 1. (A) PRR and NLR signalling co-regulate immune responses and converge at multiple points. Upon recognition of PAMPs/DAMPs, cell surface-localized PRRs recruit co-receptors to form receptor complexes and activate downstream RLCKs or CPKs, which subsequently phosphorylate downstream

components (e.g., RBOHD, CNGCs/OSCA1.3, MAPKKKs, WRKYs) to trigger ROS burst, Ca^{2+} influx, MAPK activation, phytohormone production and transcriptional reprograming. NLRs form resistosomes upon activation, which eventually lead to overlapping immune responses but with different dynamics (B) and usually stronger amplitudes compared to PTI. Various studies showed that components such as RBOHD, RLCKs, CPKs and MAPK cascade contribute to and are regulated by both PTI and ETI. Additional PTI-ETI converging points are also indicated. Importantly, ETI resistance and responses are dependent on PTI pathway components and ETI potentiates PTI. Therefore, the two signalling cascades work together in a collaborative manner to ensure effective immunity. Solid arrows indicate direct effects and dashed arrows indicate indirect effects. Question marks indicate unknown mechanisms. **(B) Dynamics and amplitudes of immune responses under different conditions (i.e., PTI alone, ETI alone, or PTI+ETI).** The x-axis indicates time after treatment of elicitors or inoculation of pathogens to activate different signalling. "PTI" refers to responses upon application of PAMP alone (PTI) and "ETI" refers to responses upon conditional expression of ETI-eliciting effectors in transgenic plants. "PTI+ETI" refers to responses upon application/expression of both PAMP and ETI-eliciting effectors or inoculation of avirulent pathogens on plants. ROS production in ETI and MAPK activation in TNL-initiated ETI requires PRR signalling, while MAPK activation in CNL-initiated ETI seems to be independent of PTI. Whether calcium flux in ETI relies on PRR signalling is unknown.

Conclusions

It has been long postulated that ETI is an "accelerated and amplified PTI response" [1]. Indeed, recent studies have provided experimental evidence for the intricate crosstalk between PRR- and NLR-mediated immune signaling and started to unravel increasing points of connections between PTI and ETI. These results suggest a need for a refinement of the "zig-zag" model (Figure 2). It appears that PTI acts as the primary defense mechanism against pathogens (and the vast number of commensal microbes). Virulent pathogens use effectors to suppress PTI as a major mechanism of pathogenesis. NLR signaling upregulates key components of PRR signaling, compensating for the attenuation of PTI components by pathogens or endogenous negative feedback by plants [9,32,56]. In this refined model, ETI is not a separate immune pathway, but rather an amplification module that depends on the PTI machinery to function effectively. Many unsolved questions remain. Importantly, it is not clear how NLR signalling mechanistically converges onto PRR signalling. Resolving this question is critical to understand the co-regulation of PTI and ETI on many immune outputs, as shown in previous studies [78,104-107]. Similarly, it will be important to examine whether the relationship between PTI and ETI mostly discovered in Arabidopsis broadly applies to other host-pathogen systems. Lastly, it remains to be seen whether the increased understanding of the PTI-ETI relationship could stimulate innovative strategies to boost ETI through manipulation of PTI components and set a foundation for efficient and broad-spectrum disease control in modern agriculture.



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304 **Figure 2. An updated model of the plant immune system.** PTI acts as the primary defence mechanism
305 against pathogens and commensal microbes, and PTI components are under negative control by the
306 endogenous "braking" mechanisms of plants to prevent over-activation and, profoundly, by effectors
307 secreted by pathogens (blue blunt arrows). Activated NLRs trigger ETI, which potentiates and restores PTI
308 through upregulation of PTI components (red arrow). The two immune branches function in cohort to
309 provide robust resistance against pathogens. The final resistance output is the combination of i) inhibition of
310 PTI by ETS or endogenous "braking" mechanisms and ii) potentiation of PTI by ETI. ETS, effector triggered
311 susceptibility. "PTI+ETS" is usually associated with compatible interactions (on the left) and "PTI+ETI"
312 incompatible interactions (on the right).

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314 Conflict of interest statement

315 The authors declare no conflict of interest.

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108. Jiang C, Fan Z, Li Z, Niu D, Li Y, Zheng M, Wang Q, Jin H, Guo J: ***Bacillus cereus* AR156 triggers induced systemic resistance against *Pseudomonas syringae* pv. *tomato* DC3000 by**

suppressing miR472 and activating CNLs-mediated basal immunity in Arabidopsis. *Mol Plant Pathol* 2020, **21**:854-870.

109. Wu Y, Gao Y, Zhan Y, Kui H, Liu H, Yan L, Kemmerling B, Zhou JM, He K, Li J: **Loss of the common immune coreceptor BAK1 leads to NLR-dependent cell death**. *Proc Natl Acad Sci U S A* 2020, **117**:27044-27053.

110. Zhang Z, Wu Y, Gao M, Zhang J, Kong Q, Liu Y, Ba H, Zhou J, Zhang Y: **Disruption of PAMP-induced MAP kinase cascade by a *Pseudomonas syringae* effector activates plant immunity mediated by the NB-LRR protein SUMM2**. *Cell Host Microbe* 2012, **11**:253-263.

111. Takagi M, Hamano K, Takagi H, Morimoto T, Akimitsu K, Terauchi R, Shirasu K, Ichimura K: **Disruption of the MAMP-Induced MEKK1-MKK1/MKK2-MPK4 Pathway Activates the TNL Immune Receptor SMN1/RPS6**. *Plant Cell Physiol* 2019, **60**:778-787.

Dear Editors and Reviewers,

Thank you very much for your editorial guidance and constructive comments. We have revised our manuscript accordingly to 1) improve clarity, 2) include more "opinions" and comments of cited papers and 3) better convey the central message.

Here is a summary of the modifications made in the revised manuscript:

1. Discussions and analysis of cited papers have been added in a few sections to facilitate thinking and point out important questions remaining to be addressed in the future. Please see point-by-point responses below for details.
2. Figure 1 and 2 (including the legends) have been modified to clarify and better illustrate the "crosstalk and convergence between PTI and ETI pathways".
3. A new table has been added to summarize the substrate proteins of the RLCK family and the associated physiological outputs in PTI and ETI.
4. Species names in different studies have been clarified.
5. Editorial errors have been corrected.

Again, we really appreciate the great suggestions, which improved our manuscript. Below are responses to each of reviewers' questions. We hope that you find the revised manuscript now meets your expectation for publication in COPB.

Comments from the Editors and Reviewers:

You will note that both reviewers feel that while you have done a good job listing the facts of your topic, there is not enough synthesis of ideas in the current version of the paper. It is very important that you carefully consider each of these points and generate a major revision of your review. Please include a document summarizing your revisions as well.

Reviewer 1: This is a very timely review that addresses the crosstalk between pattern- and effector-triggered immunity in plants. It is of high interest as the question of whether and how the two layers of plant immunity are linked has been addressed for approximately twenty years now. Emerging evidence suggests mechanistic connection as ETI and PTI immune outputs are overlapping. Likewise, full resistance (ETI) depends on PTI. Thus, I fully support this review as it raises awareness in the community for the fact that a major knowledge gap is going to be narrowed down pretty soon. I say that despite the fact that a mechanistic understanding of how this

interdependence works is still lacking. The authors have done a great job in compiling existing literature. My impression is though that the authors have (in addition to their meticulous listing of literature) shied away from at least some informative speculation on how such link(s) could be brought about. In other words, what I miss to a certain extent here is the author's OPINION. I believe this should be an important element of Current Opinion reviews.

Response: Thank you very much for your positive comments and constructive suggestions. We have now added more discussion and opinions in a few places (lines 70-75, 96-99, 130-133, 191-199, 212-213, 232-234, 249-251, and 257-259).

I list a few more queries that the authors may want to address to improve their nice work.

1. Title: I wonder whether the view is rather comprehensive than 'united'?

Response: We intended to highlight the interdependence between the two layers of plant immunity in the title, and have now changed “united” to “integrative”, to better convey the point.

2. line 46: The authors state that '..PTI and ETI involve activation of two distinct classes of receptors.' In this absoluteness this is not correct. Rice Xa21 (a LRR-RK) and tomato Cf receptors (LRR-RPs) confer ETI similar to that brought about by NLRs.

Response: Thank you for pointing this out. Indeed, there is blurred distinction between ligands perceived (and the corresponding receptors) to trigger PTI or ETI. This review discusses “NLR-mediated ETI” and “PRR-mediated PTI”. We have clarified this in the Introduction (Page 2, lines 47-48 and lines 53-54).

3. line 105-109. This sentence should be split into two as it is not understandable.

4. line 123: 'convoluted interactions'? Should it better read 'complex interactions'?

Response: Corrected.

Figure 1: I don't understand the term 'collaborated immunity'.

Response: Sorry for the miscommunication. “Collaborated immunity” refers to the co-regulation of many immune components by PRR and NLR signaling. Furthermore, ETI resistance and responses (e.g. HR, ROS production) are dependent on PTI pathway components and ETI potentiates PTI. Therefore, PRR and NLR signaling cascades work together in a collaborative manner to ensure a robust immunity. We have revised the legend accordingly and made some additional modifications in Fig. 1 (Page 8-9, lines 263-273), to highlight the “co-regulation and convergence of PTI and ETI pathways” (as commented in the next question).

Figure 2: I have to admit that I do not understand this figure at all. I admit that it is probably difficult to depict mutual potentiation of ETI and PTI pathways. However, the idea that both pathways merge at some point (the key idea of this review) does not become clear to me.

Response: We appreciate the referee’s criticism. The model in Figure 2 provided an updated view of the relationship between PTI, effectors and ETI (based on the zig-zag model proposed in 2006), with new elements (e.g., ETI potentiation of PTI, endogenous negative control of PTI by plants) added. We have modified Fig. 2 and legend to make it clearer (page 10).

It would be difficult to depict multiple converging points of PTI and ETI in one single model, without appearing over-complicated. We have modified Fig. 1 and legend to highlight the “convergence of PTI and ETI pathways”, as described above.

The reference list must be completed. Ref's 6, 38, 40, 46, 47, 48, 67, 71, and 98 are incomplete (lacking issue and page numbers).

Ref's 68 and 95 give journal names in full, whereas journal names are abbreviated in all other citations.

Response: Corrected.

Ref. 51 is highlighted (one star). I wonder whether a ref from 2011 needs particular attention?

Response: We have removed the star.

Thank you very much!

Reviewer 2: The review by Yuan, et al. presents progress from several recent papers in our understanding of how plant signaling traditionally defined as separate PTI and ETI pathways are in fact intertwined. This is an exciting area with several recent developments, and a timely topic for a review. The review by Yuan, et al. is a useful resource for categorizing these recent findings, but could do more to synthesize findings from different papers and clarify important questions moving forward.

Response: We appreciate this reviewer's comments and great suggestions. We have added discussions and analysis of cited papers in a few places, as detailed below.

One area that has been highlighted by recent papers which could be analyzed here is the nature of ETI responses outside of cell death/the hypersensitive response. Both recent NLR structure papers suggested mechanisms of NLR activation leading to cell death, but HR is not the only output of ETI. PTI components have been shown to be required for ETI-induced HR, but may be not required or even inhibitory for some other responses (e.g. EMPIS), in addition to differences depending on the class of NLR which is activated. Analysis of the cited papers with this distinction might bring clarity and/or highlight questions for the field moving forward.

Response: Many thanks for the good suggestion. Indeed, PTI signaling regulates HR and other ETI responses to different degrees and depending on specific NLR types. While HR is discussed in the same section with ETI resistance, the involvement of PTI in other ETI responses such as ROS and MAPK activation is described in separate sections ("Overlapping immune responses in PTI and ETI"). We have added a statement for clarification (Pages 3-4, lines 130-133) and more discussion in each section of immune response (ROS, Ca^{2+}, MAPK etc), as also described below.

In several places, a sentence or two at the end of a paragraph could transform it from a list of recent discoveries to a guide for future research. For example:

Paragraph lines 137-145: This paragraph incorporates 3 ideas (ETI effect on PTI is/isn't SA mediated, translational regulation in ETI/PTI, and transient PTI activation) but it is not clear to me if or how they are connected. Maybe the authors are arguing that ETI circumvents transience of PTI activation by stabilizing mRNA/translation of PTI components? This would be an interesting proposal but the SA connection is still not clear.

Response: Sorry about the confusion. This paragraph discusses possible mechanisms of ETI upregulation of PTI components. Experimental evidence suggests that this is not mediated by SA *per se* (we have added a sentence to make the transition better; page 4, lines 148-149). This reviewer is correct that we propose rescuing

mRNA/translation of PTI components by ETI as a potential mechanism, which remain to be determined in the future.

Paragraph lines 169-184: Given that the CNL ZAR1 may form a Ca2+-permeable channel alone, do CNLs/TNLs have different requirements for CNGC2/4? Also, there it is important to highlight that CNGC2/4 seem only important for PTI signaling under specific (high) Ca2+ concentrations, and thus are unlikely to represent the 'core' Ca2+-permeable channels involved in PTI.

Response: We have added more discussion about calcium influx in ETI mediated by different types of NLRs (Page 6, lines 191-199). The conditional role of CNGC2/4 in PTI-associated calcium influx was described in an earlier section (Page 2, lines 71-72).

Paragraph lines 186-194. Does the difference in MPK activation requirements by different families line up with knowledge of the different NLR's activated forms/protein interaction requirements? If yes or no, what would be a next major question to answer?

Response: This is an interesting point. So far, studies demonstrated phosphorylation of MAPKs during CNL-, but not TNL-activation. We have also highlighted that EDS1 overexpression does not lead to MAPK activation (Cui et al., *New Phytol.* 2016). In our opinion, it is important to determine how CNLs activate MAPKs and whether it overlaps with PRR-signaling pathway in the future. Thus, we have modified the paragraph accordingly (Page 7, lines 212-213).

Section lines 196-217: What might be the meaning or mechanism of differential requirements for PTI activation in ETI-induced cell death vs. ETI-induced transcriptional reprogramming?

Response: This is a good point. The differential requirements of PTI signaling in HR and ETI-associated global transcriptional reprogramming suggest that the transcriptional activation of PTI-associated genes is not sufficient to trigger normal ETI responses. Post-translational regulation of key components (by PTI signaling) is important (Yuan *et al*., *bioxiv*., 2020). We have added a sentence to clarify (Page 7, lines 232-234).

The authors certainly do not need to highlight the questions mentioned here, but the review would benefit from more discussion of the implications of putting individual papers' conclusions together.

I also have several specific comments:

- Abstract: given the blurred lines between PAMPs and effectors (which both can be ligands for PRRs), it would be better to replace the terms 'PTI' with 'PRR-trigered immunity)' and 'ETI' with 'NLR-triggered immunity (NTI)'.

Response: This a good point. Indeed, this review discusses "NLR-mediated ETI" and "PRR-mediated PTI". We have clarified this in the Introduction (Page 2, lines 53-54).

- Line 34: 'immune system' should be 'the immune system'

- Line 39: 'ranging from' requires a 'to'. 'ranging from' could be replaced with 'including'

Response: Corrected.

- Lines 57-58: PEPRs are not recognizing "ligands derived from microbes".

Response: We have modified to "ligands derived from microbes or plants".

- Line 59: some RLPs do not have a cytoplasmic tail, but are GPI-anchored proteins (e.g. CeBIP).

- Line 61: SOBIR is not a co-receptor but is a signaling adapter, as it does not involved in ligand-binding.

Response: We have modified (Page 2, lines 62-63).

- Line 62: would suggest writing "The activated heteromeric receptor complex...".

Response: We have modified.

- Line 65: BIK1 is not the founding member of the RLCK family, as other RLCK were previously identified, e.g. Pto, ACIK1, etc...

Response: Thank you for pointing this out. We have changed to "one of the best-studied members of the RLCK family".

- Lines 66-67: highlight that CNGC2/4 seem only important for PTI signaling under specific (high) Ca2+ concentrations, and thus are unlikely to represent the 'core' Ca2+-permeable channels involved in PTI.

Response: We have modified to demonstrate the conditional role of CNGC2/4 in mediating calcium influx in PTI and added a discussion about PTI-involved calcium channels (Page 2, lines 71-72).

- section on OSCA1.3 could be move up to follow the part on CNGCs.

Response: We have moved this up (Page 2, lines 71-73).

- Line 75: "activates" should be 'activate'

Response: Corrected.

- Lines 64-79: A long list of RLCK targets is difficult to internalize, a table of RLCK, target, and reference would make this information more readily digestible and allow the text to focus on comparing PTI downstream signaling mechanisms (largely through RLCK transduction) to ETI signaling mechanisms (largely unknown).

Response: Great suggestion. We have added a table (Table 1) to describe the different substrate proteins of RLCKs and the associated physiological outputs in PTI and ETI. The role of RLCKs in PTI and ETI was also commented (Page 2-3, lines 67-78).

- Line 91: also mention recent Science paper on the TNL RPP1.

- Line 94: shouldn't be "in early signaling of PTI"?

- Line 137: 'is' should by 'are'

Response: Corrected.

- Lines 142-143: This statement should have a citation.

Response: We have cited one review (Couto and Zipfel., 2016., *Nat Rev Immunol*).

- Lines 153-154: RBOHD has already been introduced as NADPH oxidase (line 70)

- Line 155: 'ROBHD' should be 'RBOHD'

Response: Corrected.

- Line 163: should this be 'upregulated' or 'activated'?

Response: Here we refer to “ETI upregulation of RBOHD transcript and protein”. Therefore, it should be “upregulated”. We have clarified (Page 5, line 170).

- Line 165: 'plant' is redundant

- Lines 174, 177: 'constitutive SA level' should be 'constitutively elevated SA (level)'

- Line 203: 'are not easy to disentangle' should be 'cannot easily disentangle'

- Line 207: 'gene' should be 'genes'

Response: Corrected.

- Line 212: which Arabidopsis PRR/co-receptor mutant?

Response: We have clarified (Page 7, lines 231-232).

- Line 229: what are "RLP-type co-receptors"? LRR-RLP use BAK1/SERKs (which are LRR-RLKs) as co-receptors.

Response: Thank you for pointing this out. We have modified the sentence (Page 8, lines 255-257).

- Line 233: the function of BAK1 and MPK4 is not to be "guardees". As such, the sentence should be "...BAK1 and MPK4, are guarded by NLRs".

- Line 238: the figure appears to depict LRR domains of receptors within the plasma membrane, as well as the ZAR resistosome pore not fully crossing the plasma membrane.

- Line 271: 'converge' should be 'converges'

Response: Corrected.

- Line 274: the extension from Arabidopsis to other species is indeed important. In several cases throughout the review, results from other species were mentioned, but it would be beneficial to make clear in each section of the review whether the results discussed are thus far from Arabidopsis alone, or have already been shown to be applicable in at least one other species.

Response: We have specified species names and/or highlighted studies in other species in a few places (lines 75-77, 89, 91, 124, 162, 187, 239, 253). For studies in other species, species names were clarified in the previous version (e.g., lines 139-141, 215-217, 248-251).

Thank you very much!

declaration of competing interests

Declaration of interests

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

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: