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# Diversified host target families mediate convergently evolved effector recognition across plant species

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**Abstract**

Recognition of pathogen effectors is a crucial step for triggering plant immunity. Resistance (R) genes often encode for nucleotide-binding leucine-rich repeat receptors (NLRs), and NLRs detect effectors from pathogens to trigger effector-triggered immunity (ETI). NLR recognition of effectors is observed in diverse forms where NLRs directly interact with effectors or indirectly detect effectors by monitoring host guardees/decoys (HGDs). HGDs undergo different biochemical modifications by diverse effectors and expand the effector recognition spectrum of NLRs, contributing robustness to plant immunity. Interestingly, in many cases of the indirect recognition of effectors, HGD families targeted by effectors are conserved across the plant species while NLRs are not. Notably, a family of diversified HGDs can activate multiple non-orthologous NLRs across plant species. Further investigation on HGDs would reveal the mechanistic basis of how the diversification of HGDs confers novel effector recognition by NLRs.

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**Keywords**

Decoy/Guardee, Effector, NLR, ETI, Convergent evolution, Plant immunity.

## Introduction

The recognition of non-self is a key step for plant survival against invading pathogens. Plants utilize extracellular and intracellular immune receptors for the surveillance of pathogen-derived molecules [1,2]. Pathogen-associated molecular patterns (PAMPs) such as conserved peptides derived from bacterial flagellin or carbohydrates released from fungal cell walls are recognized by pattern recognition receptors (PRRs) at the plant cell surface. Subsequently, the activation of PRRs leads to pattern-triggered immunity (PTI) which is followed by $Ca^{2+}$ influx, ROS production, MAP kinase cascade activation, and transcriptional reprogramming for defense gene expression and hormonal regulation [3]. To overcome PTI, pathogens secrete specialized proteins known as effectors. To counter this, plants have evolved intracellular nucleotide-binding leucine-rich repeats receptors (NLRs) that recognize the presence of corresponding effectors inside the cell [4]. Effectors can be delivered by various means, but, in this review, we focus on effectors secreted by bacterial pathogens via Type III secretion system. Upon the recognition of effectors, NLRs induce effector-triggered immunity (ETI), which potentiates PTI and can be associated with local programmed cell death [5–8]. Sensor NLRs recognize effectors directly or indirectly, and helper NLRs relay the immune signals to downstream signaling components and oligomerize to regulate cell death by forming cell membrane pores [9–13].

While some NLRs directly bind to effectors, others indirectly sense the activity of effectors. In latter case, NLRs detect the effector-directed modifications of host targets known as 'guardees' or 'decoys.' A key conceptual difference is that guardees can contribute to disease susceptibility in the absence of corresponding NLRs whereas decoys do not appear to be necessary for basal plant immunity [14–16]. Both guardees and decoys are widely accepted terminology in the field of plant

immunity, but in this review, we coin the term HGDs as an abbreviation for Host Guardees and Decoys for readability. One interesting observation in the study of NLRs, HGDs, and effectors is the convergent effector recognition: the same pathogen effector can be recognized by non-orthologous NLRs in distantly related plant species [17–21]. The detailed evolutionary and mechanistic bases of convergently evolved pathogen recognition are yet unclear, leading to questions such as 1) What is the role of HGDs in convergently evolved NLR function? 2) Do HGDs encoded in distantly related plant species function similarly for the activation of convergently evolved NLRs? In this review, we summarize examples of the convergent evolution of effector recognition by non-orthologous NLRs. We categorize each case into the types of modification on HGDs: proteolysis, phosphorylation, acetylation, and ADP-ribosylation. We note that, in many cases, HGDs targeted by the same effector fall into the same protein family. Interestingly, some of these protein families are diversified while maintaining the core sequences and structure critical for effector-directed modification. In addition, the effector can target not only the HGDs but also other members of the same protein family. The HGDs and additional proteins that are targeted by the effector can establish novel effector recognition by NLRs. Thus, we propose that the diversification of HGD families gives rise to non-orthologous NLRs that can recognize the same effector, and diversified HGDs function as a hub for effector recognition by NLRs in distantly related plant species.

## HGD modifications that activate NLRs

### Proteolysis

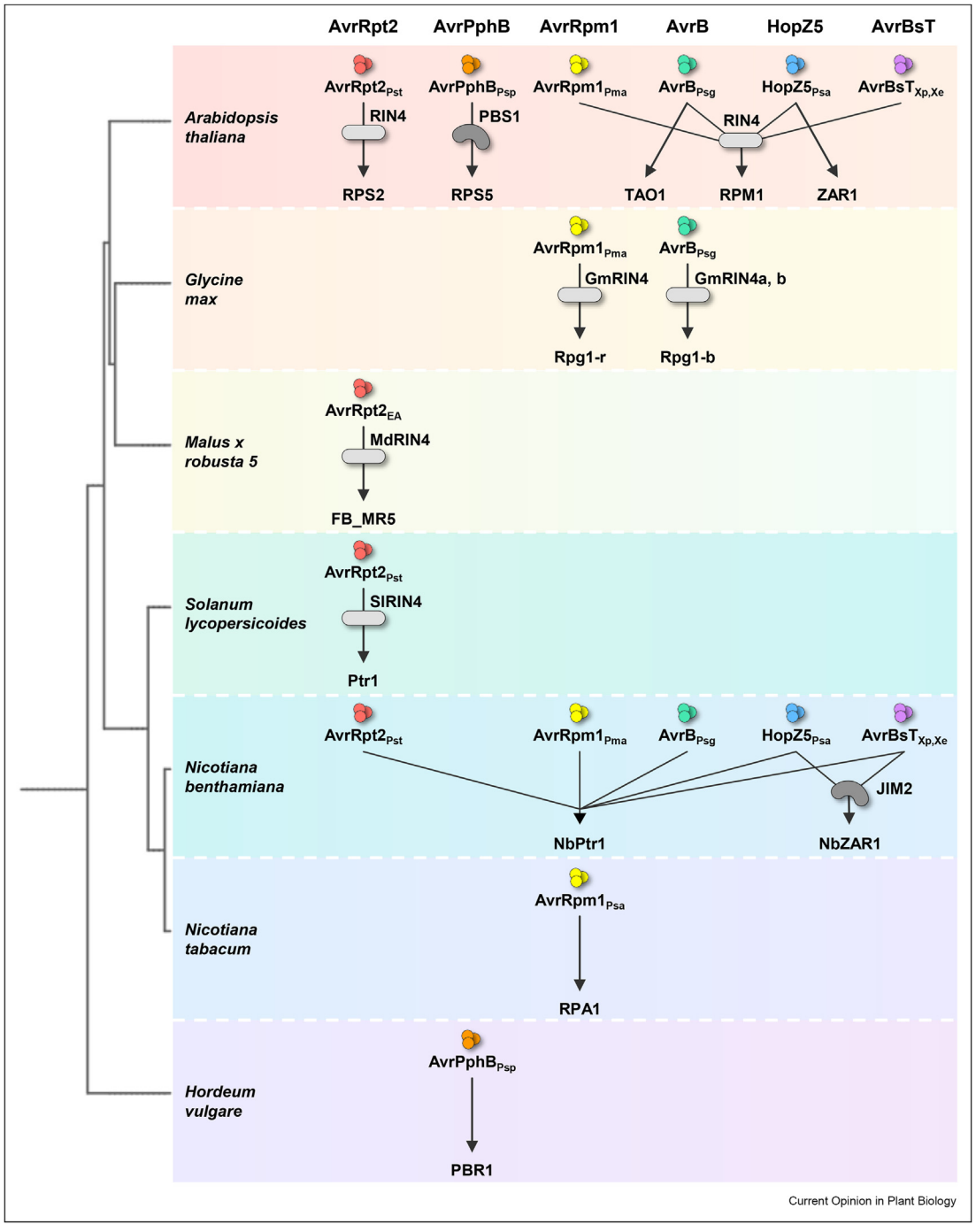
AvrRpt2 is a cysteine protease from *Pseudomonas syringae* pv. *tomato* which causes bacterial speck disease in tomatoes [22,23] (Figure 1). In *Arabidopsis thaliana* (hereafter, Arabidopsis), AvrRpt2 cleaves the immune signaling hub protein RPM1 INTERACTING PROTEIN 4 (RIN4) at the two sites located in the nitrate-induced (NOI) domains producing three AvrRpt2-cleaved products [24–27]. RIN4 is a negative regulator of the Arabidopsis NLR RESISTANCE TO PSEUDOMONAS SYRINGAE 2 (RPS2), and the degradation of Arabidopsis RIN4 causes RPS2-mediated ETI [28,29]. In a wild relative of tomato, *Solanum lycopersicoides*, AvrRpt2 is recognized by the tomato NLR Pseudomonas tomato race 1 (Ptr1). Similar to Arabidopsis RIN4, tomato RIN4 homologs can suppress Ptr1 activity, and the cleavage of tomato RIN4 homologs by AvrRpt2 leads to Ptr1 activation [18,30]. In *Nicotiana benthamiana*, AvrRpt2 is recognized by an *N. benthamiana* homolog of Ptr1 (NbPtr1) [20]. Notably, NbPtr1 shares 88.8% amino acid sequence identity with Ptr1, suggesting the parallel evolution to detect the cleavage activity of AvrRpt2. An *Erwinia amylovora* ortholog of AvrRpt2 also cleaves apple RIN4 homologs resulting in the activation of the apple NLR FB_MR5 [17]. Interestingly, unlike RPS2 activation in Arabidopsis, only the apple RIN4 C-terminal cleaved product is necessary and sufficient for FB_MR5 activation [17] (Figure 1).

Another bacterial effector with proteolytic activity is AvrPphB from *P. syringae* pv. *phaseolicola* which causes halo blight in the common bean, *Phaseolus vulgaris* [31] (Figure 1). In Arabidopsis, AvrPphB cleaves the serine/threonine kinase AVRPPHB SUSCEPTIBLE 1 (PBS1) [32–34]. Subsequently, the cleavage of PBS1 is recognized by the Arabidopsis NLR RESISTANCE TO PSEUDOMONAS SYRINGAE PV. PHASEOLICOLA (RPS5) [35,36]. RPS5 and PBS1 form a pre-activation complex at the plasma membrane [37]. Once PBS1 is cleaved by AvrPphB, the conformational change in the PBS1-RPS5 complex activates RPS5-mediated immunity [38]. PBS1 belongs to the receptor-like cytoplasmic kinase (RLCK) VII family, and several members of PBS1-like (PBL) proteins can be cleaved by AvrPphB [39]. In barley, at least two PBS1 orthologs are cleaved by AvrPphB. Also, the cleavage activity of AvrPphB is detected by the barley NLR AVRPPHB RESPONSE 1 (PBR1), which is non-orthologous to RPS5 [19] (Figure 1).

### Phosphorylation

As shown in early studies, two sequence-unrelated effectors, AvrB and AvrRpm1, induce the phosphorylation of Arabidopsis RIN4 [40]. AvrB and AvrRpm1 are effectors from *P. syringae* pv. *glycinea* and *P. syringae* pv. *maculicola* (*Psm*), which causes bacterial blight in soybean and bacterial leaf spot in Brassicaceae, respectively [41–44] (Figure 1). AvrB and AvrRpm1 are recognized by the Arabidopsis NLR RESISTANCE TO PSEUDOMONAS SYRINGAE PV. MACULICOLA 1 (RPM1) [45–47]. Both AvrB and AvrRpm1 recruit RPM1-induced protein kinases (RIPKs) and induce phosphorylation at multiple sites on RIN4 which are targeted by AvrRpt2 [40,48,49]. The specific phosphorylation at Thr166 residue on RIN4 is critical in the activation of RPM1-mediated immunity [40]. Another Arabidopsis NLR TARGET OF AVRB OPERATION 1 (TAO1) is required for the recognition of AvrB but not AvrRpm1 [50] (Figure 1). Interestingly, the RIN4 is not required for the TAO1-dependent recognition of AvrB indicating a distinct mode of function between RPM1 and TAO1 [51]. In soybean, AvrB and AvrRpm1 are recognized by the soybean NLR RESISTANCE TO PSEUDOMONAS SYRINGAE PV. GLYCINEA1-B (Rpg1-b) and Rpg1-r, respectively [21,52–54] (Figure 1). Rpg1-b and Rpg1-r share high amino acid similarity indicating their close evolutionary relationship [21,55]. However, phylogenetic analysis showed that neither Rpg1-b nor Rpg1-r is orthologous to RPM1 [21,54]. Different sets of soybean RIN4 homologs are sufficient for Rpg1-b recognition of AvrB and Rpg1-r recognition of AvrRpm1, respectively [56,57]. In contrast, AvrB and AvrRpm1 are recognized by the

**Figure 1**




**Convergently evolved effector recognition across plant species.** AvrRpt2, AvrPphB, AvrRpm1, AvrB, HopZ5, and AvrBsT are recognized in distantly related plant species. The same effector is recognized in multiple plant species by non-orthologous NLRs which suggests a convergent evolution of effector recognition. The effectors are distinguished by colors and labels to demonstrate the strain of each effector identified or studied. The arrows indicate recognition of the effectors by corresponding NLRs. RIN4 homologs are shown as grey ellipses, and RLCK members are shown as dark grey bean shapes if they are experimentally validated to be required in the effector recognition pathways. GmRIN4a and GmRIN4b are shown to be required for Rpg1-b recognition of AvrB based on the two independent studies [57,73], while the requirement of each GmRIN4 homolog for the Rpg1-r-mediated recognition of AvrRpm1 is still unclear [49,54]. The phylogenetic tree on the left shows the relatedness between the plant species, and it was generated by using phyloT (https://phylot.biobyte.de/) and visualized by iTOL (https://itol.embl.de/).

aforementioned tobacco NLR, NbPtr1, in *N. benthamiana* [20] (Figure 1). *P. syringae* pv. *actinidiae* (*Psa*) homolog of AvrRpm1 (AvrRpm1$_{Psa}$) has 58.8% amino acid identity with AvrRpm1 from *Psm* (AvrRpm1$_{Psm}$). Interestingly, the *Psa* but not *Psm* allele of AvrRpm1 is recognized by the tobacco NLR RESISTANCE TO PSEUDOMONAS SYRINGAE PV. ACTINIDIAE (RPA1) in *Nicotiana tabacum* [58]. RPA1 only shares ~20% amino acid identity with NbPtr1 or RPM1, indicating that these NLRs are non-orthologous to each other (Figure 1). Further investigation will reveal whether the NLRs described above recognize conserved phosphorylation on the HGDs targeted by AvrB or AvrRpm1 in soybean and *Nicotiana* species.

### Acetylation

HopZ5 and AvrBsT are members of the YopJ acetyltransferase family, and YopJ family effectors are prevalent across the bacterial kingdom [59] (Figure 1). HopZ5 is only present in *Psa* strains causing bacterial canker in kiwifruit [60]. AvrBsT is one of the key effectors that contribute to virulence in emerging strains of *Xanthomonas perforans* and *X. euvesicatoria* causing bacterial spot diseases in tomato and pepper [61,62]. In Arabidopsis, HopZ5 and AvrBsT acetylate Thr166 residue of RIN4 which is also indirectly targeted by AvrB and AvrRpm1 for phosphorylation [63]. Interestingly, either acetylation or phosphorylation of Thr166 of RIN4 activates RPM1. HopZ5 is also recognized by Arabidopsis NLR HOPZ ACTIVATED RESISTANCE 1 (ZAR1) [64]. In *N. benthamiana*, HopZ5 and AvrBsT are recognized independently by NbPtr1 and *N. benthamiana* homolog of ZAR1 (NbZAR1) [20]. NbZAR1 recognition of HopZ5 and AvrBsT requires XOPJ4 IMMUNITY 2 (JIM2), a pseudokinase from the RLCK XII family [65]. Investigation of *N. benthamiana* RLCKs in addition to JIM2 may reveal a similar mechanism of effector recognition by NbZAR1 as observed in Arabidopsis.

### ADP-ribosylation

Recent studies revealed that AvrRpm1 is an ADP-ribosyltransferase [49,66]. In addition to Arabidopsis RIN4, AvrRpm1 induces ADP-ribosylation in at least ten RIN4 paralogs (NOI proteins) [49]. AvrRpm1-mediated ADP-ribosylation is a critical step prior to the subsequent phosphorylation of Arabidopsis RIN4 [49]. HopF2b is another ADP-ribosyltransferase secreted from *P. syringae* pv. *tomato*. Interestingly, HopF2b also interacts with Arabidopsis RIN4 and prevents AvrRpt2-mediated cleavage [67]. Notably, AvrRpt2-mediated cleavage of Arabidopsis RIN4 also inhibits the RPM1-mediated recognition of AvrRpm1 [29]. Dynamic modifications on Arabidopsis RIN4 indicate its role as a recognition hub [27]. HopF1r (formerly known as HopF2a) is another effector with ADP-ribosyltransferase activity. HopF1r ADP-ribosylates PBL27 and enhances the interaction with RLCK XII ZED1-Related Kinase 3 (ZRK3) leading to ZAR1-mediated recognition [68].

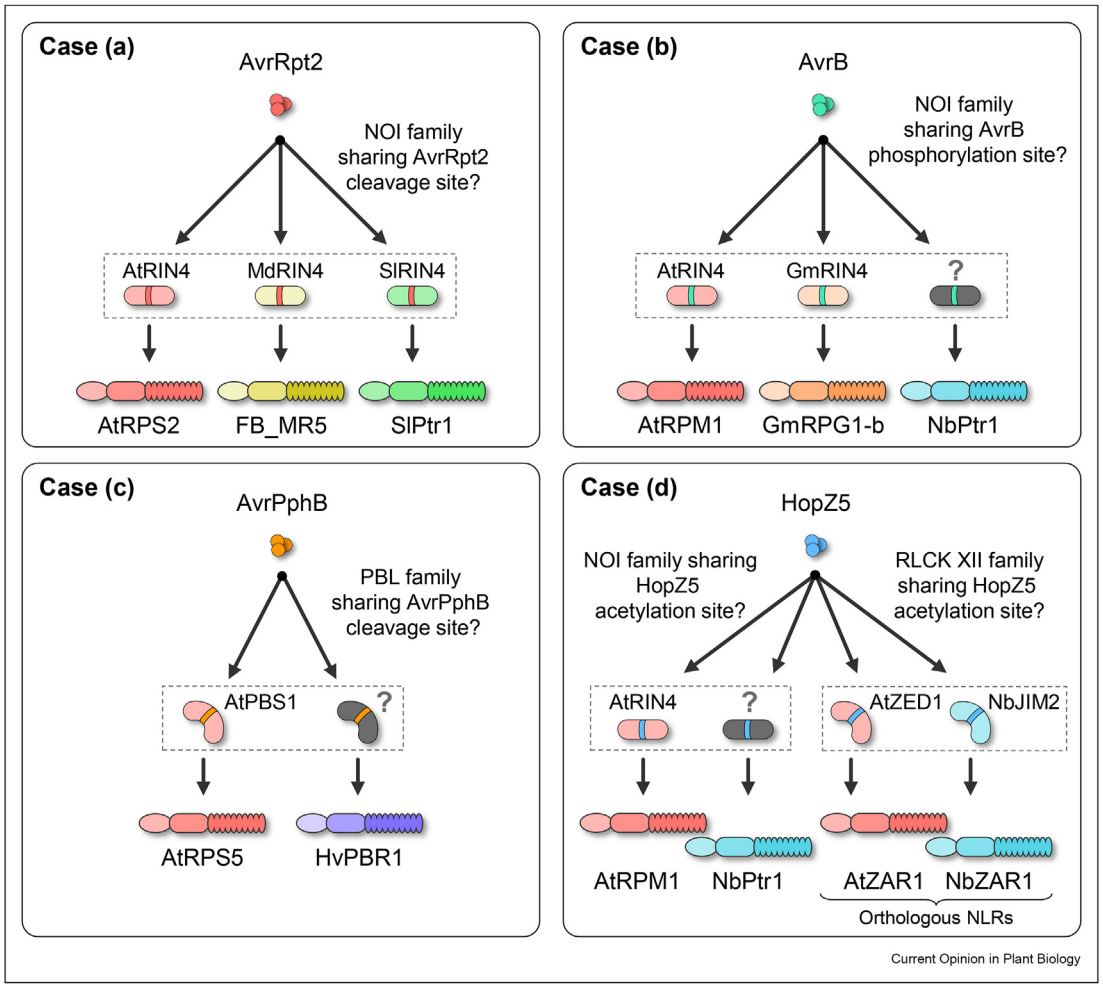
ZAR1 is an exceptional NLR among NLRs introduced in this review in that ZAR1 orthologs rather than non-orthologous NLRs recognize effectors across the distantly related plant species. Similar to the HopF1r recognition through ZRK3/PBL27, there are defined sets of interacting PBLs for each ZRK, and a diverse combination of PBLs and ZRKs, which likely function as decoys, can associate with ZAR1. ZAR1-recognized effectors modify PBLs and ZRKs as well as their virulence targets. Modifications by the effector enhance multiple ZRK/PBL interactions, but only specific ZRK/PBL interactions trigger ZAR1-mediated immunity [69]. The conservation of ZRK orthologs corresponds to cognate ZAR1 orthologs in angiosperm lineages [70,71]. This may suggest that ZAR1 recognition specificities can be defined by the distribution of RLCKs, and the diversification of RLCKs drives the expansion of effector recognition by ZAR1.

## Most HGDs mediating the convergent recognition of effectors belong to the same protein family

Non-orthologous NLRs from different plant species recognize the same type of effector by detecting the modifications on HGDs. Interestingly, these HGDs often share conserved domains and belong to the same protein family (Figure 2). For example, the recognition of AvrRpt2 by RPS2 and FB_MR5 requires the corresponding RIN4 variant from Arabidopsis and apple, respectively (Figure 2 Case (a)) [17]. Both Arabidopsis and apple RIN4 variants carry conserved AvrRpt2-cleavage sites and belong to the family of NOI proteins despite their low amino acid similarity [72]. Similarly, the two non-orthologous NLRs, RPM1 and Rpg1-b, confer recognition of AvrB by detecting the phosphorylation of conserved residues on Arabidopsis and soybean RIN4 proteins, respectively (Figure 2 Case (b)) [48,56]. However, RIN4 or NOI proteins are not the only HGDs that mediate effector recognition by non-orthologous NLRs. Arabidopsis PBS1 and barley PBS1 homologs are subjected to AvrPphB-mediated proteolysis and subsequently two non-orthologous NLRs, RPS5 and PBR1, recognize AvrPphB by detecting the protease activity of AvrPphB (Figure 2 Case (c)) [19,34,38]. These observations propose that convergently evolved effector recognition in different plant species is frequently mediated by the conservation of HGD families rather than NLRs.

According to the recent studies, HopZ5 shows a unique pattern of HGD requirement in the convergent effector recognition [20,63,64]. Remarkably, HopZ5 recognition is mediated by HGDs from two distinct families, NOI and RLCK XII family (Figure 2 Case (d)) [20,63,64]. Moreover, HopZ5 is recognized independently by RPM1 and ZAR1 in Arabidopsis and by NbPtr1 and NbZAR1 in *N. benthamiana* (Figure 2 Case (d))

**Figure 2**



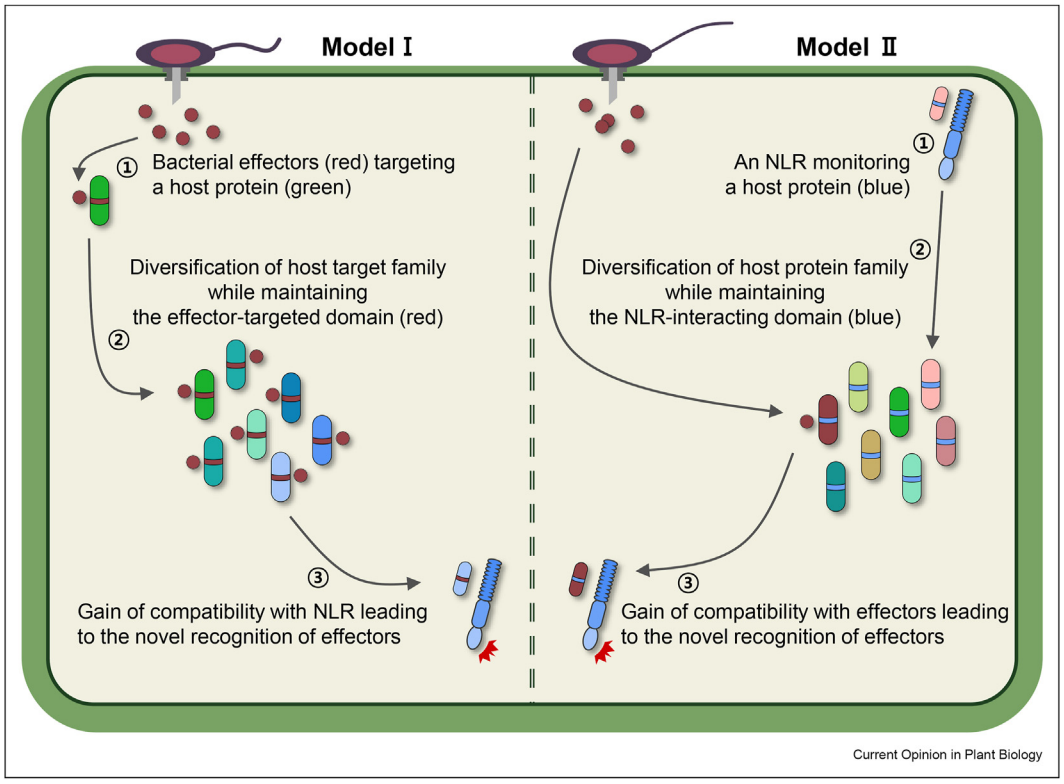

**Different plant species have convergently evolved to share HGD family to recognize effectors.** Representative cases of effector recognition by non-orthologous NLRs are shown. HGD families shared among different plant species are framed by dashed boxes. RIN4 orthologs and paralogs are shown as colored ellipses, and RLCK members are shown as colored bean shapes. The colored line on HGD represents a conserved domain targeted by effectors. NLRs are color-coded by plant species (Case (a)) Arabidopsis, apple, and tomato contain NOI proteins targeted by AvrRpt2. Cleavage activity of AvrRpt2 is recognized by AtRPS2, FB_MR5, and SlPtr1 in respective plant species (Case (b)) Arabidopsis, soybean, and tobacco carry NOI proteins targeted by AvrB. Phosphorylation induced by AvrB is recognized by AtRPM1, GmRPG1-b, and NbPtr1 in respective plant species (Case (c)) Arabidopsis and barley encode PBL proteins targeted by AvrPphB. Cleavage activity of AvrPphB is detected by AtRPS5 and HvPBR1 in respective plant species (Case (d)) Arabidopsis and tobacco carry NOI proteins and RLCK XII proteins targeted by HopZ5. The acetylation activity of HopZ5 is recognized by AtRPM1, AtZAR1, NbPtr1, and NbZAR1 in respective plant species.

[20,63,64]. Unlike RPM1 and NbPtr1, Arabidopsis ZAR1 is orthologous to NbZAR1 and senses effector-induced modification of multiple RLCKs [69]. The recognition of AvrAC and HopZ1a by ZAR1 is mediated through two different branches of RLCK interactions: PBL2-ZRK1/RKS and SZE1/2-ZED1, respectively [69]. In this perspective, the RLCK-ZAR1 recognition system might be comparable to the sensor-helper NLR network where recognition of an effector by a corresponding sensor NLR is followed by the recruitment of the common helper NLRs [69]. Thus, it is plausible that ZAR1 functions as a helper NLR while RLCKs confer the recognition specificity. Therefore, we infer that ZAR1 is one of the exceptional cases to date where the NLR remains conserved, while other NLRs are under diversifying selection.

## Diversification of HGDs potentially contributes to the convergent recognition of effectors

HGDs often belong to highly diversified protein families. For example, Arabidopsis NOI and PBL families include at least 14 and 29 members, respectively [39,72]. Recent studies also report that additional members of HGD families are subjected to effector-

**Figure 3**




**Diversification of HGD potentially leads to novel recognition of effectors** (Model I) The diversification of HGDs, while retaining the domain targeted by effectors, eventually results in association with novel NLRs. The dark red line on HGD represents a conserved domain targeted by effectors. The blue ellipse represents a member of the diversified HGD family that gained compatibility with NLR (Model II) The diversification of HGDs, while keeping association with NLRs, leads to interaction with novel effectors. The blue line on HGD represents the domain required for NLR association. The red ellipse represents a member of the diversified HGD family that gained compatibility with the effector.

induced modification. For instance, AvrRpm1 ADP-ribosylates not only RIN4 but also other members of the NOI family [49]. In this regard, the HGD families may diversify to establish novel compatibility with an existing NLR while maintaining effector-targeted conserved domains (Figure 3 Model I). This is supported by a recent study showing that the changes at two polymorphic amino acid residues in RIN4 determine compatibility with convergently evolved NLRs in different plant species [17]. An additional driving force for the diversification of HGDs might be caused by the structural diversity of effectors. Diversification of HGDs while maintaining association with NLR would increase the structural compatibility with novel or rapidly evolving effectors and lead to expanded effector recognition specificity (Figure 3 Model II). However, the models described above are not exclusive to each other. In nature, HGDs have diversified under different contexts of co-evolution driven by multiple factors. Therefore, further investigation on the HGD diversification and function would significantly advance our understanding of the evolution of the plant immune system.

## 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.

## Data availability

No data was used for the research described in the article.

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