



# Exciting times in plant biotic interactions

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As much as half of all of the calories produced by plant crops is lost to pathogens. Efforts to strengthen plant immunity have led to a deeper understanding of how plants recognize and defend against pathogens while at the same time facilitating symbiotic interactions. Over the past several decades, key interactors on the pathogen side (effectors) and plant side (intracellular and cell-surface immune receptors) have been identified. The field of plant biotic interactions appears to have entered a golden age over the last three years with multiple major discoveries being reported, beginning with the first three-dimensional structure of a plant “resistosome” in 2019 (Wang et al., 2019), followed by the structures of two more in 2020 (Ma et al., 2020; Martin et al., 2020). Resistosomes are protein complexes that form upon recognition of pathogen effector proteins and mediate immune signaling and cell death. The core of a resistosome is formed by multimers (four or five copies) of nucleotide-binding leucine-rich repeat (NLR) proteins. Although NLR proteins were reported to mediate pathogen recognition nearly 30 years ago, it had remained enigmatic as to how these proteins triggered immune responses. Structural analyses of resistosomes have greatly accelerated our understanding of their functions, including formation of a calcium permeable channel (Bi et al., 2021) and catalyzing NAD<sup>+</sup> cleavage to generate signaling molecules (Horsefield et al., 2019; Wan et al., 2019). Major advances have also been made in understanding how diverse immune signaling pathways are integrated in plant cells, with two papers describing how immune responses triggered by cell surface receptors (pattern recognition receptor-triggered immunity or PTI) are integrated with immune responses triggered by intracellular resistosomes (Ngou et al., 2021; Yuan et al., 2021). Other recent exciting advances include the demonstration that plant

small RNAs are translocated into fungal cells (Cai et al., 2018; He et al., 2021), a clear understanding of how the defense hormone salicylic acid (SA) is perceived (Liu et al., 2020; Wang et al., 2020), and how immune signals move systemically through a plant (Chen et al., 2018; Hartmann et al., 2018). With such major advancements in our molecular and cellular understanding of plant biotic interactions, and the rapid acceleration in research on this topic, this Focus Issue on Plant Biotic Interactions is timely. The 8 reviews and 14 original research articles in this issue exemplify this rapid pace of discovery and highlight the breadth of research being pursued.

Jonathan Jones and colleagues (Ngou et al., 2022) review nearly 30 years of research since the first plant NLR gene was cloned, providing a broad perspective of immune receptors (both cell surface and intracellular) and the downstream integration of their signaling pathways. Building on this review, Dmitry Lapin and colleagues (Lapin et al., 2022) provide a deep dive into the evolution, structure, and function of Toll and interleukin-1 receptor-like domains, which mediate immune signaling in organisms as diverse as bacteria, humans, and plants. Although the bulk of research on immune receptors has focused on detection of microbial pathogens, Adam Steinbrener and colleagues (Snoeck et al., 2022) give us the latest insights into how plants use similar receptors and signaling pathways to detect herbivorous insects via detection of herbivore-associated molecular patterns (HAMPs), and how, similar to microbial pathogens, insects secrete effector proteins to suppress immune responses. Besides releasing effector proteins, many insects serve as vectors of viral pathogens, which in turn use their own effectors to modify plant physiology and influence vector performance. Swayamjit Ray and Clare Casteel (Ray and

Casteel, 2022) review these intriguing interactions from the viewpoint of molecular synergisms between insect vector and virus.

In addition to activation by HAMPs, many cell surface immune receptors are activated by plant cell wall fragments released by degradative enzymes employed by microbes. The interplay between plant cell walls and microbes is reviewed by Clara Sánchez Rodríguez and colleagues (Dora et al., 2022). Once immune receptors of all types are activated, a near universal response is an influx of  $\text{Ca}^{2+}$  ions from the apoplast. The channels that control this influx have been enigmatic. Libo Shan and colleagues (Xu et al., 2022) review exciting new work that is at last pulling back the curtain on how calcium flux is regulated during immune responses. One recently discovered response to activation of immune receptors is the formation of biomolecular condensates, a phenomenon by which biomolecules (e.g., proteins and nucleic acids) are concentrated in a non-membrane bound compartment, similar to phase separation of oil and water. This phenomenon and its potential roles in regulating plant immune responses are reviewed by Wang and Gu (2022). Lastly, the exquisite communication that occurs between beneficial microbes (nitrogen-fixing bacteria and mycorrhizal fungi) and plants is reviewed by Ertao Wang and colleagues (Wang et al., 2022), who address the one of the major outstanding questions in plant–microbe interactions, namely how plants distinguish between friend and foe.

These eight reviews cover an impressive breadth of topics in plant–biotic interactions, but the 14 primary research papers in this focus issue are even more diverse. Three of the papers focus on the evolution, structure, and/or function of NLR proteins. Gong et al. (2022) report on the evolution of an ancient NLR, ZAR1, which happens to have been the first plant NLR to yield a structure. Although most NLRs are known for their rapid evolution, ZAR1 is a major exception, with clear orthologs identifiable throughout the angiosperm tree, including in many monocots and dicots. In addition to providing a phylogenetic analysis of the origins of ZAR1, Gong et al. show through functional assays that ZAR1 has co-evolved from its earliest days with a family of receptor-like cytoplasmic kinases that physically associate with ZAR1, and often function as effector targets. In the second NLR paper, Wu et al. (2022) characterize an ancient subfamily of “helper” NLRs that function downstream of pathogen perception, the N REQUIREMENT GENE 1 (NRG1) family. While full-length NRG proteins are required for activation of defense by a large number of “sensor” NLRs, Wu et al. show that N-terminally truncated NRGs have evolved independently in *Arabidopsis thaliana* and *Nicotiana benthamiana* to antagonize signaling by full-length NRGs, thus putting on the brakes to prevent auto-immunity. In the third NLR paper, Lee et al. (2022) describe an NLR gene in sorghum (ANTHRACNOSE RESISTANCE GENE1 [ARG1]) that confers broad-spectrum resistance to several fungal

pathogens, including 11 geographically diverse isolates of *Colletotrichum sublineola* (a hemibiotrophic pathogen), *Puccinia purpurea* (a biotrophic pathogen), and *Bipolaris sorghicola*, a necrotrophic pathogen. There are few, if any, prior examples of NLR genes conferring resistance to necrotrophic pathogens, making ARG1 uniquely broad in its specificity.

Moving to the pathogen and pest side of plant biotic interactions, three of the papers in this issue focus on effector proteins and their targets inside host cells. Qi et al. (2022) show that the type III effector RipAB from the bacterial pathogen *Ralstonia solanacearum* targets Arabidopsis TGACG SEQUENCE-SPECIFIC BINDING PROTEIN (TGA) transcription factors, the central regulators of SA-mediated plant defense. The authors further demonstrate that RipAB inhibits TGA activity, thus suppressing the SA regulons and expression of RBOHD and RBOHF, which mediate reactive oxygen species (ROS) production. Raffener et al. (2022) show that another type III effector, XopS from *Xanthomonas campestris* targets and regulates the stability of WRKY40, a transcriptional regulator of defense gene expression in pepper (*Capsicum annuum*). By targeting WRKY40, XopS interferes with the phytohormone SA and jasmonic acid (JA) crosstalk and prevents stomatal closure in response to biotic elicitors, an important apoplastic defense mechanism. Kitazawa et al. (2022) describe the molecular mechanism by which an effector from a phytoplasma bacterium induces a switch from flower petals to leaves. Phytoplasmas are a group of phloem-limited bacteria that lack a cell wall. They often induce changes in flower morphology that are thought to help mediate their transmission by phloem-feeding insects. In this work, the authors show that a phytoplasma effector known as PHYL<sub>oy</sub> induces proteasomal degradation of specific MADS-box transcription factors that are required for petal and sepal formation. Interestingly, it appears that this is accomplished by PHYL<sub>oy</sub> binding directly to these transcription factors and functionally mimicking ubiquitin, a novel virulence mechanism for targeting proteins for degradation. These studies exemplify the diverse virulence strategies used by virulence effectors to hijack plant defense and physiology.

Although effector proteins are powerful tools for identifying regulators of immune signaling, another informative approach is to identify and characterize autoimmune mutants. Two papers in this issue illustrate the utility of this approach. Peng et al. (2022) show that the autoimmune activation caused by mutations in the nucleoporin CONSTITUTIVE EXPRESSER OF PATHOGENESIS-RELATED GENES 5 (CPR5) can be suppressed by disrupting the mRNA processing machinery. The authors further demonstrated that CPR5 contains a bona fide RNA-binding motif and may participate in regulating mRNA maturation for proper immune activation. This result also suggests that the nuclear pore complex is functionally associated with mRNA processing. In another paper, Yang et al. (2022) find that

disruption of a glycolytic enzyme enolase named LOW EXPRESSION OF OSMOTICALLY RESPONSIVE GENE 2 (LOS2) activates NLR-mediated immune responses and propose an interesting link between perturbed sugar metabolism and immune induction.

These papers point to the complexity of immune regulation. Two papers further explore this complexity. [Shi et al. \(2022\)](#) uncover an important signaling component downstream of the receptor-like cytoplasmic kinase BRASSINOSTEROID-SIGNALING KINASE 1 (BSK1) and demonstrate its critical contribution to resistance against powdery mildew pathogens. Taking a step further downstream, [Wang et al. \(2022\)](#) report that the transcription factor TaWRKY19 negatively regulates wheat resistance against stripe rust by repressing the expression of an NADPH oxidase and thus suppressing ROS production.

An important class of biotic interactions also covered in this issue is plant–virus interactions, which are addressed in two papers. [Chiu et al. \(2022\)](#) extended our knowledge of the amazingly complex strategies used by viruses to maximize the protein coding capacity of a limited genome. Using ribosome profiling, they mapped translation initiation sites in a Begomovirus, tomato yellow leaf curl Thailand virus, and identified new genes that are conserved across Begomovirus lineages that altered viral performance in planta. [Fu et al. \(2022\)](#) moved to connect viral defenses to other biotic levels by identifying an interaction between MEL, a microtubule-associated C4HC3-type E3 ligase, and mitochondrial serine hydroxymethyltransferase, a key enzyme in one-carbon metabolic flux, which could initiate a ROS dependent defense cascade. This led to altered interactions with viral, fungal, and bacterial pathogens in multiple plant species, raising the intriguing question, if a common signaling system induces broad resistance is this via a common resistance output?

As yet another class of biotic interactions covered in this issue, [Misawa et al. \(2022\)](#) describe how legume plants precisely control the amount of infection they allow in their roots by rhizobium bacteria according to their need for nitrogen. It has long been known that application of exogenous nitrogen fertilizer to legumes will cause the plants to reduce the number of nitrogen-fixing nodules formed in symbiosis with rhizobia. This makes energetic sense from the plant perspective as there is no reason to be feeding these bacteria under nitrogen-replete conditions. However, the molecular mechanisms by which plants can sense nitrate levels in the soil and then translate that into nodule number has been unclear. In this work, Misawa et al. show that high intracellular nitrate levels activate a transcription factor (LjNLP4) that functions to induce expression of a negative regulator of nodulation genes (LjCLE-RS2) while shutting down expression of several positive regulators.

Finally, in what appears to be a newly recognized mechanism for plant biotic interaction, [Zand Karimi et al. \(2022\)](#) report on the surprising discovery that the apoplast inside Arabidopsis leaves contains abundant long non-coding

RNAs as well as small RNAs. The majority of the former were found to be associated with proteinaceous particles that pelleted at 40,000 g. These particles did not appear to be vesicular in nature, though, based on the observation that treatments with protease then RNase A eliminated the RNA without the need of adding detergent. These findings beg the question as to why plants secrete long non-coding RNAs, with the authors speculating that they might function to sequester small RNAs secreted by pathogens.

In summary, this focus issue is chalk full of exciting new work that answers some long-standing questions, while raising many new ones. Please read and enjoy.

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