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Achieving a more robust antiviral RNAi via subverting a viral virulence protein

ANTIVIRAL RNAi AND VSR

As submicroscopic organisms with small genomes, viruses can only replicate inside living cells. In fact, viruses can infect all forms of life, from animals and plants to microorganisms including fungi, bacteria, and archaea. Besides well-known human diseases such as human acquired immune deficiency syndrome, common cold, influenza, hepatitis, and coronavirus disease 2019, viral pathogens also cause more than $30 billion crop yield losses annually worldwide (Chauhan et al., 2019).

RNA interference (RNAi), which is conserved in plants, nematodes, fungi, vertebrates, and invertebrates, has been proven to play a major role in host defense against viral pathogens. RNAi is also known as post-transcriptional gene silencing, co-suppression, or quelling. Gene silencing was first discovered in plants (Lindbo and Dougherty, 2005). Dicer or Dicer-like proteins process viral RNAs into small RNAs, which then guide Argonaute-containing RNAi-induced silencing complex (RISC) to the targets in viral RNAs through Watson-Crick base pairing, resulting in viral repression (Wilson and Doudna, 2013). Host-adapted viral pathogens, however, developed suppressor of RNAi (VSR), which antagonizes antiviral RNAi. For example, the nucleocapsid protein from SARS-CoV-1/2 suppresses the production of viral-derived small interfering RNAs (vsiRNAs) through double-stranded RNA sequestration (Li and Ding, 2022). On the other hand, the HC-Pro VSR of potyviruses binds vsiRNAs and inhibits their loading onto Argonaute proteins (Valli et al., 2018).

Despite many years of research, how plants and animals deal with the challenges from VSRs is still poorly understood and remains a fascinating area for scientists to study. Recently, Jin et al. (2022) reported that a pair of barley kinases convert a VSR into an enhancer of antiviral RNAi through phosphorylation (Jin et al., 2022), which reveals a novel strategy that host cells employ to counteract the VSR activity of plant viruses.

DIVERSE FUNCTIONS OF BYDV 17K PROTEIN

Since viruses have small genomes, a single viral protein often possesses multiple functions. One such example is the 17K protein conserved in plant luteoviruses including several closely related barley yellow dwarf viruses (BYDVs). BYDVs, carrying a single-stranded positive-sense RNA genome, elicit yellow dwarf disease in major cereal crops including wheat, barley, maize, rye, and oats, with different members having dissimilar aphid vector specificities (Miller and Lozier, 2022). To date, seven open reading frames have been identified in BYDV genome (Figure 1A). The 17K protein encoded by open reading frame 4 has been found to contribute to viral systemic movement, suppression of antiviral RNAi, and disruption of host mitosis through interfering with the Wee1–Cdc25–Cdk1 mitotic entry switch (Nass et al., 1998; Fusaro et al., 2017; Jin et al., 2020) (Figure 1A). Clearly, 17K is an important virulence protein with multiple functions in BYDV pathogenesis. It is of great interest to understand how host plants may regulate 17K to keep BYDV proliferation under control.

PHOSPHORYLATION OF BYDV 17K BY BARLEY GRIK1–SnRK1 KINASES

Jin et al. (2022) identified two potential phosphorylation motifs of sucrose non-fermenting 1-related protein kinase 1 in 17K protein and subsequently demonstrated that barley HvGRIK1–HvSnRK1 kinases interacted with 17K and phosphorylated five residues of 17K *in vitro* (Figure 1A) (Jin et al., 2022). When the authors infected transgenic plants overexpressing SnRK1–YFP or $SnRK1^{K139R}$–YFP, with the latter inhibiting endogenous SnRK1 activity in a dominant negative manner (Han et al., 2020), with BYDV, they found that 17K phosphorylation was increased in the former but decreased in the latter genotype, thus validating the phosphorylation of 17K by HvSnRK1 in BYDV-infected host cells.

TURNING AN ENEMY INTO A FRIEND

To investigate the biological significance of 17K phosphorylation by HvGRIK1–HvSnRK1 kinases, the authors compared disease symptoms of SnRK1-YFP transgenic plants with wild-type plants after they were infected with BYDV (Jin et al., 2022). The data showed that the SnRK1–YFP transgenic barley plants were more resistant to BYDV than wild-type controls, indicating that the phosphorylated 17K (P17K thereafter) upregulates barley resistance to BYDV.

Since 17K is an RNAi suppressor, Jin et al. (2022), examined the effects of phosphorylation on 17K's VSR activity by conducting a series of complementary experiments. Remarkably, they found that P17K lost VSR function while gaining the ability to enhance RNAi through elevating vsiRNA levels (Jin et al., 2022). Furthermore, P17K, but not 17K, showed the ability to bind vsiRNAs and to interact with a small RNA-degrading nuclease HvSDN1 (Figure 1A). Through optimizing small RNA cleavage assays, the authors demonstrated that recombinant HvSDN1 could degrade BYDV vsiRNAs, which was, however, efficiently inhibited by $17K^{5D}$ (a phosphomimic mutant of 17K) but not

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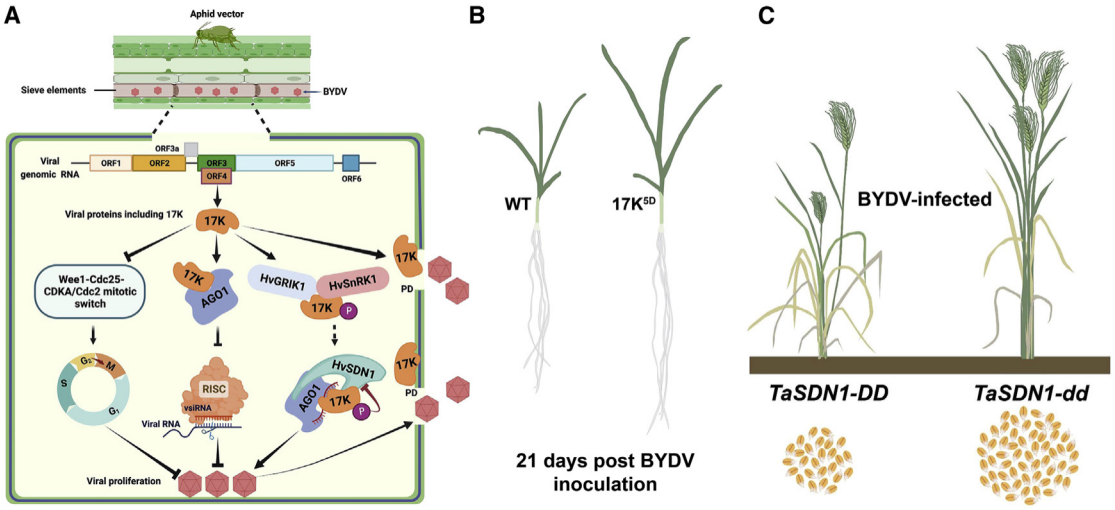

Figure 1. Novel ways for controlling barley yellow dwarf virus through enhancing antiviral RNAi.

(A) Functions of BYDV 17K protein and its phosphorylated form (P17K) in host cells. BYDVs are transmitted by aphids in a persistent manner and are restricted to the sieve elements of phloem tissue. The single-stranded RNA genome of BYDV contains seven open reading frames, with open reading frame 4 encoding the 17K protein. 17K performs multiple virulence functions to aid virus proliferation and spread, i.e., interruption of the mitotic entry switch Wee1–Cdc25–CDKA/Cdc2 to inhibit host mitosis, suppression of antiviral RNAi likely through interfering with RNAi-induced silencing complex (RISC) by interacting with AGO1, and promotion of cell-to-cell movement of viral particles via modifying plasmodesmata (PD). However, HvGRIK1 and HvSnRK1 kinases covert a portion of the 17K to phosphorylated 17K (P17K), which elevates antiviral RNAi through enhancing vsiRNA abundance via decreasing the degradation of AGO1-associated vsiRNAs by HvSDN1. Thus, P17K, a product of host-mediated subversion of the viral virulence protein 17K, helps host cells to achieve a more robust anti-BYDV RNAi.

(B) Transgenic wheat plants overexpressing $17K^{5D}$ (mimicking P17K) show enhanced resistance against BYDV.

(C) Knocking out *TaSDN1-DD* homoeolog through genome editing yields a novel wheat material (*TaSDN1-dd*) with elevated resistance to BYDV.

(A) was created with the software BioRender (BioRender.com).

17K (Figure 1A) (Jin et al., 2022). Finally, and most importantly, the authors showed that P17K formed a complex with vsiRNA-carrying HvAGO1 and the HvSDN1 enzyme in BYDV-infected cells; within this complex, P17K inhibited HvSDN1's function in degrading vsiRNAs through direct protein–protein interaction as well as by weakening the interaction between HvSDN1 and HvAGO1 (Figure 1A). Noteworthy, 17K was also found to bind to HvAGO1; this may disrupt the function of HvAGO1 in antiviral RNAi, thus representing a mechanism underlying 17K's VSR activity (Jin et al., 2022).

NOVEL WAYS FOR ENGINEERING BYDV-RESISTANT CROPS

The data presented in this work not only revealed a novel mechanism of achieving a more robust RNAi via phosphorylation of a viral RNAi suppressor but also provide effective ways for engineering BYDV-resistant crops. Jin et al. (2022) demonstrated that overexpressing $17K^{5D}$ in common wheat, or silencing of *HvSDN1* in barley, boosted the resistance of these plants to BYDV through elevating vsiRNA abundance (Figure 1B) (Jin et al., 2022). Consistently, they obtained a novel common wheat material (*TaSDN1-dd*) showing improved resistance to BYDV through genome editing of *TaSDN1-DD* homoeolog (Figure 1C).

FUTURE PERSPECTIVES

The study by Jin and colleagues suggest that plants may have evolved an effective counter-counter defense against viral VSRs through post-translational modifications of these pivotal virulence proteins. Since there are now increasing reports of host-mediated modifications of VSRs (Zhuang et al., 2022), future studies will tell if the conversion of VSRs into RNAi enhancers, as demonstrated by Jin et al. (2022), may represent a general strategy for host cells to combat VSRs.

Transgenic plants overexpressing hairpin RNA or artificial microRNA have been shown to be valuable for controlling viral diseases (Gaffar and Koch, 2019). As SDN1 degrades vsiRNA and mutation of SDN1 enhances antiviral RNAi via elevating vsiRNA abundance (Jin et al., 2022), appropriate manipulation of SDN1 and homologs, which are highly conserved in eukaryotes (Ramachandran and Chen, 2008; Chen et al., 2018), may gain a wide application in engineering antiviral resistance in the future.

Many studies in plant–pathogen interactions have mainly focused on how pathogens manipulate plant physiology to cause diseases; our understanding on how plants fight against the virulence systems of adapted pathogens and keep them under control remains limited. This study illustrates that plant has evolved the ability to subvert a key pathogen virulence protein to boost plant defense, which may stimulate further basic and applied research along this new direction.

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