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Plant Antiviral *Resistance* Genes May Have Undergone Dissimilar Selection in Nature and in Crop Fields

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Abstract

Plant genomes contain more than 100 copies of *Resistance* (*R*) genes that encode receptor proteins. Each gene product directly or indirectly recognizes pathogen infection to induce resistance against the pathogen. The product of the *Arabidopsis thaliana R* gene *RCY1* recognizes the capsid protein of cucumber mosaic virus (CMV) and induces a hypersensitive response (HR), which leads to programmed cell death of infected cells and containment of the virus in inoculated leaves. We recently demonstrated that the average number of CMV genomes that established cell infection [*i.e.*, multiplicity of infection (MOI)] after cell-to-cell movement decreased by ~23% upon induction of HR. In contrast, infection by a CMV mutant that had a smaller reduction in MOI (~10%) upon *R*-gene-mediated recognition resulted in a systemic HR in the plant, leading to plant death. This finding suggested that inefficient induction of resistance allows a virus to spread, causing the death of the infected plant. A simulation suggested that this death of an infected individual may function as a suicide strategy to protect neighboring plants that are often "kin" of the infected plant, by reducing the source of infection. Thus, systemic host death, caused by inefficient *R*-gene-mediated induction of resistance against viruses, can be positively selected in nature; this type of death serves as population-level resistance against the pathogen and the starting point for further adaptation toward more efficient resistance. During plant domestication, traits facilitating population-level resistance may have undergone negative selection, resulting in the loss of associated *R* genes in our most common crops.

Introduction

Plants are threatened by many pathogens, including fungi, bacteria, and viruses. In response to these pathogens, plants have evolved many resistance mechanisms, including the well-studied *resistance* (*R*)-gene-mediated resistance (Villena *et al*., 2018). Plant genomes carry many *R* genes, which encode receptor proteins that share nucleotide-binding (NB) and leucine-rich repeat (LRR) domains. Each *R* gene induces resistance upon recognition of specific pathogen(s), via direct or indirect interaction between the gene product (*i.e.*, receptor protein) and pathogen-derived protein(s) (reviewed in Collier and Moffett, 2009, and elsewhere). Most well-studied *R* genes induce a hypersensitive response (HR) upon pathogen recognition; HRs are characterized by containment of the pathogen in the infected leaves and the induction of programmed cell death (PCD) in infected tissue. Although many *R* genes have been found to induce HR upon virus infection, interactions between viruses and *R* genes sometimes lead to different consequences. One such consequence is the induction of extreme resistance, in which virus replication and movement to adjacent cells is suppressed in the initially infected cell without causing PCD. The *Rx* gene, an *R* gene introgressed from a wild relative to cultivated potato, induces extreme resistance against potato virus X (Bendahmane *et al*., 1999); the *RCY1* gene, an HR-inducing *R* gene of *Arabidopsis thaliana* against cucumber mosaic virus (CMV), induces extreme resistance when overexpressed in transformant plants

(Sekine *et al*., 2008). An alternative consequence of *R*-gene-mediated recognition of a virus is systemic necrosis, which has long been considered a result of aggressive virus infection; however, recent studies have suggested that, at least in some instances, systemic necrosis is caused by mechanisms that are similar to HR. Systemic necrosis is established by means of the same pathway as HR (Komatsu *et al*., 2010); notably, viruses that cause HR in certain crop cultivars can cause systemic necrosis in other cultivars (Jones and Vincent, 2018). Furthermore, viral amino-acid substitutions in the capsid protein (CP) of turnip crinkle virus have been shown to change the host response from HR to systemic necrosis (Kang *et al*., 2015). These observations suggest that systemic necrosis is sometimes a result of imperfect HR induction, when the virus infection is uncontrolled despite induction of PCD, resulting in plant death. Thus, *R*-gene-mediated systemic necrosis is sometimes regarded as systemic HR (SHR); however, several issues remain unresolved, including how and whether the strength of resistance can be compared between HR and SHR, as well as whether the fitness of plants that induce SHR is higher than that of fully susceptible plants. Our recent study enabled us to answer these questions and discuss the evolution of antiviral *R* genes.

Reduced viral MOI in the *RCY1*-CMV(Y) system

Resistance to CMV-Y (*RCY1*) is a CC-NB-LRR-type *R* gene that was originally found in *A. thaliana* ecotype C24 (Takahashi *et al*., 1994, 2002; reviewed in Ando *et al*., 2019). The RCY1 protein is believed to recognize the CP of CMV (Takahashi *et al*., 2001), although it is unclear whether RCY1 and CP interact directly or indirectly. The gene products of allelic *RCY1* homologs in *A. thaliana* ecotypes L*er* and Di-17 (i.e., *RPP8* and *HRT*, respectively) recognize distinct plant pathogens, *Hyaloperonospora parasitica* and turnip crinkle virus (Cooley *et al*., 2000). We have found that *Nicotiana benthamiana* plants transformed with a chimeric *R* gene containing the CC-NB domains from *RPP8* and an LRR domain from *RCY1* can recognize CMV and induce HR; we have also found that a CMV mutant carrying an N31T (asparagine 31 to threonine) substitution in its CP escapes recognition by RCY1 (Takahashi *et al*., unpublished). In a more recent study, we found that a T45M CP mutant caused SHR in the *RPP8-RCY1* chimeric *R*-gene transformant *N. benthamiana* and *RCY1*-carrying *A. thaliana* (Abebe *et al*., unpublished) (**Fig. 1**). Multiplicity of infection (MOI) comprises the average number of viral genomes that establish infection in a host cell. Previously, we established a method for estimation of MOI during cell-to-cell infection by plant viruses, based on fluorescent microscopy observations and statistical analysis (Miyashita and Kishino, 2010; Miyashita *et al*., 2015). Briefly, two viral derivatives that carry a *yellow-fluorescent-protein* (*YFP*) gene or a *cyan-fluorescent-protein* (*CFP*) gene are co-inoculated on plant leaves and the stochastic separation of the two derivatives is observed by fluorescent microscopy (**Fig. 2AB**). Because a smaller MOI will result in quicker separation of viral derivatives encoding *YFP* or *CFP*, MOI can be estimated from the frequency of singly infected cells after a certain number of cell-to-cell infection cycles. Using this method, we estimated that MOIs in the first cell-to-cell infections of Japanese soil-borne wheat mosaic virus and tomato mosaic virus were approximately 6 and 4, respectively (Miyashita and Kishino, 2010; Miyashita *et al*., 2015). We used this method to estimate the CMV MOI in first cell-to-cell infections in *N. benthamiana* leaf tissue, in the presence and absence of the *R* gene (**Fig. 2C**). CMV with the wild-type *CP* gene exhibited a ~23% reduction in MOI in plants with the *R* gene, compared to plants without the *R* gene; CMV with the T45M CP substitution exhibited a ~10% reduction in MOI, while CMV with the N31T CP substitution did not

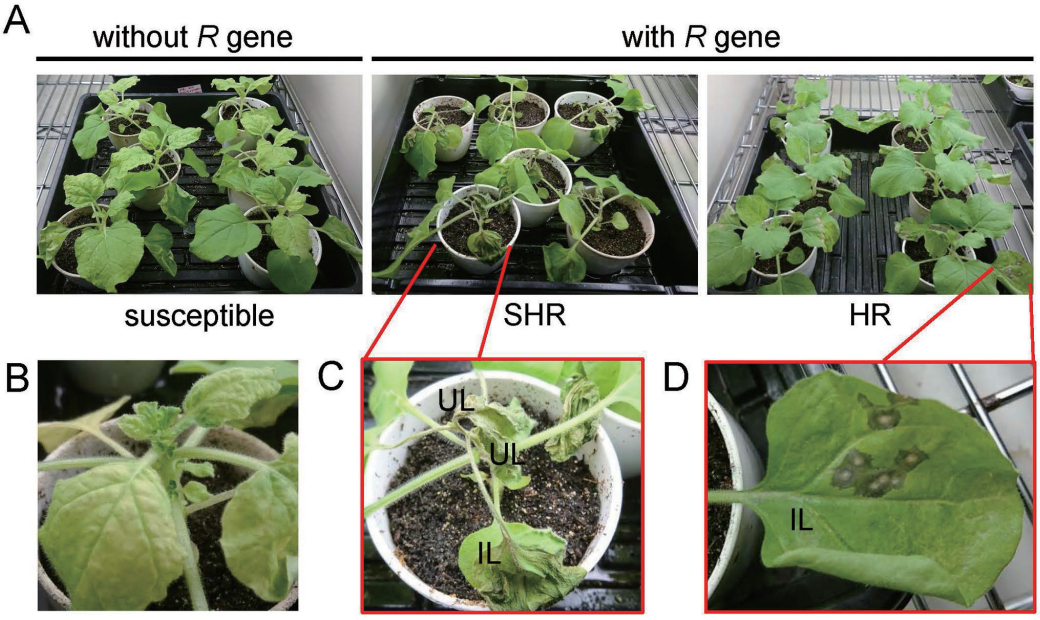

Fig. 1. Susceptible interaction and SHR/HR induction in the CMV–*N. benthamiana* system. (A) [left] Susceptible interaction between wild-type cucumber mosaic virus and wild-type *Nicotiana benthamiana*, demonstrating mosaic symptoms; [middle] SHR induction by CP T45M variant CMV in *R*-gene transformant *N. benthamiana*; [right] HR induction by wild-type CMV in *R*-gene transformant *N. benthamiana*. (B) Mosaic symptoms in a susceptible interaction. (C) Systemic necrosis caused by SHR. In addition to the inoculated leaves (IL), uninoculated upper leaves (UL) are dying. (D) HR lesions formed in an inoculated leaf. PCD did not spread beyond the leaf.

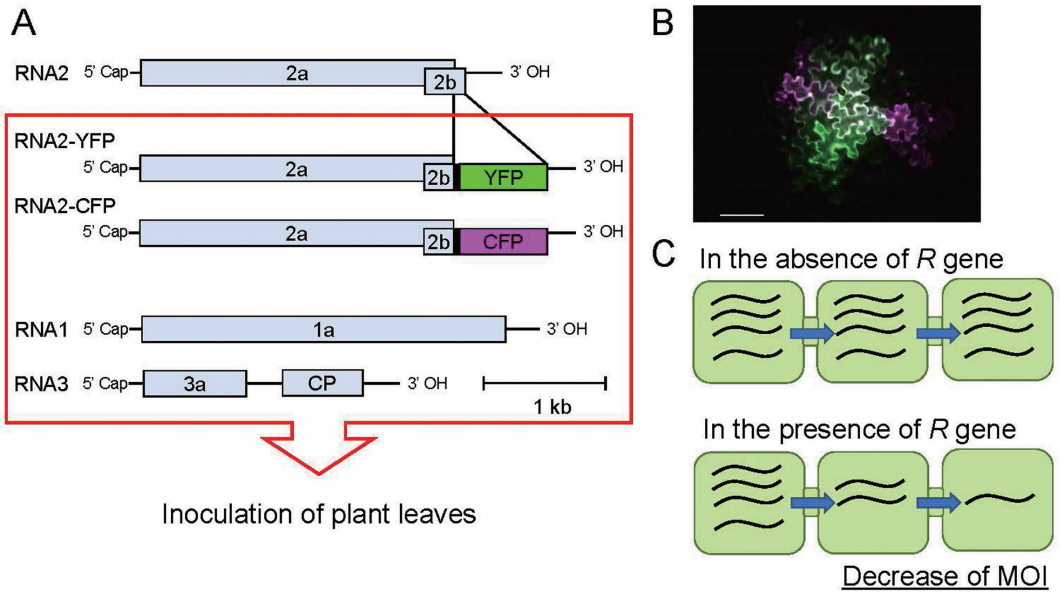

Fig. 2. Estimation of plant viral MOI during cell-to-cell movement. (A) MOI estimation for CMV. RNA2 derivatives carrying *YFP* or *CFP* genes were mixed with wild-type RNA1 and RNA3, then used to inoculate *N. benthamiana* leaves. (B) Observation of stochastic separation of the two derivatives by means of fluorescent microscopy. (C) Schematic explanation for the reduction in MOI.

exhibit a significant reduction in MOI between plants with and without the *R* gene (Abebe *et al*., unpublished). A reduction in MOI directly results in the stochastic failure of cell-to-cell infections. Because PCD is observed only after several cycles of cell-to-cell infection, a reduction in MOI in the first cell-to-cell infections suggests PCD-independent resistance against CMV. This is consistent with observations that PCD and virus containment occur via independent pathways (Komatsu *et al*., 2010; Takahashi *et al*., 2012). We previously suggested that a small MOI during cell-to-cell infection is necessary for plant viruses to enhance selection on their *trans*-acting genes or elements (Miyashita and Kishino, 2010; Miyashita 2018). In a host that recognizes a particular virus through a corresponding *R* gene, MOI may decrease to a level at which the virus cannot continue spreading through the host tissue. Furthermore, our MOI estimates provided the first direct evidence that resistance induction is weaker in SHR than in HR; in addition, HR changes to SHR due to small differences in viral MOI, which reflect small differences in the level of resistance induced by *R*-gene-mediated pathogen recognition.

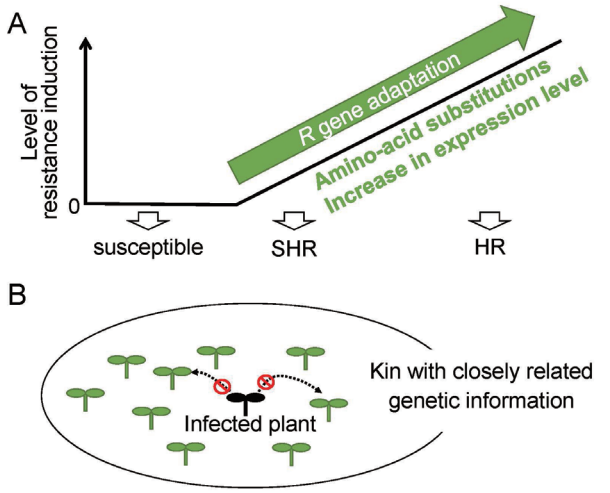

Fig. 3. Possible evolutionary trajectory of *R* genes and kin selection for SHR. (A) Possible evolutionary trajectory of *R* genes. (B) Systemic death caused by SHR can be adaptive when kin selection occurs.

Natural selection may favor SHR, while selection in crop fields may not

These findings led us to reflect on the general evolutional trajectory of antiviral *R* genes (**Fig. 3A**). When an *R* gene product recognizes a new viral protein, the level of resistance induced is not initially sufficient to contain the virus, resulting in SHR; improved recognition compatibility with amino-acid substitutions in the *R* gene and an increase in its expression gradually enhance the resistance induction level, thus changing the phenotype from SHR to HR. This concept implicitly includes the unproven assumption that SHR is more adaptive than the absence of an *R* gene to recognize the virus. However, given that SHR brings death to the plant, it is important to consider how this outcome could be adaptive. We suggest that kin selection (reviewed in Birch, 2019) can address this issue (**Fig. 3B**). Most land plants propagate by means of seeds, sometimes by means of vegetative reproduction. Thus, propagation occurs locally; individuals that are closely related genetically (i.e., "kin") are in close proximity with each other. In this context, SHR may help infected plants to avoid serving as an infection source for their kin, at the cost of the infected plant's life. Kim *et al*. (2008) mentioned the concept of kin selection for SHR; however, no study has yet analyzed whether this selection is adaptive. Accordingly, we developed a mathematical model for natural selection involving SHR, in which the locality of propagation is parameterized. Simulations based on this model revealed that SHR can be adaptive when propagation occurs locally, whereas it cannot be adaptive when propagation occurs in a more dispersed manner (Miyashita *et al*., unpublished). These results imply that individual death by SHR can serve as a suicide strategy by which plants save

their kin; however, the strategy is not applicable to organisms in which propagation does not occur locally, such as animals. SHR might not have been favored in crop domestication by our human ancestors; this might have resulted in the deletion of some *R* genes from crop genomes. Future studies focusing on the possible deletion of *R* genes may reveal the effects of human activity on crop–pathogen interactions and provide novel strategies for identification of unknown *R* genes.

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