# Title

The Resistance Awakens: Natural diversity informs engineering of plant immune receptors at the DNA, RNA, and protein levels

# Authors

Chandler A. Sutherland, Danielle M. Stevens, Kyungyong Seong, Wei Wei, and Ksenia V. Krasileva

# Summary

Plants are natural bioengineers, and we illustrate how the stunning diversity in plant immune receptors can be translated into engineering efforts from Arabidopsis to crops.

# Abstract

Plants rely on germline-encoded, innate immune receptors to sense pathogens and initiate the defense response. The exponential increase in quality and quantity of genomes, RNA-seq datasets, and protein structures has underscored the incredible diversity of plant immunity. Arabidopsis continues to serve as a valuable model and the theoretical foundation of our understanding of wild plant diversity of immune receptors, but expansion of study into agricultural crops has also revealed distinct evolutionary trajectories and challenges. Here, we provide the classical context for study of both intracellular nucleotide-binding, leucine-rich repeat receptors (NLRs) and surface-localized pattern recognition receptors (PRRs) at the levels of DNA sequences, transcriptional regulation, and protein structures. We then examine how recent technology has shaped our understanding of immune receptor evolution and informed our ability to efficiently engineer resistance. We summarize current literature and provide an outlook on how researchers take inspiration from natural diversity in bioengineering efforts for disease resistance in crops.

# Introduction

Like all organisms, plants can sense the presence of pathogens and induce immune responses. In the absence of adaptive immunity, plant innate immune receptors provide sufficient diversity at a population level to recognize rapidly evolving pathogens, including viruses, bacteria, nematodes, fungi, oomycetes, insects, and parasitic plants (Hamilton et al. 1990; Dangl and Jones 2001; Chisholm et al. 2006). Understanding natural diversity remains essential for uncovering how plants evolve detection of new pathogens and for successful deployment of disease resistance in crops (Barragan and Weigel 2021).

Plant immune receptors come in two main forms based on their domain architectures and cellular localization: surface-localized pattern recognition receptors (PRRs) and intracellular nucleotide-binding, leucine-rich repeat receptors (NLRs) (Jones and Dangl 2006; Ngou et al. 2022a). PRRs include receptor-like kinases (RLKs) and receptor-like proteins (RLPs) that bind to host-derived molecules, microbe-derived molecules, or pathogen-derived effector proteins present outside of the plant cell (Shiu and Bleecker 2003). Upon ligand binding, a protein complex is formed between primary receptor, ligand, and RLK co-receptor(s), leading to kinase activation and downstream immune activation (Li et al. 2024b).

NLRs are structurally and functionally conserved across plants, animals, fungi, and bacteria, in that they are able to perceive pathogen-derived effector proteins and induce immune signaling (Jones et al. 2016; Wojciechowski et al. 2022; Kibby et al. 2023). Plant NLRs are defined by their regulatory central NB-ARC (nucleotide-binding adaptor shared by Apaf-1, R proteins, and Ced-4) domain and series of C-terminal leucine-rich repeats (LRRs) mostly implicated in ligand binding (Baggs et al. 2017). N-terminal signaling domains include coiled coils (CC), Resistance to Powdery Mildew 8 (RPW8) domains or Toll-Interleukin receptor (TIR) domains. Upon activation, NLRs form oligomeric structures bringing their signaling domains into activation (Jones et al. 2016; Lolle et al. 2020). Both CC-NLRs and RPW8-NLRs assemble into ion channel pores, while tetrameric TIR-NLRs enable NADase enzymatic activities (Huang et al. 2022; Jia et al. 2022; Yu et al. 2022).

Functionally, NLRs are and PRRs could be classified as sensors and helpers. Sensor NLRs can either directly bind pathogen effector proteins translocated into the host, guard host proteins targeted by effectors, or 'bait' for the effectors with integrated domains (IDs) (Baggs et al. 2017; Bailey et al. 2018). Helper NLRs aid the activation of immune responses for a subset of sensor NLRs that belong to their regulatory networks or for their specific pairs (Adachi et al. 2019). Similarly, some PRRs act as sensors by binding to a particular epitope or ligand, whereas co-receptors from the SERK (somatic embryogenesis receptor kinase) family act as helpers, activating subsequent immune signaling (Snoeck et al. 2023). Immune signaling after PRR and NLR activation includes mitogen activated protein kinase (MAPK) signaling, $Ca^{2+}$ burst, production of reactive oxygen species (ROS), transcriptional reprogramming, and, in case of most NLRs, programmed cell death called hypersensitive response (HR), allowing propagation of responses (Lolle et al. 2020; Wang et al. 2023). Pathways activated by PRRs and NLRs work synergistically, with PRRs typically having a broader pathogen recognition range while NLRs have a more narrow, strain-specific but faster evolving response (Tian et al. 2021; Yuan et al. 2021; Ngou et al. 2022a).

Identifying functional PRRs and NLRs, defining their recognition range, and quantifying their diversity across evolutionary scales have led to multiple strategies of rational crop improvement (Jones et al. 2024). In this review, we focus on the 'central dogma' as it relates to plant immune receptors and their associated diversity at the DNA, RNA, and protein level. We describe how available data has expanded from single genes to pangenomes and from individual gene expression studies to multi-tissue RNA-seq datasets. We propose that extreme combinatorial amino acid diversity in innate immune receptor repertoires on the plant pan-genome level fits the model of 'anticipatory immunity', in which diversity is rapidly generated on a population level in anticipation of new pathogen challenge, leading to new recognition specificities and selection of functional alleles across generations. Accumulation of expression datasets together with advances in synthetic biology allow for new possibilities in engineering inducible disease resistance. Evolutionary, experimental, and computational structural analyses have facilitated creation of designer receptors with resurrected, broader binding capabilities, or fully synthetic recognition specificities. With accumulation of training data, the future of

incorporating generative machine learning could greatly expedite engineering efforts. Modern technologies have accelerated translation from model species into diverse crops, providing practical solutions for sustainable agriculture.

## Episode 1: Hope for Tomorrow: DNA Diversity and Anticipatory Immunity

*By Chandler A. Sutherland and Ksenia V. Krasileva*

"A long time ago, in 119 million nucleotides far, far away…" we start our journey with DNA sequences. Most mutations are deleterious (Eyre-Walker and Keightley 2007; Chen et al. 2022), but immune systems present an evolutionary edge case in which high mutation is beneficial and even required to keep pace with rapidly evolving pathogens (Martincorena and Luscombe 2013; Müller et al. 2018). Across the tree of life, this has led to several radical evolutionary innovations, including emergence of molecular mechanisms of self-mutation and clonal selection on antibodies in vertebrates and CRISPR/Cas-based incorporation of extragenomic DNA in bacteria (Figure 1) (Müller et al. 2018). The plant immune system is no exception in the need for rapid evolution. The stunning genetic diversity of plant immune receptors has long been appreciated and inspired multiple hypotheses and questions about its generation and maintenance.

### Mystery is Irresistible: Early clues towards immune receptor diversity generation

The molecular study of NLRs and PRRs began with single gene cloning and diversity analysis in *Arabidopsis thaliana* (hereafter as Arabidopsis) (Bent et al. 1994; Grant et al. 1995; Gómez-Gómez and Boller 2000; Zipfel et al. 2006), tobacco (Whitham et al. 1994), and in crops (Martin et al. 1993; Jones et al. 1994; Song et al. 1995; Meyers et al. 1998; Dodds et al. 2001). These established systems of immune receptors and their pathogen complements provided molecular evidence for the gene-for-gene theory, where each functional plant immune gene is paired with a corresponding gene in the pathogen (Flor 1971). In this framework, the presence and activity of both gene partners is required for immunity. Even in this early work, the importance of population-level polymorphism in

immune receptors to mediate co-evolution of plants and their pathogens was recognized (Mode 1958; Flor 1971; Dodds 2023).

Genetic maps based on molecular markers revealed that many NLRs and PRRs in Arabidopsis (Kunkel 1996; Botella et al. 1997) and in crops (Farrara et al. 1987; Islam and Shepherd 1991; Jones et al. 1993; Kesseli et al. 1994) are organized in genomic clusters. This clustered organization was hypothesized to increase the likelihood of tandem duplication, unequal crossing over, and gene conversion, driving structural and copy number variations (Parker et al. 1997; Parniske et al. 1997; Dixon et al. 1998; Michelmore and Meyers 1998; Thomas et al. 1998; Fritz-Laylin et al. 2005). However, clustered status is not a requirement for diversity, with singleton Arabidopsis NLR RPP13 maintaining extremely high amino acid diversity (Bittner-Eddy et al. 2000; Rose et al. 2004). Generally high amino acid diversity of the LRR domain of both NLRs and PRRs was highlighted, and hypothesized to be maintained by diversifying and/or balancing selection (Hamilton et al. 1990; Parniske et al. 1997; Michelmore and Meyers 1998).

The first plant genome sequenced was Arabidopsis, allowing for genome-wide quantification of NLR and PRR copy numbers, types, and organization (The Arabidopsis Genome Initiative 2000; Dangl and Jones 2001; Shiu and Bleecker 2001). Characterization of NLRs and PRRs within and between species revealed high nucleotide diversity in the LRR region primarily driven by nonsynonymous mutations (Nordborg et al. 2005; Bakker et al. 2006; Li et al. 2024b). The NLR gene family in Arabidopsis includes the most polymorphic loci and contains the highest frequency of major effect mutations across the genome (Clark et al. 2007; Gan et al. 2011). For many immune-associated PRRs, diversity analysis has lagged behind genome generation due in part to the lack of known targets, making it difficult to functionally group receptors across species (Shiu and Bleecker 2001; Li et al. 2024b). However, mutational frequency is higher in PRRs associated with immunity than those involved in development (Ngou et al. 2024). It was proposed that the high copy number and clustered organization of immune receptors allowed for maintenance of functional recognition despite accumulation of the observed high polymorphism (Figure 1) (Dangl and Jones 2001).

Non-model and crop reference genomes have become increasingly available with over 4,000 reference plant genomes currently deposited, allowing for interspecies quantification and comparison of PRR and NLRs across plants (Gao et al. 2018; Baggs et al. 2020; Man et al. 2020; Ngou et al. 2022b, 2024)**.** The interspecies perspective on the evolution of immune receptors has shown lineage-specific expansions and contractions, potentially mediated by lifestyle, pathogen pressure, and genome organization (Baggs et al. 2020; Giolai and Laine 2024). Integration of IDs in particular has occurred unevenly across and within species (Kroj et al. 2016; Bailey et al. 2018; Van de Weyer et al. 2019). Thus far, the majority of studies in NLR-IDs have focused on RRS1 with the C-terminal WRKY domain in Arabidopsis (Narusaka et al. 2014; Le Roux et al. 2015; Sarris et al. 2015; Zhang et al. 2017; Mukhi et al. 2021) and RGA5 and Pik-1 in rice, which both contain heavy metal-associated (HMA) domains (Ashikawa et al. 2008; Cesari et al. 2013; Maqbool et al. 2015; Ortiz et al. 2017; Guo et al. 2018; Sugihara et al. 2023). The expansion of genomic resources will likely facilitate identifications of novel functional NLR-IDs and their applications in the near future.

## Change is the essential process of all existence: pangenomic dimensions of diversity

The clustered organization, high incidence of structural variation, and polymorphic nature of immune receptors make them challenging to anchor to reference genomes (Figure 1) (Jiao and Schneeberger 2020; Barragan and Weigel 2021). To overcome this, Resistance Gene Enrichment Sequencing (RenSeq) uses a combination of hybrid probe-based enrichment of NLR loci and long read sequencing to allow for examination of NLR diversity across and within species (Jupe et al. 2013). Application of RenSeq across 64 *Arabidopsis* accessions enabled a comprehensive, intraspecies view of NLR diversity (Van de Weyer et al. 2019). The portability of this technique has quickly translated to crop species and their wild relatives, enabling rapid cloning of resistance genes (Witek et al. 2016; Arora et al. 2019; Vendelbo et al. 2022), investigation of selective pressures related to domestication (Seong et al. 2020; Gladieux et al. 2024), and study of the relationship between ecological pressures and NLR gene content (Keepers et al. 2024).

If RenSeq unlocked the door to comprehensive immune receptor diversity characterization, recent long read, *de novo* assembled pan-genomes have thrown it wide open. Long read pan-genomes of Arabidopsis now cover 167 distinct accessions (Jiao and Schneeberger 2020; Kang et al. 2023; Wlodzimierz et al. 2023; Lian et al. 2024; Teasdale et al. 2024), and pan-genome scale long read assemblies are now available for many crops including maize (Hufford et al. 2021), rice (Shang et al. 2022), and their availability has recently been reviewed (Pucker et al. 2022; Shi et al. 2023). Long-read genomes provide the opportunity to investigate intraspecies PRR evolution, an opportunity unavailable with RenSeq data. Researchers are no longer limited in the availability and accuracy of sequences of plant immune receptors, but in the ability to meaningfully analyze and draw conclusions from them in the pangenomic dimension (Barragan and Weigel 2021).

Phylogenetic grouping of pangenome sequences of PRRs and NLRs has shown high diversity at the solvent-exposed residues of the LRR domains, confirming single gene studies on a genomic level (Man et al. 2020; Prigozhin and Krasileva 2021; Trinh et al. 2023; Li et al. 2024b). Quantification of amino acid diversity by Shannon entropy in the model species of Arabidopsis, Brachypodium (Prigozhin and Krasileva 2021), and maize (Prigozhin et al. 2024) reveal in each a subset of NLRs that are evolving quite rapidly. While rice NLRs show signatures of balancing selection (Gladieux et al. 2024), Arabidopsis NLR diversity is driven by positive, diversifying selection (Van de Weyer et al. 2019; Sutherland et al. 2024). In Arabidopsis, this high diversity is associated with distinct genomic features and a predicted higher likelihood of mutation (Sutherland et al. 2024). Use of graph-based methods to define the genomic regions surrounding NLRs in 17 Arabidopsis accessions allowed for a holistic view of NLR evolution, reaffirming previous emphasis on local copy number variation, association of variable NLRs with transposable elements (TEs), and high polymorphism (Teasdale et al. 2024). Application of these tools developed in Arabidopsis to other crop species will reveal the extent to which these observations are conserved.

While mechanistic questions remain, long read, pangenome approaches have supported and unified many early hypotheses on the maintenance and generation of immune

receptor diversity. In Arabidopsis, a “diversity in diversification” explanation helps to combine the contributions of large structural variations, fusions, deletions, gene conversion, point mutation, and TE insertions (Teasdale et al. 2024). Understanding the mechanisms of diversity generation, the sequence space of receptors, and the associated genomic contexts will allow us to boost the efficiency and success rate of receptor engineering, inspired by plants’ natural genome engineering efforts.

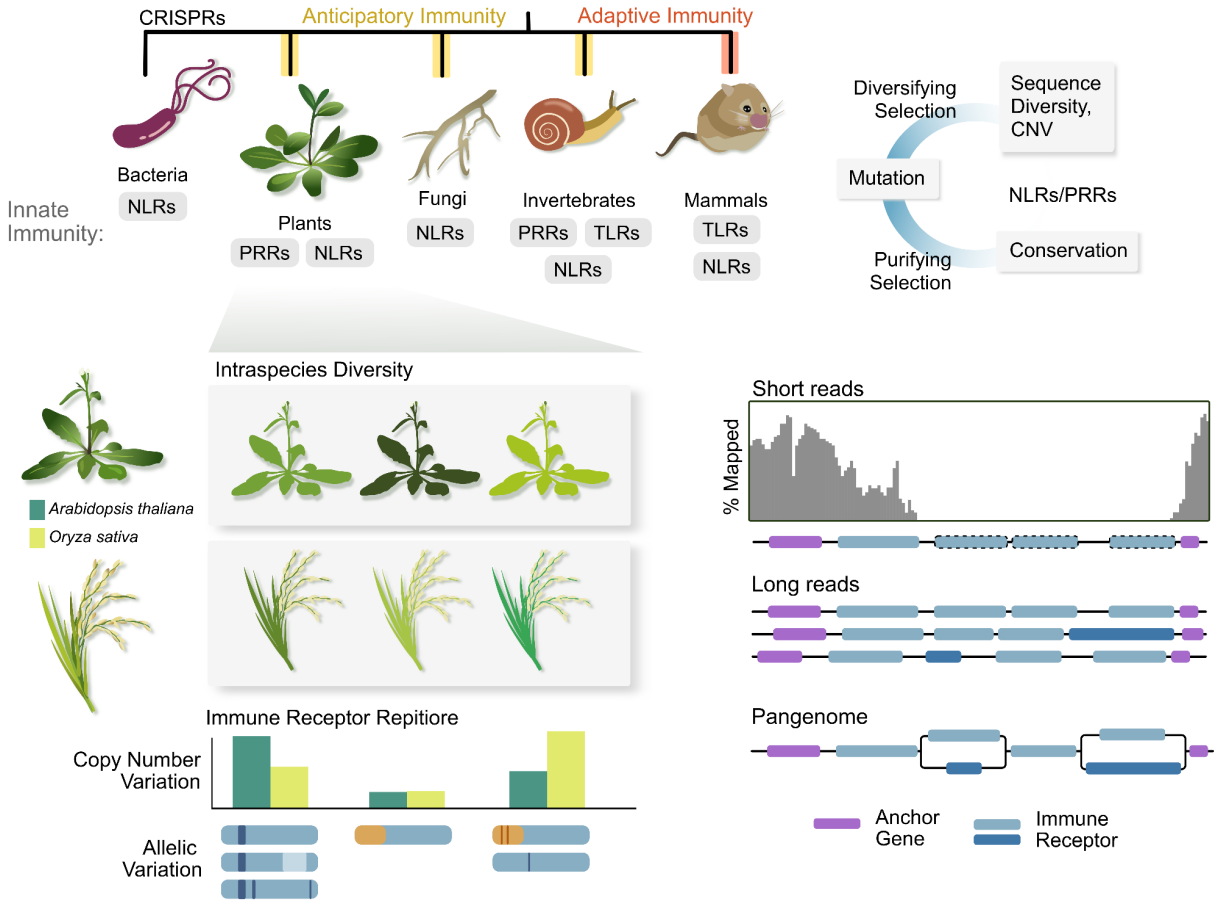



**Figure 1: Immune receptors generate and maintain high diversity to manage diverse pathogenic threats**. Across the tree of life, the pressure for diversity in immune receptors has resulted in multiple mechanisms for its generation including CRISPR/Cas-based systems in bacteria, adaptive immunity in vertebrates, and anticipatory immunity in invertebrates, plants, and fungi. Innate immune receptors include NLRs, PRRs and toll-like receptors (TLRs). Within or across species, receptor number and allelic variation can

be measured using genomic sequencing. Long-read sequencing and *de novo* reference assembly enables capture of diverse immune receptor content that was previously missed in reference-based genome assemblies using short-read sequencing.

## Your focus determines your reality: exploiting *de novo* mutation events for the study of immune receptor evolution

Plant immune receptor diversity is maintained at the population level to buffer against pathogens (Hamilton et al. 1990; Dangl and Jones 2001; Bakker et al. 2006; Teasdale et al. 2024). We propose that plant immune systems, placed in the context of innate immune system evolution across the tree of life, follow a pattern of anticipatory immunity (Müller et al. 2018), where immune receptors are both more likely to mutate and are selected for high diversity. Mutation here is used in the broadest sense, encompassing structural variation and sequence polymorphism, reflecting all possible outcomes of DNA damage and repair. This immune system is anticipatory as opposed to adaptive because it includes higher mutational likelihood at immune receptors in anticipation of pathogen attack with classical darwinian, population-scale selection as opposed to clonal, individual-scale selection. This phenomenon has been described in invertebrate innate immune receptors (Zhang et al. 2004; Ghosh et al. 2011; Adema 2015), but has not been extended to plants.

An opportunity to test this hypothesis is highly accurate quantification of low frequency, *de novo* mutational events, as new mutations have minimal opportunity for selection to act. This allows for analyses of the underlying mutation distribution. Mutations are rare events on the population scale, but occur constantly throughout plant development and growth. Previous applications of somatic mutation sequencing in Arabidopsis were used to compare mutations between tissues and characterize the mutational rate and spectrum of DNA repair knockouts (Wang et al. 2019c; Wu et al. 2020; Quiroz et al. 2024). However, improvements in ultra-accurate sequencing technology have decreased the variant calling error rate such that *de novo* mutations can be confidently quantified to the nanoscale (Abascal et al. 2021; Bae et al. 2023). Application of this technique to animal tissue has revealed several distinct signatures and rates of mutation associated with tissue types and age (Abascal et al. 2021; Moore et al. 2021; Cagan et al. 2022), but it

has yet to be applied to plant nuclear DNA or broadly to innate immune gene evolution. Ultra-accurate mutation sequencing can be applied to both somatic and gametophytic tissues, with an opportunity to use pollen as an accessible representative of germline mutations. Structural variation can additionally be quantified de novo, posing the opportunity to observe the birth and death of immune receptors by copy number variation (Liu et al. 2024b). These improvements in sequencing technology will allow for direct quantification of mutation in immune receptors and help identify the mechanisms driving this diversity.

## Episode 2: Temptation of Response: Expression Variation and its Regulation

*By Wei Wei and Chandler A. Sutherland*

Constitutively activated defense responses can cause severe cell damage and reduce plant growth, making precise regulation of immune receptor expression critical for balancing immunity and growth. While lineage-specific patterns in expression are observable even within species, common pathways for evolution of regulation can be surmised from studies across model plants and crops. Editing transcriptional regulation provides a promising opportunity for engineering broad-spectrum resistance.

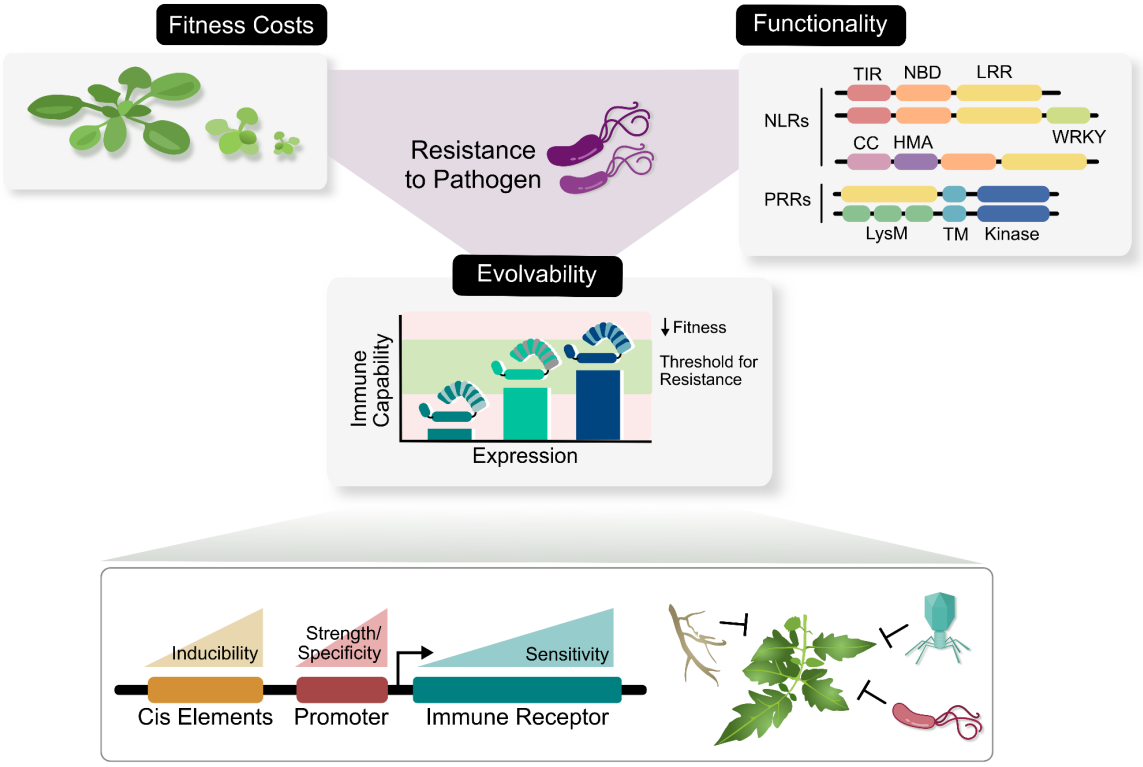
### Logic is the beginning of wisdom: immune receptors are induced by stimuli and can be tightly regulated in their expression

The first observed expression pattern for NLRs was induction in response to pathogens or other stress stimuli. In Arabidopsis, 74 out of 124 NLRs surveyed using microarray exhibited significant inductions by at least one pathogen, pathogen-related defense elicitors, or hormone treatment (Mohr et al. 2010). The 74 NLR genes varied in the types of pathogens or treatments they responded to, suggesting diverse regulatory pathways mediating NLR induction. Similarly, characterization of the NLR gene family in tomatoes revealed that 15 and 74 NLRs out of 321 were differentially expressed in early and late blight-infected leaves, respectively (Bashir et al. 2022). NLRs in monocot crop species such as maize and rice also exhibited expression changes induced by biotic and abiotic

stresses (Ding et al. 2020; Hayford et al. 2024). When responding to pathogens, some NLRs showed differential expression between resistant and susceptible genotypes, pointing to the relevance of expression regulation to immunity (Bashir et al. 2022; Fick et al. 2022a). Defense signaling triggered by a specific NLR also induces global NLR expression, suggesting a potential positive feedback loop to boost immunity. In both NLR-based hybrid incompatibilities (Bomblies et al. 2007; Atanasov et al. 2018; Barragan et al. 2021) and autoactive immune mutants (Yang et al. 2020, 2021), a large proportion of non-causal NLRs are expressed.

Gene expression is in part regulated by the cis-regulatory elements located in promoters. A motif search of all Arabidopsis NLR promoters revealed enrichment of W-boxes, the binding site for WRKY transcription factors implicated in defense responses (Mohr et al. 2010). Mutagenesis of three W-boxes in the *RPP8* promoter greatly diminished its inducibility and resistance to pathogens, indicating their importance in regulating NLR expression (Mohr et al. 2010). NLR gene expression regulation has also been widely reported on the pre-transcription level (histone modification and DNA methylation) and post-transcription level (alternative splicing, premature transcription termination and small RNAs), which were recently reviewed (Lai and Eulgem 2017; Fick et al. 2022b).

Most immune associated PRRs appear to be developmentally and spatially regulated, particularly in the roots and shoots. In Arabidopsis, the flagellin receptor FLS2 and elongation factor Tu receptor EFR are expressed in the shoots as early as 3 weeks of age. In roots, only FLS2 was induced specifically in endodermis of the differentiated root, whereas EFR was not (Beck et al. 2014; Wyrsch et al. 2015; Emonet et al. 2021). In tomato, the flagellin receptor FLS3 and cold shock protein receptor CORE are expressed in foliar tissue as early as four and six weeks of age, respectively (Clarke et al. 2013; Wang et al. 2016). Conversely in the roots, FLS2, FLS3, and CORE displayed enhanced expression in seedlings during the early, but not late, differentiation state (Leuschen-Kohl et al. 2024). We hypothesize receptor sequences–and more likely, expression variations–would explain the differential immune response across plant tissues or species previously observed (Clarke et al. 2013; Wei et al. 2018; Trinh et al. 2023).




300

301 **Figure 2: Different selective pressures on immune receptors can lead to diverse**
302 **expression patterns and inform engineering efforts for resistance to pathogens**.
303 Each receptor has a minimal threshold of expression to effectively monitor for the
304 pathogen, and an upper threshold of expression associated with fitness costs. Different
305 receptors have diverse modularity that can alter functionality and tolerable range of
306 expression. Precise control of expression by fine-tuning cis-elements provides a platform
307 for engineering broad-spectrum resistance against pathogenic threats. For functionality,
308 domains are labeled as the following: TIR = Toll-Interleukin receptor, CC = coiledcoil, NB
309 = nucleotide-binding domain, LRR = leucine-rich repeat, HMA = heavy-metal-associated,
310 TM = transmembrane.

311 **The Force is a balance between extremes: immune receptors exist across axes of**
312 **regulation**

313 With the increase of RNA-seq datasets across species and tissue types, the precise
314 dynamics of immune receptor expression can be examined at scale. It has been recently
315 observed that high NLR expression in the absence of a pathogen is more common than

previously recognized in Arabidopsis, tomato, barley, and wild wheat progenitor leaf tissues (Figure 2) (Brabham et al. 2024b). High steady state expression of rapidly evolving NLRs has been observed across tissues in Arabidopsis Col-0 (Sutherland et al. 2024), and several NLRs across the pangenome of maize show constitutive expression (Prigozhin et al. 2024). RLK expression in Arabidopsis and tomato has shown constitutive, tissue specific, and developmentally regulated expression, whereas RLPs in Arabidopsis show constitutive and pathogen inducible expression (Chuberre et al. 2018; Emonet et al. 2021; Steidele and Stam 2021). Therefore, while some immune receptors may be under tight transcriptional control to prevent autoimmunity or more general reductions in fitness, there are many NLRs and some PRRs that are expressed highly in the absence of pathogens, and this expression is in some cases required for their function as sensors of effectors (Bieri et al. 2004; Brabham et al. 2024b).

Restricting expression of NLRs to the tissues with the highest pathogen pressure would reduce fitness costs associated with high receptor expression (Contreras et al. 2023; Lüdke et al. 2023). Two examples in tomato support this hypothesis: the *I-2* gene provides immunity against vascular wilt and shows vascular tissue-specific expression (Mes et al. 2000), and the *NRC6* gene cluster that confers resistance to cyst and root-knot nematodes has root-exclusive expression (Lüdke et al. 2023). Spatial regulation of expression can also be driven by developmental tradeoffs: altering of *FLS2*-expressing cell types in Arabidopsis caused collapsed meristem cells and inhibited root growth (Emonet et al. 2021). At the gene family level, NLRs in tomato show root skewed expression, while Arabidopsis has reduced expression of NLRs in roots relative to shoots, consistent with a lack of association with arbuscular mycorrhizal fungi and therefore decreased pathogen pressure in the roots (Munch et al. 2018).

Immune receptor expression level is shaped by downward selective pressure driven by fitness costs associated with excessive expression and upward selective pressure of the minimum dosage required for functionality (Figure 2). Depending on the mode of action and likelihood of autoactivity, individual receptors have different upper and lower bounds of tolerable expression. The NLRs Sr33 and Sr50 are moderately autoactive on the protein level and constitutively high gene expression exacerbates the autoactivity

(Tamborski et al. 2023). In this case, pathogen-specific induction of NLRs, in combination with low basal expression, would prevent autoimmunity while maintaining their functionality for defense activation upon pathogen attack. NLRs in sensor-helper pairs are in tight co-evolutionary relationships, as unbalanced expression of the two NLRs can spuriously activate the immune system. These NLR pairs are usually found to be co-localized in the genome, with head-to-head orientation, supposedly being co-regulated by the same promoter (Yang et al. 2024; Shimizu et al. 2022). However, *Mla7* in Barley is a singleton direct recognition NLR that requires multiple active copies with high expression for resistance (Brabham et al. 2024b), indicating a requirement of high expression for functionality. Understanding the relationship between expression, mechanism, and functionality of NLRs will greatly assist engineering efforts and successful implementation in crops (Figure 2).

Two theories have emerged for the relationship between expression and evolvability of immune receptors. For an immune receptor under diversifying selection to evolve new recognition specificity, it can either be lowly expressed while accumulating mutations and then be de-repressed to higher expression once a functional allele evolves (Shivaprasad et al. 2012; Zhang et al. 2016; Brabham et al. 2024b), or begin constitutively expressed and evolve regulation after functionality (Prigozhin and Krasileva 2021; Sutherland et al. 2024). Low expression of a rapidly evolving receptor would allow for a higher tolerance of mutations with slightly deleterious effects until functionality is achieved, as supported by the presence of microRNA families capable of silencing multiple NLRs simultaneously in pathogen-free tissues (Shivaprasad et al. 2012; Zhang et al. 2016) and the enrichment of functional, presumably not deleterious NLRs in constitutive expression states (Brabham et al. 2024b). Alternatively, high expression levels observed across highly variable NLRs (Brabham et al. 2024b; Sutherland et al. 2024) may increase evolvability through increased likelihood of mutation associated with transcription (Staunton et al. 2023) and strengthened effect of individual mutations on fitness, increasing the efficacy of selection (Bódi et al. 2017; Payne and Wagner 2019). However, it is likely the variation in expression drives evolvability of immune receptors (Capp 2021; Prigozhin et al. 2024), with both theories acting in parallel on different receptors depending on the initial expression state and the strength of selection. High throughput analysis of the

relationship between expression levels and fitness of immune receptors of multiple types will provide the evidence required to resolve this thorny evolutionary trajectory.

## I am one with the Force, and the Force is with me: opportunities and challenges in immune receptor expression

Recent advancements in understanding the evolution of spatial and temporal expression can assist in the identification and engineering of functional immune receptors. For identification, constitutive expression can be used as a selection criteria to enrich for functional NLRs genome-wide or in map-based NLR cloning (Kawashima et al. 2016; Brabham et al. 2024b), and tissue specificity can be related to pathogen specificity, narrowing the search space for resistance (Contreras et al. 2023; Lüdke et al. 2023). For example, shoot-specific expression of an engineered defense-related gene in rice largely mitigated the fitness cost of constitutive defense, as it concentrated the immune responses to tissues threatened by their target pathogens (Molla et al. 2016). The development of spatial and single cell RNA-seq will provide high-resolution expression atlases (Zhu et al. 2023), with the opportunity to bring immune receptor engineering to unprecedented levels of precision.

The specificity of interaction between NLRs and effectors makes it challenging to engineer the receptors for broad spectrum resistance. As an alternative strategy to protein engineering, several studies have explored control of transcription. For example, autoactive NLRs or elicitors of NLRs can be deployed in plants under pathogen-inducible (PI) promoters (Figure 2). This strategy has been successful against pathogens harboring transcription activator-like (TAL) effectors, such as *Xanthomonas* and *Ralstonia* (Zeng et al. 2015; Gallas et al. 2024). Stacking effector binding elements in the promoter drives the expression of a cell death-inducing NLR gene *Xa10* in rice, enabling induction of resistance by multiple different *Xanthomonas oryzae* strains (Zeng et al. 2015). However, it is challenging to apply these synthetic cassettes against other pathogens because the transcription induction depends on TAL effectors. The tobacco promoter *hsr203j* shows conserved induction by pathogens of several classes and was harnessed to regulate an

HR-inducing elicitor in tobacco, producing broad-spectrum resistance (Keller et al. 1999). Recently, an autoactive mutant of *Sr33* and the intrinsically autoactive *Sr50* were fused with PI promoters in tomato for conferring broad-spectrum resistance (Wei et al. 2024). Critical to the success of this effort was pairing autoactive NLRs of varying sensitivities with PI promoters optimized for strength and inducibility to balance immunity and fitness costs (Figure 2).

Widely adopting a transcriptional engineering strategy in crops requires precise transcriptional regulation specifically responsive to pathogens and not other stressors. Some PI promoters are also developmentally regulated, leading to unintended induction of NLR expression and autoimmunity at a specific developmental stages (Honee et al. 1998; Wei et al. 2024). Comprehensive examination across different tissues, developmental stages, and stress responses is necessary to mine for specifically PI promoters. In addition, the advancement of synthetic biology tools has empowered engineering of transcriptional regulation. For example, promoter engineering at different scales could fine tune the expression precisely to balance leaky expression and induced immunity (Jores et al. 2021; Wei et al. 2024). Designing transcription circuits that display both pathogen inducibility and tissue specificity would further reduce the fitness cost of defense (Brophy et al. 2022). Several successful cases of engineering induced resistance also suggest adding post-transcriptional or translational regulations in addition to inducible promoters might be required to minimize undesired expression (Gonzalez et al. 2015; Xu et al. 2017). Manipulation of spatial, temporal, and inducibility of immune receptor expression, aided by characterized promoter libraries, represents an exciting opportunity for the future of plant immune engineering.

## Episode 3. The Rise of Ultimate Power: Immune Receptor Engineering

*By Kyungyong Seong and Danielle M. Stevens*

Both genetic and regulatory changes are reflected in protein structure and function. In an endless armsrace, pathogens evolve effectors to disarm plants, often adapting more rapidly and divergently than plants can respond. In monocultural fields, the breakdown of resistance can destroy entire populations, threatening global food security. The ultimate

power to overcome these threats rises through precise introduction of desired changes in immune receptors.

**A little more knowledge lights our way: paving the path to receptor engineering**

Since the discovery of the first immune receptors, a central goal of our community has been to engineer receptors for improved, altered, or broadened ligand specificity. Early engineering endeavors were driven by classical techniques including random mutagenesis, chimeras, and domain swaps leading to altered ligand perception (Wulff et al. 2001; Mueller et al. 2012; Helft et al. 2016), enhanced immune responses (Harris et al. 2013; Sueldo et al. 2015) and gain of effector recognition (Segretin et al. 2014; Huang et al. 2021). Studies of allele variants provided further insight into receptor function, residues potentially required for ligand binding, and protein complex formation and stability (Dunning et al. 2007). The overarching aim remains to amplify intended immune outcomes while minimizing unintended consequences (Figure 3). This requires continued accumulation of knowledge in protein stability during complex formation for PRRs (Dunning et al. 2007; Li et al. 2024a) and addressing challenges posed by intricate interactions of NLRs, in order to allow precise and effective engineering strategies.

**Table 1. Representative structures of NLRs and PRRs**

| PDB Accession* | NLR/ PRR | Type | Organism | Effector/ Ligand | Organism | Reference |
|---|---|---|---|---|---|---|
| 4EBZ | CERK1 | LysM-RLK | *Arabidopsis thaliana* | chitin | | (Liu et al. 2012) |
| 4MN8 | FLS2 | LRR-RLK | *Arabidopsis thaliana* | flg22 | | (Sun et al. 2013) |
| 6J5T | ZAR1 | CC-NLR | *Arabidopsis thaliana* | | | (Wang et al. 2019a, 2019b) |
| 7CRC | RPP1 | TIR-NLR | *Arabidopsis thaliana* | ATR1 | *Hyaloperonospora arabidopsidis* | (Ma et al. 2020) |
| 7JLU | Roq1 | TIR-NLR | *Nicotiana benthamiana* | XopQ | *Xanthomonas euvesicatoria* | (Martin et al. 2020) |
| 7DRC | RXEG1 | LRR-RLP | *Nicotiana benthamiana* | XEG1 | | (Sun et al. 2022) |
| 7XE0 | Sr35 | CC-NLR | *Triticum* | AvrSr35 | *Puccinia* | (Zhao et al. 2022) |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | *monococcum* | | *graminis* f. sp . *tritici* | |
| 7XC2 | Sr35 | CC-NLR | *Triticum monococcum* | AvrSr35 | *Puccinia graminis* f. sp . *tritici* | (Förderer et al. 2022) |
| N/A | MLA13 | CC-NLR | *Hordeum vulgare* | $AVR_{A13}$-1 | *Blumeria hordei* | (Lawson et al. 2024) |
| 8RFH | NRC2 | CC-NLR | *Nicotiana benthamiana* | | | (Selvaraj et al. 2024) |
| 9FP6 | NRC2 | CC-NLR | *Nicotiana benthamiana* | | | (Madhuprakash et al. 2024) |
| 8XUV | NRC2 | CC-NLR | *Solanum lycopersicum* | | | (Ma et al. 2024) |
| 9CC9 | NRC4 | CC-NLR | *Nicotiana benthamiana* | | | (Liu et al. 2024a) |
| 8ZF0 | ADR1 | RPW8-NLR | *Oryza sativa* | | | (Wu et al. 2024) |
| 8ZW9 | ADR1 | RPW8-NLR | *Arabidopsis thaliana* | | | (Yu et al. 2024b) |

*Only one structure is associated with each study in this table. Additional structures can be identified within each entry in the Protein Data Bank (PDB).

## We are what they grew beyond: pushing the boundaries of receptor engineering

Numerous past studies have laid foundations for recent advances in sequence and structural analyses. For engineering the LRR domain, evolution-guided targeted swaps of highly variable LRR residues from Sr50 to closely related Sr33 demonstrated precise transfer of effector recognition specificity in wheat (Tamborski et al. 2023). Similarly, phylogenetic comparisons and ancestral reconstruction of the bean receptor INR enabled altered perception of the caterpillar-associated molecule inceptin (Snoeck et al. 2022). In contrast to the evolution guided design, high-throughput random screening can be an alternative strategy. This approach involves generating shuffled DNA libraries of receptors and screening recombinants against effectors through yeast two-hybrids (Zhang et al. 2024b). A successful example includes the identification of a single substitution between barley MLA7 and MLA13 that allowed MLA7 to recognize MLA13's cognate effector, $Avr_{A13}$-1 (Zhang et al. 2024b).

In parallel, structural insights have accelerated receptor engineering (Table 1). FLS2 from Arabidopsis has provided fundamental knowledge to engineer recognition of flg22 epitope variants (Sun et al. 2013; Li et al. 2024a; Zhang et al. 2024a). Discovery of diverse FLS2 homologs and structure-guided residue swaps improved flg22 binding affinity and gained perception of previously evaded ligands (Fürst et al. 2020; Wei et al. 2020). Unlike PRRs, the number of NLR structures has gradually grown (Table 1). The resistosome structures of indirect binder ZAR1 and direct binder TIR-NLR RPP1 in Arabidopsis (Wang et al. 2019a, 2019b; Ma et al. 2020) propelled the determination of CC-NLRs capable of directly binding to their effectors. The einkorn wheat Sr35 resistosome structure was solved (Förderer et al. 2022; Zhao et al. 2022), allowing identification of its effector binding site and direct transfer of its specificity to wheat TaSh1 and barley HvSh1 (Förderer et al. 2022). Similarly, the heterodimer structure of barley MLA13 and its cognate effector $Avr_{A13}$-1 pinpointed a single amino acid change that can broaden recognition specificity of MLA7 (Lawson et al. 2024). These examples demonstrated that receptor engineering can be accelerated through experimental determination of PRR-ligand and NLR-effector complex structures.

Given the challenges in determining structures via X-ray crystallography and Cryogenic electron microscopy, AlphaFold has become a valuable alternative in both PRRs and NLRs. Two independent studies have shown that using AlphaFold can improve engineering and rational design for expanded ligand recognition in FLS2, where a small number of substitutions was required to gain recognition to a target set of flg22 epitopes (Li et al. 2024a; Zhang et al. 2024a). The use of AlphaFold also allowed the prediction of MLA3's binding site in barley (Gómez De La Cruz et al. 2024), uncovering its similarity to that of OsHIPP43, a rice protein targeted by the effector Pwl2 (Brabham et al. 2024a; Zdrzałek et al. 2024). This molecular mimicking was akin to Tsw in pepper, the LRR of which showed structural similarity to COI1 and TIR1 commonly targeted by pathogens (Chen et al. 2023). AlphaFold enabled precise transfer of the Pwl2 binding site from MLA3 to Sr50 without hindering Sr50's ability to recognize its effector AvrSr50 (Gómez De La Cruz et al. 2024). Another study utilized AlphaFold to iteratively refine crude molecular docking models of Sr50 and AvrSr50 and guide the selection of LRR residues responsible for effector binding (Seong et al. 2024). Eventually, Sr50 could be resurrected to induce

immune responses to the escape effector mutant AvrSr50$^{QCMJC}$, which no naturally occurring immune receptors are known to recognize (Ortiz et al. 2022; Seong et al. 2024).

Structural information and precise modification of IDs has been an alternative avenue to LRR engineering (Cesari et al. 2022; Zhang et al. 2024c). The crystal structure of the effector AVR-Pik bound to OsHIPP19 in rice led to mutations that expanded recognition specificity of Pik-1 against stealthy effector variants (Maidment et al. 2021, 2023). Similarly, the complex structure of Pwl2 bound to its host protein OsHIPP43 in rice enabled the generation of Pikm-1 with its HMA domain replaced with OsHIPP43 for broad pathogen recognition (Zdrzałek et al. 2024). The identification of additional host proteins, such as OsHIPP20 (Oikawa et al. 2024), and their interactions with effectors would further offer additional ID engineering strategies. The manageable size of IDs has also facilitated scalable, high-throughput approaches for ID engineering. For instance, mutagenized HMA domains of Pik1 were displayed on the yeast cell surface to screen against diverse variants of Avr-Pik (Rim et al. 2024). Selection based on interaction by fluorescence-activated cell sorting enabled directed evolution of the HMA domain with broad-spectrum binding capabilities. Challenges remain in effective integration of IDs without autoimmunity and compromised immune responses (Marchal et al. 2022; Zdrzałek et al. 2023). Together with the pikobody approach integrating nanobodies from mammalian adaptive immune systems into NLRs (Kourelis et al. 2023), development of novel methodologies continues to push the boundaries of immune receptor engineering.

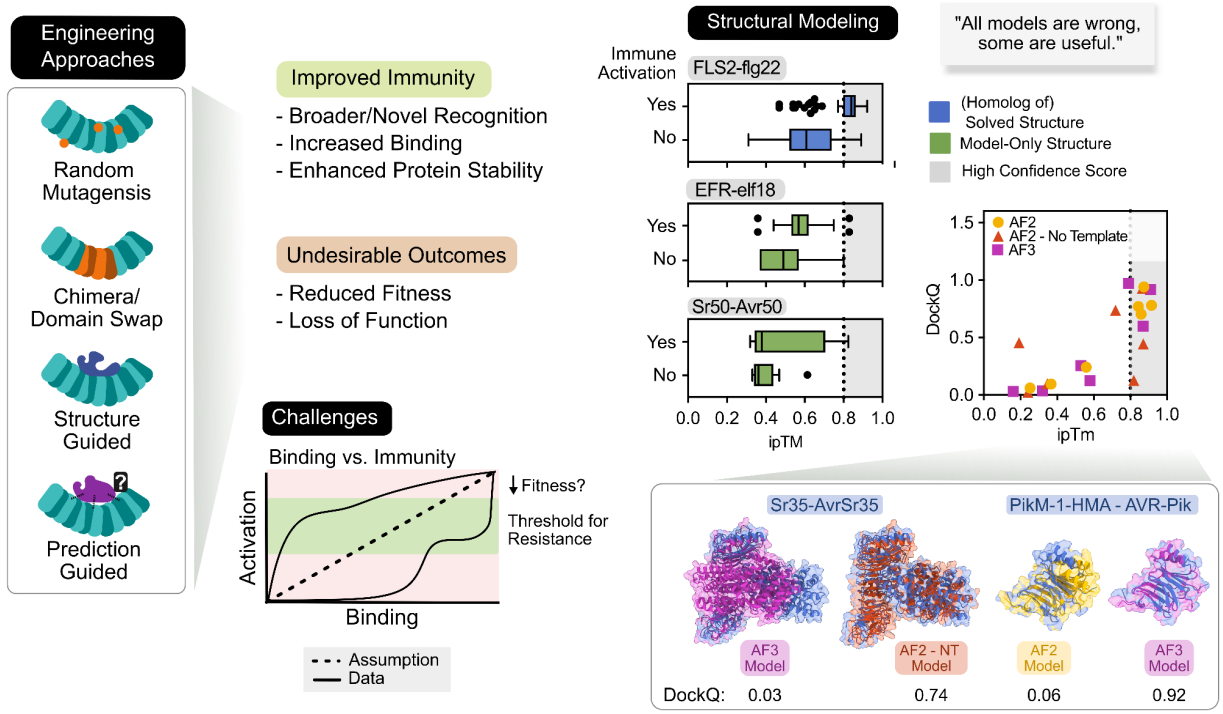



**Figure 3: Combination of techniques including structural modeling enable engineering of immune receptors.** Engineering approaches aim to improve immune responses but could lead to undesirable outcomes. In particular, our understanding of the relationship between ligand binding and immune activation is yet incomplete, making it challenging to establish strategies to overcome the unintended consequences. Structural prediction enables hypothesis generation of receptor-ligand interactions and may guide engineering endeavors, as some models show high accuracy. Nevertheless, predicted structures should be used with caution, as they occasionally display notable accuracy, and correlations between the model accuracy and immune responses need to be further examined.

## May the Fold be with you: structural quest for immune receptors and effectors

AlphaFold has markedly expanded our understanding of immune receptors, effectors, ligands, and their interactions (Evans et al. 2021, 202; Jumper et al. 2021; Abramson et al. 2024; Li et al. 2024b). Recent studies have explored the structural diversity of N-terminal domains of NLRs within *Plantae* (Chia et al. 2024) and LRR domains in maize (Prigozhin et al. 2024). Putative resistosome structures of CC-NLRs were constructed

with AlphaFold 3 with high confidence (Madhuprakash et al. 2024). In parallel, our community has gained an additional dimension of insights in effector diversity, regulation, evolution and interaction that was difficult to uncover with primary sequences alone (Seong and Krasileva 2021, 2023; Rocafort et al. 2022; Derbyshire and Raffaele 2023; Homma et al. 2023; Yan et al. 2023; Asghar et al. 2024; Mukhopadhyay et al. 2024; Yu et al. 2024a).

In certain contexts, AlphaFold achieves accurate binding interaction predictions, highlighting its potential for computational screening (Figure 3). In particular, relatively small sizes of IDs and effectors enable scalable all-vs-all interaction predictions. Combined with experimental validation, this computational approach creates opportunities to rapidly uncover novel effector targets and identify functional IDs suitable for engineering. Known NLR-IDs, such as the WRKY domain found in RPS1 and *Triticum urartu*'s YrU1 (Le Roux et al. 2015; Sarris et al. 2015; Wang et al. 2020; Mukhi et al. 2021) or the zinc-finger BED domain in Yr5 and Yr7 in wheat (Marchal et al. 2018), could be included for further expansion of our understanding of their functions. Despite such promises, AlphaFold's predictions on some LRR-ligand or LRR-effector complexes show notable inaccuracy (Figure 3). For PRRs, previously characterized FLS2-flg22 interactions have proposed using ipTM as a proxy for binding, in which accuracy of immune prediction was based on an ipTM threshold of 0.8 (Figure 3) (Li et al. 2024a). However, when assessing recognition prediction for other receptor-ligand/effector interactions using the same threshold, accuracy is very low (Figure 3) (Colaianni et al. 2021; Parys et al. 2021; Seong et al. 2024; Stevens et al. 2024; Trinh et al. 2024). Similarly, the two LRR-effector complexes, MLA13 and $AVR_{A13}$-1 (Lawson et al. 2024) as well as Sr50 and AvrSr50 (Seong et al. 2024), could not be predicted correctly. The low accuracy is likely due to a combination of factors including the lack of underlying solved structure AlphaFold was trained on and the large binding face along the concave surface. Therefore, while AlphaFold has enabled structural modeling attempts for many known NLR-effector pairs lacking experimental structures (Fick et al. 2024; Wang et al. 2024), these predictions require careful validation through additional experimental data.

Our understanding of AlphaFold is yet incomplete. Although AlphaFold could not predict the complex structure for Sr50 and AvrSr50, experimentally supported dimeric structures could be modeled for Sr50 variants, $Sr50^{K711D}$ and $SR50^{3BI}$, and AvrSr50 (Figure 3) (Gómez De La Cruz et al. 2024; Seong et al. 2024). These results suggest that single amino acid changes in NLRs can significantly impact AlphaFold's predictions, and that the altered prediction possibly reflects biological relevance. This underscores the potential for computational mutational screening to explore how sequence variations influence predictive accuracy and experimental assessment of biological impacts in plants. Advances in prediction algorithms, such as AlphaFold 3, will also continue to address the challenges of accurate structural modeling. However, despite the release of AlphaFold 3, AlphaFold 2 remains a viable option, as we still require experimentally determined templates to improve prediction accuracy, particularly for effector structures like AvrSr27 and AvrSr50 (Figure 3) (Ortiz et al. 2022; Outram et al. 2024). AlphaFold 2, along with the more accessible ColabFold (Mirdita et al. 2022), offers tunable parameters that could enhance the prediction successes. For instance, increasing the number of random seeds, recycles, and ensembles can enhance the predictive power (Jumper et al. 2021; Agarwal and McShan 2024).

Looking ahead, refining AlphaFold predictions for PRR-ligand and NLR-effector complexes will greatly benefit from broader community collaboration. By standardizing benchmarking protocols and validating predicted structures experimentally, our community can more effectively assess AlphaFold's performance and identify pathways for improvement, particularly for complex cases. Structural investigations into these predictions offer a promising avenue to accelerate our understanding of plant immune responses and advance predictive capabilities in protein design, while experimental structures provide essential ground-truth over time. Embracing not only successes but also failures in this collective structural endeavor is essential to complete our structural quests. After all, all models are wrong, but some are useful.

## To boldly go where no one has gone before: leveraging deep learning in plant immunity

Beyond protein structure prediction, machine learning—particularly deep learning—has rapidly expanded our toolkit for exploring protein biology. Geometric deep learning enables analysis of protein surfaces to identify protein-protein interaction sites (Gainza et al. 2020; Tubiana et al. 2022; Krapp et al. 2023). Advances in algorithms like ProteinMPNN and RFDiffusion have made it possible to design primary sequences and protein structures (Dauparas et al. 2022; Watson et al. 2023). Protein language models have also shown remarkable promise with applications in illuminating virtual mutations and aiding antibody design (Hie et al. 2021, 2022, 2023; Lin et al. 2023; Ruffolo and Madani 2024). Applications can potentially extend to NLRs and effectors, as well as PRRs and ligands, allowing association of mutational likelihood with specific roles, functions, and interactions. Notably, our community has already uncovered insights into evolutionary variability in residues within LRR domains of NLRs and PRRs (Prigozhin and Krasileva 2021; Li et al. 2024a; Seong et al. 2024), as well as functional diversity within closely related receptors (Lu et al. 2016; Saur et al. 2019; Bauer et al. 2021). These findings lay the groundwork for using machine learning models to deepen our understanding of natural diversity and its role in functional diversification, potentially enhancing our ability to engineer plant immune receptors.

One common theme for the future is the need to generate and utilize good underlying experimental data for more accurate hypothesis generation and model training. For antibody structure prediction, over 3,400 antibody structures and 550 million antibody sequences were used for training (Ruffolo et al. 2023). Building such models for PRR-ligand or NLR-effector interactions will require well-organized and accessible datasets. Recent comparative genomic approaches have curated a naturally diverse PRR ligand dataset, much of which has been experimentally tested in Arabidopsis or tomato (Colaianni et al. 2021; Parys et al. 2021; Stevens et al. 2024). Similarly, comprehensive resources cataloging NLRs and detailing plant-pathogen interactions have been developed, including RefPlantNLR, ANNA, NLRscape, PlantNLRatlas, and PHI-base (Urban et al. 2019; Kourelis et al. 2021; Liu et al. 2021; Li et al. 2023; Martin et al. 2023). However, we may need to broaden these databases to include diverse data types, such as predicted immune receptors, effectors or ligands, and their complex structures, along with quantitative and qualitative interaction data. These resources could facilitate models

for predicting mutation impacts and support the design of functional assays to capture binding, recognition, and immune outcomes. Standardizing data types and reporting methods will be crucial to make these resources broadly usable and to support machine learning applications. In this new journey, our community stands ready to explore new frontiers, boldly going where no one has gone before in plant immunity.

## Conclusions

The growth of the availability and quality of data at the DNA, RNA, and protein levels have provided insights into the diversity generation and maintenance of plant immune receptors and have begun to answer the long-standing question of how strictly innate immune systems cope with rapidly evolving pathogens. Natural diversity uncovered at each layer poses challenges and opportunities for receptor engineering. Further understanding of the mechanisms behind high nucleotide diversity, transcriptional regulation, and immune receptors' ligand binding and activation will not only lead to new engineering strategies but also aid in continued development of computational predictive and generative models.

We posit that there is sufficient evidence to suggest that plant immune systems fit an anticipatory model of immune evolution, with a high likelihood of individual mutation maintained by population level-selection. The expression of immune receptors exists along multiple axes of regulation and is increasingly relevant to functional receptor prioritization and optimized control in engineering efforts. The new hope of protein structure prediction has revolutionized immune receptor design, but poses new opportunities and challenges for ensuring accurate and relevant structures. As we gain additional high throughput datasets through community-coordinated efforts, we can better leverage emerging machine learning approaches. Along with progress in understanding plant disease resistance, we face regulatory challenges, in particular which bioengineering efforts will be allowed in scalable agricultural settings and when they will be accepted by the general public. "Difficult to see. Always in motion is the future"—it is not yet clear how far we can apply synthetic immune receptors in crops. However, we must do our best to communicate that plants are natural engineers, and we have and continue to learn from them in our efforts.

## Contributions

CAS led conceptualization of the manuscript and drafting of the DNA diversity and contributed to expression variation. DMS added PRR content across each section, led figure generation, and contributed to the immune receptor engineering section. WW led expression variation and its regulation. KS led immune receptor engineering. KVK wrote the introduction, contributed to conceptualization of the manuscript, and contributed to the DNA section. All authors were involved in reviewing and editing.

## Conflict of Interest Statement

There are no conflicts of interest.

## Acknowledgements

We would like to thank all the members of the Krasileva lab for their feedback on the conceptualization and editing of this manuscript. We would like to acknowledge George Lucas and the Star Wars franchise for inspiration in the thematic organization of the manuscript, and to ChatGPT for assistance in incorporating his work into the headings. ChatGPT was not otherwise used in the conceptualization, drafting, or editing of this manuscript. CAS has been supported by the Grace Kase-Tsujimoto Graduate Fellowship. KS has been supported by the Berkeley BioEnginuity Fellowship. All authors have received support from the NIH Director's Award (1DP2AT011967-01) awarded to KVK, Gordon and Betty Moore Inventor Fellowship (grant number: 8802), and the Innovative Genomics Institute.

# References

**Abascal F, Harvey LMR, Mitchell E, Lawson ARJ, Lensing SV, Ellis P, Russell AJC, Alcantara RE, Baez-Ortega A, Wang Y, et al.** Somatic mutation landscapes at single-molecule resolution. Nature. 2021:**593**(7859):405–410. https://doi.org/10.1038/s41586-021-03477-4

**Abramson J, Adler J, Dunger J, Evans R, Green T, Pritzel A, Ronneberger O, Willmore L, Ballard AJ, Bambrick J, et al.** Accurate structure prediction of biomolecular interactions with AlphaFold 3. Nature. 2024:**630**(8016):493–500. https://doi.org/10.1038/s41586-024-07487-w

**Adachi H, Derevnina L, and Kamoun S**. NLR singletons, pairs, and networks: evolution, assembly, and regulation of the intracellular immunoreceptor circuitry of plants. Curr Opin Plant Biol. 2019:**50**:121–131. https://doi.org/10.1016/j.pbi.2019.04.007

**Adema CM**. Fibrinogen-Related Proteins (FREPs) in Mollusks. Results Probl Cell Differ. 2015:**57**:111–129. https://doi.org/10.1007/978-3-319-20819-0_5

**Agarwal V and McShan AC**. The power and pitfalls of AlphaFold2 for structure prediction beyond rigid globular proteins. Nat Chem Biol. 2024:**20**(8):950–959. https://doi.org/10.1038/s41589-024-01638-w

**Arora S, Steuernagel B, Gaurav K, Chandramohan S, Long Y, Matny O, Johnson R, Enk J, Periyannan S, Singh N, et al.** Resistance gene cloning from a wild crop relative by sequence capture and association genetics. Nat Biotechnol. 2019:**37**(2):139–143. https://doi.org/10.1038/s41587-018-0007-9

**Asghar R, Wu N, Ali N, Wang Y, and Akkaya M**. Computational studies reveal structural characterization and novel families of Puccinia striiformis f. sp. tritici effectors. 2024. https://doi.org/10.1101/2024.09.12.612600

**Ashikawa I, Hayashi N, Yamane H, Kanamori H, Wu J, Matsumoto T, Ono K, and Yano M**. Two Adjacent Nucleotide-Binding Site–Leucine-Rich Repeat Class Genes Are Required to Confer Pikm -Specific Rice Blast Resistance. Genetics. 2008:**180**(4):2267–2276. https://doi.org/10.1534/genetics.108.095034

**Atanasov KE, Liu C, Erban A, Kopka J, Parker JE, and Alcázar R**. NLR Mutations Suppressing Immune Hybrid Incompatibility and Their Effects on Disease

Resistance. Plant Physiol. 2018:**177**(3):1152–1169. https://doi.org/10.1104/pp.18.00462

**Bae JH, Liu R, Roberts E, Nguyen E, Tabrizi S, Rhoades J, Blewett T, Xiong K, Gydush G, Shea D, et al.** Single duplex DNA sequencing with CODEC detects mutations with high sensitivity. Nat Genet. 2023:**55**(5):871–879. https://doi.org/10.1038/s41588-023-01376-0

**Baggs E, Dagdas G, and Krasileva K**. NLR diversity, helpers and integrated domains: making sense of the NLR IDentity. Curr Opin Plant Biol. 2017:**38**:59–67. https://doi.org/10.1016/j.pbi.2017.04.012

**Baggs EL, Monroe JG, Thanki AS, O'Grady R, Schudoma C, Haerty W, and Krasileva KV**. Convergent Loss of an EDS1/PAD4 Signaling Pathway in Several Plant Lineages Reveals Coevolved Components of Plant Immunity and Drought Response[OPEN]. Plant Cell. 2020:**32**(7):2158–2177. https://doi.org/10.1105/tpc.19.00903

**Bailey PC, Schudoma C, Jackson W, Baggs E, Dagdas G, Haerty W, Moscou M, and Krasileva KV**. Dominant integration locus drives continuous diversification of plant immune receptors with exogenous domain fusions. Genome Biol. 2018:**19**(1):23. https://doi.org/10.1186/s13059-018-1392-6

**Bakker EG, Toomajian C, Kreitman M, and Bergelson J**. A Genome-Wide Survey of *R* Gene Polymorphisms in *Arabidopsis*. Plant Cell. 2006:**18**(8):1803–1818. https://doi.org/10.1105/tpc.106.042614

**Barragan AC, Collenberg M, Wang J, Lee RRQ, Cher WY, Rabanal FA, Ashkenazy H, Weigel D, and Chae E**. A Truncated Singleton NLR Causes Hybrid Necrosis in Arabidopsis thaliana. Mol Biol Evol. 2021:**38**(2):557–574. https://doi.org/10.1093/molbev/msaa245

**Barragan AC and Weigel D**. Plant NLR diversity: the known unknowns of pan-NLRomes. Plant Cell. 2021:**33**(4):814–831. https://doi.org/10.1093/plcell/koaa002

**Bashir S, Rehman N, Fakhar Zaman F, Naeem MK, Jamal A, Tellier A, Ilyas M, Silva Arias GA, and Khan MR**. Genome-wide characterization of the NLR gene family in tomato (Solanum lycopersicum) and their relatedness to disease

resistance. Front Genet. 2022:**13**:931580. https://doi.org/10.3389/fgene.2022.931580

**Bauer S, Yu D, Lawson AW, Saur IML, Frantzeskakis L, Kracher B, Logemann E, Chai J, Maekawa T, and Schulze-Lefert P**. The leucine-rich repeats in allelic barley MLA immune receptors define specificity towards sequence-unrelated powdery mildew avirulence effectors with a predicted common RNase-like fold. PLOS Pathog. 2021:**17**(2):e1009223. https://doi.org/10.1371/journal.ppat.1009223

**Beck M, Wyrsch I, Strutt J, Wimalasekera R, Webb A, Boller T, and Robatzek S**. Expression patterns of FLAGELLIN SENSING 2 map to bacterial entry sites in plant shoots and roots. J Exp Bot. 2014:**65**(22):6487–6498. https://doi.org/10.1093/jxb/eru366

**Bent AF, Kunkel BN, Dahlbeck D, Brown KL, Schmidt R, Giraudat J, Leung J, and Staskawicz BJ**. RPS2 of Arabidopsis thaliana: a Leucine-Rich Repeat Class of Plant Disease Resistance Genes. Science. 1994:**265**(5180):1856–1860. https://doi.org/10.1126/science.8091210

**Bieri S, Mauch S, Shen Q-H, Peart J, Devoto A, Casais C, Ceron F, Schulze S, Steinbiß H-H, Shirasu K, et al.** RAR1 Positively Controls Steady State Levels of Barley MLA Resistance Proteins and Enables Sufficient MLA6 Accumulation for Effective Resistance. Plant Cell. 2004:**16**(12):3480–3495. https://doi.org/10.1105/tpc.104.026682

**Bittner-Eddy PD, Crute IR, Holub EB, and Beynon JL**. RPP13 is a simple locus in Arabidopsis thaliana for alleles that specify downy mildew resistance to different avirulence determinants in Peronospora parasitica. Plant J. 2000:**21**(2):177–188. https://doi.org/10.1046/j.1365-313x.2000.00664.x

**Bódi Z, Farkas Z, Nevozhay D, Kalapis D, Lázár V, Csörgő B, Nyerges Á, Szamecz B, Fekete G, Papp B, et al.** Phenotypic heterogeneity promotes adaptive evolution. PLOS Biol. 2017:**15**(5):e2000644. https://doi.org/10.1371/journal.pbio.2000644

**Bomblies K, Lempe J, Epple P, Warthmann N, Lanz C, Dangl JL, and Weigel D**. Autoimmune Response as a Mechanism for a Dobzhansky-Muller-Type

Incompatibility Syndrome in Plants. PLOS Biol. 2007:**5**(9):e236. https://doi.org/10.1371/journal.pbio.0050236

**Botella MA, Coleman MJ, Hughes DE, Nishimura MT, Jones JDG, and Somerville SC**. Map positions of 47 Arabidopsis sequences with sequence similarity to disease resistance genes. Plant J. 1997:**12**(5):1197–1211. https://doi.org/10.1046/j.1365-313X.1997.12051197.x

**Brabham HJ, Gómez De La Cruz D, Were V, Shimizu M, Saitoh H, Hernández-Pinzón I, Green P, Lorang J, Fujisaki K, Sato K, et al.** Barley MLA3 recognizes the host-specificity effector Pwl2 from Magnaporthe oryzae. Plant Cell. 2024a:**36**(2):447–470. https://doi.org/10.1093/plcell/koad266

**Brabham HJ, Hernández-Pinzón I, Yanagihara C, Ishikawa N, Komori T, Matny ON, Hubbard A, Witek K, Feist A, Numazawa H, et al.** Discovery of functional NLRs using expression level, high-throughput transformation, and large-scale phenotyping. 2024b:2024.06.25.599845. https://doi.org/10.1101/2024.06.25.599845

**Brophy JAN, Magallon KJ, Duan L, Zhong V, Ramachandran P, Kniazev K, and Dinneny JR**. Synthetic genetic circuits as a means of reprogramming plant roots. Science. 2022:**377**(6607):747–751. https://doi.org/10.1126/science.abo4326

**Cagan A, Baez-Ortega A, Brzozowska N, Abascal F, Coorens THH, Sanders MA, Lawson ARJ, Harvey LMR, Bhosle S, Jones D, et al.** Somatic mutation rates scale with lifespan across mammals. Nature. 2022:1–8. https://doi.org/10.1038/s41586-022-04618-z

**Capp J-P**. Interplay between genetic, epigenetic, and gene expression variability: Considering complexity in evolvability. Evol Appl. 2021:**14**(4):893–901. https://doi.org/10.1111/eva.13204

**Cesari S, Thilliez G, Ribot C, Chalvon V, Michel C, Jauneau A, Rivas S, Alaux L, Kanzaki H, Okuyama Y, et al.** The Rice Resistance Protein Pair RGA4/RGA5 Recognizes the Magnaporthe oryzae Effectors AVR-Pia and AVR1-CO39 by Direct Binding. Plant Cell. 2013:**25**(4):1463–1481. https://doi.org/10.1105/tpc.112.107201

**Cesari S, Xi Y, Declerck N, Chalvon V, Mammri L, Pugnière M, Henriquet C, De**

**Guillen K, Chochois V, Padilla A, et al.** New recognition specificity in a plant immune receptor by molecular engineering of its integrated domain. Nat Commun. 2022:**13**(1):1524. https://doi.org/10.1038/s41467-022-29196-6

**Chen J, Bataillon T, Glémin S, and Lascoux M**. What does the distribution of fitness effects of new mutations reflect? Insights from plants. New Phytol. 2022:**233**(4):1613–1619. https://doi.org/10.1111/nph.17826

**Chen J, Zhao Y, Luo X, Hong H, Yang T, Huang S, Wang C, Chen H, Qian X, Feng M, et al.** NLR surveillance of pathogen interference with hormone receptors induces immunity. Nature. 2023:**613**(7942):145–152. https://doi.org/10.1038/s41586-022-05529-9

**Chia K-S, Kourelis J, Teulet A, Vickers M, Sakai T, Walker JF, Schornack S, Kamoun S, and Carella P**. The N-terminal domains of NLR immune receptors exhibit structural and functional similarities across divergent plant lineages. Plant Cell. 2024:**36**(7):2491–2511. https://doi.org/10.1093/plcell/koae113

**Chisholm ST, Coaker G, Day B, and Staskawicz BJ**. Host-Microbe Interactions: Shaping the Evolution of the Plant Immune Response. Cell. 2006:**124**(4):803–814. https://doi.org/10.1016/j.cell.2006.02.008

**Chuberre C, Plancot B, Driouich A, Moore JP, Bardor M, Gügi B, and Vicré M**. Plant Immunity Is Compartmentalized and Specialized in Roots. Front Plant Sci. 2018:**9**. https://doi.org/10.3389/fpls.2018.01692

**Clark RM, Schweikert G, Toomajian C, Ossowski S, Zeller G, Shinn P, Warthmann N, Hu TT, Fu G, Hinds DA, et al.** Common Sequence Polymorphisms Shaping Genetic Diversity in Arabidopsis thaliana. Science. 2007:**317**(5836):338–342. https://doi.org/10.1126/science.1138632

**Clarke CR, Chinchilla D, Hind SR, Taguchi F, Miki R, Ichinose Y, Martin GB, Leman S, Felix G, and Vinatzer BA**. Allelic variation in two distinct Pseudomonas syringae flagellin epitopes modulates the strength of plant immune responses but not bacterial motility. New Phytol. 2013:**200**(3):847–860. https://doi.org/10.1111/nph.12408

**Colaianni NR, Parys K, Lee H-S, Conway JM, Kim NH, Edelbacher N, Mucyn TS, Madalinski M, Law TF, Jones CD, et al.** A complex immune response to

flagellin epitope variation in commensal communities. Cell Host Microbe. 2021:**29**(4):635-649.e9. https://doi.org/10.1016/j.chom.2021.02.006

**Contreras MP, Lüdke D, Pai H, Toghani A, and Kamoun S**. NLR receptors in plant immunity: making sense of the alphabet soup. EMBO Rep. 2023:**24**(10):e57495. https://doi.org/10.15252/embr.202357495

**Dangl JL and Jones JDG**. Plant pathogens and integrated defence responses to infection. Nature. 2001:**411**(6839):826–833. https://doi.org/10.1038/35081161

**Dauparas J, Anishchenko I, Bennett N, Bai H, Ragotte RJ, Milles LF, Wicky BIM, Courbet A, De Haas RJ, Bethel N, et al.** Robust deep learning–based protein sequence design using ProteinMPNN. Science. 2022:**378**(6615):49–56. https://doi.org/10.1126/science.add2187

**Derbyshire MC and Raffaele S**. Surface frustration re-patterning underlies the structural landscape and evolvability of fungal orphan candidate effectors. Nat Commun. 2023:**14**(1):5244. https://doi.org/10.1038/s41467-023-40949-9

**Ding L, Xu X, Kong W, Xia X, Zhang S, Liu L-W, Liu A, and Zou L**. Genome-wide identification and expression analysis of rice *NLR* genes responsive to the infections of *Xanthomonas oryzae* pv. *oryzae* and *Magnaporthe oryzae*. Physiol Mol Plant Pathol. 2020:**111**:101488. https://doi.org/10.1016/j.pmpp.2020.101488

**Dixon MS, Hatzixanthis K, Jones DA, Harrison K, and Jones JD**. The tomato Cf-5 disease resistance gene and six homologs show pronounced allelic variation in leucine-rich repeat copy number. Plant Cell. 1998:**10**(11):1915. https://doi.org/10.1105/tpc.10.11.1915

**Dodds PN**. From Gene-for-Gene to Resistosomes: Flor's Enduring Legacy. Mol Plant-Microbe Interactions®. 2023:**36**(8):461–467. https://doi.org/10.1094/MPMI-06-23-0081-HH

**Dodds PN, Lawrence GJ, and Ellis JG**. Six Amino Acid Changes Confined to the Leucine-Rich Repeat β-Strand/β-Turn Motif Determine the Difference between the P and P2 Rust Resistance Specificities in Flax. Plant Cell. 2001:**13**(1):163–178. https://doi.org/10.1105/tpc.13.1.163

**Dunning FM, Sun W, Jansen KL, Helft L, and Bent AF**. Identification and Mutational Analysis of Arabidopsis FLS2 Leucine-Rich Repeat Domain Residues That

Contribute to Flagellin Perception. Plant Cell. 2007:**19**(10):3297–3313. https://doi.org/10.1105/tpc.106.048801

**Emonet A, Zhou F, Vacheron J, Heiman CM, Dénervaud Tendon V, Ma K-W, Schulze-Lefert P, Keel C, and Geldner N**. Spatially Restricted Immune Responses Are Required for Maintaining Root Meristematic Activity upon Detection of Bacteria. Curr Biol. 2021:**31**(5):1012-1028.e7. https://doi.org/10.1016/j.cub.2020.12.048

**Evans R, O'Neill M, Pritzel A, Antropova N, Senior A, Green T, Žídek A, Bates R, Blackwell S, Yim J, et al.** Protein complex prediction with AlphaFold-Multimer. 2021. https://doi.org/10.1101/2021.10.04.463034

**Eyre-Walker A and Keightley PD**. The distribution of fitness effects of new mutations. Nat Rev Genet. 2007:**8**(8):610–618. https://doi.org/10.1038/nrg2146

**Farrara BF, Ilott TW, and Michelmore RW**. Genetic analysis of factors for resistance to downy mildew (Bremia lactucae) in species of lettuce (Lactuca sativa and L. serriola). Plant Pathol. 1987:**36**(4):499–514. https://doi.org/10.1111/j.1365-3059.1987.tb02267.x

**Fick A, Fick JLM, Swart V, and Van Den Berg N**. What NLR you recognizing? Predicted binding affinities- and energies may be used to identify novel NLR-effector interactions. 2024. https://doi.org/10.1101/2024.07.26.605369

**Fick A, Swart V, Backer R, Bombarely A, Engelbrecht J, and van den Berg N**. Partially Resistant Avocado Rootstock Dusa® Shows Prolonged Upregulation of Nucleotide Binding-Leucine Rich Repeat Genes in Response to Phytophthora cinnamomi Infection. Front Plant Sci. 2022a:**13**. https://doi.org/10.3389/fpls.2022.793644

**Fick A, Swart V, and van den Berg N**. The Ups and Downs of Plant NLR Expression During Pathogen Infection. Front Plant Sci. 2022b:**13**. https://doi.org/10.3389/fpls.2022.921148

**Flor HH**. Current Status of the Gene-For-Gene Concept. Annu Rev Phytopathol. 1971:**9**(Volume 9, 1971):275–296. https://doi.org/10.1146/annurev.py.09.090171.001423

**Förderer A, Li E, Lawson AW, Deng Y, Sun Y, Logemann E, Zhang X, Wen J, Han**

**Z, Chang J, et al.** A wheat resistosome defines common principles of immune receptor channels. Nature. 2022:**610**(7932):532–539. https://doi.org/10.1038/s41586-022-05231-w

**Fritz-Laylin LK, Krishnamurthy N, Tör M, Sjölander KV, and Jones JDG**. Phylogenomic Analysis of the Receptor-Like Proteins of Rice and Arabidopsis. Plant Physiol. 2005:**138**(2):611–623. https://doi.org/10.1104/pp.104.054452

**Fürst U, Zeng Y, Albert M, Witte AK, Fliegmann J, and Felix G**. Perception of Agrobacterium tumefaciens flagellin by FLS2XL confers resistance to crown gall disease. Nat Plants. 2020:**6**(1):22–27. https://doi.org/10.1038/s41477-019-0578-6

**Gainza P, Sverrisson F, Monti F, Rodolà E, Boscaini D, Bronstein MM, and Correia BE**. Deciphering interaction fingerprints from protein molecular surfaces using geometric deep learning. Nat Methods. 2020:**17**(2):184–192. https://doi.org/10.1038/s41592-019-0666-6

**Gallas N, Li X, von Roepenack-Lahaye E, Schandry N, Jiang Y, Wu D, and Lahaye T**. An ancient cis-element targeted by Ralstonia solanacearum TALE-like effectors facilitates the development of a promoter trap that could confer broad-spectrum wilt resistance. Plant Biotechnol J. 2024:**22**(3):602–616. https://doi.org/10.1111/pbi.14208

**Gan X, Stegle O, Behr J, Steffen JG, Drewe P, Hildebrand KL, Lyngsoe R, Schultheiss SJ, Osborne EJ, Sreedharan VT, et al.** Multiple reference genomes and transcriptomes for Arabidopsis thaliana. Nature. 2011:**477**(7365):419–423. https://doi.org/10.1038/nature10414

**Gao Y, Wang W, Zhang T, Gong Z, Zhao H, and Han G-Z**. Out of Water: The Origin and Early Diversification of Plant R-Genes. Plant Physiol. 2018:**177**(1):82–89. https://doi.org/10.1104/pp.18.00185

**Ghosh J, Lun CM, Majeske AJ, Sacchi S, Schrankel CS, and Smith LC**. Invertebrate immune diversity. Dev Comp Immunol. 2011:**35**(9):959–974. https://doi.org/10.1016/j.dci.2010.12.009

**Giolai M and Laine A-L**. A trade-off between investment in molecular defense repertoires and growth in plants. Science. 2024:**386**(6722):677–680.

https://doi.org/10.1126/science.adn2779

**Gladieux P, van Oosterhout C, Fairhead S, Jouet A, Ortiz D, Ravel S, Shrestha R-K, Frouin J, He X, Zhu Y, et al.** Extensive immune receptor repertoire diversity in disease-resistant rice landraces. Curr Biol. 2024:**34**(17):3983-3995.e6. https://doi.org/10.1016/j.cub.2024.07.061

**Gómez De La Cruz D, Zdrzałek R, Banfield MJ, Talbot NJ, and Moscou MJ**. Molecular mimicry of a pathogen virulence target by a plant immune receptor. 2024. https://doi.org/10.1101/2024.07.26.605320

**Gómez-Gómez L and Boller T**. FLS2: An LRR Receptor–like Kinase Involved in the Perception of the Bacterial Elicitor Flagellin in Arabidopsis. Mol Cell. 2000:**5**(6):1003–1011. https://doi.org/10.1016/S1097-2765(00)80265-8

**Gonzalez TL, Liang Y, Nguyen BN, Staskawicz BJ, Loqué D, and Hammond MC**. Tight regulation of plant immune responses by combining promoter and suicide exon elements. Nucleic Acids Res. 2015:**43**(14):7152–7161. https://doi.org/10.1093/nar/gkv655

**Grant MR, Godiard L, Straube E, Ashfield T, Lewald J, Sattler A, Innes RW, and Dangl JL**. Structure of the Arabidopsis RPM1 Gene Enabling Dual Specificity Disease Resistance. Science. 1995:**269**(5225):843–846.

**Guo L, Cesari S, De Guillen K, Chalvon V, Mammri L, Ma M, Meusnier I, Bonnot F, Padilla A, Peng Y-L, et al.** Specific recognition of two MAX effectors by integrated HMA domains in plant immune receptors involves distinct binding surfaces. Proc Natl Acad Sci. 2018:**115**(45):11637–11642. https://doi.org/10.1073/pnas.1810705115

**Hamilton WD, Axelrod R, and Tanese R**. Sexual reproduction as an adaptation to resist parasites (a review). Proc Natl Acad Sci U S A. 1990:**87**(9):3566–3573.

**Harris CJ, Slootweg EJ, Goverse A, and Baulcombe DC**. Stepwise artificial evolution of a plant disease resistance gene. Proc Natl Acad Sci. 2013:**110**(52):21189–21194. https://doi.org/10.1073/pnas.1311134110

**Hayford RK, Haley OC, Cannon EK, Portwood JL, Gardiner JM, Andorf CM, and Woodhouse MR**. Functional annotation and meta-analysis of maize transcriptomes reveal genes involved in biotic and abiotic stress. BMC

Genomics. 2024:**25**(1):533. https://doi.org/10.1186/s12864-024-10443-7

**Helft L, Thompson M, and Bent AF**. Directed Evolution of FLS2 towards Novel Flagellin Peptide Recognition. PLOS ONE. 2016:**11**(6):e0157155. https://doi.org/10.1371/journal.pone.0157155

**Hie B, Zhong ED, Berger B, and Bryson B**. Learning the language of viral evolution and escape. Science. 2021:**371**(6526):284–288. https://doi.org/10.1126/science.abd7331

**Hie BL, Shanker VR, Xu D, Bruun TUJ, Weidenbacher PA, Tang S, Wu W, Pak JE, and Kim PS**. Efficient evolution of human antibodies from general protein language models. Nat Biotechnol. 2023. https://doi.org/10.1038/s41587-023-01763-2

**Hie BL, Yang KK, and Kim PS**. Evolutionary velocity with protein language models predicts evolutionary dynamics of diverse proteins. Cell Syst. 2022:**13**(4):274-285.e6. https://doi.org/10.1016/j.cels.2022.01.003

**Homma F, Huang J, and Van Der Hoorn RAL**. AlphaFold-Multimer predicts cross-kingdom interactions at the plant-pathogen interface. Nat Commun. 2023:**14**(1):6040. https://doi.org/10.1038/s41467-023-41721-9

**Honee G, Buitink J, Jabs T, De Kloe J, Sijbolts F, Apotheker M, Weide R, Sijen T, Stuiver M, and De Wit PJ**. Induction of defense-related responses in Cf9 tomato cells by the AVR9 elicitor peptide of cladosporium fulvum is developmentally regulated. Plant Physiol. 1998:**117**(3):809–820. https://doi.org/10.1104/pp.117.3.809

**Huang H, Huang S, Li J, Wang H, Zhao Y, Feng M, Dai J, Wang T, Zhu M, and Tao X**. Stepwise artificial evolution of an Sw-5b immune receptor extends its resistance spectrum against resistance-breaking isolates of Tomato spotted wilt virus. Plant Biotechnol J. 2021:**19**(11):2164–2176. https://doi.org/10.1111/pbi.13641

**Huang S, Jia A, Song W, Hessler G, Meng Y, Sun Y, Xu L, Laessle H, Jirschitzka J, Ma S, et al.** Identification and receptor mechanism of TIR-catalyzed small molecules in plant immunity. Science. 2022:**377**(6605):eabq3297. https://doi.org/10.1126/science.abq3297

**Hufford MB, Seetharam AS, Woodhouse MR, Chougule KM, Ou S, Liu J, Ricci WA, Guo T, Olson A, Qiu Y, et al.** De novo assembly, annotation, and comparative analysis of 26 diverse maize genomes. Science. 2021:**373**(6555):655–662. https://doi.org/10.1126/science.abg5289

**Islam MR and Shepherd KW**. Present status of genetics of rust resistance in flax. Euphytica. 1991:**55**(3):255–267. https://doi.org/10.1007/BF00021246

**Jia A, Huang S, Song W, Wang J, Meng Y, Sun Y, Xu L, Laessle H, Jirschitzka J, Hou J, et al.** TIR-catalyzed ADP-ribosylation reactions produce signaling molecules for plant immunity. Science. 2022:**377**(6605):eabq8180. https://doi.org/10.1126/science.abq8180

**Jiao W-B and Schneeberger K**. Chromosome-level assemblies of multiple Arabidopsis genomes reveal hotspots of rearrangements with altered evolutionary dynamics. Nat Commun. 2020:**11**(1):989. https://doi.org/10.1038/s41467-020-14779-y

**Jones DA, Dickinson MJ, Balint-Kurti PJ, Dixon MS, and Jones JDG**. Two complex resistance loci revealed in tomato by classical and RFLP mapping of the Cf-2, Cf-4, Cf-5, and Cf-9 genes for resistance to Cladosporium fulvum. Mol Plant Microbe Interact. 1993:**6**:348–348.

**Jones DA, Thomas CM, Hammond-Kosack KE, Balint-Kurti PJ, and Jones JDG**. Isolation of the Tomato Cf-9 Gene for Resistance to Cladosporium fulvum by Transposon Tagging. Science. 1994:**266**(5186):789–793.

**Jones JDG and Dangl JL**. The plant immune system. Nature. 2006:**444**(7117):323–329. https://doi.org/10.1038/nature05286

**Jones JDG, Staskawicz BJ, and Dangl JL**. The plant immune system: From discovery to deployment. Cell. 2024:**187**(9):2095–2116. https://doi.org/10.1016/j.cell.2024.03.045

**Jones JDG, Vance RE, and Dangl JL**. Intracellular innate immune surveillance devices in plants and animals. Science. 2016:**354**(6316):aaf6395. https://doi.org/10.1126/science.aaf6395

**Jores T, Tonnies J, Wrightsman T, Buckler ES, Cuperus JT, Fields S, and Queitsch C**. Synthetic promoter designs enabled by a comprehensive analysis of plant core promoters. Nat Plants. 2021:**7**(6):842–855.

https://doi.org/10.1038/s41477-021-00932-y

**Jumper J, Evans R, Pritzel A, Green T, Figurnov M, Ronneberger O, Tunyasuvunakool K, Bates R, Žídek A, Potapenko A, et al.** Highly accurate protein structure prediction with AlphaFold. Nature. 2021:**596**(7873):583–589. https://doi.org/10.1038/s41586-021-03819-2

**Jupe F, Witek K, Verweij W, Sliwka J, Pritchard L, Etherington GJ, Maclean D, Cock PJ, Leggett RM, Bryan GJ, et al.** Resistance gene enrichment sequencing (RenSeq) enables reannotation of the NB-LRR gene family from sequenced plant genomes and rapid mapping of resistance loci in segregating populations. Plant J Cell Mol Biol. 2013:**76**(3):530–544. https://doi.org/10.1111/tpj.12307

**Kang M, Wu H, Liu H, Liu W, Zhu M, Han Y, Liu W, Chen C, Song Y, Tan L, et al.** The pan-genome and local adaptation of Arabidopsis thaliana. Nat Commun. 2023:**14**(1):6259. https://doi.org/10.1038/s41467-023-42029-4

**Kawashima CG, Guimarães GA, Nogueira SR, MacLean D, Cook DR, Steuernagel B, Baek J, Bouyioukos C, Melo B do V, and Tristão G**. A pigeonpea gene confers resistance to Asian soybean rust in soybean. Nat Biotechnol. 2016:**34**(6):661–665.

**Keepers K, Peterson K, Raduski A, Turner KM, Van Tassel D, Smith K, Harkess A, Bever JD, and Brandvain Y**. Disease resistance gene count increases with rainfall in Silphium integrifolium. Ecol Evol. 2024:**14**(9):e11143. https://doi.org/10.1002/ece3.11143

**Keller H, Pamboukdjian N, Ponchet M, Poupet A, Delon R, Verrier J-L, Roby D, and Ricci P**. Pathogen-induced elicitin production in transgenic tobacco generates a hypersensitive response and nonspecific disease resistance. Plant Cell. 1999:**11**(2):223. https://doi.org/10.2307/3870852

**Kesseli RV, Paran I, and Michelmore RW**. Analysis of a detailed genetic linkage map of Lactuca sativa (lettuce) constructed from RFLP and RAPD markers. Genetics. 1994:**136**(4):1435–1446. https://doi.org/10.1093/genetics/136.4.1435

**Kibby EM, Conte AN, Burroughs AM, Nagy TA, Vargas JA, Whalen LA, Aravind L, and Whiteley AT**. Bacterial NLR-related proteins protect against phage. Cell.

2023:**186**(11):2410-2424.e18. https://doi.org/10.1016/j.cell.2023.04.015

**Kourelis J, Marchal C, Posbeyikian A, Harant A, and Kamoun S**. NLR immune receptor–nanobody fusions confer plant disease resistance. Science. 2023:**379**(6635):934–939. https://doi.org/10.1126/science.abn4116

**Kourelis J, Sakai T, Adachi H, and Kamoun S**. RefPlantNLR is a comprehensive collection of experimentally validated plant disease resistance proteins from the NLR family. PLOS Biol. 2021:**19**(10):e3001124. https://doi.org/10.1371/journal.pbio.3001124

**Krapp LF, Abriata LA, Cortés Rodriguez F, and Dal Peraro M**. PeSTo: parameter-free geometric deep learning for accurate prediction of protein binding interfaces. Nat Commun. 2023:**14**(1):2175. https://doi.org/10.1038/s41467-023-37701-8

**Kroj T, Chanclud E, Michel-Romiti C, Grand X, and Morel J-B**. Integration of decoy domains derived from protein targets of pathogen effectors into plant immune receptors is widespread. New Phytol. 2016:**210**(2):618–626. https://doi.org/10.1111/nph.13869

**Kunkel BN**. A useful weed put to work: genetic analysis of disease resistance in *Arabidopsis thaliana*. Trends Genet. 1996:**12**(2):63–69. https://doi.org/10.1016/0168-9525(96)81402-8

**Lai Y and Eulgem T**. Transcript-level expression control of plant NLR genes. Mol Plant Pathol. 2017:**19**(5):1267–1281. https://doi.org/10.1111/mpp.12607

**Lawson AW, Flores-Ibarra A, Cao Y, An C, Neumann U, Gunkel M, Saur IML, Chai J, Behrmann E, and Schulze-Lefert P**. The barley MLA13-AVRA13 heterodimer reveals principles for immunoreceptor recognition of RNase-like powdery mildew effectors. 2024. https://doi.org/10.1101/2024.07.14.603419

**Le Roux C, Huet G, Jauneau A, Camborde L, Trémousaygue D, Kraut A, Zhou B, Levaillant M, Adachi H, Yoshioka H, et al.** A Receptor Pair with an Integrated Decoy Converts Pathogen Disabling of Transcription Factors to Immunity. Cell. 2015:**161**(5):1074–1088. https://doi.org/10.1016/j.cell.2015.04.025

**Leuschen-Kohl R, Roberts R, Stevens DM, Zhang N, Buchanan S, Pilkey B, Coaker G, and Iyer-Pascuzzi AS**. Tomato roots exhibit distinct, development-specific responses to bacterial-derived peptides. 2024:2024.11.04.621969.

https://doi.org/10.1101/2024.11.04.621969

**Li T, Bolaños EJ, Stevens DM, Sha H, Prigozhin DM, and Coaker G**. Unlocking Expanded Flagellin Perception Through Rational Receptor Engineering. bioRxiv. 2024a:2024.09.09.612155. https://doi.org/10.1101/2024.09.09.612155

**Li T, Moreno-Pérez A, and Coaker G**. Plant Pattern recognition receptors: Exploring their evolution, diversification, and spatiotemporal regulation. Curr Opin Plant Biol. 2024b:**82**:102631. https://doi.org/10.1016/j.pbi.2024.102631

**Li X, Ma L, Wang Y, Ye C, Guo C, Li Y, Mei X, Du F, and Huang H**. PlantNLRatlas: a comprehensive dataset of full- and partial-length NLR resistance genes across 100 chromosome-level plant genomes. Front Plant Sci. 2023:**14**:1178069. https://doi.org/10.3389/fpls.2023.1178069

**Lian Q, Huettel B, Walkemeier B, Mayjonade B, Lopez-Roques C, Gil L, Roux F, Schneeberger K, and Mercier R**. A pan-genome of 69 Arabidopsis thaliana accessions reveals a conserved genome structure throughout the global species range. Nat Genet. 2024:**56**(5):982–991. https://doi.org/10.1038/s41588-024-01715-9

**Lin Z, Akin H, Rao R, Hie B, Zhu Z, Lu W, Smetanin N, Verkuil R, Kabeli O, Shmueli Y, et al.** Evolutionary-scale prediction of atomic-level protein structure with a language model. Science. 2023:**379**(6637):1123–1130. https://doi.org/10.1126/science.ade2574

**Liu F, Yang Z, Wang C, You Z, Martin R, Qiao W, Huang J, Jacob P, Dangl JL, Carette JE, et al.** Activation of the helper NRC4 immune receptor forms a hexameric resistosome. Cell. 2024a:**187**(18):4877-4889.e15. https://doi.org/10.1016/j.cell.2024.07.013

**Liu MH, Costa BM, Bianchini EC, Choi U, Bandler RC, Lassen E, Grońska-Pęski M, Schwing A, Murphy ZR, Rosenkjær D, et al.** DNA mismatch and damage patterns revealed by single-molecule sequencing. Nature. 2024b:1–10. https://doi.org/10.1038/s41586-024-07532-8

**Liu T, Liu Z, Song C, Hu Y, Han Z, She J, Fan F, Wang J, Jin C, Chang J, et al.** Chitin-Induced Dimerization Activates a Plant Immune Receptor. Science. 2012:**336**(6085):1160–1164. https://doi.org/10.1126/science.1218867

**Liu Y, Zeng Z, Zhang Y-M, Li Q, Jiang X-M, Jiang Z, Tang J-H, Chen D, Wang Q, Chen J-Q, et al.** An angiosperm NLR Atlas reveals that NLR gene reduction is associated with ecological specialization and signal transduction component deletion. Mol Plant. 2021:**14**(12):2015–2031. https://doi.org/10.1016/j.molp.2021.08.001

**Lolle S, Stevens D, and Coaker G**. Plant NLR-triggered immunity: from receptor activation to downstream signaling. Curr Opin Immunol. 2020:**62**:99–105. https://doi.org/10.1016/j.coi.2019.12.007

**Lu X, Kracher B, Saur IML, Bauer S, Ellwood SR, Wise R, Yaeno T, Maekawa T, and Schulze-Lefert P**. Allelic barley MLA immune receptors recognize sequence-unrelated avirulence effectors of the powdery mildew pathogen. Proc Natl Acad Sci. 2016:**113**(42). https://doi.org/10.1073/pnas.1612947113

**Lüdke D, Sakai T, Kourelis J, Toghani A, Adachi H, Posbeyikian A, Frijters R, Pai H, Harant A, Ernst K, et al.** A root-specific NLR network confers resistance to plant parasitic nematodes. 2023:2023.12.14.571630. https://doi.org/10.1101/2023.12.14.571630

**Ma S, An C, Lawson AW, Cao Y, Sun Y, Tan EYJ, Pan J, Jirschitzka J, Kümmel F, Mukhi N, et al.** Oligomerization-mediated autoinhibition and cofactor binding of a plant NLR. Nature. 2024:**632**(8026):869–876. https://doi.org/10.1038/s41586-024-07668-7

**Ma S, Lapin D, Liu L, Sun Y, Song W, Zhang X, Logemann E, Yu D, Wang J, Jirschitzka J, et al.** Direct pathogen-induced assembly of an NLR immune receptor complex to form a holoenzyme. Science. 2020:**370**(6521):eabe3069. https://doi.org/10.1126/science.abe3069

**Madhuprakash J, Toghani A, Contreras MP, Posbeyikian A, Richardson J, Kourelis J, Bozkurt TO, Webster MW, and Kamoun S**. A disease resistance protein triggers oligomerization of its NLR helper into a hexameric resistosome to mediate innate immunity. Sci Adv. 2024:**10**(45):eadr2594. https://doi.org/10.1126/sciadv.adr2594

**Maidment JH, Shimizu M, Bentham AR, Vera S, Franceschetti M, Longya A, Stevenson CE, De La Concepcion JC, Białas A, Kamoun S, et al.** Effector

target-guided engineering of an integrated domain expands the disease resistance profile of a rice NLR immune receptor. eLife. 2023:**12**:e81123. https://doi.org/10.7554/eLife.81123

**Maidment JHR, Franceschetti M, Maqbool A, Saitoh H, Jantasuriyarat C, Kamoun S, Terauchi R, and Banfield MJ**. Multiple variants of the fungal effector AVR-Pik bind the HMA domain of the rice protein OsHIPP19, providing a foundation to engineer plant defense. J Biol Chem. 2021:**296**:100371. https://doi.org/10.1016/j.jbc.2021.100371

**Man J, Gallagher JP, and Bartlett M**. Structural evolution drives diversification of the large LRR-RLK gene family. New Phytol. 2020:**226**(5):1492–1505. https://doi.org/10.1111/nph.16455

**Maqbool A, Saitoh H, Franceschetti M, Stevenson C, Uemura A, Kanzaki H, Kamoun S, Terauchi R, and Banfield M**. Structural basis of pathogen recognition by an integrated HMA domain in a plant NLR immune receptor. eLife. 2015:**4**:e08709. https://doi.org/10.7554/eLife.08709

**Marchal C, Pai H, Kamoun S, and Kourelis J**. Emerging principles in the design of bioengineered made-to-order plant immune receptors. Curr Opin Plant Biol. 2022:**70**:102311. https://doi.org/10.1016/j.pbi.2022.102311

**Marchal C, Zhang J, Zhang P, Fenwick P, Steuernagel B, Adamski NM, Boyd L, McIntosh R, Wulff BBH, Berry S, et al.** BED-domain-containing immune receptors confer diverse resistance spectra to yellow rust. Nat Plants. 2018:**4**(9):662–668. https://doi.org/10.1038/s41477-018-0236-4

**Martin EC, Ion CF, Ifrimescu F, Spiridon L, Bakker J, Goverse A, and Petrescu A-J**. NLRscape: an atlas of plant NLR proteins. Nucleic Acids Res. 2023:**51**(D1):D1470–D1482. https://doi.org/10.1093/nar/gkac1014

**Martin GB, Brommonschenkel SH, Chunwongse J, Frary A, Ganal MW, Spivey R, Wu T, Earle ED, and Tanksley SD**. Map-Based Cloning of a Protein Kinase Gene Conferring Disease Resistance in Tomato. Science. 1993:**262**(5138):1432–1436. https://doi.org/10.1126/science.7902614

**Martin R, Qi T, Zhang H, Liu F, King M, Toth C, Nogales E, and Staskawicz BJ**. Structure of the activated Roq1 resistosome directly recognizing the pathogen

effector XopQ. bioRxiv. 2020:2020.08.13.246413. https://doi.org/10.1101/2020.08.13.246413

**Martincorena I and Luscombe NM**. Non-random mutation: The evolution of targeted hypermutation and hypomutation. BioEssays. 2013:**35**(2):123–130. https://doi.org/10.1002/bies.201200150

**Mes JJ, Van Doorn AA, Wijbrandi J, Simons G, Cornelissen BJC, and Haring MA**. Expression of the Fusarium resistance gene I-2 colocalizes with the site of fungal containment. Plant J. 2000:**23**(2):183–193. https://doi.org/10.1046/j.1365-313x.2000.00765.x

**Meyers BC, Chin DB, Shen KA, Sivaramakrishnan S, Lavelle DO, Zhang Z, and Michelmore RW**. The major resistance gene cluster in lettuce is highly duplicated and spans several megabases. Plant Cell. 1998:**10**(11):1817. https://doi.org/10.1105/tpc.10.11.1817

**Michelmore RW and Meyers BC**. Clusters of Resistance Genes in Plants Evolve by Divergent Selection and a Birth-and-Death Process. Genome Res. 1998:**8**(11):1113–1130. https://doi.org/10.1101/gr.8.11.1113

**Mirdita M, Schütze K, Moriwaki Y, Heo L, Ovchinnikov S, and Steinegger M**. ColabFold: making protein folding accessible to all. Nat Methods. 2022:**19**(6):679–682. https://doi.org/10.1038/s41592-022-01488-1

**Mode CJ**. A Mathematical Model for the Co-Evolution of Obligate Parasites and Their Hosts. Evolution. 1958:**12**(2):158–165. https://doi.org/10.2307/2406026

**Mohr TJ, Mammarella ND, Hoff T, Woffenden BJ, Jelesko JG, and McDowell JM**. The Arabidopsis Downy Mildew Resistance Gene RPP8 Is Induced by Pathogens and Salicylic Acid and Is Regulated by W Box cis Elements. Mol Plant-Microbe Interactions®. 2010:**23**(10):1303–1315. https://doi.org/10.1094/MPMI-01-10-0022

**Molla KA, Karmakar S, Chanda PK, Sarkar SN, Datta SK, and Datta K**. Tissue-specific expression of Arabidopsis NPR1 gene in rice for sheath blight resistance without compromising phenotypic cost. Plant Sci. 2016:**250**:105–114. https://doi.org/10.1016/j.plantsci.2016.06.005

**Moore L, Cagan A, Coorens THH, Neville MDC, Sanghvi R, Sanders MA, Oliver**

**TRW, Leongamornlert D, Ellis P, Noorani A, et al.** The mutational landscape of human somatic and germline cells. Nature. 2021:**597**(7876):381–386. https://doi.org/10.1038/s41586-021-03822-7

**Mueller K, Bittel P, Chinchilla D, Jehle AK, Albert M, Boller T, and Felix G**. Chimeric FLS2 Receptors Reveal the Basis for Differential Flagellin Perception in Arabidopsis and Tomato. Plant Cell. 2012:**24**(5):2213–2224. https://doi.org/10.1105/tpc.112.096073

**Mukhi N, Brown H, Gorenkin D, Ding P, Bentham AR, Stevenson CEM, Jones JDG, and Banfield MJ**. Perception of structurally distinct effectors by the integrated WRKY domain of a plant immune receptor. Proc Natl Acad Sci. 2021:**118**(50):e2113996118. https://doi.org/10.1073/pnas.2113996118

**Mukhopadhyay S, Javed MA, Wu J, and Pérez-López E**. Structure-guided secretome analysis of gall-forming microbes offers insights into effector diversity and evolution. 2024. https://doi.org/10.1101/2024.09.03.609900

**Müller V, de Boer RJ, Bonhoeffer S, and Szathmáry E**. An evolutionary perspective on the systems of adaptive immunity. Biol Rev. 2018:**93**(1):505–528. https://doi.org/10.1111/brv.12355

**Munch D, Gupta V, Bachmann A, Busch W, Kelly S, Mun T, and Andersen SU**. The Brassicaceae Family Displays Divergent, Shoot-Skewed NLR Resistance Gene Expression. Plant Physiol. 2018:**176**(2):1598–1609. https://doi.org/10.1104/pp.17.01606

**Narusaka M, Hatakeyama K, Shirasu K, and Narusaka Y**. Arabidopsis dual resistance proteins, both RPS4 and RRS1, are required for resistance to bacterial wilt in transgenic Brassica crops. Plant Signal Behav. 2014:**9**(7):e29130. https://doi.org/10.4161/psb.29130

**Ngou BPM, Ding P, and Jones JDG**. Thirty years of resistance: Zig-zag through the plant immune system. Plant Cell. 2022a:1447–1478. https://doi.org/10.1093/plcell/koac041

**Ngou BPM, Heal R, Wyler M, Schmid MW, and Jones JDG**. Concerted expansion and contraction of immune receptor gene repertoires in plant genomes. Nat Plants. 2022b:**8**(10):1146–1152. https://doi.org/10.1038/s41477-022-01260-5

**Ngou BPM, Wyler M, Schmid MW, Kadota Y, and Shirasu K**. Evolutionary trajectory of pattern recognition receptors in plants. Nat Commun. 2024:**15**:308. https://doi.org/10.1038/s41467-023-44408-3

**Nordborg M, Hu TT, Ishino Y, Jhaveri J, Toomajian C, Zheng H, Bakker E, Calabrese P, Gladstone J, Goyal R, et al.** The Pattern of Polymorphism in Arabidopsis thaliana. PLOS Biol. 2005:**3**(7):e196. https://doi.org/10.1371/journal.pbio.0030196

**Oikawa K, Fujisaki K, Shimizu M, Takeda T, Nemoto K, Saitoh H, Hirabuchi A, Hiraka Y, Miyaji N, Białas A, et al.** The blast pathogen effector AVR-Pik binds and stabilizes rice heavy metal-associated (HMA) proteins to co-opt their function in immunity. PLOS Pathog. 2024:**20**(11):e1012647. https://doi.org/10.1371/journal.ppat.1012647

**Ortiz D, Chen J, Outram MA, Saur IML, Upadhyaya NM, Mago R, Ericsson DJ, Cesari S, Chen C, Williams SJ, et al.** The stem rust effector protein AvrSr50 escapes Sr50 recognition by a substitution in a single surface-exposed residue. New Phytol. 2022:**234**(2):592–606. https://doi.org/10.1111/nph.18011

**Ortiz D, De Guillen K, Cesari S, Chalvon V, Gracy J, Padilla A, and Kroj T**. Recognition of the Magnaporthe oryzae Effector AVR-Pia by the Decoy Domain of the Rice NLR Immune Receptor RGA5. Plant Cell. 2017:**29**(1):156–168. https://doi.org/10.1105/tpc.16.00435

**Outram MA, Chen J, Broderick S, Li Z, Aditya S, Tasneem N, Arndell T, Blundell C, Ericsson DJ, Figueroa M, et al.** AvrSr27 is a zinc-bound effector with a modular structure important for immune recognition. New Phytol. 2024:**243**(1):314–329. https://doi.org/10.1111/nph.19801

**Parker JE, Coleman MJ, Szabò V, Frost LN, Schmidt R, van der Biezen EA, Moores T, Dean C, Daniels MJ, and Jones JD**. The Arabidopsis downy mildew resistance gene RPP5 shares similarity to the toll and interleukin-1 receptors with N and L6. Plant Cell. 1997:**9**(6):879–894. https://doi.org/10.1105/tpc.9.6.879

**Parniske M, Hammond-Kosack KE, Golstein C, Thomas CM, Jones DA, Harrison K, Wulff BBH, and Jones JDG**. Novel Disease Resistance Specificities Result from Sequence Exchange between Tandemly Repeated Genes at the Cf-4/9

Locus of Tomato. Cell. 1997:**91**(6):821–832. https://doi.org/10.1016/S0092-8674(00)80470-5

**Parys K, Colaianni NR, Lee H-S, Hohmann U, Edelbacher N, Trgovcevic A, Blahovska Z, Lee D, Mechtler A, Muhari-Portik Z, et al.** Signatures of antagonistic pleiotropy in a bacterial flagellin epitope. Cell Host Microbe. 2021:**29**(4):620-634.e9. https://doi.org/10.1016/j.chom.2021.02.008

**Payne JL and Wagner A**. The causes of evolvability and their evolution. Nat Rev Genet. 2019:**20**(1):24–38. https://doi.org/10.1038/s41576-018-0069-z

**Prigozhin DM and Krasileva KV**. Analysis of intraspecies diversity reveals a subset of highly variable plant immune receptors and predicts their binding sites. Plant Cell. 2021:**33**(4):998–1015. https://doi.org/10.1093/plcell/koab013

**Prigozhin DM, Sutherland CA, Rangavajjhala S, and Krasileva KV**. Majority of the highly variable NLRs in maize share genomic location and contain additional target-binding domains. Mol Plant-Microbe Interactions®. 2024:MPMI-05-24-0047-FI. https://doi.org/10.1094/MPMI-05-24-0047-FI

**Pucker B, Irisarri I, Vries J de, and Xu B**. Plant genome sequence assembly in the era of long reads: Progress, challenges and future directions. Quant Plant Biol. 2022:**3**:e5. https://doi.org/10.1017/qpb.2021.18

**Quiroz D, Oya S, Lopez-Mateos D, Zhao K, Pierce A, Ortega L, Ali A, Carbonell-Bejerano P, Yarov-Yarovoy V, Suzuki S, et al.** H3K4me1 recruits DNA repair proteins in plants. Plant Cell. 2024:**36**(6):2410–2426. https://doi.org/10.1093/plcell/koae089

**Rim EY, Garrett OD, Howard AJ, Shim Y, Li Y, Van Dyke JE, Packer RC, Ho N, Jain RS, Stewart VJ, et al.** Directed evolution of a plant immune receptor for broad spectrum recognition of pathogen effectors. 2024. https://doi.org/10.1101/2024.09.30.614878

**Rocafort M, Bowen JK, Hassing B, Cox MP, McGreal B, De La Rosa S, Plummer KM, Bradshaw RE, and Mesarich CH**. The Venturia inaequalis effector repertoire is dominated by expanded families with predicted structural similarity, but unrelated sequence, to avirulence proteins from other plant-pathogenic fungi. BMC Biol. 2022:**20**(1):246. https://doi.org/10.1186/s12915-022-01442-9

**Rose LE, Bittner-Eddy PD, Langley CH, Holub EB, Michelmore RW, and Beynon JL**. The Maintenance of Extreme Amino Acid Diversity at the Disease Resistance Gene, RPP13, in Arabidopsis thaliana. Genetics. 2004:**166**(3):1517–1527. https://doi.org/10.1534/genetics.166.3.1517

**Ruffolo JA, Chu L-S, Mahajan SP, and Gray JJ**. Fast, accurate antibody structure prediction from deep learning on massive set of natural antibodies. Nat Commun. 2023:**14**(1):2389. https://doi.org/10.1038/s41467-023-38063-x

**Ruffolo JA and Madani A**. Designing proteins with language models. Nat Biotechnol. 2024:**42**(2):200–202. https://doi.org/10.1038/s41587-024-02123-4

**Sarris PF, Duxbury Z, Huh SU, Ma Y, Segonzac C, Sklenar J, Derbyshire P, Cevik V, Rallapalli G, Saucet SB, et al.** A Plant Immune Receptor Detects Pathogen Effectors that Target WRKY Transcription Factors. Cell. 2015:**161**(5):1089–1100. https://doi.org/10.1016/j.cell.2015.04.024

**Saur IM, Bauer S, Kracher B, Lu X, Franzeskakis L, Müller MC, Sabelleck B, Kümmel F, Panstruga R, Maekawa T, et al.** Multiple pairs of allelic MLA immune receptor-powdery mildew AVRA effectors argue for a direct recognition mechanism. eLife. 2019:**8**:e44471. https://doi.org/10.7554/eLife.44471

**Segretin ME, Pais M, Franceschetti M, Chaparro-Garcia A, Bos JIB, Banfield MJ, and Kamoun S**. Single Amino Acid Mutations in the Potato Immune Receptor R3a Expand Response to Phytophthora Effectors. Mol Plant-Microbe Interactions®. 2014:**27**(7):624–637. https://doi.org/10.1094/MPMI-02-14-0040-R

**Selvaraj M, Toghani A, Pai H, Sugihara Y, Kourelis J, Yuen ELH, Ibrahim T, Zhao H, Xie R, Maqbool A, et al.** Activation of plant immunity through conversion of a helper NLR homodimer into a resistosome. PLOS Biol. 2024:**22**(10):e3002868. https://doi.org/10.1371/journal.pbio.3002868

**Seong K and Krasileva KV**. Computational Structural Genomics Unravels Common Folds and Novel Families in the Secretome of Fungal Phytopathogen Magnaporthe oryzae. Mol Plant-Microbe Interactions®. 2021:**34**(11):1267–1280. https://doi.org/10.1094/MPMI-03-21-0071-R

**Seong K and Krasileva KV**. Prediction of effector protein structures from fungal phytopathogens enables evolutionary analyses. Nat Microbiol. 2023:**8**(1):174–

187. https://doi.org/10.1038/s41564-022-01287-6

**Seong K, Seo E, Witek K, Li M, and Staskawicz B**. Evolution of NLR resistance genes with noncanonical N-terminal domains in wild tomato species. New Phytol. 2020:**227**(5):1530–1543. https://doi.org/10.1111/nph.16628

**Seong K, Wei W, Vega B, Dee A, Ramirez-Bernardino G, Kumar R, Parra L, and Krasileva K**. Engineering the plant intracellular immune receptor Sr50 to restore recognition of the AvrSr50 escape mutant. 2024. https://doi.org/10.1101/2024.08.07.607039

**Shang L, Li X, He H, Yuan Q, Song Y, Wei Z, Lin H, Hu M, Zhao F, Zhang C, et al.** A super pan-genomic landscape of rice. Cell Res. 2022:**32**(10):878–896. https://doi.org/10.1038/s41422-022-00685-z

**Shi J, Tian Z, Lai J, and Huang X**. Plant pan-genomics and its applications. Mol Plant. 2023:**16**(1):168–186. https://doi.org/10.1016/j.molp.2022.12.009

**Shiu SH and Bleecker AB**. Plant receptor-like kinase gene family: diversity, function, and signaling. Sci STKE Signal Transduct Knowl Environ. 2001:**2001**(113):re22. https://doi.org/10.1126/stke.2001.113.re22

**Shiu S-H and Bleecker AB**. Expansion of the Receptor-Like Kinase/Pelle Gene Family and Receptor-Like Proteins in Arabidopsis. Plant Physiol. 2003:**132**(2):530–543. https://doi.org/10.1104/pp.103.021964

**Shivaprasad PV, Chen H-M, Patel K, Bond DM, Santos BACM, and Baulcombe DC**. A microRNA superfamily regulates nucleotide binding site-leucine-rich repeats and other mRNAs. Plant Cell. 2012:**24**(3):859–874. https://doi.org/10.1105/tpc.111.095380

**Snoeck S, Abramson BW, Garcia AGK, Egan AN, Michael TP, and Steinbrenner AD**. Evolutionary gain and loss of a plant pattern-recognition receptor for HAMP recognition. 2022:2022.03.30.484633. https://doi.org/10.1101/2022.03.30.484633

**Song W-Y, Wang G-L, Chen L-L, Kim H-S, Pi L-Y, Holsten T, Gardner J, Wang B, Zhai W-X, Zhu L-H, et al.** A Receptor Kinase-Like Protein Encoded by the Rice Disease Resistance Gene, Xa21. Science. 1995:**270**(5243):1804–1806. https://doi.org/10.1126/science.270.5243.1804

**Staunton PM, Peters AJ, and Seoighe C**. Somatic mutations inferred from RNA-seq

data highlight the contribution of replication timing to mutation rate variation in a model plant. Genetics. 2023:iyad128. https://doi.org/10.1093/genetics/iyad128

**Steidele CE and Stam R**. Multi-omics approach highlights differences between RLP classes in Arabidopsis thaliana. BMC Genomics. 2021:**22**(1):557. https://doi.org/10.1186/s12864-021-07855-0

**Stevens DM, Moreno-Pérez A, Weisberg AJ, Ramsing C, Fliegmann J, Zhang N, Madrigal M, Martin G, Steinbrenner A, Felix G, et al.** Natural variation of immune epitopes reveals intrabacterial antagonism. Proc Natl Acad Sci. 2024:**121**(23):e2319499121. https://doi.org/10.1073/pnas.2319499121

**Sueldo DJ, Shimels M, Spiridon LN, Caldararu O, Petrescu A-J, Joosten MHAJ, and Tameling WIL**. Random mutagenesis of the nucleotide-binding domain of NRC1 (NB-LRR Required for Hypersensitive Response-Associated Cell Death-1), a downstream signalling nucleotide-binding, leucine-rich repeat (NB-LRR) protein, identifies gain-of-function mutations in the nucleotide-binding pocket. New Phytol. 2015:**208**(1):210–223. https://doi.org/10.1111/nph.13459

**Sugihara Y, Abe Y, Takagi H, Abe A, Shimizu M, Ito K, Kanzaki E, Oikawa K, Kourelis J, Langner T, et al.** Disentangling the complex gene interaction networks between rice and the blast fungus identifies a new pathogen effector. PLOS Biol. 2023:**21**(1):e3001945. https://doi.org/10.1371/journal.pbio.3001945

**Sun Y, Li L, Macho AP, Han Z, Hu Z, Zipfel C, Zhou J-M, and Chai J**. Structural Basis for flg22-Induced Activation of the Arabidopsis FLS2-BAK1 Immune Complex. Science. 2013:**342**(6158):624–628. https://doi.org/10.1126/science.1243825

**Sun Y, Wang Y, Zhang X, Chen Z, Xia Y, Wang L, Sun Y, Zhang M, Xiao Y, Han Z, et al.** Plant receptor-like protein activation by a microbial glycoside hydrolase. Nature. 2022:**610**(7931):335–342. https://doi.org/10.1038/s41586-022-05214-x

**Sutherland CA, Prigozhin DM, Monroe JG, and Krasileva KV**. High allelic diversity in Arabidopsis NLRs is associated with distinct genomic features. EMBO Rep. 2024:**25**(5):2306–2322. https://doi.org/10.1038/s44319-024-00122-9

**Tamborski J, Seong K, Liu F, Staskawicz BJ, and Krasileva KV**. Altering Specificity and Autoactivity of Plant Immune Receptors Sr33 and Sr50 Via a Rational

Engineering Approach. Mol Plant-Microbe Interactions®. 2023:**36**(7):434–446. https://doi.org/10.1094/MPMI-07-22-0154-R

**Teasdale LC, Murray KD, Collenberg M, Contreras-Garrido A, Schlegel T, Ess L van, Jüttner J, Lanz C, Deusch O, Fitz J, et al.** Pangenomic context reveals the extent of intraspecific plant NLR evolution. 2024:2024.09.02.610789. https://doi.org/10.1101/2024.09.02.610789

**The Arabidopsis Genome Initiative**. Analysis of the genome sequence of the flowering plant Arabidopsis thaliana. Nature. 2000:**408**(6814):796–815. https://doi.org/10.1038/35048692

**Thomas CM, Dixon MS, Parniske M, Golstein C, and Jones JD**. Genetic and molecular analysis of tomato Cf genes for resistance to Cladosporium fulvum. Philos Trans R Soc B Biol Sci. 1998:**353**(1374):1413. https://doi.org/10.1098/rstb.1998.0296

**Tian H, Wu Z, Chen S, Ao K, Huang W, Yaghmaiean H, Sun T, Xu F, Zhang Y, Wang S, et al.** Activation of TIR signalling boosts pattern-triggered immunity. Nature. 2021:**598**(7881):500–503. https://doi.org/10.1038/s41586-021-03987-1

**Trinh J, Li T, Franco JY, Toruño TY, Stevens DM, Thapa SP, Wong J, Pineda R, de Dios EÁ, Kahn TL, et al.** Variation in microbial feature perception in the Rutaceae family with immune receptor conservation in citrus. Plant Physiol. 2023:**193**(1):689–707. https://doi.org/10.1093/plphys/kiad263

**Trinh J, Tran M, and Coaker G**. The perception and evolution of flagellin, cold shock protein and elongation factor Tu from vector-borne bacterial plant pathogens. Mol Plant Pathol. 2024:**25**(10):e70019. https://doi.org/10.1111/mpp.70019

**Tubiana J, Schneidman-Duhovny D, and Wolfson HJ**. ScanNet: an interpretable geometric deep learning model for structure-based protein binding site prediction. Nat Methods. 2022:**19**(6):730–739. https://doi.org/10.1038/s41592-022-01490-7

**Urban M, Cuzick A, Seager J, Wood V, Rutherford K, Venkatesh SY, De Silva N, Martinez MC, Pedro H, Yates AD, et al.** PHI-base: the pathogen–host interactions database. Nucleic Acids Res. 2019:gkz904. https://doi.org/10.1093/nar/gkz904

**Van de Weyer A-L, Monteiro F, Furzer OJ, Nishimura MT, Cevik V, Witek K, Jones**

**JDG, Dangl JL, Weigel D, and Bemm F**. A Species-Wide Inventory of NLR Genes and Alleles in Arabidopsis thaliana. Cell. 2019:**178**(5):1260-1272.e14. https://doi.org/10.1016/j.cell.2019.07.038

**Vendelbo NM, Mahmood K, Steuernagel B, Wulff BBH, Sarup P, Hovmøller MS, Justesen AF, Kristensen PS, Orabi J, and Jahoor A**. Discovery of Resistance Genes in Rye by Targeted Long-Read Sequencing and Association Genetics. Cells. 2022:**11**(8):1273. https://doi.org/10.3390/cells11081273

**Wang H, Zou S, Li Y, Lin F, and Tang D**. An ankyrin-repeat and WRKY-domain-containing immune receptor confers stripe rust resistance in wheat. Nat Commun. 2020:**11**(1):1353. https://doi.org/10.1038/s41467-020-15139-6

**Wang J, Hu M, Wang J, Qi J, Han Z, Wang G, Qi Y, Wang H-W, Zhou J-M, and Chai J**. Reconstitution and structure of a plant NLR resistosome conferring immunity. Science. 2019a:**364**(6435):eaav5870. https://doi.org/10.1126/science.aav5870

**Wang J, Song W, and Chai J**. Structure, biochemical function, and signaling mechanism of plant NLRs. Mol Plant. 2023:**16**(1):75–95. https://doi.org/10.1016/j.molp.2022.11.011

**Wang J, Wang J, Hu M, Wu S, Qi J, Wang G, Han Z, Qi Y, Gao N, Wang H-W, et al.** Ligand-triggered allosteric ADP release primes a plant NLR complex. Science. 2019b:**364**(6435):eaav5868. https://doi.org/10.1126/science.aav5868

**Wang L, Albert M, Einig E, Fürst U, Krust D, and Felix G**. The pattern-recognition receptor CORE of Solanaceae detects bacterial cold-shock protein. Nat Plants. 2016:**2**(12):1–9. https://doi.org/10.1038/nplants.2016.185

**Wang L, Ji Y, Hu Y, Hu H, Jia X, Jiang M, Zhang X, Zhao L, Zhang Y, Jia Y, et al.** The architecture of intra-organism mutation rate variation in plants. PLOS Biol. 2019c:**17**(4):e3000191. https://doi.org/10.1371/journal.pbio.3000191

**Wang L, Jia Y, Osakina A, Olsen KM, Huang Y, Jia MH, Ponniah S, Pedrozo R, Nicolli C, and Edwards JD**. Receptor- ligand interactions in plant inmate immunity revealed by AlphaFold protein structure prediction. 2024. https://doi.org/10.1101/2024.06.12.598632

**Watson JL, Juergens D, Bennett NR, Trippe BL, Yim J, Eisenach HE, Ahern W, Borst AJ, Ragotte RJ, Milles LF, et al.** De novo design of protein structure and

function with RFdiffusion. Nature. 2023:**620**(7976):1089–1100. https://doi.org/10.1038/s41586-023-06415-8

**Wei W, Kim D, Koehler N, Bendl A, Cho M-J, and Krasileva K**. Engineering pathogen-inducible promoters for conferring disease resistance in tomato. Synth Biol. 2024.

**Wei Y, Balaceanu A, Rufian JS, Segonzac C, Zhao A, Morcillo RJL, and Macho AP**. An immune receptor complex evolved in soybean to perceive a polymorphic bacterial flagellin. Nat Commun. 2020:**11**(1):3763. https://doi.org/10.1038/s41467-020-17573-y

**Wei Y, Caceres-Moreno C, Jimenez-Gongora T, Wang K, Sang Y, Lozano-Duran R, and Macho AP**. The Ralstonia solanacearum csp22 peptide, but not flagellin-derived peptides, is perceived by plants from the Solanaceae family. Plant Biotechnol J. 2018:**16**(7):1349–1362. https://doi.org/10.1111/pbi.12874

**Whitham S, Dinesh-Kumar SP, Choi D, Hehl R, Corr C, and Baker B**. The product of the tobacco mosaic virus resistance gene N: Similarity to toll and the interleukin-1 receptor. Cell. 1994:**78**(6):1101–1115. https://doi.org/10.1016/0092-8674(94)90283-6

**Witek K, Jupe F, Witek AI, Baker D, Clark MD, and Jones JDG**. Accelerated cloning of a potato late blight–resistance gene using RenSeq and SMRT sequencing. Nat Biotechnol. 2016:**34**(6):656–660. https://doi.org/10.1038/nbt.3540

**Wlodzimierz P, Rabanal FA, Burns R, Naish M, Primetis E, Scott A, Mandáková T, Gorringe N, Tock AJ, Holland D, et al.** Cycles of satellite and transposon evolution in Arabidopsis centromeres. Nature. 2023:**618**(7965):557–565. https://doi.org/10.1038/s41586-023-06062-z

**Wojciechowski JW, Tekoglu E, Gąsior-Głogowska M, Coustou V, Szulc N, Szefczyk M, Kopaczyńska M, Saupe SJ, and Dyrka W**. Exploring a diverse world of effector domains and amyloid signaling motifs in fungal NLR proteins. PLOS Comput Biol. 2022:**18**(12):e1010787. https://doi.org/10.1371/journal.pcbi.1010787

**Wu Y, Xu W, Zhao G, Lei Z, Li K, Liu J, Huang S, Wang J, Zhong X, Yin X, et al.** A canonical protein complex controls immune homeostasis and multipathogen

resistance. Science. 2024:eadr2138. https://doi.org/10.1126/science.adr2138

**Wu Z, Waneka G, Broz AK, King CR, and Sloan DB**. MSH1 is required for maintenance of the low mutation rates in plant mitochondrial and plastid genomes. Proc Natl Acad Sci. 2020:**117**(28):16448–16455. https://doi.org/10.1073/pnas.2001998117

**Wulff BBH, Thomas CM, Smoker M, Grant M, and Jones JDG**. Domain Swapping and Gene Shuffling Identify Sequences Required for Induction of an Avr-Dependent Hypersensitive Response by the Tomato Cf-4 and Cf-9 Proteins. Plant Cell. 2001:**13**(2):255–272. https://doi.org/10.1105/tpc.13.2.255

**Wyrsch I, Domínguez-Ferreras A, Geldner N, and Boller T**. Tissue-specific FLAGELLIN-SENSING 2 (FLS2) expression in roots restores immune responses in Arabidopsis fls2 mutants. New Phytol. 2015:**206**(2):774–784. https://doi.org/10.1111/nph.13280

**Xu G, Yuan M, Ai C, Liu L, Zhuang E, Karapetyan S, Wang S, and Dong X**. uORF-mediated translation allows engineered plant disease resistance without fitness costs. Nature. 2017:**545**(7655):491–494. https://doi.org/10.1038/nature22372

**Yan X, Tang B, Ryder LS, MacLean D, Were VM, Eseola AB, Cruz-Mireles N, Ma W, Foster AJ, Osés-Ruiz M, et al.** The transcriptional landscape of plant infection by the rice blast fungus Magnaporthe oryzae reveals distinct families of temporally co-regulated and structurally conserved effectors. Plant Cell. 2023:**35**(5):1360–1385. https://doi.org/10.1093/plcell/koad036

**Yang L, Chen X, Wang Z, Sun Q, Hong A, Zhang A, Zhong X, and Hua J**. HOS15 and HDA9 negatively regulate immunity through histone deacetylation of intracellular immune receptor NLR genes in Arabidopsis. New Phytol. 2020:**226**(2):507–522. https://doi.org/10.1111/nph.16380

**Yang L, Wang Z, and Hua J**. A Meta-Analysis Reveals Opposite Effects of Biotic and Abiotic Stresses on Transcript Levels of Arabidopsis Intracellular Immune Receptor Genes. Front Plant Sci. 2021:**12**:625729. https://doi.org/10.3389/fpls.2021.625729

**Yu D, Song W, Tan EYJ, Liu L, Cao Y, Jirschitzka J, Li E, Logemann E, Xu C, Huang S, et al.** TIR domains of plant immune receptors are 2′,3′-cAMP/cGMP

synthetases mediating cell death. Cell. 2022:**185**(13):2370-2386.e18. https://doi.org/10.1016/j.cell.2022.04.032

**Yu DS, Outram MA, Smith A, McCombe CL, Khambalkar PB, Rima SA, Sun X, Ma L, Ericsson DJ, Jones DA, et al.** The structural repertoire of Fusarium oxysporum f. sp. lycopersici effectors revealed by experimental and computational studies. eLife. 2024a:**12**:RP89280. https://doi.org/10.7554/eLife.89280.3

**Yu H, Xu W, Chen S, Wu X, Rao W, Liu X, Xu X, Chen J, Nishimura MT, Zhang Y, et al.** Activation of a helper NLR by plant and bacterial TIR immune signaling. Science. 2024b:eadr3150. https://doi.org/10.1126/science.adr3150

**Yuan M, Jiang Z, Bi G, Nomura K, Liu M, Wang Y, Cai B, Zhou J-M, He SY, and Xin X-F**. Pattern-recognition receptors are required for NLR-mediated plant immunity. Nature. 2021:**592**(7852):105–109. https://doi.org/10.1038/s41586-021-03316-6

**Zdrzałek R, Stone C, De La Concepcion JC, Banfield MJ, and Bentham AR**. Pathways to engineering plant intracellular NLR immune receptors. Curr Opin Plant Biol. 2023:**74**:102380. https://doi.org/10.1016/j.pbi.2023.102380

**Zdrzałek R, Xi Y, Langner T, Bentham AR, Petit-Houdenot Y, De La Concepcion JC, Harant A, Shimizu M, Were V, Talbot NJ, et al.** Bioengineering a plant NLR immune receptor with a robust binding interface toward a conserved fungal pathogen effector. Proc Natl Acad Sci. 2024:**121**(28):e2402872121. https://doi.org/10.1073/pnas.2402872121

**Zeng X, Tian D, Gu K, Zhou Z, Yang X, Luo Y, White FF, and Yin Z**. Genetic engineering of the Xa10 promoter for broad-spectrum and durable resistance to Xanthomonas oryzae pv. oryzae. Plant Biotechnol J. 2015:**13**(7):993–1001. https://doi.org/10.1111/pbi.12342

**Zhang S, Liu S, Lai H-F, Caflisch A, and Zipfel C**. Reverse engineering of the pattern recognition receptor FLS2 reveals key design principles of broader recognition spectra against evading flg22 epitopes. bioRxiv. 2024a:2024.10.10.617594. https://doi.org/10.1101/2024.10.10.617594

**Zhang S-M, Adema CM, Kepler TB, and Loker ES**. Diversification of Ig Superfamily Genes in an Invertebrate. Science. 2004:**305**(5681):251–254.

https://doi.org/10.1126/science.1088069

**Zhang X, Gao J, Molloy LM, Crean LM, Williams SJ, and Rathjen JP**. Identification of a key gain-of-function residue for effector binding by in vitro shuffling of barley Mla NLR genes. 2024b. https://doi.org/10.1101/2024.10.27.619561

**Zhang X, Liu Y, Yuan G, Wang S, Wang D, Zhu T, Wu X, Ma M, Guo L, Guo H, et al.** The synthetic NLR RGA5HMA5 requires multiple interfaces within and outside the integrated domain for effector recognition. Nat Commun. 2024c:**15**(1):1104. https://doi.org/10.1038/s41467-024-45380-2

**Zhang Y, Xia R, Kuang H, and Meyers BC**. The Diversification of Plant NBS-LRR Defense Genes Directs the Evolution of MicroRNAs That Target Them. Mol Biol Evol. 2016:**33**(10):2692–2705. https://doi.org/10.1093/molbev/msw154

**Zhang Z-M, Ma K-W, Gao L, Hu Z, Schwizer S, Ma W, and Song J**. Mechanism of host substrate acetylation by a YopJ family effector. Nat Plants. 2017:**3**(8):1–10. https://doi.org/10.1038/nplants.2017.115

**Zhao Y-B, Liu M-X, Chen T-T, Ma X, Li Z-K, Zheng Z, Zheng S-R, Chen L, Li Y-Z, Tang L-R, et al.** Pathogen effector AvrSr35 triggers Sr35 resistosome assembly via a direct recognition mechanism. Sci Adv. 2022:**8**(36):eabq5108. https://doi.org/10.1126/sciadv.abq5108

**Zhu J, Moreno-Pérez A, and Coaker G**. Understanding plant pathogen interactions using spatial and single-cell technologies. Commun Biol. 2023:**6**(1):814. https://doi.org/10.1038/s42003-023-05156-8

**Zipfel C, Kunze G, Chinchilla D, Caniard A, Jones JDG, Boller T, and Felix G**. Perception of the Bacterial PAMP EF-Tu by the Receptor EFR Restricts Agrobacterium-Mediated Transformation. Cell. 2006:**125**(4):749–760. https://doi.org/10.1016/j.cell.2006.03.037