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Views & Comments

Equipping NLRs with Multifunctional Modules to Outpace Pathogen Evolution

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1. Introduction

Plant diseases pose a significant threat to global agricultural production, particularly oomycete diseases such as soybean root rot and potato late blight, which severely impact food security and farmer incomes. Potato late blight, caused by the oomycete pathogen *Phytophthora infestans* (*P. infestans*), is among the most devastating diseases affecting potato crops worldwide. It is notorious for its rapid spread and severe impact on yield and tuber quality, often resulting in significant economic losses [1].

As the cornerstone of plant immune systems, nucleotide-binding leucine-rich repeat (NLR) proteins recognize pathogen effector proteins and trigger immune responses. In recent years, NLR-based disease resistance engineering has emerged as a key technological approach for improving crop resistance. A recent study conducted by Wang et al. [2] analyzed the NLRome of 52 wild and cultivated species and identified three new late blight resistance genes (*Rpi-cph1*, *Rpi-cjm1*, and *Rpi-brk1*) through three different systematic methods. Furthermore, the team developed a modular "plug-in" strategy for engineering disease resistance in potatoes, demonstrating that integrating functional domains into NLR proteins can expand their recognition capabilities and thus broaden the resistance spectrum.

2. From breeding challenges to NLR engineering: Strategies for accelerated late blight resistance

Multiple challenges persist in the use of conventional breeding methods for potato late blight management. As a model autotetraploid species, potato possesses four sets of highly homologous chromosomes, complicating the establishment of homozygosity and stable inheritance for target disease resistance genes (resistance to *P. infestans*, Rpi genes). This process is time-consuming and requires multiple generations of backcrossing to establish stable breeding lines. To address these constraints, Cheng et al. [3] and Zhang et al. [4] pioneered a hybrid breeding strategy. This innovative approach shifts potato propagation from clonal tetraploid reproduction to true seed-based diploid hybridization, accelerating the development of inbred lines while also facilitating the effective accumulation of disease resistance genes through multiple rounds of hybridization. Crucially, it significantly shortened the breeding cycle from the traditional 10–12 years to a remarkable 3–5 years.

Most late blight R genes encode NLRs, but many have been overcome by the rapid evolution of *P. infestans* [5]. Many crops face a similar challenge: owing to the rapid evolution of pathogens in the field, new physiological races frequently emerge, which can quickly overcome single disease resistance genes, leading to the rapid loss of resistance in crop varieties. Identifying reliable NLR genes and utilizing them for genetic engineering represents one of the key solutions to address this challenge. For example, plant disease resistance can be engineered by fusing a pathogen protease cleavage site to autoactive NLRs, enabling broad-spectrum durable immunity [5]. Stacking multiple NLR genes in potatoes can trigger calcium-dependent protein kinase (CDPK) signaling, thereby increasing broad-spectrum resistance against late blight [6]. Engineering the C-terminal domain of the *Arabidopsis* pattern recognition receptor receptor-like protein 23 (RLP23) enables broad-spectrum resistance against bacterial, fungal, and oomycete pathogens in tomato, rice, and poplar without compromising yield [7]. In addition to the rapid evolution of the pathogen, regulatory constraints on genetically modified organisms (GMOs) pose another significant challenge, underscoring the critical importance of identifying reliable NLR genes. This raises a key question: Although engineering NLR genes can increase plant resistance, how can we identify reliable NLRs for this purpose?

3. Three novel methods for the identification of new NLRs

In this context, Wang et al. [2] (*Nature*, 2025) provides new methods and innovative perspectives (Fig. 1). They selected 52 high-quality monoploid genome assemblies (seven newly sequenced in this study, 44 from Tang et al. [8], and the DM1-3 516 R44 (DM) reference genome), representing wild and cultivated species across *Solanum* section *Petota*. Then, 39 211 NLR genes were reannotated across all the genomes using a standardized NLR-annotator pipeline. They classified NLRs into six superclades on the basis of domain architecture: toll/interleukin-1 receptor (TIR)-NLRs, resistance to powdery mildew 8 -NLRs (RNLs),

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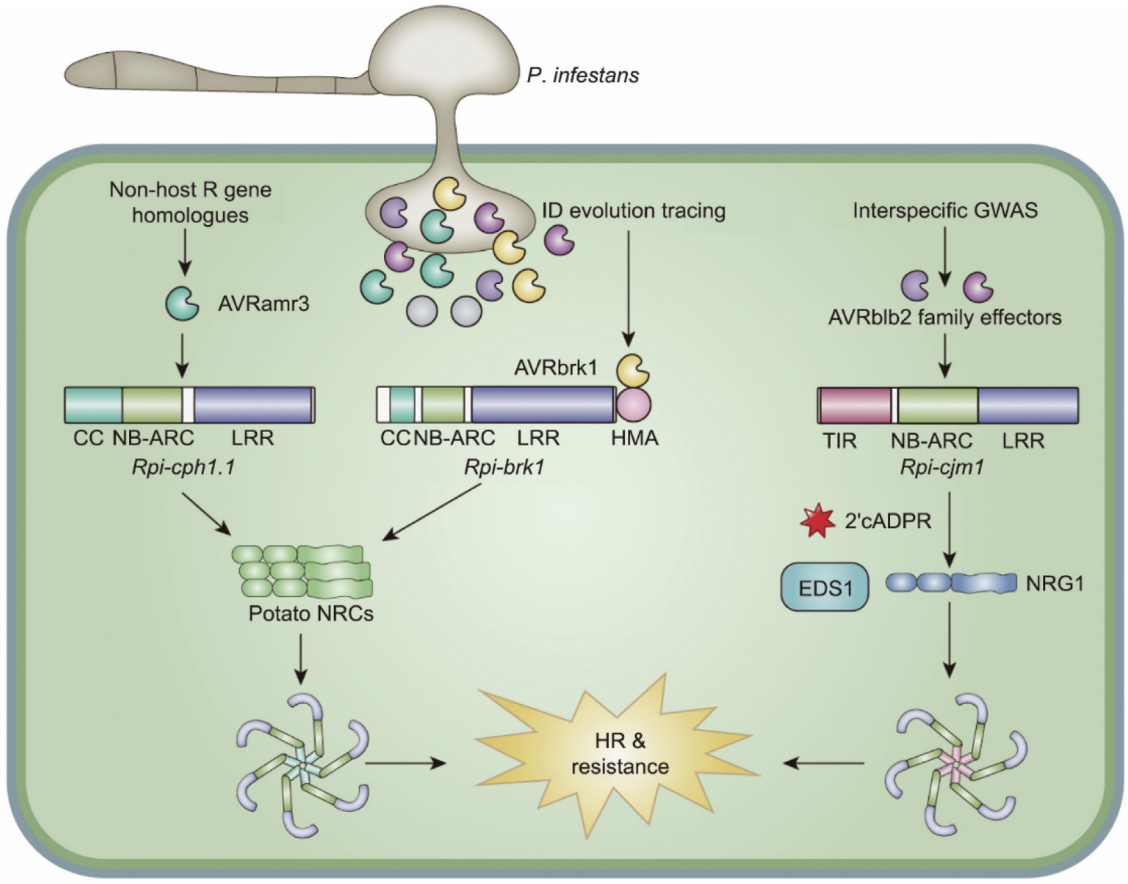

Fig. 1. Working models of *Rpi-cph1*, *Rpi-brk1*, and *Rpi-cjm1*. *Rpi-cph1* was discovered by searching for homologs of non-host resistance genes in wild potato relatives. *Rpi-brk1* was identified through evolutionary analysis of noncanonical IDs in the potato NLRome, revealing its HMA domain and effector recognition specificity. *Rpi-cjm1* was identified via a combined strategy of pathogen effector screening and interspecific GWASs. All three genes recognize specific *P. infestans* effectors—AVRamr3, AVRbrk1, and AVRblb2 family members, respectively—and trigger downstream immune responses. *Rpi-cph1* and *Rpi-brk1* are CNL proteins that function with NRC helper proteins and resistosome complexes. *Rpi-cjm1* generates 2′cADPR signaling molecules via its TIR domain, with EDS1 and NRG1 being required for cell death and resistance execution. LRR: leucine-rich repeat; NB-ARC: nucleotide-binding adaptor shared by Apaf-1, R proteins, and CED4; HR: hypersensitive response.

coiled-coil group 10 (CC_{G10})-NLRs, coiled-coil (CC)-NLR-required for cell death (NRC)-helpers, CC-NRC-sensors, and CC-NLR-others. Using phylogenetic relationships and sequence similarity, they further clustered NLRs into 489 clades and categorized the clades as type I or type II. Type I NLRs are characterized by rapid evolution, as evidenced by high copy number variation and extensive gene duplication. Although they constitute only 7.2% of all NLR clades, they account for 67.1% of the total NLR gene count, indicating significant expansion capacity. Functionally, type I NLRs are predominantly associated with the sensor role, recognizing fast-evolving pathogens. This is strongly supported by the finding that 17 out of 18 (94.4%) known sensor NLR (e.g., *R1* [9], *Rpi-blb2* [10], *R3a* [11], and *R8* [12]) homologs targeting oomycetes, nematodes, and viruses reside in type I clades. In contrast, type II NLRs are evolutionarily conserved and typically present as single or low-copy genes. They represent 92.8% of all NLR clades but only 32.1% of the total NLR genes, with most clades having zero to two copies per genome. Functionally, they are more closely linked to the helper role; ten out of eleven known helper NLR homologs belong to type II clades. Additionally, sensor NLRs that recognize bacterial and fungal pathogens are primarily found within type II clades (eight out of nine). These findings reveal the relationship between NLR evolution and function, providing evolutionary evidence for predicting the function of disease resistance genes.

Notably, this study provides three effective methods for identifying new NLR genes. First, by searching for *Rpi-amr3*, the Rpi gene in *Solanum americanum*, which is considered a non-host with high resistance to late blight, they successfully discovered a coiled-coil nucleotide-binding leucine-rich repeat (CNL) gene from the wild potato *Solanum cardiophyllum* (which can be crossed with cultivated potatoes) and named it *Rpi-cph1*. Second, they discovered *Rpi-cjm1*, the first TIR-type NLR detected in potato, by combining pathogen effector screening with a cross-species genome-wide association study (GWAS) that leverages natural genetic variation across 101 wild *Solanum* accessions, enabling rapid, high-resolution R gene discovery. Third, through evolutionary analysis of noncanonical integrated domains (IDs) in the potato NLRome, they reported that the heavy metal-associated (HMA) domain evolves rapidly within NLRs and identified an NLR gene carrying the HMA domain, which was named *Rpi-brk1*. Experimental validation conducted in a growth chamber confirmed that these three newly discovered NLRs confer resistance to six different *P. infestans* isolates, including JH19, 88069, NL07434, EC1, T30-4, and HB1501. They further investigated the resistance mechanisms of these three NLR genes. *Rpi-cph1*-mediated cell death requires potato NRC helpers. Compared with the well-known NLR *Rpi-blb2*, *Rpi-cjm1* recognizes multiple AVRblb2 family effectors and exhibits broader effector recognition specificity. *Rpi-cjm1* produces 2′-cyclic adenosine diphosphate-ribose (2′cADPR) signaling molecules through the enzymatic activity of its TIR domains, and the resistance mediated by *Rpi-cjm1* depends on enhanced disease susceptibility 1 (EDS1) and N requirement gene 1 (NRG1). *Rpi-brk1* confers disease

resistance through its integrated HMA domain, which directly binds to the pathogen effector avirulence protein brk1 (AVRbrk1), triggering immune responses that are dependent on NRC helper proteins (Fig. 1). Structural modeling and mutagenesis experiments revealed a specific interaction interface formed mainly by the α1 and α2 helices of the HMA domain. Site-directed mutagenesis of seven key residues within this interface (E1125, D1126, R1133, D1161, E1162, Q1164, and E1168) completely abolished both HMA^{brk1}-AVRbrk1 binding and *Rpi-brk1*-mediated cell death, confirming that effector recognition occurs precisely within a defined molecular interface of the HMA module.

4. Breakthroughs in plant disease resistance breeding

Finally, after identifying *Rpi-brk1* and its HMA-mediated recognition mechanism, the researchers next asked whether the HMA domain could function as a portable unit. They fused the HMA from *Rpi-brk1* to the C-terminus of the potato NLR R1, a protein previously inactive against late blight. They named this the "plug-in" strategy. Surprisingly , this engineered receptor not only gained the ability to recognize the *P. infestans* effector AVRbrk1 (which the original R1 protein does not recognize) but also retained its ability to recognize AVR1. This successful engineering exemplifies the "plug-in" strategy based on a modular design principle. The HMA domain serves as an interchangeable recognition module that can be functionally detached from its native NLR context (*Rpi-brk1*) and integrated into a compatible NLR backbone (R1). This approach leverages the natural modularity of NLRs, where IDs evolve as autonomous effector-binding units. The finding that R1-HMA^{brk1} gains AVRbrk1 recognition while retaining AVR1 recognition demonstrates that the grafted HMA module operates independently and does not interfere with the preexisting recognition apparatus of the receptor. This modular logic presents a scalable framework for engineering resistance: in theory, various pathogen-specific recognition modules (different IDs) could be "plug-in" to suitable NLR backbones to construct custom resistance traits.

The "plug-in" strategy represents a breakthrough approach for engineering disease resistance in crops, particularly in addressing the challenge of rapidly evolving pathogens such as *P. infestans*. The "plug-in" strategy can mitigate the significant impact of potato late blight and provides a scalable solution for global food security.

5. Future perspectives

In addition to oomycete pathogens, plants also face threats from numerous viruses, bacteria, fungi, and nematodes. Owing to the rapid evolution of pathogens, we cannot remain passive. This innovative research provides not only a novel solution for controlling potato late blight but also a promising new path for the entire field of crop disease resistance breeding. For example, rice (*Oryza sativa* L.), one of the world's most important staple crops, has long been plagued by various pathogens. Notably, bacterial diseases such as bacterial blight of rice [13], fungal diseases such as rice blast, sheath blight, and false smut [14], and various viral diseases, particularly Rice stripe virus (RSV) and Rice black-streaked dwarf virus (RBSDV) [15], are problematic. These diseases can cause severe yield losses and quality deterioration in rice. Traditional disease resistance breeding largely relies on screening natural variants, but the rapid mutation of pathogens slows the identification of effective resistance genes and leads to the continual emergence of new pathogen strains. Against this backdrop, the "plug-in" strategy offers a groundbreaking approach to research resistance in rice. By drawing inspiration from the design of the potato NLRome, can we construct a diverse library of decoy domains and insert these modules into rice NLR proteins, thereby expanding their recognition spectrum against various pathogen effectors. To translate this strategy to rice, we propose an actionable framework. First, we will construct a diverse library of decoy domains (e.g., HMA, WRKY, and other pathogen-interacting modules) from natural rice NLRs or synthetic sources. Second, we will insert these modules into rice NLR proteins (e.g., *Oryza sativa* nucleotide-binding leucine-rich repeat protein 1 (OsNLR1)) via genetic engineering. The insertion will be guided by structural and functional analyses to ensure compatibility with the NLR backbone. Finally, we will validate the engineered NLRs in controlled environments (e.g., greenhouse assays) and field trials to ensure that they confer broad-spectrum resistance against bacterial, fungal, and viral effectors without compromising plant fitness. Beyond this, similar to the discovery of *Rpi-cph1* from wild potato relatives, mining R gene homologs from non-host species has proven instrumental in providing durable, broad-spectrum resistance against rapidly evolving fungal pathogens, such as rice blast. This parallel underscores a core strategy: leveraging NLR genes or their close homologs derived from wild or non-host species offers a powerful approach to introduce robust and potentially more durable resistance into cultivated crops.

Equipping NLRs with multifunctional defense mechanisms to overcome various constraints and tailor plants for efficient, broad-spectrum, and durable disease resistance. This modular design may not only broaden the spectrum of rice NLR proteins but also effectively avoid the side effects of autoactivation, ensuring normal rice growth. This approach not only increases the efficiency of resistance breeding but also provides new horizons for precision and intelligent disease resistance breeding in rice. Furthermore, it provides a scalable framework for combating emerging pathogens in other crops. For instance, similar strategies could be applied to wheat, maize, or soybean by identifying and integrating appropriate decoy domains into their respective NLR proteins.

6. Conclusions

Wang et al. [2] presented a comprehensive NLRome of 39 211 NLR genes from 31 wild and 21 cultivated potato genomes in the tuber-bearing section *Petota*. This represents the most comprehensive and largest-scale repository of disease resistance genes currently available in the plant kingdom. On the basis of the NLRome database, the authors successfully cloned three late blight resistance genes (*Rpi-cph1*, *Rpi-cjm1*, and *Rpi-brk1*) in potato using comparative and evolutionary genomics approaches. Excitingly, the study also innovatively proposed a "plug-in" disease resistance breeding strategy. This work provides not only new insights for the future mining of resistance genes but also novel strategies for crop disease resistance breeding, offering robust scientific and technological support for safeguarding food security.

CRediT authorship contribution statement

Qinglun Li: Writing – original draft, Conceptualization. **Jianping Chen:** Writing – review & editing, Conceptualization. **Zongtao Sun:** Writing – review & editing, Writing – original draft, Project administration, Conceptualization.

Declaration of competing interest

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

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