Plant Physiology®

Update

Plant autoimmunity—fresh insights into an old phenomenon

Matthias Freh [ID] ,[1] Jinlan Gao,[2] Morten Petersen [ID] [2] and Ralph Panstruga [ID] [1,*,†]

1   Institute for Biology I, Unit of Plant Molecular Cell Biology, RWTH Aachen University, Aachen 52056, Germany
2   Institute of Biology, Functional Genomics, Copenhagen University, Copenhagen 2200, Denmark

*Author for communication: panstruga@bio1.rwth-aachen.de
†Senior author
R.P. designed the research. M.F., J.G., M.P., and R.P. wrote the paper.
The author responsible for distribution of materials integral to the findings presented in this article in accordance with the policy described in the Instructions for Authors (https://academic.oup.com/plphys/pages/general-instructions) is Ralph Panstruga (panstruga@bio1.rwth-aachen.de).

Abstract

The plant immune system is well equipped to ward off the attacks of different types of phytopathogens. It primarily relies on two types of immune sensors—plasma membrane-resident receptor-like kinases and intracellular nucleotide-binding domain leucine-rich repeat (NLRs) receptors that engage preferentially in pattern- and effector-triggered immunity, respectively. Delicate fine-tuning, in particular of the NLR-governed branch of immunity, is key to prevent inappropriate and deleterious activation of plant immune responses. Inadequate *NLR* allele constellations, such as in the case of hybrid incompatibility, and the mis-activation of NLRs or the absence or modification of proteins guarded by these NLRs can result in the spontaneous initiation of plant defense responses and cell death—a phenomenon referred to as plant autoimmunity. Here, we review recent insights augmenting our mechanistic comprehension of plant autoimmunity. The recent findings broaden our understanding regarding hybrid incompatibility, unravel candidates for proteins likely guarded by NLRs and underline the necessity for the fine-tuning of NLR expression at various levels to avoid autoimmunity. We further present recently emerged tools to study plant autoimmunity and draw a cross-kingdom comparison to the role of NLRs in animal autoimmune conditions.

Plant immunity at a glance

The plant immune system comprises two major realms: constitutive defense components and induced immune responses. The former encompasses preformed mechanical barriers like the waxy cuticle as well as chemical components such as constitutively produced antimicrobials (Wittstock and Gershenzon, 2002; Yeats and Rose, 2013). The latter rests on multiple interconnected cellular reactions in response to the perception of specific pathogen-linked molecules, which ultimately slows down or fully impedes pathogen proliferation, sometimes in association with localized host cell death (Zhou and Zhang, 2020). The induced immune responses can be further subdivided into two major branches: those triggered by extracellularly occurring molecules, both from the pathogen (pathogen-associated molecular patterns [PAMPs], Box 1) and the plant itself (damage-associated molecular patterns [DAMPs], Box 1), and those triggered by intracellularly delivered pathogen effector proteins (Box 1). Accordingly, these branches are referred to as PAMP-triggered immunity (PTI) and effector-triggered immunity (ETI), respectively (Box 1; Jones and Dangl, 2006; Zhou and Zhang, 2020). PTI mainly relies on membrane-resident receptor-like kinases (RLKs) as immune sensors, whereas ETI is primarily based on nucleotide-binding domain leucine-rich repeat (NLR) receptors that according to their N-terminal domain can be further

Open Access

ADVANCES

- Lesion mimic and autoimmune mutants exhibit activation of immunity in the absence of infection.
- Leading causes of autoimmunity include hybrid necrosis seen in intraspecies crosses involving incompatible alleles of immune genes, mutations in *NLR* genes leading to increased expression or gain-of-function that trigger ETI, mutations in effector targets sensed by specific NLRs that trigger ETI, and mutations in negative regulators of immunity (e.g. transcriptional repressors and proteasomal components).
- Fine-tuning of *NLR* expression at all levels is crucial to avoid autoimmunity.
- Advanced tools to study NLR-mediated autoimmunity have recently emerged.

subdivided into Toll/interleukin receptor type NLRs (TNLs) and coiled-coil type NLRs (CNLs) (Box 1). Apart from "full-length" TNLs, C-terminally truncated versions without leucine-rich repeats exist. These TN proteins are thought to co-operate with TNLs as complexes (Zhang and Gassmann, 2003; Liang et al., 2019; Cai et al., 2021; Chen et al., 2021).

While some NLRs perceive pathogen effectors by direct binding, others monitor ("guard") the status of host proteins (the "guardees") including their possible biochemical manipulation by pathogen effectors (Figure 1, A and B). In case of manipulation or even degradation of the guardee by pathogen effectors, the respective NLR gets activated and induces downstream signaling, which ultimately leads to ETI-associated plant defense responses (Khan et al., 2016; Ngou et al., 2021). NLR activation often leads to a hypersensitive response (HR), frequently culminating in cell death at the site of pathogen

ingress, to inhibit the growth of (hemi-)biotrophic pathogens that rely on living host cells (Balint-Kurti, 2019).

Although it was originally assumed that individual NLR proteins are sufficient for the induction of resistance via ETI (Flor, 1971; Hammond-Kosack and Jones, 1997; Jones and Dangl, 2006; Rafiqi et al., 2009), it was recently discovered that the situation can be more complex. While indeed some "singleton" NLRs operate autonomously, such as the CNLs Mildew locus a 10 (Mla10) from barley (*Hordeum vulgare*) and HOPZ-ACTIVATED RESISTANCE 1 (ZAR1) from Arabidopsis (*Arabidopsis thaliana*; Maekawa et al., 2011a; Seto et al., 2017), many NLRs seem to work either in pairs or as components of complex networks (Adachi et al., 2019). In the latter case, "sensor" NLRs either directly or indirectly recognize pathogen effectors, while their associated "helper" NLRs (such as members of the ACTIVATED DISEASE RESISTANCE 1 [ADR1] and N REQUIREMENT GENE 1 [NRG1] families in Arabidopsis) mediate signaling downstream of "sensor" NLRs, thereby inducing strong defense responses and HR, probably by forming Ca^{2+}-permeable cation channels (Saile et al., 2020; Bi et al., 2021; Jacob et al., 2021).

Autoimmunity/lesion mimic phenotypes

Plant autoimmunity can generally be described as the genetically conditioned inappropriate (over-)activation of plant immune responses, leading to severe disadvantages of the affected plants (Bruggeman et al., 2015; van Wersch et al., 2016). Characteristic phenotypes associated with autoimmunity are (sometimes severely) stunted growth (dwarfism), leaf chlorosis and necrosis, runaway cell death, reduced reproductive fitness, and occasionally even plant lethality (Table 1). An overactivation of immune responses may be caused by mutations or downregulation of negative regulators of plant immunity or the misactivation or overactivation of immune sensors. The latter might be

Box 1 IMPORTANT ACRONYMS IN PLANT IMMUNITY

- CNL: NLR with a coiled-coiled type N-terminal (head-) domain
- DAMP: molecule derived from plant—endogenous structures (e.g. the cell wall) that is released upon attack by pathogens or herbivores; can be recognized by specific plant cell surface receptors
- Effector protein: protein delivered by a pathogen; often interferes with the host defense response or metabolism
- ETI: plant immune response triggered upon recognition of pathogen effectors by intracellular NLR receptors
- NLR: Member of a plant protein family of intracellular immune receptors that either directly or indirectly recognizes pathogen effectors and subsequently induces immune reactions
- PAMP: pathogen-derived molecule, often from the cell wall or outer structures of pathogens, such as bacterial flagellin or fungal chitin. Can be recognized by specific plant cell surface receptors. Sometimes also referred to as microbe-associated molecular pattern (MAMP)
- PTI: plant immune response triggered upon recognition of extracellular PAMPs or DAMPs by cell surface receptors
- TNL: plant NLR with an N-terminal (head-) domain that resembles the intracellular domain of animal Toll and interleukin receptors
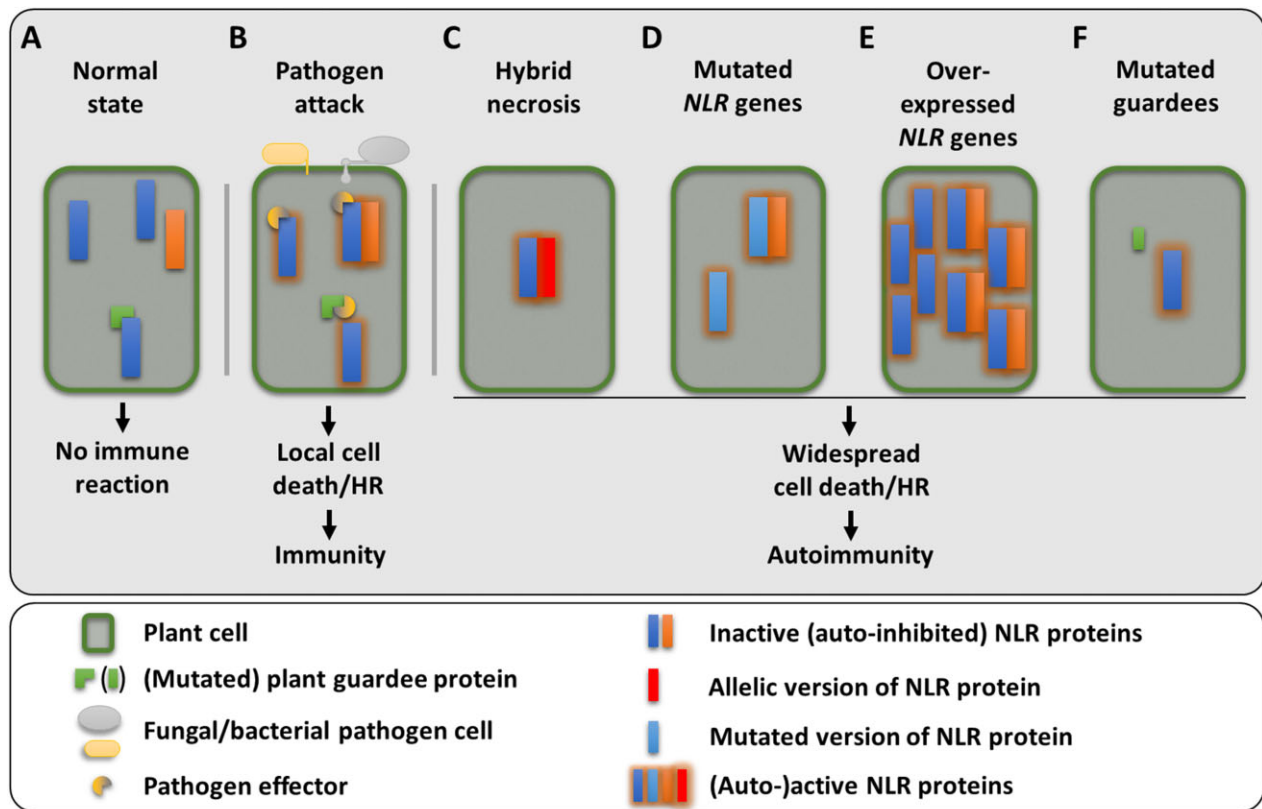
Figure 1 Mechanisms of pathogen perception and involvement of NLRs in plant autoimmunity. A, Inactive immune state without immune reaction mediated by NLRs. B, Immune reaction induced by effectors delivered during pathogen attack. The effectors are either directly recognized by NLRs or interact with guardee proteins guarded by NLRs. C–F, Autoimmunity induced by NLRs: (C) hybrid necrosis, (D) mutations in *NLR* genes, (E) overexpression of *NLR* genes, or (F) mutation of guardees that are monitored by NLRs.

caused by the absence or mutational modification of sensor NLR-monitored guardees (Chakraborty et al., 2018). In crop plants, autoimmune phenotypes have been classically described as "lesion mimic" phenotypes as they often resemble the lesions that plants develop in interactions with biotrophic pathogens (Walbot et al., 1983; Table 2). Later the term was also applied to mutants in Arabidopsis, and several of these were shown to be affected in the control of phytohormone and cell death pathways (Lorrain et al., 2003; Moeder and Yoshioka, 2008; Bruggeman et al., 2015). Hallmarks of autoimmunity comprise the spontaneous yet sometimes developmentally regulated accumulation of defense-related phytohormones such as salicylic acid (SA) and the constitutive expression of defense marker genes (Disch et al., 2016; Radojičić et al., 2018; Castel et al., 2019). Accordingly, second-site mutations in key regulators of plant immunity often relieve the autoimmune phenotypes due to a block in downstream immune signaling (Wang et al., 2013; Zhao et al., 2015; Rodriguez et al., 2016). Typical examples of the latter are components that play a key role in SA-dependent amplification of immunity such as ENHANCED DISEASE SUSCEPTIBILITY 1 (EDS1) and PHYTOALEXIN DEFICIENT 4 (PAD4; Vogelmann et al., 2012; Disch et al., 2016; Jia et al., 2021).

Hybrid incompatibility/necrosis

A classic form of autoimmunity is the so-called hybrid incompatibility or hybrid necrosis. This type of autoimmunity, which phenotypically was first reported 100 years ago (reviewed in Li and Weigel, 2021), can occur in heterozygous progeny of different genotypes of the same plant species that carry incompatible alleles of polymorphic immune signaling components (Bomblies and Weigel, 2007; Wan et al., 2020; Figure 1C). It is thought that within-species hybrid incompatibility represents an intermediate state along the trajectory from population isolation to speciation. Such intraspecific incompatibilities can involve unsuited interactions between *NLR* genes (Atanasov et al., 2018; Barragan et al., 2021), *NLRs* with *RLKs* (Alcázar et al., 2010), *NLRs* with other genes (Barragan et al., 2019), or even rely on non-*NLR* components of the immune system (Chen et al., 2014). An example of hybrid necrosis that has been intensively studied involves the *DANGEROUS MIX 2* (*DM2* also *RECOGNITION OF PERONOSPORA PARASITICA 1* [*RPP1*]-like) locus in Arabidopsis (Bomblies et al., 2007; Alcázar et al., 2014) (Table 1). This locus harbors seven to eight *NLR* genes (depending on the ecotype) whereof only one seems to be required and sufficient for the occurrence of hybrid incompatibility in several ecotype combinations, according to

Table 1 Recent examples of autoimmune phenotypes triggered by mutations or hybrid necrosis in *A. thaliana*

Responsible Gene	Phenotype	Protein Function/Annotation	Comment	References
acd6 (At4G14400)	Dwarfism and leaf necrosis	Resistance to *Pseudomonas syringae*, putative ankyrin and transmembrane domains	Dependent on SNC1 and SA-dependent	Rate et al. (1999); Zhu et al. (2018)
bak1 bkk1 (At4G33430, At2G13790)	Stunted growth and necrosis	Receptor-like kinases that act as co-receptors in PTI	Dependent on ADR1 family	Wu et al. (2020a)
cad1 (At1G29690)	Dwarfism and leaf necrosis	MACPF protein; putative negative regulator of SA-mediated programmed cell death	No guard protein known so far	Holmes et al. (2021)
camta3/sr1 (At5G01820)	Dwarfism and early leaf chlorosis	Putative calmodulin-binding transcription factor, acts in cold response pathway	Dependent on DSC1/2	Du et al. (2009); Lolle et al. (2017); Yuan et al. (2018)
cbp60b (At5G57580)	Stunted growth and delayed, bushy flowering	Calmodulin binding protein; putative activator of immunity	Dependent on SNC1	Li et al. (2021)
cngc20 (At3G17700)	Stunted growth	Cyclic nucleotide-gated channel	No guard protein known so far	Zhao et al. (2021)
DM2/RPP1-like (At3G44670)	Dwarfism	TNL, confers resistance to *Hyaloperonospora arabidopsidis*	Dependent on SULKI1/2, hybrid incompatibility between L*er* and Kas2, only expressed under low temperatures (14–16°C)	Atanasov et al. (2018); Ordon et al. (2021)
DM10 (At5G58120)	Lethal hybrid necrosis (~3 weeks)	Truncated allele of a TIR-NLR from Arabidopsis accession Cdm-0	Interacts with specific alleles of DM11	Barragan et al. (2021)
HR4 (At3G50480)	Impaired growth/development	Involved in pathogen resistance, interaction with RPP7	Hybrid necrosis in specific allele combinations with itself or RPP7	Xiao et al. (2005); Barragan et al. (2019); Li et al. (2020)
ka120 (At3G08960)	Severe dwarfism	Nuclear import receptor protein	Constrains the nuclear activity of SNC1	Jia et al. (2021)
lsd1 (At4G20380)	Runaway cell death	Negative regulation of basal defense, contains zinc-finger motifs	Induced under long-day conditions and low temperature	Dietrich et al. (1994, 1997); Huang et al. (2010b); Lv et al. (2019)
mekk1, mkk2, mpk4 (At4G08500, At4G29810, At4G01370)	From stunted growth up to extreme dwarfism	Components of a MAP kinase cascade involved in response to pathogens and abiotic stress	Guarded by SUMM2 and RPS6	Zhang et al. (2017b); Takagi et al. (2019)
saul1 (At1G20780)	Seedling lethality	E3 ubiquitin ligase, associated with senescence	Dependent on SOC3 together with CHS1 or TN2, as well as SUSA2	Tong et al. (2017); Liang et al. (2019, 2020)
srfr1 (At4G37460)	Dwarfism	Shows similarity to transcriptional repressors; putative negative regulator of defense responses	Dependent on SNC1; suppressed under elevated temperature	Kim et al. (2010); Garner et al. (2021)
topp4 (At2G39840)	Dwarfism and curly leaves	Serine/threonine phosphatase	Dependent on SUT1, HSP70, and RAR1	Yan et al. (2019)
tpr2, tpr3 (At3G16830, At5G27030)	Enhanced dwarfism in *srfr1* and *snc1* background	TOPLESS family proteins; negative regulators of SNC1 dependent autoimmune phenotypes	Work antagonistically to TPR1 in modulating SNC1	Garner et al. (2021)
zed1-D (At3G57750)	Dwarfism/compressed inflorescence growth	Pseudokinase that might act as a decoy for the *P. syringae* effector HopZ1a	Dependent on ZAR1, gain-of-function, dose-dependent effect	Lewis et al. (2013); Wang et al. (2017, 2019b)

recent findings (Ordon et al., 2021). An interesting case is the recently reported allele-specific incompatibility between the *RPP7 NLR* gene cluster and the non-*NLR* gene cluster encoding RESISTANCE TO POWDERY MILDEW 8/ HOMOLOG OF RPW8 (RPW8/HR) proteins. For this protein combination, the activation of autoimmunity is dependent on the number of HET-S-like repeats and the identity of the C-terminal tails in the RPW8/HR proteins in combination

Table 2 Examples of autoimmune phenotypes triggered by mutations in various crop plants

Organism	Responsible Gene	Phenotype	Comment	References
Soybean	dlm	Necrotic leaf speckles	Developing with the age of the leaf, light-dependent	Chung et al. (1998); Kim et al. (2005)
	spl-1/lm1 (Gm04g242300)	Necrotic leaf speckles	Copper ion binding protein, phenotype developing with the age of the leaf, light-dependent (enhanced under summer-planting conditions)	Al Amin et al. (2019)
	lmm2-1 (Gm14g003200)	Small chlorotic lesions, subsequently turning necrotic	Light-dependent, gene encodes a coproporphyrinogen III oxidase that participates in tetrapyrrole biosynthesis	Ma et al. (2020a)
Rice	lmi	Necrotic leaf lesions	Starting from the leaf-tip, lethal before the complete spike is ripened (few seeds), light-dependent	Liu et al. (2003)
	lmm9150/aba2 (Os03g59610)	Necrotic leaf lesions, preharvest sprouting, enhanced resistance to bacterial blight and rice blast, H_2O_2 accumulation	Alcohol dehydrogenase, abscisic acid biosynthesis	Liao et al. (2018)
	oscul3a	Red/brown scattered leaf lesions, ROS burst, lipid accumulation, increased resistance against bacterial blight and rice blast, reduced growth and yield	Light-sensitive (delayed development in the greenhouse versus the field)	Liu et al. (2017); Gao et al. (2019)
	osvoz1/osvoz2	Stunted growth, necrotic leaf lesions, and seedling lethality, in RNAi knockdown lines accompanied by ROS overaccumulation and enhanced resistance to rice blast	Transcription factors that modulate immunity against MoO	Wang et al. (2021)
	spl2	Spontaneous lesions and lesions triggered by wounding	Ozone hypersensitive, light-dependent	Kojo et al. (2006)
	spl3-t (Os03g06630)	Necrotic spots, connecting over the whole leaf during development, decreased plant height and seed setting rate, increased ear length	Light-dependent/induced	YuChun et al. (2018)
	spl11	Small spot-like necrotic lesions	E3 ubiquitin ligase protein	Zeng et al. (2004)
	spl36	Necrotic leaf lesions	Light-dependent	Yuchun et al. (2021)
	wlml1	Yellow to white patches and lesions, plant height, and grain weight reduced	Temperature-dependent (reduced under high temperatures)	Chen et al. (2018)
Barley	bspl1	Necrotic lesions on the whole leaf, H_2O_2 accumulation	Light-dependent/induced	Zhang et al. (2019)
	mlo	Necrotic leaf spots, early leaf senescence, spontaneous formation of callose-containing call wall appositions, durable resistance to powdery mildew		Wolter et al. (1993); Peterhänsel et al. (1997)
Cotton	Le4	Necrotic lesion leading to leaf shedding, abnormal thylakoid-structure, H_2O_2 accumulation in early leaf stages, infertile flowers	Hybrid necrosis, photoperiod-sensitive	Deng et al. (2019)

with specific RPP7 partners, which triggers the formation of a resistosome-like RPP7 oligomeric complex (Barragan et al., 2019; Li et al., 2020). Although the occurrence of hybrid necrosis is often linked to the presence of *NLR* clusters, typically exhibiting copy number variation of highly polymorphic *NLR* genes between genotypes within a species, a recent example demonstrates that this is not necessarily the case: *DM10* codes for a C-terminally truncated singleton NLR involved in hybrid incompatibility (Barragan et al., 2021). Given that hybrid necrosis and heterosis (enhanced trait performance of hybrids) could be considered as the extreme phenotypic outcomes regarding the evolutionary tradeoff between growth and immunity, breeding efforts should aim at balancing these two forces, for example, based on genomic data-driven reverse breeding (Calvo-Baltanás et al., 2021).

Autoimmunity based on constitutive activation or overexpression of NLRs

Apart from hybrid necrosis/hybrid incompatibility, autoimmunity can originate from activating mutations in *NLR* genes or overexpression of these (Figure 1D and E), often in the context of the above-mentioned sensor and helper NLR pairs or networks. Several of these mutants originally resulted from genetic screens in Arabidopsis. A prominent example of this is a gain of function mutation in *SUPPRESSOR OF NPR1-1, CONSTITUTIVE 1* (*SNC1*), a gene encoding a TNL protein (Zhang et al., 2003). In addition, mutations in several other genes (e.g. *BONZAI 1* [*BON1*], *BON ASSOCIATION PROTEIN 1* [*BAP1*], *BRI1-ASSOCIATED RECEPTOR KINASE 1* [*BAK1*]-*INTERACTING RECEPTOR-LIKE KINASE 1* [*BIR1*], *SUPPRESSOR OF RESISTANT TO PSEUDOMONAS SYRINGAE 4* [*RPS4*]-*RLD 1* [*SRFR1*], *CONSTITUTIVE EXPRESSER OF PR GENES 1* [*CPR1*], and *MITOGEN-ACTIVATED PROTEIN* [*MAP*] *KINASE PHOSPHATASE 1* [*MKP1*]) can result in SNC1-dependent autoimmunity, possibly via an SA-dependent upregulation of *SNC1* transcript levels in some of these mutants (Gou and Hua, 2012). Alternatively, some of the proteins encoded by these genes might be guarded by SNC1 (Rodriguez et al., 2016). Another recent addition to the list of putative SNC1 guardees is CALMODULIN-BINDING PROTEIN 60b (CBP60b), a calmodulin-binding protein that serves a role as a transcriptional activator of immunity. Unexpectedly, loss of function of CBP60b results in autoimmunity, and this EDS1-PAD4-dependent phenotype can be partially rescued by a lack of SNC1 (Li et al., 2021). The nucleocytoplasmically distributed SNC1 protein requires the nuclear import receptor IMPORTIN-α3/MODIFIER OF SNC1, 6 for nuclear translocation, and its nuclear localization is a prerequisite for autoimmunity (Lüdke et al., 2021). Yet another nuclear transport receptor, the karyopherin KA120, seems to modulate the nucleocytoplasmic homoeostasis of SNC1 or may serve as a further guardee of the NLR (Jia et al., 2021). SNC1 appears to exert its immunity-activating activity through a complex interplay with several regulators of plant immunity,

including SRFR1, a negative regulator of effector-triggered immunity, and TOPLESS-RELATED 2 (TPR2) and TPR3, two antagonistically acting members of the TOPLESS family of transcriptional co-repressors (Garner et al., 2021). Another example of a gain-of-function mutant is provided by the *TN* gene *CHILLING SENSITIVE 1* (*CHS1*), which induces temperature-dependent autoimmunity by enhanced protein interaction with the TIR domain of the TNL encoded by the neighboring *SUPPRESSOR OF CHS1-2, 3* (*SOC3*) gene (Zhang et al., 2017a). Similarly, autoimmunity conferred by the *chs3-2D* mutant, which is based on a dominant mutation in the TNL-encoding *CHS3* gene, relies on *CONSTITUTIVE SHADE-AVOIDANCE 1* (*CSA1*), another TNL-encoding gene that resides in very close (<4 kb) chromosomal proximity to *CHS3* (Xu et al., 2015).

Apart from activating gain-of-function mutations, autoimmunity can be triggered by overexpression of *NLR* genes or truncated versions thereof (Lai and Eulgem, 2018; Figure 1E). A typical example is the temperature-dependent autoimmunity conditioned by the overexpression of full-length *RPS4* (Heidrich et al., 2013). However, also overexpression of truncated NLR variants can trigger cell death and autoimmunity. For example, expression of a version of the NLR RPS5 lacking the C-terminal leucine-rich repeats results in constitutively activated programmed cell death, possibly due to a lack of sufficient autoinhibition in its truncated form (Ade et al., 2007). Notably, not only the overexpression of NLRs can result in autoimmunity but also the combined overexpression of EDS1 and PAD4 or the overexpression of the common RLK co-receptor BAK1 or its membrane-associated ectodomain, each triggering similar downstream defense responses as overactivated or overexpressed NLRs (Domínguez-Ferreras et al., 2015; Cui et al., 2017). Since accumulating evidence points to a mutual interplay between ETI and PTI (Ngou et al., 2021; Yuan et al., 2021), the constitutive activation of PTI pathways may be similar to the inadequate activation of NLRs result in autoimmunity.

Autoimmunity based on the lack or modification of an NLR-guarded protein

Mutations that affect a guardee can also condition autoimmunity. Because of the absence or modification of a guardee protein, the respective guarding NLR might become activated, resulting in constitutive defense responses (Figure 1F). An example of this scenario is the RESISTANCE TO PSEUDOMONAS SYRINGAE PV MACULICOLA 1 (RPM1)-INTERACTING PROTEIN 4 (RIN4), which is guarded by two NLRs (RPS2 and RPM1). Lack of RIN4 results in seedling lethality that can be rescued by second-site mutations in *RPS2* and other immune signaling components (Mackey et al., 2003). Another case is the monitoring of the E3 ubiquitin ligase SENESCENCE-ASSOCIATED E3 UBIQUITIN LIGASE 1 (SAUL1) by the TNL SOC3. After SOC3 was found earlier in a suppressor screen for autoimmunity caused by the *saul1-1* mutation (Tong et al., 2017), it was recently shown that SOC3 pairs with the genetically linked TN-type

proteins CHS1 and TIR-NBS2 (TN2) to monitor SAUL1 homeostasis (Liang et al., 2019). The SOC3-CHS1 and SOC3-TN2 complexes additionally require the F-Box protein SUPPRESSOR OF SAUL1, 2 (SUSA2) for downstream activation of immune responses, linking these responses to the degradation of cellular proteins via polyubiquitination by E3 ligases (Liang et al., 2020). TN2 appears to guard additionally the exocyst complex subunit EXOCYST SUBUNIT EXO70 FAMILY PROTEIN B1 (EXO70B1). Loss-of-function *exo70b1* mutants exhibit hyperactivated defense responses, including spontaneous cell death. This phenotype is dependent on TN2, which also interacts with EXO70B1 in yeast and in planta (Zhao et al., 2015). A similar model was proposed for the negative regulator of immunity, CALMODULIN-BINDING TRANSCRIPTION ACTIVATOR 3 (CAMTA3), and its guard NLRs DOMINANT SUPRESSOR OF CAMTA3 NUMBER 1 (DSC1) and DSC2. The latter were identified in a screen based on the expression of dominant-negative (DN) P-loop mutants of these NLRs in the background of the autoimmune *camta3* mutant (Lolle et al., 2017; Yuan et al., 2018). Yet another presumed guard–guardee pair are the TYPE ONE SERINE/THREONINE PROTEIN PHOSPHATASE 4 (TOPP4) phosphatase and the CNL SUPPRESSORS OF TOPP4-1 (SUT1). Autoimmunity conditioned by the *topp4* mutant is dependent on the immune receptor chaperones HEAT SHOCK PROTEIN 70 (HSP70) and REQUIRED FOR MLA RESISTANCE 1 (RAR1), and it is suppressed by lack of SUT1. Furthermore, SUT1 physically interacts with TOPP4 (Yan et al., 2019), while the previously mentioned CNL ZAR1 guards HOPZ-ETI-DEFICIENT 1 (ZED1) and several other temperature-dependent (pseudo-)kinases, that in turn modulate the transcription of *SNC1* (see also above—(Wang et al., 2017)).

A complex situation is provided by the monitoring of CALMODULIN-BINDING RECEPTOR-LIKE CYTOPLASMIC KINASE 3 (CRCK3) via the NLRs SUPPRESSOR OF MKK1 MKK2 2 (SUMM2) and RPS6. CRCK3 is a constitutively phosphorylated substrate of the MAPK/ERK KINASE KINASE 1 (MEKK1)-MAP KINASE (MPK)/ERK KINASE 1/2 (MKK1/2)-MAP KINASE 4 (MPK4) MAP kinase cascade. Functional disruption of this cascade results in SUMM2-dependent autoimmunity (Zhang et al., 2017b). The activation of SUMM2 by yet another MPK kinase kinase, MEKK2, requires a trimeric complex of two RLKs and a glycosylphosphatidylinositol-anchored protein (Huang et al., 2020). In addition to SUMM2, RPS6 also monitors the same MPK cascade comprised of MEKK1, MKK1/2, and MPK4 (Takagi et al., 2019). Proper *RPS6* transcript accumulation requires the RNA helicase HUA ENHANCER 2 (HEN2), and, accordingly, *hen2* mutants partially rescue the MPK cascade-associated autoimmune phenotype (Takagi et al., 2020). Interestingly, mutations in the mRNA decay genes *PAT1* (a gene with homology to yeast *PAT1*) and *SMG7* (a gene with homology to human *SMG7*) lead to autoimmune phenotypes, which in *smg7* depend on a specific allele of *RPS6* while autoimmunity in *pat1* is suppressed by mutations in

SUMM2 (Gloggnitzer et al., 2014; Roux et al., 2015). Thus, RPS6 and SUMM2 apparently also monitor the integrity of mRNA decay pathways, suggesting that these pathways are likely targets of phytopathogen effectors. We note that mutations in other decay components also lead to autoimmunity (Rayson et al., 2012; Chantarachot et al., 2020), raising the possibility that they may also be guarded by NLRs.

In addition to these well-studied cases, there are some recent reports of additional mutants that exhibit EDS1-dependent autoimmunity, suggesting that also in these instances a (guarding?) NLR protein might be involved. These comprise a recessive gain-of-function mutation in the *CYCLIC NUCLEOTIDE-GATED CHANNEL 20* (*CNGC20*) gene (Zhao et al., 2021) and a missense mutation in the *CONSTITUTIVE ACTIVE DEFENSE 1* (*CAD1*) gene (Holmes et al., 2021). Interestingly, double mutants of the genes coding for the common ETI-associated microbe-associated molecular pattern (MAMP) co-receptor BAK1 and its paralog BAK1-LIKE 1 (BKK1) exhibit spontaneous cell death reminiscent of autoimmunity. This phenotype is dependent on the ADR1 family of helper NLRs, suggesting that BAK1 and BKK1 might also be guarded by a yet unrecognized sensor NLR (Wu et al., 2020a). Another example is the double mutant of *PENETRATION 1* (*PEN1*) and its paralog, *SYNTAXIN OF PLANTS 122* (*SYP122*), encoding plasma membrane-resident syntaxins, which also exhibits a severe autoimmune phenotype (Zhang et al., 2008). This requires the deubiquitinase-ASSOCIATED MOLECULE WITH THE SH3 DOMAIN OF STAM 3 (AMSH3), but intriguingly, *amsh3* knockout mutants are likewise lesion mimic mutants, and lesions in both *PEN1/SYP122* and *AMSH3* could at least be partially rescued by mutations in *EDS1* and/or *PAD4* (Schultz-Larsen et al., 2018). Mutations in several genes implicated in ceramide metabolism also result in autoimmunity. This includes mutations in *ACCELERATED CELL DEATH 5* (*ACD5*), coding for a ceramide kinase (Liang, 2003; Zeng et al., 2021), and in *NEUTRAL CERAMIDASE 2*, a ceramidase-encoding gene (Zeng et al., 2021). Given that the disruption of multiple components involved in ceramide metabolism causes EDS1/PAD4/ SA INDUCTION DEFICIENT 2 (SID2)-dependent autoimmune phenotypes, several NLRs may monitor ceramide metabolism and/or membrane structure and integrity. In line with this idea, disruption of *INOSITOLPHOSPHORYLCERAMIDE SYNTHASE 2* (*IPCS2*) displays *RPW8*-dependent spontaneous HR-like cell death (Wang et al., 2008) and knockout of the ceramide-1-phosphate transfer protein *ACD11* results in autoimmunity that depends on the NLR LAZARUS 5 (LAZ5; Palma et al., 2010). In summary, there is a rapidly growing list of guard–guardee pairs that can be linked to autoimmunity in Arabidopsis.

Fine-tuning of NLR expression is essential to prevent autoimmunity

The *NLR* gene family is highly expanded in plants and the Arabidopsis genome encodes some 150 NLRs

(Meyers eal., 2003) while around 500 are found in rice (*Oryza sativa*; Zhou et al., 2004b; Li et al., 2010). *NLRs* tend to pair or appear in genomic clusters, which can expand or shrink rapidly probably as a consequence of adaptation to evolving pathogens. While singleton NLRs usually form homo-oligomers, paired and clustered NLRs are likely to co-function by hetero-dimerization or oligo-merization (van Wersch and Li, 2019). To keep pace with an almost unlimited number of pathogen effectors with a limited set of *NLR* genes while at the same time avoid-ing the inappropriate activation of immune responses, *NLR* transcript integrity and abundance must be tightly regulated. Accumulating evidence indicates that NLR ho-meostasis is not only regulated transcriptionally and posttranscriptionally (e.g. by [alternative] mRNA splic-ing) but also at the translational and posttranslational level (Li et al., 2015; Lai and Eulgem, 2018; Richard et al., 2018; Sun et al., 2020; Ma et al., 2020b; Wu et al., 2020b; Parker et al., 2021), and that malfunctions in any of these processes can give rise to autoimmunity. The analysis of the Arabidopsis *acd11* lesion mimic mutant led to the identification of *LAZ2*, encoding a histone ly-sine methyltransferase likely implicated in the epigenetic regulation of the *LAZ5* gene, which encodes an RPS4-like NLR protein that is responsible for triggering cell death in the absence of ACD11 (Palma *et al.* 2010). *NLR* genes are generally under tight transcriptional control (Borrelli et al., 2018). A loss of negative regulation can result in autoimmunity, as evidenced by loss of SRFR1-mediated fine-tuning of *SNC1* expression (Garner et al., 2021). An interesting case is the histone-binding protein ENHANCED DOWNY MILDEW 2 (EDM2), which pro-motes expression of some *NLR* genes while suppressing the expression of others, thereby balancing *NLR* tran-script levels (Lai et al., 2020). *NLR* genes are further pref-erentially subject to alternative poly(A) site selection and associated premature termination of transcription, which is mediated by the RNA-binding protein FLOWERING PROTEIN A (FPA; Borrelli et al., 2018; Parker et al., 2021) and can give rise to nonsense-mediated RNA decay. In some cases, *NLR* transcript ac-cumulation is further adjusted by small RNAs (miRNA-derived siRNAs; Zhai et al., 2011). NLR protein homeo-stasis is controlled via ubiquitination-based and proteasome-dependent protein turnover, as suggested by the recent identification of two master E3 ubiquitin ligases (SNIPER1 and SNIPER2) in Arabidopsis that can ubiquitinate the nucleotide-binding domain of sensor NLRs, thereby ensuring their adequate levels (Wu et al., 2020b). A contribution of the proteasome in adjusting NLR abundance was revealed by the role of the protea-some regulator PROTEASOME REGULATOR 1 (PTRE1) in controlling SNC1 accumulation (Thulasi Devendrakumar et al., 2019). At the posttranslational level, NLR proteins are autoinhibited in the resting state in nonchallenged plant cells and only become active upon suitable triggers (reviewed in Chiang and Coaker, 2015).

Modulation of autoimmunity by environmental conditions

Independent of the species, many plant autoimmune phe-notypes seem to be strongly modulated by external abiotic factors. These include temperature, light irradiance, photo-period, and humidity. Most frequently described have been influences by light and temperature, where dependence on light intensity has been observed in many crop autoimmune mutants (Table 2), while many Arabidopsis autoimmune mutants show sensitivity toward elevated or reduced tem-perature. Autoimmunity in the Arabidopsis *saul1-1* mutant conferred by the TNL SOC3, for example, is suppressed un-der high temperature ($>24°C$; Disch et al., 2016). Additionally, several of the "chilling-sensitive" (*chs*) mutants identified in genetic screenings for sensitivity at low temper-ature turned out to be dependent on TNL or TN-type fam-ily members, and expression of the autoimmune phenotype often involves additional neighboring TNLs (Schneider et al., 1995; Yang et al., 2010; Huang et al., 2010a; Zbierzak et al., 2013; Xu et al., 2015; Liang et al., 2019). Conversely, autoim-munity conferred by the *zed1-D* mutation in a gene encod-ing a heat-induced pseudokinase, is only expressed under higher temperatures (Wang et al., 2017). Similar to heat, high humidity may also suppress autoimmunity. Stunted growth and constitutive expression of *RPW8* and *EDS1* in *suppressor of SA insensitive 4* (*ssi4*) mutant plants are, for ex-ample, suppressed by high humidity (Zhou et al., 2004a). In addition, spontaneous HR-like lesions in *RPW8* overexpres-sion lines could also be delayed or suppressed by high hu-midity, low light, and high temperature, probably by disrupting an EDS1/SA-dependent amplification circuit (Xiao et al., 2003).

Although environmental conditions influence autoimmu-nity in many instances, the underlying mechanisms remain poorly understood. There is a general tendency that PTI is elevated at higher temperatures, while ETI, which typically relies on NLR activity, on the contrary is reduced (Cheng et al., 2013). Accordingly, HR-like cell death, which is an im-portant aspect of many autoimmune phenotypes, is typically abolished under high temperatures and on top of that is also often light-dependent (Balint-Kurti, 2019). Only few exceptions such as the *zed1-D* mutant behave differently, possibly due to specific characteristics of the involved guar-dees. A recent meta-analysis of whole-genome shotgun (RNA-sequencing) data sets revealed that generally the ex-pression of *NLR* genes is increased upon biotic stress (or treatment with SA) while their expression is mostly reduced under abiotic stress such as heat, but also drought or treat-ment with the phytohormone abscisic acid (Yang et al., 2021). This could explain why NLR-dependent autoimmune phenotypes are generally reduced at higher temperatures and often depend on the availability of light. One may also speculate that temperature-dependent alterations in NLR

conformation could be a reason for the temperature sensitivity of some autoimmune phenotypes. Several NLR proteins need the help of (co-)chaperones such as HSP90, RAR1, and SUPPRESSOR OF THE G2 ALLELES OF SKP1 (SGT1) for stability, function, and possibly oligomerization, which may fail at elevated temperatures (Seo et al., 2008; Kadota et al., 2010). Alternatively, or in addition, the temperature sensitivity of autoimmunity may relate to cell biological abnormalities in the respective mutants. A decrease in temperature leads to chloroplast and cell wall changes in the *saul1*-1 mutant and enhances its autoimmune phenotype. This was also seen in the *chs1* mutant, pointing at low temperature-induced alterations in chloroplast homeostasis and cell wall integrity, which may either directly or indirectly impact the activation of defense responses (Zbierzak et al., 2013; Disch et al., 2016). The erratic formation of (often cellose- and lignin-containing) cell wall deposits is a common characteristic of lesion mimic mutants. Data of a recent study now suggest that such cell wall deposition is a consequence and not the cause of temperature-induced autoimmunity, since reduction of cell wall deposition in *saul1*-1 *pmr4*-1 double mutants, which lack the callose synthase POWDERY MILDEW RESISTANCE 4 (PMR4), did not affect autoimmunity triggered upon exposure of these plants to low temperature (Hessler et al., 2021).

Autoimmunity in crop plants

While autoimmune mutants and their mechanistic link to plant immunity have been well studied in the past in Arabidopsis (van Wersch et al., 2016), crop plants have been investigated less thoroughly in this respect. Next to the original discovery of so-called "lesion mimic mutants" in maize (*Zea mays*; Walbot et al., 1983; Johal et al., 1995), over the years many examples for this type of autoimmune mutants have been discovered in crop plants like soybean (*Glycine max*), rice, barley, and cotton (*Gossypium hirsutum*; Table 2; Walbot et al., 1983; Wolter et al., 1993; Johal et al., 1995; Chung et al., 1998; Rostoks et al., 2003; McGrann et al., 2015; Chen et al., 2018; Liao et al., 2018; YuChun et al., 2018; Al Amin et al., 2019). In crop plants, extreme phenotypes such as dwarfism and stunted development have been rarely reported, probably because these phenotypes are already excluded during the initial steps of breeding. The same applies to hybrid incompatibility in crops. While this phenomenon is probably of high interest as it can severely hinder breeding between specific crop lines and natural accessions, such interactions would be excluded in early stages of the breeding process. Although there is very little known about the biochemical function of the proteins encoded by the genes that underlie these autoimmune phenotypes, there still are several traits that many of these mutants share. This includes the spontaneous formation of necrotic leaf spots and the accumulation of reactive oxygen species (ROS) (Table 2). On top of that, lesion mimic mutants mostly show increased resistance to obligate biotrophic pathogens, while the interaction with facultative or hemi-biotrophic

pathogens is more differentiated. For those, the interactions with lesion mimic mutants range from enhanced resistance to hyper-susceptibility (McGrann et al., 2015; Liao et al., 2018; Gruner et al., 2020). A prominent example of a lesion mimic phenotype in crop plants is the mutation of the so-called *Mlo* gene in barley. While the *mlo* mutant was originally discovered in the context of broad-spectrum powdery mildew resistance (Wiberg, 1973), it was later shown to also cause a lesion mimic phenotype whose intensity seems to depend on the genetic background (Bjørnstad and Aastveit, 1990; Wolter et al., 1993). As for many of the crop lesion mimic mutants, neither the biochemical function of the Mlo protein nor the mechanism underlying the *mlo* lesion mimic phenotype has been unraveled, yet (Kusch and Panstruga, 2017). It might be speculated that at least some of the "lesion mimic genes" encode guardees that, when absent or mutated, result in autoimmune responses due to the inadequate activation of their guarding NLRs.

As in Arabidopsis, the lesion mimic autoimmune mutants in crop plants could be used to examine the presumptive defense pathways that get (over-)activated in these mutants. The availability of high-quality genome sequences and considerable improvements in gene cloning technologies have enabled the identification of the underlying genes in several instances (Lolle et al., 2017; Liang et al., 2019; Yan et al., 2019). Rice *spotted leaf 11* (*spl11*) mutants, for example, display spontaneous cell death and increased resistance to both fungal and bacterial pathogens. *SPL11* encodes an E3 ubiquitin ligase that can be phosphorylated by a monocot-specific RLK (SPL11 CELL DEATH SUPPRESSOR 2 (SDS2). Phosphorylated SPL11 acts in two branches to regulate cell death and immunity via the ROS-producing NADPH oxidase RESPIRATORY BURST OXIDASE HOMOLOG B (OsRBOHB): either by mediating ubiquitination and degradation of SDS2, which interacts with and phosphorylates the receptor-like cytoplasmic kinases OsRLCK118/176, or by promoting degradation of SPL11-INTERACTING PROTEIN 6 (SPIN6), which is a Rho GTPase-activating protein also involved in OsRBOHB-dependent immunity (Zeng et al., 2004; Liu et al., 2015; Fan et al., 2018). Another E3-related autoimmune phenotype in rice is conferred by the *vascular plant one zinc-finger 1/2* (*osvoz1/osvoz2*) double mutant. The two transcription factors OsVOZ1 and OsVOZ2 co-function as positive regulators of the *NLR* gene *PYRICULARIA ORYZAE RESISTANCE Z-T* (*PIZ-T*) and are subject to ubiquitination by the E3 ligase AVRPIZ-T INTERACTING PROTEIN 10 (APIP10; Wang et al., 2021). SPL11 and APIP10 thus function differently than the Arabidopsis SNIPER E3 ligases, which regulate the turnover of NLRs by direct binding and ubiquitination (Wu et al., 2020b). It remains an open question if SNIPER-like E3 ligases exist in rice (and monocots in general) and whether SPL11 and APIP10 represent guardees of yet unrecognized NLRs. A very recent addition to the suite of crop lesion mimic mutants is the rice *spl36* mutant. The wild-type gene encodes a receptor-like protein kinase that regulates defense responses in rice (Yuchun et al., 2021). Despite these
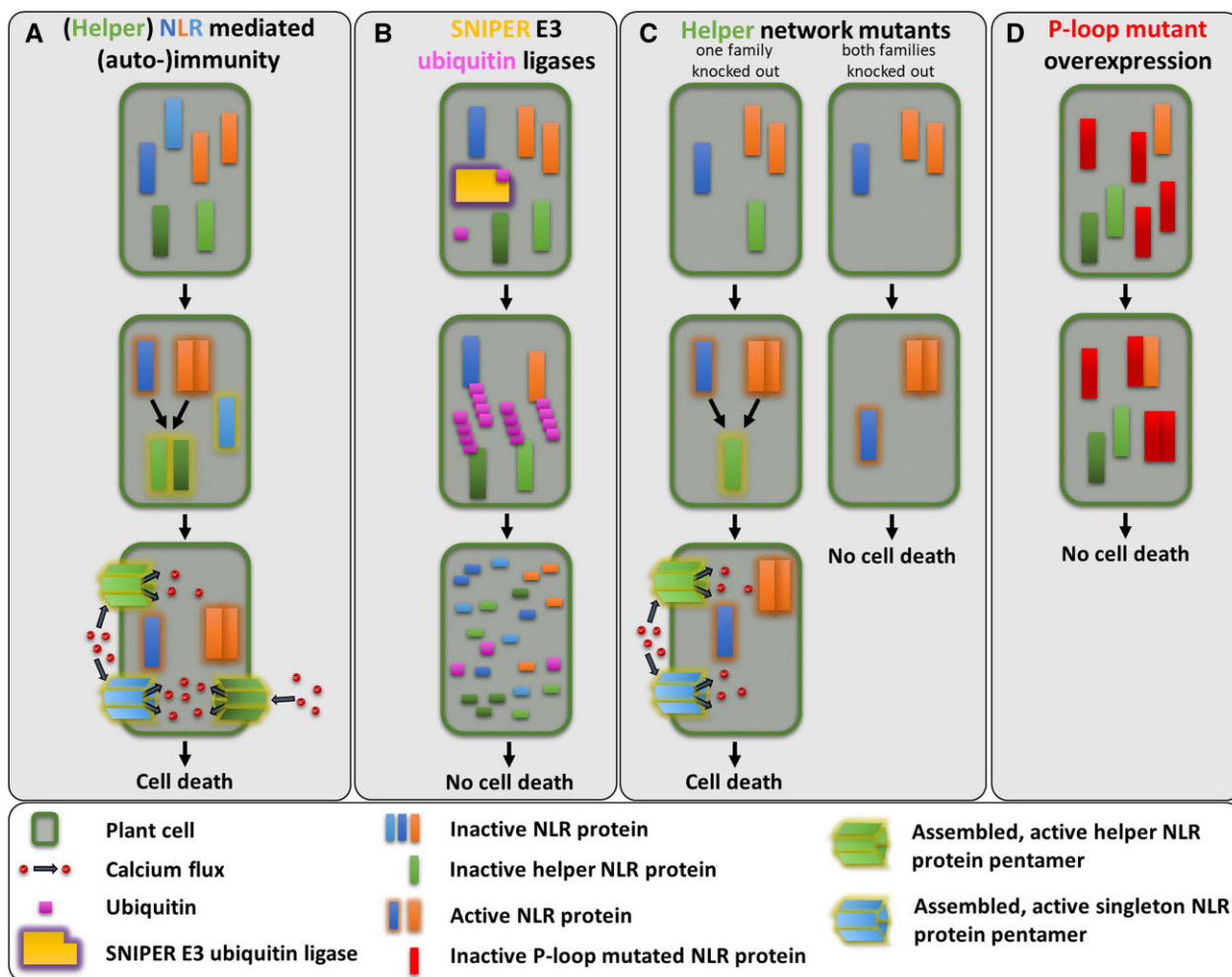
Figure 2 Mechanistic principles of three methods to investigate the involvement of NLRs in plant autoimmune mutants. A, (Auto-)immunity mediated by NLRs with or without the contribution of helper NLRs. Upon pathogen recognition or autoimmune induction by sensor NLRs, the sensor NLR itself or associated helper NLRs can form cation-permeable channels in the cell membrane. Influx of extracellular Ca^{2+} leads to cell death. B, SNIPER E3 ubiquitin ligases specifically target NLRs for proteasome-dependent degradation by poly-ubiquitination. Overexpression of *SNIPER* genes in autoimmune mutants is predicted to suppress the phenotype when NLRs are involved. C, Many sensor NLRs rely on the downstream signaling of helper NLRs (see (A)). Loss-of-function mutations in one or both families of helper NLRs can be a way to demonstrate the likely involvement of sensor NLRs relying on these families in autoimmune mutants. D, P-loop mutants are DN versions of specific NLRs with mutations in the P-loop motif of the nucleotide-binding domain. Expression of the respective DN allele in autoimmune mutants that depend on a specific NLR was shown to suppress the autoimmunity phenotype.

advancements, the longer generation times and more challenging transformation and regeneration protocols in crop plants often still represent severe bottlenecks for detailed functional analyses compared to the model plant Arabidopsis (Altpeter et al., 2016). Thus, it remains that the extensive research that is done in Arabidopsis continues to keep informing the understanding of autoimmune mutants in crop plants.

Tools to analyze NLR-mediated autoimmunity

Although NLRs often contribute in one or the other way to plant autoimmunity (Figures 1 and 2, A), their participation is not always easy to demonstrate. Experimental approaches

to interrogate a putative contribution of NLRs to an autoimmune phenotype are, thus, highly valuable and are best developed in Arabidopsis. Introgression of second-site mutations in *EDS1*, *PAD4*, and *NONRACE-SPECIFIC DISEASE RESISTANCE 1 (NDR1)* into the genetic background of lesion mimic mutants is widely used to gain a glimpse of whether TNLs or CNLs could be involved. A recently described tool to inquire the involvement of NLRs in autoimmunity is based on the so-called SNIPER E3 ubiquitin ligases. These Arabidopsis proteins specifically interact with the nucleotide-binding domains of sensor NLRs such as the CNL SUMM2, the TNL RPP4, and the TN protein CHS1, but not helper NLRs, tagging them with ubiquitin for proteasomal degradation (Figure 2B). *SNIPER* overexpression has been shown to suppress NLR-mediated autoimmunity in many

mutants including *snc1*, *chs1*-2, *chs2*-1, and *chs3*-2D (Tong et al., 2017; Huang et al., 2018; Wu et al., 2020b). Accordingly, transformation of *SNIPER* overexpression constructs into autoimmune mutants could be used to probe the contribution of any sensor NLRs in the process. Another approach to explore the putative role of NLRs in plant autoimmunity is to take advantage of the recently discovered helper NLR networks. Mutants that are deficient in one or several helper NLRs can compromise the activity of entire groups of sensor NLRs (Feehan et al., 2020; Saile et al., 2020). Such helper NLR mutants could suppress sensor NLR-dependent autoimmune phenotypes, thereby narrowing down the involved type of sensor NLR (Figure 2C).

Forward genetic screens represent a classic route to identify suppressors of any mutant phenotype, and *summ2* and *soc3* mutants were found to suppress autoimmunity in *mkk1/2* and *saul-1*, respectively, via such an approach (Zhang et al., 2012; Tong et al., 2017). However, this kind of screens can lead to the identification of any type of suppressor genes (not only *NLRs*) because they are unbiased. To search for NLRs involved in autoimmunity in a targeted manner, Jia and colleagues performed a genetic screen using an artificial microRNA library targeting 108 *NLRs* for gene silencing and identified *SNC1* to be responsible for the autoimmunity seen in the *ka120* mutant (Jia et al., 2021). However, in some instances, more than one NLR may contribute to autoimmunity. DN NLRs that carry an inhibitory mutation in the P-loop, the phosphate-binding motif in the NLR ATPase domain, are another way of identifying NLRs involved in autoimmunity that may surpass the issue of functional redundancy. Lolle and co-workers introduced P-loop mutations into 108 Arabidopsis *NLRs* and tested whether their (over-)expression in the genetic background of an autoimmune mutant (*camta3*) can result in a wild type-like phenotype. This approach led to the identification of two unrelated dominant suppressors of *camta3*, *DSC1* and *DSC2*, that could not be identified using single *NLR* knockout or knockdown approaches (Lolle et al., 2017; Figure 2D). Importantly, *DN-DSC1* or *DN-DSC2* expression is sufficient to bypass *camta3*-associated autoimmunity while *dsc1*/*camta3* and *dsc2*/*camta3* double mutants exhibit autoimmunity and both *NLRs* need to be knocked out to suppress the *camta3* phenotype. The mechanistic basis of the P-loop-associated DN effect is, however, still poorly understood but may relate to the known homo- and hetero-oligomerization of activated NLR proteins (Adachi et al., 2019). Finally, chemical genetics can be used to discover new players related to plant autoimmunity. Testing of 13600 low-molecular-weight compounds led to the identification of a substance (Ro 8-4304) that suppresses both the stunted growth and constitutive immune responses of the *chs3*-2D mutant. A subsequent genetic screen for *chs3*-2D-derived mutants that are insensitive to Ro 8-4304 uncovered several methylosome components involved in mRNA splicing as negative regulators of plant immunity (Huang et al., 2016).

NLR-mediated autoimmunity in animals

Just as in plants, animals deploy NLR proteins for the intracellular perception of non-self and modified-self molecules. In contrast to plants, where NLRs typically mediate the direct or indirect detection of pathogen effector proteins, at least in vertebrates they are primarily implicated in MAMP and toxin recognition. Mammalian NLRs function in cell-autonomous immunity, mediating proinflammatory cytokine responses, and triggering cell death (Motta et al., 2015). While the overall domain architecture of plant and animal NLRs is very similar, especially the N-terminal head- and central nucleotide-binding domains have kingdom-specific characteristics (Maekawa et al., 2011b). For mammalian NLRs, it has been known for a while that they can assemble into large apoptosome or inflammasome complexes via their head domains when activated (Cain et al., 2002; Zhao et al., 2011), and only recently, similar homo-oligomerization was also demonstrated for activated plant NLRs (Burdett et al., 2019; Martin et al., 2020). While these mammalian complexes activate downstream signaling by activating caspases, a group of proteases implicated in initiating apoptotic cell death (Mermigka et al., 2020), the recently discovered ZAR1 and RECOGNITION OF XopQ 1 (ROQ1) plant "resistosomes" are hypothesized to mediate membrane pore formation and cleavage of nicotinamide-adenine dinucleotide (NAD^+) for downstream signaling and cell death execution, respectively (Wang et al., 2019a; Martin et al., 2020).

Similar to plants, mutations in animal NLRs are associated with a broad range of autoimmune-type disorders, which are best studied in humans and mice. Mutations in the genes encoding the human Nucleotide-binding oligomerization domain-containing protein 1 (NOD1) and NOD2 receptors, for example, are linked to chronic autoinflammatory and autoimmune diseases. Notably, there seems to be no domain specificity regarding the mutations and the associated conditions caused, and the sites of mutations linked to autoimmunity seem to be different in plant and animal NLRs. While mutations in the leucine-rich repeat regions of NOD1 and NOD2 are related to asthma and inflammatory bowel disease or Crohn's disease, respectively, a mutation in the NOD2 nucleotide-binding domain could be connected to Blau syndrome (an inflammatory disorder that primarily affects the skin, joints, and eyes), for example, Motta et al. (2015).

Concluding remarks

The investigation of the molecular principles underlying plant autoimmunity and lesion mimic phenotypes continues to provide knowledge that is key for a full comprehension of the plant immune system. The studies provide important details regarding NLR expression, intracellular trafficking, and activity, and reveal candidate proteins likely safeguarded by NLRs. Several components that were previously thought to represent "negative regulators" of plant immunity upon closer inspection turned out to be in fact proteins surveilled by NLRs, which explains the autoimmunity phenotypes associated with their absence or mutation. Hence, advanced

OUTSTANDING QUESTIONS

- What can we learn from autoimmune and lesion mimic mutants regarding plant immunity?
- Which aspects need to be considered when reporting autoimmune and lesion mimic mutants?
- Are the events downstream of NLR activation identical in authentic plant defense and autoimmunity?
- Can the constitutive activation of PTI also result in autoimmunity?
- Are there truly cases where autoimmunity is not linked to NLR activity?
- What are the best experimental approaches to identify (NLR) genes underlying an autoimmune response?
- What is the molecular basis for the frequent modulation of autoimmunity by environmental conditions?
- Will climate change (elevated temperatures) result in more cases of autoimmunity in plants?
- Can autoimmunity be exploited to increase disease resistance in crops?
- Are there means to mitigate hybrid necrosis yet retain pathogen responsiveness?

genetic and molecular tools are emerging to dissect the NLR network functions related to autoimmunity, which can also be useful for the broader community of plant scientists. However, a number of pertinent questions still need to be clarified in this field of research (see "Outstanding Questions" box). It remains a fruitful exercise to broaden the view to NLR-associated science conducted in the animal field, as NLR proteins seem to function according to a common biochemical principle, and, as in the case of other biological processes, much can be possibly learnt by a cross-kingdom perspective.

Funding

References

Adachi H, Derevnina L, Kamoun S (2019) NLR singletons, pairs, and networks: evolution, assembly, and regulation of the intracellular immunoreceptor circuitry of plants. Curr Opin Plant Biol **50**: 121–131

Ade J, DeYoung BJ, Golstein C, Innes RW (2007) Indirect activation of a plant nucleotide binding site-leucine-rich repeat protein by a bacterial protease. Proc Natl Acad Sci USA **104**: 2531–2536

Alcázar R, García A V., Kronholm I, de Meaux J, Koornneef M, Parker JE, Reymond M (2010) Natural variation at Strubbelig Receptor Kinase 3 drives immune-triggered incompatibilities between *Arabidopsis thaliana* accessions. Nat Genet **42**: 1135–1139

Alcázar R, von Reth M, Bautor J, Chae E, Weigel D, Koornneef M, Parker JE (2014) Analysis of a plant complex resistance gene locus underlying immune-related hybrid incompatibility and its occurrence in nature. PLoS Genet **10**: e1004848

Altpeter F, Springer NM, Bartley LE, Blechl AE, Brutnell TP, Citovsky V, Conrad LJ, Gelvin SB, Jackson DP, Kausch AP, et al. (2016) Advancing crop transformation in the era of genome editing. Plant Cell **28**: 1510–1520

Al Amin GM, Kong K, Sharmin RA, Kong J, Bhat JA, Zhao T (2019) Characterization and rapid gene-mapping of leaf lesion mimic phenotype of *spl-1* mutant in soybean (*Glycine max* (L.) Merr). Int J Mol Sci **20**: 2193

Atanasov KE, Liu C, Erban A, Kopka J, Parker JE, Alcázar R (2018) *NLR* mutations suppressing immune hybrid incompatibility and their effects on disease resistance. Plant Physiol **177**: 1152–1169

Balint-Kurti P (2019) The plant hypersensitive response: concepts, control and consequences. Mol Plant Pathol **20**: 1163–1178

Barragan AC, Collenberg M, Wang J, Lee RRQ, Cher WY, Rabanal FA, Ashkenazy H, Weigel D, Chae E (2021) A truncated singleton NLR causes hybrid necrosis in *Arabidopsis thaliana*. Mol Biol Evol **38**: 557–574

Barragan CA, Wu R, Kim ST, Xi W, Habring A, Hagmann J, Van de Weyer AL, Zaidem M, Ho WWH, Wang G, et al. (2019) RPW8/HR repeats control NLR activation in *Arabidopsis thaliana*. PLoS Genet **15**: e1008313

Bi G, Su M, Li N, Liang Y, Dang S, Xu J, Hu M, Wang J, Zou M, Deng Y, et al. (2021) The ZAR1 resistosome is a calcium-permeable channel triggering plant immune signaling. Cell **184**: 3528–3541.e12

Bjørnstad Å, Aastveit K (1990) Pleiotropic effects on the *ml-o* mildew resistance gene in barley in different genetical backgrounds. Euphytica **46**: 217–226

Bomblies K, Lempe J, Epple P, Warthmann N, Lanz C, Dangl JL, Weigel D (2007) Autoimmune response as a mechanism for a Dobzhansky-Muller-type incompatibility syndrome in plants. PLoS Biol **5**: 1962–1972

Bomblies K, Weigel D (2007) Hybrid necrosis: autoimmunity as a potential gene-flow barrier in plant species. Nat Rev Genet **8**: 382–393

Borrelli GM, Mazzucotelli E, Marone D, Crosatti C, Michelotti V, Valè G, Mastrangelo AM (2018) Regulation and evolution of NLR genes: a close interconnection for plant immunity. Int J Mol Sci **19**: 1662

Bruggeman Q, Raynaud C, Benhamed M, Delarue M (2015) To die or not to die? Lessons from lesion mimic mutants. Front Plant Sci **6**: 24

Burdett H, Bentham AR, Williams SJ, Dodds PN, Anderson PA, Banfield MJ, Kobe B (2019) The plant "resistosome": structural insights into immune signaling. Cell Host Microbe **26**: 193–201

Cai H, Wang W, Rui L, Han L, Luo M, Liu N, Tang D (2021) The TIR-NBS protein TN13 associates with the CC-NBS-LRR resistance protein RPS5 and contributes to RPS5-triggered immunity in Arabidopsis. Plant J **107**: 775–786

Cain K, Bratton SB, Cohen GM (2002) The Apaf-1 apoptosome: a large caspase-activating complex. Biochimie **84**: 203–214

Calvo-Baltanás V, Wang J, Chae E (2021) Hybrid incompatibility of the plant immune system: an opposite force to heterosis equilibrating hybrid performances. Front Plant Sci **11**: 576796

Castel B, Ngou PM, Cevik V, Redkar A, Kim DS, Yang Y, Ding P, Jones JDG (2019) Diverse NLR immune receptors activate defence via the RPW8-NLR NRG1. New Phytol 222: 966–980

Chakraborty J, Ghosh P, Das S (2018) Autoimmunity in plants. Planta 248: 751–767

Chantarachot T, Sorenson RS, Hummel M, Ke H, Kettenburg AT, Chen D, Aiyetiwa K, Dehesh K, Eulgem T, Sieburth LE, et al. (2020) DHH1/DDX6-like RNA helicases maintain ephemeral half-lives of stress-response mRNAs. Nat Plants 6: 675–685

Chen C, Chen H, Lin YS, Shen JB, Shan JX, Qi P, Shi M, Zhu MZ, Huang XH, Feng Q, et al. (2014) A two-locus interaction causes interspecific hybrid weakness in rice. Nat Commun 5: 3357

Chen P, Hu H, Zhang Y, Wang Z, Dong G, Cui Y, Qian Q, Ren D, Guo L (2018) Genetic analysis and fine-mapping of a new rice mutant, white and lesion mimic leaf1. Plant Growth Regul 85: 425–435

Chen Y, Zhong G, Cai H, Chen R, Liu N, Wang W, Tang D (2021) A truncated TIR-NBS protein TN10 pairs with two clustered TIR-NBS-LRR immune receptors and contributes to plant immunity in Arabidopsis. Int J Mol Sci 22: 4004

Cheng C, Gao X, Feng B, Sheen J, Shan L, He P (2013) Plant immune response to pathogens differs with changing temperatures. Nat Commun 4: 2530

Chiang YH, Coaker G (2015) Effector triggered immunity: NLR immune perception and downstream defense responses. Arab B 13: e0183

Chung J, Staswick PE, Graef GL, Wysong DS, Specht JE (1998) Inheritance of a disease lesion mimic mutant in soybean. J Hered 89: 363–365

Cui H, Gobbato E, Kracher B, Qiu J, Bautor J, Parker JE (2017) A core function of EDS1 with PAD4 is to protect the salicylic acid defense sector in Arabidopsis immunity. New Phytol 213: 1802–1817

Deng J, Fang L, Zhu X, Zhou B, Zhang T, Zhang D (2019) A CC-NBS-LRR gene induces hybrid lethality in cotton. J Exp Bot 70: 5145–5156

Dietrich RA, Delaney TP, Uknes SJ, Ward ER, Ryals JA, Dangl JL (1994) Arabidopsis mutants simulating disease resistance response. Cell 77: 565–577

Dietrich RA, Richberg MH, Schmidt R, Dean C, Dangl JL (1997) A novel zinc finger protein is encoded by the Arabidopsis LSD1 gene and functions as a negative regulator of plant cell death. Cell 88: 685–694

Disch EM, Tong M, Kotur T, Koch G, Wolf CA, Li X, Hoth S (2016) Membrane-associated ubiquitin ligase SAUL1 suppresses temperature- and humidity-dependent autoimmunity in Arabidopsis. Mol Plant-Microbe Interact 29: 69–80

Domínguez-Ferreras A, Kiss-Papp M, Jehle AK, Felix G, Chinchilla D (2015) An overdose of the Arabidopsis coreceptor BRASSINOSTEROID INSENSITIVE1-ASSOCIATED RECEPTOR KINASE1 or its ectodomain causes autoimmunity in a SUPPRESSOR OF BIR1-1-dependent manner. Plant Physiol 168: 1106–1121

Du L, Ali GS, Simons KA, Hou J, Yang T, Reddy ASN, Poovaiah BW (2009) Ca^{2+}/calmodulin regulates salicylic-acid-mediated plant immunity. Nature 457: 1154–1158

Fan J, Bai P, Ning Y, Wang J, Shi X, Xiong Y, Zhang K, He F, Zhang C, Wang R, et al. (2018) The monocot-specific receptor-like kinase SDS2 controls cell death and immunity in rice. Cell Host Microbe 23: 498–510

Feehan JM, Castel B, Bentham AR, Jones JD (2020) Plant NLRs get by with a little help from their friends. Curr Opin Plant Biol 56: 99–108

Flor HH (1971) Current status of the gene-for-gene concept. Annu Rev Cell Dev Biol 9: 275–296

Gao Z, Liu Q, Zhang Y, Fang H, Zhang Y, Sinumporn S, Abbas A, Ning Y, Wang GL, Cheng S, et al. (2019) A proteomic approach identifies novel proteins and metabolites for lesion mimic formation and disease resistance enhancement in rice. Plant Sci 287: 110182

Garner CM, Spears BJ, Su J, Cseke LJ, Smith SN, Rogan CJ, Gassmann W (2021) Opposing functions of the plant TOPLESS gene family during SNC1-mediated autoimmunity. PLoS Genet 17: e1009026

Gloggnitzer J, Akimcheva S, Srinivasan A, Kusenda B, Riehs N, Stampfl H, Bautor J, Dekrout B, Jonak C, Jiménez-Gómez JM, et al. (2014) Nonsense-mediated mRNA decay modulates immune receptor levels to regulate plant antibacterial defense. Cell Host Microbe 16: 376–390

Gou M, Hua J (2012) Complex regulation of an R gene SNC1 revealed by autoimmune mutants. Plant Signal Behav 7: 213–216

Gruner K, Esser T, Acevedo-Garcia J, Freh M, Habig M, Strugala R, Stukenbrock E, Schaffrath U, Panstruga R (2020) Evidence for allele-specific levels of enhanced susceptibility of wheat mlo mutants to the hemibiotrophic fungal pathogen Magnaporthe oryzae pv. Triticum. Genes 11: 517

Hammond-Kosack KE, Jones JDG (1997) Plant disease resistance genes. Annu Rev Plant Biol 48: 575–607

Heidrich K, Tsuda K, Blanvillain-Baufumet S, Wirthmueller L, Bautor J, Parker JE (2013) Arabidopsis TNL-WRKY domain receptor RRS1 contributes to temperature-conditioned RPS4 auto-immunity. Front Plant Sci 4: 403

Hessler G, Portheine SM, Gerlach E-M, Lienemann T, Koch G, Voigt CA, Hoth S (2021) POWDERY MILDEW RESISTENT4-dependent cell wall deposition is a consequence but not the cause of temperature-induced autoimmunity. J Exp Bot 72: 7549–7563

Holmes DR, Bredow M, Thor K, Pascetta SA, Sementchoukova I, Siegel KR, Zipfel C, Monaghan J (2021) A novel allele of the Arabidopsis thaliana MACPF protein CAD1 results in deregulated immune signaling. Genetics 217: iyab022

Huang J, Zhu C, Li X (2018) $SCF^{SNIPER4}$ controls the turnover of two redundant TRAF proteins in plant immunity. Plant J 95: 504–515

Huang X, Li J, Bao F, Zhang X, Yang S (2010a) A gain-of-function mutation in the Arabidopsis disease resistance gene RPP4 confers sensitivity to low temperature. Plant Physiol 154: 796–809

Huang X, Li Y, Zhang X, Zuo J, Yang S (2010b) The Arabidopsis LSD1 gene plays an important role in the regulation of low temperature-dependent cell death. New Phytol 187: 301–312

Huang Y, Yin C, Liu J, Feng B, Ge D, Kong L, Ortiz-Morea FA, Richter J, Hauser MT, Wang WM, et al. (2020) A trimeric CrRLK1L-LLG1 complex genetically modulates SUMM2-mediated autoimmunity. Nat Commun 11: 4859

Huang S, Balgi A, Pan Y, Li M, Zhang X, Du L, Zhou M, Roberge M, Li X (2016) Identification of methylosome components as negative regulators of plant immunity using chemical genetics. Mol Plant 9: 1620–1633

Jacob P, Kim NH, Wu F, El-Kasmi F, Chi Y, Walton WG, Furzer OJ, Lietzan AD, Sunil S, Kempthorn K, et al. (2021) Plant "helper" immune receptors are Ca^{2+}-permeable nonselective cation channels. Science 7917: eabg7917

Jia M, Shen X, Tang Y, Shi X, Gu Y (2021) A karyopherin constrains nuclear activity of the NLR protein SNC1 and is essential to prevent autoimmunity in Arabidopsis. Mol Plant 14: 1733–1744

Johal GS, Hulbert SH, Briggs SP (1995) Disease lesion mimics of maize: a model for cell death in plants. BioEssays 17: 685–692

Jones J, Dangl J (2006) The plant immune system. Nature 444: 323–329

Kadota Y, Shirasu K, Guerois R (2010) NLR sensors meet at the SGT1–HSP90 crossroad. Trends Biochem Sci 35: 199–207

Khan M, Subramaniam R, Desveaux D (2016) Of guards, decoys, baits and traps: pathogen perception in plants by type III effector sensors. Curr Opin Microbiol 29: 49–55

Kim HK, Kim YJ, Paek KH, Chung JI, Kim JK (2005) The phenotype of the soybean disease-lesion mimic (dlm) mutant is light-dependent and associated with chloroplast function. Plant Pathol J 21: 395–401

Kim SH, Gao F, Bhattacharjee S, Adiasor JA, Nam JC, Gassmann W (2010) The Arabidopsis resistance-like gene *SNC1* is activated by mutations in *SRFR1* and contributes to resistance to the bacterial effector AvrRps4. PLoS Pathog **6**: e1001172

Kojo K, Yaeno T, Kusumi K, Matsumura H, Fujisawa S, Terauchi R, Iba K (2006) Regulatory mechanisms of ROI generation are affected by rice *spl* mutations. Plant Cell Physiol **47**: 1035–1044

Kusch S, Panstruga R (2017) *mlo*-based resistance: an apparently universal "weapon" to defeat powdery mildew disease. Mol Plant-Microbe Interact **30**: 179–189

Lai Y, Eulgem T (2018) Transcript-level expression control of plant NLR genes. Mol Plant Pathol **19**: 1267–1281

Lai Y, Lu XM, Daron J, Pan S, Wang J, Wang W, Tsuchiya T, Holub E, McDowell JM, Keith Slotkin R, et al. (2020) The Arabidopsis PHD-finger protein EDM2 has multiple roles in balancing NLR immune receptor gene expression. PLoS Genet **16**: e1008993

Lewis JD, Lee AHY, Hassan JA, Wan J, Hurley B, Jhingree JR, Wang PW, Lo T, Youn JY, Guttman DS, et al. (2013) The Arabidopsis ZED1 pseudokinase is required for ZAR1-mediated immunity induced by the *Pseudomonas syringae* type III effector HopZ1a. Proc Natl Acad Sci **110**: 18722–18727

Li J, Ding J, Zhang W, Zhang Y, Tang P, Chen JQ, Tian D, Yang S (2010) Unique evolutionary pattern of numbers of gramineous NBS-LRR genes. Mol Genet Genomics **283**: 427–438

Li LS, Ying J, Li E, Ma T, Li M, Gong LM, Wei G, Zhang Y, Li S (2021) Arabidopsis CBP60b is a central transcriptional activator of immunity. Plant Physiol **186**: 1645–1659

Li L, Habring A, Wang K, Weigel D (2020) Atypical resistance protein RPW8/HR triggers oligomerization of the NLR immune receptor RPP7 and autoimmunity. Cell Host Microbe **27**: 405–417

Li L, Weigel D (2021) One hundred years of hybrid necrosis: hybrid autoimmunity as a window into the mechanisms and evolution of plant–pathogen interactions. Annu Rev Phytopathol **59**: 213–237

Li X, Kapos P, Zhang Y (2015) NLRs in plants. Curr Opin Immunol **32**: 114–121

Liang H (2003) Ceramides modulate programmed cell death in plants. Genes Dev **17**: 2636–2641

Liang W, Tong M, Li X (2020) SUSA2 is an F-box protein required for autoimmunity mediated by paired NLRs SOC3-CHS1 and SOC3-TN2. Nat Commun **11**: 5190

Liang W, Wersch S, Tong M, Li X (2019) TIR-NB-LRR immune receptor SOC 3 pairs with truncated TIR-NB protein CHS1 or TN2 to monitor the homeostasis of E3 ligase SAUL1. New Phytol **221**: 2054–2066

Liao Y, Bai Q, Xu P, Wu T, Guo D, Peng Y, Zhang H, Deng X, Chen X, Luo M, et al. (2018) Mutation in rice *Abscisic Acid2* results in cell death, enhanced disease-resistance, altered seed dormancy and development. Front Plant Sci **9**: 405

Liu D, Cheng Z, Liu G, Liu G, Wang Y, Zhao X, Zhu L (2003) Characterization and mapping of a lesion mimic mutant in rice (*Oryza sativa* L.). Chinese Sci Bull **48**: 892–896

Liu J, Park CH, He F, Nagano M, Wang M, Bellizzi M, Zhang K, Zeng X, Liu W, Ning Y, et al. (2015) The RhoGAP SPIN6 associates with SPL11 and OsRac1 and negatively regulates programmed cell death and innate immunity in rice. PLoS Pathog **11**: e1004629

Liu Q, Ning Y, Zhang Y, Yu N, Zhao C, Zhan X, Wu W, Chen D, Wei X, Wang GL, et al. (2017) OsCUL3a negatively regulates cell death and immunity by degrading OsNPR1 in rice. Plant Cell **29**: 345–359

Lolle S, Greeff C, Petersen K, Roux M, Jensen MK, Bressendorff S, Rodriguez E, Sømark K, Mundy J, Petersen M (2017) Matching NLR immune receptors to autoimmunity in *camta3* mutants using antimorphic NLR alleles. Cell Host Microbe **21**: 518–529

Lorrain S, Vailleau F, Balagué C, Roby D (2003) Lesion mimic mutants: keys for deciphering cell death and defense pathways in plants? Trends Plant Sci **8**: 263–271

Lüdke D, Roth C, Kamrad SA, Messerschmidt J, Hartken D, Appel J, Hörnich BF, Yan Q, Kusch S, Klenke M, et al. (2021) Functional requirement of the *Arabidopsis* importin-α nuclear transport receptor family in autoimmunity mediated by the NLR protein SNC1. Plant J **105**: 994–1009

Lv R, Li Z, Li M, Dogra V, Lv S, Liu R, Lee KP, Kim C (2019) Uncoupled expression of nuclear and plastid photosynthesis-associated genes contributes to cell death in a lesion mimic mutant. Plant Cell **31**: 210–230

Ma J, Yang S, Wang D, Tang K, Feng XX, Feng XZ (2020a) Genetic mapping of a light-dependent lesion mimic mutant reveals the function of coproporphyrinogen III oxidase homolog in soybean. Front Plant Sci **11**: 557

Ma S, Lapin D, Liu L, Sun Y, Song W, Zhang X, Logemann E, Yu D, Wang J, Jirschitzka J, et al. (2020b) Direct pathogen-induced assembly of an NLR immune receptor complex to form a holoenzyme. Science **370**: eabe3069

Mackey D, Belkhadir Y, Alonso JM, Ecker JR, Dangl JL (2003) *Arabidopsis* RIN4 is a target of the type III virulence effector AvrRpt2 and modulates RPS2-mediated resistance. Cell **112**: 379–389

Maekawa T, Cheng W, Spiridon LN, Töller A, Lukasik E, Saijo Y, Liu P, Shen QH, Micluta MA, Somssich IE, et al. (2011a) Coiled-coil domain-dependent homodimerization of intracellular barley immune receptors defines a minimal functional module for triggering cell death. Cell Host Microbe **9**: 187–199

Maekawa T, Kufer TA, Schulze-Lefert P (2011b) NLR functions in plant and animal immune systems: so far and yet so close. Nat Immunol **12**: 818–826

Martin R, Qi T, Zhang H, Liu F, King M, Toth C, Nogales E, Staskawicz BJ (2020) Structure of the activated ROQ1 resistosome directly recognizing the pathogen effector XopQ. Science **370**: eabd9993

McGrann GRD, Steed A, Burt C, Nicholson P, Brown JKM (2015) Differential effects of lesion mimic mutants in barley on disease development by facultative pathogens. J Exp Bot **66**: 3417–3428

Mermigka G, Amprazi M, Mentzelopoulou A, Amartolou A, Sarris PF (2020) Plant and animal innate immunity complexes: fighting different enemies with similar weapons. Trends Plant Sci **25**: 80–91

Meyers BC, Kozik A, Griego A, Kuang H, Michelmore RW (2003) Genome-wide analysis of NBS-LRR-encoding genes in Arabidopsis. Plant Cell **15**: 809–834

Moeder W, Yoshioka K (2008) Lesion mimic mutants. Plant Signal Behav **3**: 764–767

Motta V, Soares F, Sun T, Philpott DJ (2015) NOD-like receptors: versatile cytosolic sentinels. Physiol Rev **95**: 149–178

Ngou BPM, Ahn HK, Ding P, Jones JDG (2021) Mutual potentiation of plant immunity by cell-surface and intracellular receptors. Nature **592**: 110–115

Ordon J, Martin P, Erickson JL, Ferik F, Balcke G, Bonas U, Stuttmann J (2021) Disentangling cause and consequence: genetic dissection of the *DANGEROUS MIX2* risk locus, and activation of the DM2h NLR in autoimmunity. Plant J **106**: 1008–1023

Palma K, Thorgrimsen S, Malinovsky FG, Fiil BK, Nielsen HB, Brodersen P, Hofius D, Petersen M, Mundy J (2010) Autoimmunity in Arabidopsis *acd11* is mediated by epigenetic regulation of an immune receptor. PLoS Pathog **6**: e1001137

Parker MT, Knop K, Zacharaki V, Sherwood A V, Tomé D, Yu X, Martin PG, Beynon J, Michaels SD, Barton GJ, et al. (2021) Widespread premature transcription termination of *Arabidopsis thaliana* NLR genes by the spen protein FPA. eLife **10**: e65537

Peterhänsel C, Freialdenhoven A, Kurth J, Kolsch R, Schulze-Lefert P (1997) Interaction analyses of genes required for resistance responses to powdery mildew in barley reveal distinct pathways leading to leaf cell death. Plant Cell **9**: 1397–1409

Radojičić A, Li X, Zhang Y (2018) Salicylic acid: a double-edged sword for programed cell death in plants. Front Plant Sci **9**: 1133

Rafiqi M, Bernoux M, Ellis JG, Dodds PN (2009) In the trenches of plant pathogen recognition: role of NB-LRR proteins. Semin Cell Dev Biol 20: 1017–1024

Rate DN, Cuenca J V., Bowman GR, Guttman DS, Greenberg JT (1999) The gain-of-function Arabidopsis acd6 mutant reveals novel regulation and function of the salicylic acid signaling pathway in controlling cell death, defenses, and cell growth. Plant Cell 11: 1695–1708

Rayson S, Arciga-Reyes L, Wootton L, De Torres Zabala M, Truman W, Graham N, Grant M, Davies B (2012) A role for nonsense-mediated mRNA decay in plants: pathogen responses are induced in Arabidopsis thaliana NMD mutants. PLoS One 7: e31917

Richard MMS, Gratias A, Meyers BC, Geffroy V (2018) Molecular mechanisms that limit the costs of NLR-mediated resistance in plants. Mol Plant Pathol 19: 2516–2523

Rodriguez E, El Ghoul H, Mundy J, Petersen M (2016) Making sense of plant autoimmunity and 'negative regulators.' FEBS J 283: 1385–1391

Rostoks N, Schmierer D, Kudrna D, Kleinhofs A (2003) Barley putative hypersensitive induced reaction genes: genetic mapping, sequence analyses and differential expression in disease lesion mimic mutants. Theor Appl Genet 107: 1094–1101

Roux ME, Rasmussen MW, Palma K, Lolle S, Regué ÀM, Bethke G, Glazebrook J, Zhang W, Sieburth L, Larsen MR, et al. (2015) The mRNA decay factor PAT1 functions in a pathway including MAP kinase 4 and immune receptor SUMM2. EMBO J 34: 593–608

Saile SC, Jacob P, Castel B, Jubic LM, Salas-Gonzalez I, Bäcker M, Jones JDG, Dangl JL, El Kasmi F (2020) Two unequally redundant "helper" immune receptor families mediate Arabidopsis thaliana intracellular "sensor" immune receptor functions. PLoS Biol 18: e3000783

Schneider JC, Suzanne H, Somerville CR (1995) Chilling-sensitive mutants of Arabidopsis. Plant Mol Biol Rep 13: 11–17

Schultz-Larsen T, Lenk A, Kalinowska K, Vestergaard LK, Pedersen C, Isono E, Thordal-Christensen H (2018) The AMSH3 ESCRT-III-associated deubiquitinase is essential for plant immunity. Cell Rep 25: 2329–2338

Seo YS, Lee SK, Song MY, Suh JP, Hahn TR, Ronald P, Jeon JS (2008) The HSP90-SGT1-RAR1 molecular chaperone complex: a core modulator in plant immunity. J Plant Biol 51: 1–10

Seto D, Koulena N, Lo T, Menna A, Guttman DS, Desveaux D (2017) Expanded type III effector recognition by the ZAR1 NLR protein using ZED1-related kinases. Nat Plants 3: 17027

Sun Y, Zhu YX, Balint-Kurti PJ, Wang GF (2020) Fine-tuning immunity: players and regulators for plant NLRs. Trends Plant Sci 25: 695–713

Takagi M, Hamano K, Takagi H, Morimoto T, Akimitsu K, Terauchi R, Shirasu K, Ichimura K (2019) Disruption of the MAMP-induced MEKK1-MKK1/MKK2-MPK4 pathway activates the TNL immune receptor SMN1/RPS6. Plant Cell Physiol 60: 778–787

Takagi M, Iwamoto N, Kubo Y, Morimoto T, Takagi H, Takahashi F, Nishiuchi T, Tanaka K, Taji T, Kaminaka H, et al. (2020) Arabidopsis SMN2/HEN2, encoding DEAD-Box RNA helicase, governs proper expression of the resistance gene SMN1/RPS6 and is involved in dwarf, autoimmune phenotypes of mekk1 and mpk4 Mutants. Plant Cell Physiol 61: 1507–1516

Thulasi Devendrakumar K, Copeland C, Li X (2019) The proteasome regulator PTRE1 contributes to the turnover of SNC1 immune receptor. Mol Plant Pathol 20: 1566–1573

Tong M, Kotur T, Liang W, Vogelmann K, Kleine T, Leister D, Brieske C, Yang S, Lüdke D, Wiermer M, et al. (2017) E3 ligase SAUL1 serves as a positive regulator of PAMP-triggered immunity and its homeostasis is monitored by immune receptor SOC3. New Phytol 215: 1516–1532

Vogelmann K, Drechsel G, Bergler J, Subert C, Philippar K, Soll J, Engelmann JC, Engelsdorf T, Voll LM, Hoth S (2012) Early senescence and cell death in Arabidopsis saul1 mutants involves the PAD4-dependent salicylic acid pathway. Plant Physiol 159: 1477–1487

Walbot V, Hoisington DA, Neuffer MG (1983) Disease lesion mimic mutations. Genetic Engineering of Plants: An. Springer, Boston, MA, pp 431–442

Wan W, Kim S, Castel B, Charoennit N, Chae E (2020) Genetics of autoimmunity in plants: an evolutionary genetics perspective. New Phytol 231: 1215–1233

Wang W, Yang X, Tangchaiburana S, Ndeh R, Markham JE, Tsegaye Y, Dunn T M, Wang GL, Bellizzi M, Parsons JF, et al. (2008) An inositolphosphorylceramide synthase is involved in regulation of plant programmed cell death associated with defense in Arabidopsis. Plant Cell 20: 3163–3179

Wang J, Hu M, Wang J, Qi J, Han Z, Wang G, Qi Y, Wang HW, Zhou JM, Chai J (2019a) Reconstitution and structure of a plant NLR resistosome conferring immunity. Science 364: eaav5870

Wang J, Wang R, Fang H, Zhang C, Zhang F, Hao Z, You X, Shi X, Park CH, Hua K, et al. (2021) Two VOZ transcription factors link an E3 ligase and an NLR immune receptor to modulate immunity in rice. Mol Plant 14: 253–266

Wang Y, Zhang Y, Wang Z, Zhang X, Yang S (2013) A missense mutation in CHS1, a TIR-NB protein, induces chilling sensitivity in Arabidopsis. Plant J 75: 553–565

Wang Z, Cui D, Liu C, Zhao J, Liu J, Liu N, Tang D, Hu Y (2019b) TCP transcription factors interact with ZED1-related kinases as components of the temperature-regulated immunity. Plant Cell Environ 42: 2045–2056

Wang Z, Cui D, Liu J, Zhao J, Liu C, Xin W, Li Y, Liu N, Ren D, Tang D, et al. (2017) Arabidopsis ZED1-related kinases mediate the temperature-sensitive intersection of immune response and growth homeostasis. New Phytol 215: 711–724

van Wersch S, Li X (2019) Stronger when together: clustering of plant NLR disease resistance genes. Trends Plant Sci 24: 688–699

van Wersch R, Li X, Zhang Y (2016) Mighty dwarfs: Arabidopsis autoimmune mutants and their usages in genetic dissection of plant immunity. Front Plant Sci 7: 1717

Wiberg A (1973) Mutants of barley with induced resistance to powdery mildew. Hereditas 75: 83–100

Wittstock U, Gershenzon J (2002) Constitutive plant toxins and their role in defense against herbivores and pathogens. Curr Opin Plant Biol 5: 300–307

Wolter M, Hollricher K, Salamini F, Schulze-Lefert P (1993) The mlo resistance alleles to powdery mildew infection in barley trigger a developmentally controlled defence mimic phenotype. Mol Gen Genet 239: 122–128

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

Wu Z, Tong M, Tian L, Zhu C, Liu X, Zhang Y, Li X (2020b) Plant E3 ligases SNIPER1 and SNIPER2 broadly regulate the homeostasis of sensor NLR immune receptors. EMBO J 39: e104915

Xiao S, Brown S, Patrick E, Brearley C, Turner JG (2003) Enhanced transcription of the Arabidopsis disease resistance genes RPW8.1 and RPW8.2 via a salicylic acid-dependent amplification circuit is required for hypersensitive cell death. Plant Cell 15: 33–45

Xiao S, Calis O, Patrick E, Zhang G, Charoenwattana P, Muskett P, Parker JE, Turner JG (2005) The atypical resistance gene, RPW8, recruits components of basal defence for powdery mildew resistance in Arabidopsis. Plant J 42: 95–110

Xu F, Zhu C, Cevik V, Johnson K, Liu Y, Sohn K, Jones JD, Holub EB, Li X (2015) Autoimmunity conferred by chs3-2D relies on CSA1, its adjacent TNL-encoding neighbour. Sci Rep 5: 8792

Yan J, Liu Y, Huang X, Li L, Hu Z, Zhang J, Qin Q, Yan L, He K, Wang Y, et al. (2019) An unreported NB-LRR protein SUT1 is required for the autoimmune response mediated by type one

protein phosphatase 4 mutation (topp4-1) in *Arabidopsis*. Plant J **100**: 357–373

Yang H, Shi Y, Liu J, Guo L, Zhang X, Yang S (2010) A mutant CHS3 protein with TIR-NB-LRR-LIM domains modulates growth, cell death and freezing tolerance in a temperature-dependent manner in Arabidopsis. Plant J **63**: 283–296

Yang L, Wang Z, Hua J (2021) A meta-analysis reveals opposite effects of biotic and abiotic stresses on transcript levels of Arabidopsis intracellular immune receptor genes. Front Plant Sci **12**: 625729

Yeats TH, Rose JKC (2013) The formation and function of plant cuticles. Plant Physiol **163**: 5–20

Yuan M, Ngou BPM, Ding P, Xin XF (2021) PTI-ETI crosstalk: an integrative view of plant immunity. Curr Opin Plant Biol **62**: 102030

Yuan P, Tanaka K, Du L, Poovaiah BW (2018) Calcium signaling in plant autoimmunity: a guard model for AtSR1/CAMTA3-mediated immune response. Mol Plant **11**: 637–639

YuChun R, Na X, Juan H, Ran J, JiangMing X, ZhouLin S, Ling H, Mei L, YueXing W, Jiang H (2018) Fine mapping and candidate gene analysis of a lesion mimic mutant *spl-3t* in rice. Sci Sin Vitae **48**: 1101–1114

Yuchun R, Ran J, Sheng W, Xianmei W, Hanfei Y, Chenyang P, Sanfeng L, Dedong X, Weiyong Z, Gaoxing D, et al. (2021) *SPL36* encodes a receptor-like protein kinase that regulates programmed cell death and defense responses in rice. Rice **14**: 34

Zbierzak AM, Porfirova S, Griebel T, Melzer M, Parker JE, Dörmann P (2013) A TIR-NBS protein encoded by Arabidopsis *Chilling Sensitive 1* (*CHS1*) limits chloroplast damage and cell death at low temperature. Plant J **75**: 539–552

Zeng HY, Liu Y, Chen DK, Bao HN, Huang LQ, Yin J, Chen YL, Xiao S, Yao N (2021) The immune components ENHANCED DISEASE SUSCEPTIBILITY 1 and PHYTOALEXIN DEFICIENT 4 are required for cell death caused by overaccumulation of ceramides in Arabidopsis. Plant J **107**: 1447–1465

Zeng LR, Qu S, Bordeos A, Yang C, Baraoidan M, Yan H, Xie Q, Baek HN, Leung H, Wang GL (2004) *Spotted leaf11*, a negative regulator of plant cell death and defense, encodes a U-box/armadillo repeat protein endowed with E3 ubiquitin ligase activity. Plant Cell **16**: 2795–2808

Zhai J, Jeong DH, de Paoli E, Park S, Rosen BD, Li Y, González AJ, Yan Z, Kitto SL, Grusak MA, et al. (2011) MicroRNAs as master regulators of the plant NB-LRR defense gene family via the production of phased, trans-acting siRNAs. Genes Dev **25**: 2540–2553

Zhang X, Tian B, Fang Y, Tong T, Zheng J, Xue D (2019) Proteome analysis and phenotypic characterization of the lesion mimic mutant *bspl* in barley. Plant Growth Regul **87**: 329–339

Zhang XC, Gassmann W (2003) *RPS4*-mediated disease resistance requires the combined presence of *RPS4* transcripts with full-length and truncated open reading frames. Plant Cell **15**: 2333–2342

Zhang Y, Goritschnig S, Dong X, Li X (2003) A gain-of-function mutation in a plant disease resistance gene leads to constitutive activation of downstream signal transduction pathways in *suppressor of npr1-1, constitutive 1*. Plant Cell **15**: 2636–2646

Zhang Y, Wang Y, Liu J, Ding Y, Wang S, Zhang X, Liu Y, Yang S (2017a) Temperature-dependent autoimmunity mediated by *chs1* requires its neighboring *TNL* gene *SOC3*. New Phytol **213**: 1330–1345

Zhang Z, Lenk A, Andersson MX, Gjetting T, Pedersen C, Nielsen ME, Newman MA, Hou BH, Somerville SC, Thordal-Christensen H (2008) A lesion-mimic syntaxin double mutant in *Arabidopsis* reveals novel complexity of pathogen defense signaling. Mol Plant **1**: 510–527

Zhang Z, Liu Y, Huang H, Gao M, Wu D, Kong Q, Zhang Y (2017b) The NLR protein SUMM2 senses the disruption of an immune signaling MAP kinase cascade via CRCK3. EMBO Rep **18**: 292–302

Zhang Z, Wu Y, Gao M, Zhang J, Kong Q, Liu Y, Ba H, Zhou J, Zhang Y (2012) 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 **11**: 253–263

Zhao C, Tang Y, Wang J, Zeng Y, Sun H, Zheng Z, Su R, Schneeberger K, Parker JE, Cui H (2021) A mis-regulated cyclic nucleotide-gated channel mediates cytosolic calcium elevation and activates immunity in Arabidopsis. New Phytol **230**: 1078–1094

Zhao T, Rui L, Li J, Nishimura MT, Vogel JP, Liu N, Liu S, Zhao Y, Dangl JL, Tang D (2015) A truncated NLR protein, TIR-NBS2, is required for activated defense responses in the *exo70B1* mutant. PLoS Genet **11**: e1004945

Zhao Y, Yang J, Shi J, Gong YN, Lu Q, Xu H, Liu L, Shao F (2011) The NLRC4 inflammasome receptors for bacterial flagellin and type III secretion apparatus. Nature **477**: 596–602

Zhou F, Menke FLH, Yoshioka K, Moder W, Shirano Y, Klessig DF (2004a) High humidity suppresses *ssi4*-mediated cell death and disease resistance upstream of MAP kinase activation, H_2O_2 production and defense gene expression. Plant J **39**: 920–932

Zhou J, Zhang Y (2020) Plant immunity: danger perception and signaling. Cell **181**: 978–989

Zhou T, Wang Y, Chen JQ, Araki H, Jing Z, Jiang K, Shen J, Tian D (2004b) Genome-wide identification of NBS genes in *japonica* rice reveals significant expansion of divergent non-TIR NBS-LRR genes. Mol Genet Genomics **271**: 402–415

Zhu W, Zaidem M, Van de Weyer AL, Gutaker RM, Chae E, Kim ST, Bemm F, Li L, Todesco M, Schwab R, et al. (2018) Modulation of *ACD6* dependent hyperimmunity by natural alleles of an *Arabidopsis thaliana* NLR resistance gene. PLoS Genet **14**: e1007628