

COMMENTARY OPEN ACCESS

Rewiring NLRs for Broad and Cross-Species Plant Immunity

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1 | Introduction

Plant pathogens pose a persistent threat to global food security, and crops defend themselves via two major immune strategies: pattern-triggered immunity (PTI) initiated by surface-localised pattern recognition receptors (PRRs), and effector-triggered immunity (ETI) mediated by intracellular nucleotide-binding leucine-rich repeat (NLR) receptors [1]. Among these, engineering NLRs offers a powerful route to durable resistance. Yet, two critical challenges remain—most NLRs have narrow effector recognition spectra and are functionally constrained to closely related species due to their reliance on taxonomically restricted helper NLRs.

Recent advances have begun to address these bottlenecks. Wang et al. introduced a synthetic NLR strategy in which engineered immune receptors are activated by conserved pathogen-derived proteases, conferring broad-spectrum viral resistance [2]. In parallel, Du et al. circumvented taxonomic barriers by co-expressing Solanaceae sensor NLRs with their cognate NRC helper NLRs in rice, soybean, and *Arabidopsis*, thereby overcoming restricted taxonomic functionality (RTF) and enabling functional immune transfer across distant lineages [3].

2 | Broadening Effector Recognition: Synthetic NLRs Activated by Pathogen Proteases

Proteases are widely deployed by plant pathogens—including viruses, bacteria, and fungi—to facilitate infection. Potyviruses, which comprise approximately 30% of known plant

viruses, encode a nuclear inclusion a (NIa) protease that cleaves itself into VPg and NIa-Pro, both possessing enzymatic activity [4, 5]. Importantly, NLRs such as coiled-coil (CC) domain-containing NLRs (CNLs) and RESISTANCE TO POWDERY MILDEW 8-like CC (CC_R) domain-containing NLRs (RNLs) require a free, unmodified N-terminus for immune activation, and N-terminal tags typically inactivate this functionality [6]. Building on this insight, Wang et al. engineered NLRs with an appended viral protease cleavage site (PCS) positioned before the N-terminus of an autoactive NLR (aNLR), creating immune switches that are conditionally activated by viral protease activity.

To test this concept, the authors constructed a chimaeric receptor, HA-PCS^{PVY}-aTm-2², combining the NIa protease cleavage site from potato virus Y (PVY) with the autoactive CNL protein Tm-2². Expression of this construct in *Nicotiana benthamiana* (*Nb*) triggered cell death in response to NIa protease and conferred complete PVY resistance. Notably, the transgenic plants also exhibited immunity to several related potyviruses, including turnip mosaic virus (TuMV), pepper mottle virus (PepMoV), chilli veinal mottle virus (ChiVMV), and plum pox virus (PPV), underscoring the potential for broad-spectrum resistance via conserved protease recognition.

The strategy was further extended to RNL-type receptors. Protease-responsive variants of autoactive AtNRG1.1 (D485V) and autoactive NbNRG1 (D519V) recapitulated the function of their CNL counterpart, triggering cell death upon NIa^{PVY} protease co-expression and conferring robust resistance to multiple potyviruses in transgenic plants. However, the initial constructs

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failed to recognise the NIa protease of tobacco etch virus (TEV), rendering the plants susceptible. To address this, the authors developed a tandem cleavage design—HA-PCS^{TEV}-PCS^{PVY}-aA_tNRG1.1—that responded to both PVY and TEV proteases. Transgenic lines expressing this construct exhibited full immunity to PVY and its relatives, and partial-to-complete resistance against TEV.

To demonstrate translational potential in crops, Wang et al. generated transgenic soybean plants expressing HA-PCS^{SMV}-aA_tNRG1.1, which conferred complete resistance to soybean mosaic virus (SMV). This proof-of-concept highlights the practical utility of protease-cleavable NLRs in engineering broad-spectrum resistance in economically important crops (Figure 1).

This platform offers several advantages: (1) a simple design requiring only a single autoactive NLR without additional decoy components; (2) broad-spectrum pathogen recognition through conserved or tandem cleavage sites; (3) cross-crop compatibility; and (4) evolutionary durability, as protease mutations that escape detection would likely compromise pathogen fitness.

3 | Overcoming Taxonomic Barriers: Co-Expression of Sensor and Helper NLRs

While broadening effector recognition addresses one major limitation, functional constraints across plant taxa present another. NLRs often fail to function outside their native taxonomic group due to their dependence on specific helper proteins [7]. Du et al. tackled this challenge by co-transferring Solanaceae sensor NLRs and their NRC helpers into distantly related species, thereby restoring ETI function in previously non-permissive hosts [3].

They selected two Solanaceae sensors: pepper Bs2 and *Solanum americanum* Rpi-amr3. Bs2 recognises AvrBs2, a conserved effector in *Xanthomonas* species, including *Xanthomonas oryzae* pv. *Oryzicola* (*Xoc*), the causal agent of bacterial leaf streak (BLS)—a major threat to rice. However, Bs2 functions only in Solanaceae and fails in distantly related species like *Arabidopsis* [8]. As for Rpi-amr3, it recognises a *Phytophthora infestans* effector (AvrAmr3) that is found in many *Phytophthora* pathogen species infecting rosoid crops [9, 10]. However, NRC-dependent Rpi-amr3 is absent from rosoid crops.

Transient assays in *Nb* confirmed that Bs2 can recognise AvrBs2^{*Xoc*} to trigger a hypersensitive response. Then they showed Bs2 alone failed to induce cell death upon AvrBs2^{*Xoc*} recognition while co-delivery of Bs2 with *Nb* helper NLRs (*Nb*NRC2/3/4) restored ETI in rice protoplasts. Similarly, Bs2 with *Solanum* NRCs or Rpi-amr1/3 with *Sa*NRCs conferred NLRs function in soybean and *Arabidopsis* protoplasts.

Further, they generated transgenic *Arabidopsis* or rice plants that either carry sensor NLRs or sensor plus helper NLRs, and found that stable *Arabidopsis* lines carrying sensor and helper NLRs show effector-dependent cell death. Expression of Bs2 in rice confers resistance against *Xoc* in an NRC2/3/4-dependent manner. Inoculation with the *Xoc* can induce oligomerisation of NRC2/4 and defence gene expression in rice, demonstrating that the co-transfer of sensor-helper NLR pairs is sufficient to fully reconstitute the ETI signalling pathway. Besides, results also revealed that the effector is recognized in transgenic plants carrying the Solanaceae sensor and helper NLRs but not in sensor NLR-alone lines. Importantly, heterologous expression of these NLR combinations did not adversely affect plant growth or yield. Evaluations of basal resistance markers and susceptibility to *Magnaporthe oryzae* infection revealed no signs of autoimmunity or compromised plant fitness.

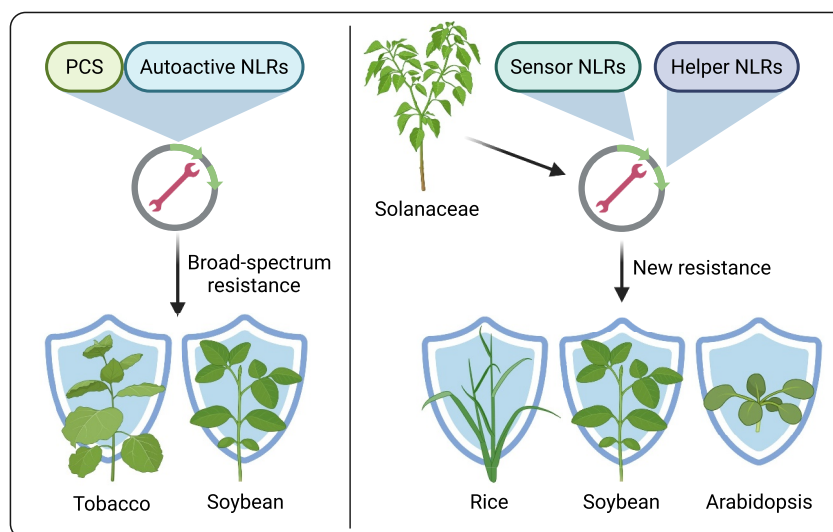


FIGURE 1 | Synthetic design and cross-species transfer strategies to broaden NLR-mediated plant immunity. Left panel: Wang et al. engineered NLRs with viral protease cleavage sites (PCS) that are activated upon cleavage by pathogen-derived proteases, enabling broad-spectrum resistance against multiple potyviruses in diverse hosts, including tobacco (*Nb*) and soybean. Right panel: Du et al. overcame restricted taxonomic functionality (RTF) by co-expressing Solanaceae sensor NLRs with their NRC clade helper NLRs, reconstituting functional immunity in distantly related species including rice, soybean, and *Arabidopsis*, and conferring novel pathogen resistance. The graphical model was created with [BioRender.com](https://www.biorender.com).

In conclusion, they showed that the co-transfer of Bs2 and NRC helper NLRs (but neither alone) confers strong resistance against *Xoc* in rice, proving that restricted RTF can be overcome. Their approach highlights the vast potential of NLRs from distantly related plants as untapped genetic resources for expanding crop disease resistance. Moreover, such NLRs may offer more durable resistance, as non-adapted pathogens are less likely to have evolved effector-mediated suppression mechanisms against them compared to host-derived NLRs (Figure 1).

4 | Comparative Insights and Future Directions

Collectively, these studies employ NLR immune receptors to overcome limitations of natural disease resistance through distinct strategies. Wang et al. harness pathogen-derived proteases—conserved across kingdoms—to activate NLRs, reducing reliance on effector recognition. This approach shows promise against diverse protease-secreting pathogens, including viruses, bacteria, oomycetes, fungi, nematodes, and piercing-sucking insects. Nevertheless, it has limitations: (1) it is effective only against protease-producing pathogens; (2) unknown cleavage sites preclude targeted NLR design; and (3) field efficacy and potential yield impacts require further evaluation.

On the other hand, Du et al. bypass RTF by interfamily co-expressing sensor NLRs with NRC helpers. While this approach minimises fitness costs and counters pathogen adaptation, several questions remain. For example, the performance of sensor–NRC combinations in non-asterid crops needs further investigation. The concurrent expression of multiple NLRs raises the possibility of unintended autoactivation and autoimmune responses. High sequence similarity in leucine-rich repeat (LRR) domains may also trigger gene silencing and transcriptional instability. Reliable co-expression of exogenous helpers across genetic backgrounds remains challenging. Thus, large-scale, multi-environment trials are essential to evaluate the durability and agronomic performance of NLR-engineered crops.

Given that pathogens often co-infect plants, integrating both strategies offers a promising avenue for designing crops with broad and durable resistance. For example, co-expressing a PCS-based synthetic NLR with an NLR/NRC helper system could confer simultaneous resistance to multiple pathogens in a single crop. Future research should prioritise using CRISPR–Cas genome editing to precisely modify endogenous NLR genes, fine-tune immune activation, and stack resistance traits without compromising plant growth. Mining NLR genes from wild relatives and non-host species, combined with smart breeding technologies, will accelerate the development of high-yielding, disease-resistant crops. Such integrated approaches are critical for building sustainable agricultural systems resilient to diverse and co-infecting pathogen communities.

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Conflicts of Interest

The authors declare no conflicts of interest.

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Author Contributions

Q.G. conceptualized the commentary. Q.G. and J.J.L. prepared the first draft of the commentary and figure. Q.G. and J.J.L. corrected and