Efficient Dicer processing of virus-derived double-stranded RNAs and its modulation by RIG-I-like receptor LGP2.
Identification of positive and negative regulators of antiviral RNA interference in *Arabidopsis thaliana*

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Virus-host coevolution often drives virus immune escape. However, it remains unknown whether natural variations of plant virus resistance are enriched in genes of RNA interference (RNAi) pathway known to confer essential antiviral defense in plants. Here, we report two genome-wide association study screens to interrogate natural variation among wild-collected *Arabidopsis thaliana* accessions in quantitative resistance to the endemic cucumber mosaic virus (CMV). We demonstrate that the highest-ranked gene significantly associated with resistance from both screens acts to regulate antiviral RNAi in ecotype Columbia-0. One gene, corresponding to *Reduced Dormancy 5* (*RDO5*), enhances resistance by promoting amplification of the virus-derived small interfering RNAs (vsiRNAs). Interestingly, the second gene, designated *Antiviral RNAi Regulator 1* (*VIR1*), dampens antiviral RNAi so its genetic inactivation by CRISPR/Cas9 editing enhances both vsiRNA production and CMV resistance. Our findings identify positive and negative regulators of the antiviral RNAi defense that may play important roles in virus-host coevolution.

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Diverse antiviral defense mechanisms have been documented in higher plants. Several lines of evidence show that the RNA interference (RNAi) pathway mediates an essential antiviral immunity mechanism in plants. In antiviral RNAi, host cells process viral long dsRNA into small interfering RNAs (siRNAs) by a Dicer nuclease to trigger specific virus clearance in RNA-induced silencing complex (RISC) by an Argonaute (AGO) protein. Both Dicer and AGO gene families appear to have expanded in plants for the control of virus infection. In the model plant species *Arabidopsis thaliana*, for example, 3 of the 4 Dicer-like genes (DCI1) and 7 of the 10 AGO genes participate in the RNAi-mediated antiviral immunity. Moreover, among the 6 RNA-dependent RNA polymerase (RdRP) genes of *A. thaliana*, RdRP1 (RDR1), RDR2 and RDR6 have all been shown to direct viral siRNA amplification. Interestingly, the 19-member AGO family of rice plants includes a hormone-inducible AGO18 that promotes antiviral RNAi by enhancing the expression of AGO1, which is necessary for vsiRNA-RISC assembly. Notably, viral suppressors of RNAi (VSRs) are essential virulence proteins of plant RNA and DNA viruses. Studies on the origins and variability of VSR genes have shown that antiviral RNAi exerts selection pressure against plant virus genomes.

Viruses with an RNA genome possess extraordinary adaptive abilities because of their error-prone replication mechanisms. Less is known about the natural variation that wild host plants in ecosystems with little human intervention accumulate and select to inherit in their progeny in response to virus infection. Recently, whole genome information has become available for 1135 natural inbred lines from worldwide wild-collected accessions of *A. thaliana*. Cucumber mosaic virus (CMV) is a natural pathogen of *A. thaliana* with incidence reaching up to 80% in some localities. CMV contains a tripartite positive-strand RNA genome coding for 5 proteins required for genome replication, virion assembly, in-planta movement and RNAi suppression. VSR protein 2b is encoded by an out-of-frame gene that overlaps the more ancient viral RdRP gene and is the most variable viral protein among CMV strains. CMV is transmissible by mechanical contacts and many species of aphids as well as less efficiently through the seed.

In this work, we investigate whether single nucleotide polymorphisms (SNPs) significantly associated with virus resistance among wild plant populations are enriched in specific pathways known to confer antiviral protection in plants. We use genome-wide association studies (GWAS) approach to interrogate natural variation among re-sequenced wild *A. thaliana* populations in quantitative resistance to CMV. For a better survey of host natural variation in antiviral responses, we conduct independent GWAS screens with two distantly related CMV strains, one unmodified and the other rendered defective in counter-defense against antiviral RNAi. We show that the highest-ranked gene significantly associated with quantitative virus resistance identified from each of two independent GWAS screens functions in antiviral RNAi. Notably, we demonstrate opposing roles in antiviral RNAi for the two genes evolved in ecotype Columbia-0, indicating that these regulatory genes of antiviral RNAi defense may play important roles in virus-host coevolution.

**Results**

Mapping host natural variation identifies a quantitative virus resistance gene. We first assessed natural variation among the fully re-sequenced *A. thaliana* accessions in quantitative resistance to CMV-Δ2b, a previously characterized mutant of the highly virulent subgroup I CMV strain Fny that is rendered susceptible to antiviral RNAi by introducing nucleotide substitutions to prevent VSR-2b expression. Virus accumulation levels in the upper non-inoculated leaves were measured by enzyme-linked immunosorbent assay (ELISA) of the viral coat protein (CP) two weeks after mechanical inoculation of *A. thaliana* seedlings.

The virus titers in 496 accessions displayed a nearly normal distribution after log-transformation (Fig. 1a and Supplementary Data 1). Analyzing the data with the easyGWAS pipeline found that none of the SNPs significantly associated with quantitative virus resistance mapped to any of the known antiviral RNAi genes. The most significantly associated SNP resided in a region of chromosome 4 that codes for the gene At4g11040 (Fig. 1b, c and Supplementary Fig. 1a), corresponding to the previously reported Reduced Dormancy 5 (RDO5)/Delay of Germination 18 (DOG18). We found that CMV-Δ2b titers were significantly different between the accessions classified as haplotypes T and G according to the SNP (Supplementary Fig. 1b). Northern blot analysis further verified that CMV-Δ2b replicated to lower levels in 4 selected accessions of haplotype T including Col-0 than the 3 accessions of haplotype G (Supplementary Fig. 1c). We then compared CMV-Δ2b infection in accession Antwerpen-1 (An-1) carrying a single base-pair (bp) frameshifting deletion in RDO5 with accession Columbia-0 (Col-0), classified in the resistant haplotype along with 380 additional accessions among those examined (Fig. 1c and Supplementary Fig. 2b). Western blot analysis of the viral CP showed that CMV-Δ2b accumulated to much higher levels in An-1 plants than either Col-0 plants or the two independent lines of An-1 transgenically complemented with the RDO5 gene from Col-0 (Fig. 1d). These results suggest suppression of CMV-Δ2b accumulation by RDO5 from Col-0 plants.

To verify the role of RDO5 in Col-0 plants, we obtained two Col-0 mutants carrying a T-DNA insertion at different positions in the second exon of RDO5, designated rdo5-4 and rdo5-5 (Fig. 1c). We also generated two independent transgene complementation lines of rdo5-4 with the same RDO5 gene driven by its native promoter described above. ELISA detection of the viral CP revealed significantly enhanced accumulation of CMV-Δ2b in both rdo5-4 and rdo5-5 mutant plants compared to either wild-type Col-0 plants or either of the two RDO5-complemented lines of rdo5-4 plants (Fig. 1e). Moreover, both Western blotting detection of CP and Northern blotting detection of the viral genomic RNAs showed that CMV-Δ2b accumulated to higher levels in rdo5-4 and rdo5-5 mutant plants than wild-type Col-0 plants and the complemented lines of rdo5-4 mutant (Fig. 1f, f). By comparison, CMV-Δ2b replicated to higher levels in rdr6 mutant plants than rdo5-4 and rdo5-5 mutant plants (Fig. 1f, f), which may explain the absence of clear symptomatic differences between the infected Col-0 and rdo5 mutant plants (Supplementary Fig. 2a).

To further verify the role of RDO5 by an independent approach, we performed gene knockout via CRISPR/Cas9 in Col-0 plants and obtained another homozygous rdo5 mutant containing a deletion of 344 bp starting from the 12th codon of RDO5, designated cr15 (Fig. 1c and Supplementary Fig. 2b). We found that cr15 plants also supported significantly enhanced replication of CMV-Δ2b compared to its wild-type Col-0 and the two transgene complemented lines of rdo5-4 mutant (Fig. 1e, f). Together, our findings show that the highest-ranked gene in the GWAS screen confers quantitative virus resistance in Col-0 accession.

**RDO5 enhances antiviral RNAi by promoting viral siRNA amplification.** The seed-specific RDO5 codes for a nuclear pseudophosphatase that promotes seed dormancy in a manner independent of the biosynthesis of phytohormone abscisic acid. Consistently, rdo5-4, rdo5-5 and cr15 mutants exhibited...
significantly enhanced seed germination rates than Col-0 and the complementation lines of rdo5-4 and An-1 plants carrying the wild-type RDO5 transgene derived from Col-0 (Supplementary Fig. 2c), providing evidence for functional rescue by an additional phenotype. Notably, we found that CMV-Δ2b infection induced expression of RDO5 in leaves, suggesting a different function of RDO5 in vegetative tissues in response to virus infection (Supplementary Fig. 2d).

A. thaliana resistance to CMV-Δ2b is mediated by antiviral RNAi dependent mainly on the DCL4 and RDR6 pathway. Five A. thaliana chromosomes were depicted in different colors. The horizontal dash-dot line corresponds to the significance threshold (p = 5.48 × 10^{-7}). The black triangle above the threshold indicates the most significantly associated locus. c Regional Manhattan plot (from 6745 kb to 6747 kb), structure of RDO5, and positions of T-DNA insertion alleles (rdo5-4 and rdo5-5), CRISPR/Cas9 deletion allele (cr15) and the single base pair deletion in accession An-1. d Western detection of viral coat protein (CP) accumulation in An-1 and two RDO5-complemented lines. Detection of tubulin alpha chain was shown as loading control. e, f ELISA and Western detection (e) of viral CP accumulation with the titer in Col-0 set as 1 and Northern detection (f) of CMV-Δ2b genomic RNAs 1-3 (gRNAs), subgenomic RNA4 and vsiRNAs as well as endogenous miRNA 173 (miR173) and trans-acting siRNA 255 (tasi255) in wild-type (Col-0), rdo5 mutants and complemented lines. 25 S rRNA was stained and U6 RNA probed on the same membrane as loading controls. g Ratios of vsiRNAs/gRNAs were calculated from Phosphor-imager readings of Northern hybridization signals in (f) with the ratio in Col-0 set as 1. The experiments in (d) and (f) were repeated three times independently with similar results. Data presented are means ± SEM from three replicates (e) or independent experiments (g), letters indicate significant differences (one-way ANOVA, Duncan, p < 0.05) and black dots represent the individual values. The source data underlying blots in (d), (e) and (f), ELISA data in (e) and ratio data in (g) are provided as a Source Data file.
Consistent with a predominantly RDR6-dependent antiviral RNAi against CMV-Δ2b described previously, CMV-Δ2b replicated to significantly lower levels in both wild-type Col-0 and rdr1 plants than rdr6 or rdr1 rdr6 plants (Fig. 2a, b) and vsiRNA amplification in rdr1 plants was as efficient as in Col-0 plants in contrast to defective vsiRNA amplification in rdr6 or rdr1 rdr6 plants (Fig. 2b, c). We found that CMV-Δ2b accumulation was significantly enhanced and the vsiRNAs/gRNAs ratio was significantly decreased in rdo5 rdr1 double mutant plants compared to rdr1 single mutant plants (Fig. 2a–c). By contrast, no significant differences in either CMV-Δ2b accumulation or the vsiRNAs/gRNAs ratio were observed between rdr6 and rdo5 rdr6 plants (Fig. 2a–c). Moreover, neither CMV-Δ2b accumulation nor the vsiRNAs/gRNAs ratio was significantly different between rdr1 rdr6 double mutant plants and rdo5 rdr1 rdr6 triple mutant plants (Fig. 2a–c). These findings indicate that RDO5 acts specifically in the antiviral RNAi defense mechanism by enhancing vsiRNA amplification in an RDR6-dependent pathway.

We performed an additional set of infection experiments to verify the proposed RDR6-dependent antiviral activity of RDO5 using CMV-2aTΔ2b, which contains a 295-nt deletion in RNA 2 of Fny-CMV resulting in both the loss of VSR-2b expression and a C-terminal truncation of the viral RdRP protein. Unlike CMV-Δ2b, efficient CMV-2aTΔ2b infection occurs only in rdr1 rdr6 double mutant plants because it triggers potent amplification of vsiRNAs by both RDR1 and RDR6 pathways. ELISA and Western blotting detection of the viral CP as well as Northern blotting detection of the viral RNAs found no significant differences in either CMV-2aTΔ2b or the vsiRNAs/gRNAs ratios between either wild-type plants (Col-0) or rdr6 mutant plants with rdo5 mutant plants (rdo5-4, rdo5-5, cr15) or the two complemented lines of rdo5-4 plants (Supplementary Fig. 3). These results indicate that RDO5 is dispensable for RDR1-dependent amplification of the vsiRNAs. Consistently, enhanced accumulation of CMV-2aTΔ2b and decreased vsiRNAs/gRNAs ratio were observed in rdo5 rdr1 plants compared to rdr1 plants, but no significant differences in either CMV-2aTΔ2b accumulation or the vsiRNAs/gRNAs ratio were observed between rdr6 and rdo5 rdr6 plants (Fig. 2a, c, d). Moreover, we detected no significant differences in either CMV-2aTΔ2b accumulation or the vsiRNAs/gRNAs ratio between rdr1

Fig. 2 RDO5 promotes antiviral RNAi. a–h Accumulation of CMV-Δ2b (a, b, f, g) or CMV-2aTΔ2b (a, d, g, h) detected in wild-type (Col-0), single, double or triple mutant plants as indicated at 2 weeks post-infection by ELISA (a, e, g) and Western blot analysis of the viral coat protein (CP) or Northern blot analysis of the viral RNAs 1-4 (b, d, f, h). Detection of the vsiRNAs and plant endogenous small RNAs (b, d), and the calculation of vsiRNAs/gRNAs ratios (c) were as described in the legend to Fig. 1. Note the reduced sample loading (to ½) for total proteins and total RNAs (e). Detection of the vsiRNAs and plant endogenous small RNAs (f) from agoI/2 double mutant plants and rdo5/agoI/2 triple mutant plants infected with CMV-Δ2b. Data presented are means ± SEM from three replicates (a, e, g) or independent experiments (c), letters indicate groups with significant differences (one-way ANOVA, Duncan, \( \alpha \) < 0.05) and black dots represent the individual values. The experiments in (b), (d), (f) and (h) were repeated three times independently with similar results. The source data underlying blots in (b), (d), (f) and (h), ELISA data in (a), (e) and (g), and ratio data in (c) are provided as a Source Data file.
Natural variation identifies a host gene that inhibits antiviral defense. In a parallel GWAS screen, we used a wild-type isolate of subgroup II CMV strain Q (Q-CMV) that is not modified in counter-defense against antiviral RNAi, but causes much weaker disease symptoms than Fny-CMV in Col-0 plants. We measured the accumulation levels of Q-CMV in 500 accessions of *A. thaliana* by detecting the viral genomic RNA3 with quantitative reverse transcription-polymerase chain reaction (RT-qPCR) (Supplementary Fig. 4a and Supplementary Data 1). Using the easyGWAS pipeline, we did not identify SNPs significantly associated with quantitative resistance in any of the known antiviral RNAi genes as found in the above GWAS screen. The SNP associated most significantly with quantitative resistance against Q-CMV resided in a region of chromosome 5 between genes At5g05130 and At5g05140 (Fig. 3a, b and Supplementary Fig. 4b), neither of which was characterized previously. We found that Q-CMV accumulation levels as measured by RT-qPCR were significantly different between the accessions classified as haplotype A and G according to the SNP (Supplementary Fig. 4c). Northern blot analysis further verified that Q-CMV replicated to lower levels in 3 selected accessions of haplotype G than the 4 accessions of haplotype A including Col-0 (Supplementary Fig. 4d).

Functional studies in Col-0 plants identified At5g05140 as a regulator of antiviral RNAi, designated *Antiviral RNAi Regulator 1* (*VIR1*). Firstly, we found that expression of *VIR1*, but not At5g05130, was induced in Col-0 plants by infection with Q-CMV, Q-CMV–Δ2b or CMV-2aTa2b (Supplementary Fig. 5a). *VIR1* expression levels were also significantly higher in the selected accessions of haplotype A than those from haplotype G after Q-CMV infection (Supplementary Fig. 5b). As shown in Fig. 3b, we obtained *VIR1* and At5g05130 knockout mutants of Col-0 plants. *vir1-1* mutant contained a T-DNA insertion in the eighth exon of *VIR1*. *cr5* mutant was generated by CRISPR/Cas9 genome editing to delete a 215-bp fragment in the first exon of At5g05130. Neither *vir1-1* nor *cr5* exhibited visible developmental defects (Supplementary Fig. 6a). RT-qPCR analysis showed that *VIR1* is a negative regulator of antiviral RNAi. The N-terminal region of *VIR1* protein shares strong homology with Arabidopsis transcript elongation factor IIS (AtTFIIS) conserved broadly in eukaryotes (Supplementary Fig. 5c). However, most of the critical residues in the C-terminal domain of TFIIS essential to facilitate mRNA synthesis by RNA polymerase II complex are not conserved in *VIR1* protein. Similar to *rdo5* mutant plants, *rdo2* mutant plants that lack AtTFIIS display clearly reduced seed dormancy. We found that *vir1-1* mutant plants exhibited significantly enhanced seed dormancy and that *VIR1* expression was detectable in seeds (Supplementary Fig. 6d, e), suggesting *VIR1* as a negative regulator of seed dormancy.

To determine whether *VIR1* interferes with vsiRNA biogenesis, we constructed double and triple mutants by genetic crosses of *vir1-1* plants with *dcl2-1* and/or *dcl4-2* mutant plants characterized previously. We challenged this panel of mutant plants with CMV-2aTa2b and CMV-Δ2b derived from Fny-CMV since our previous studies have defined the genetic pathways in the biogenesis of vsiRNAs triggered by these mutant viruses. RT-qPCR and Northern blotting analysis showed that CMV-2aTa2b replicated to significantly lower levels in *vir1-1* mutant plants than Col-0 plants (Fig. 4a, b), as did Q-CMV and Q-CMV–Δ2b (Fig. 3c–f). Notably, whereas no significant differences were detected in virus accumulation between *dcl2* and *vir1 dcl2* mutant plants, CMV-2aTa2b replicated to significantly higher levels in *vir1 dcl4* double mutant plants than *dcl4* single mutant plants (Fig. 4a, b). Consistent with the known dominant role of DCL4 over DCL2 in the biogenesis of vsiRNAs, 21-nt vsiRNAs made by DCL4 were undetectable whereas 22-nt vsiRNAs by DCL2 accumulated to high levels in either *dcl4* plants or *vir1 dcl4* plants (Fig. 4b). By comparison, however, 22-nt vsiRNAs accumulated to markedly lower levels in *vir1 dcl4* plants than Q-CMV accumulated to significantly lower levels in *vir1-1* mutant plants than Col-0 (Fig. 3c). In contrast, no statistically significant differences in Q-CMV accumulation were found between Col-0 and At5g05130–*cr5* mutant plants or two independent *VIR1*-transgene complementation lines of *vir1-1* (Fig. 3c). Moreover, *vir1-1* plants, but not *cr5* plants or the transgene-complemented lines of *vir1-1*, supported significantly reduced replication of Q-CMV–Δ2b (Fig. 3c), a 2b-deletion mutant of Q-CMV defective in suppressing antiviral RNAi initiated by either DCL2 or DCL4 shown by a previous study. We next calculated and compared the ratio of the vsiRNAs/gRNAs detected by Northern blotting (Fig. 3d–f). The results indicated that production of the vsiRNAs triggered by either Q-CMV or Q-CMV–Δ2b was significantly enhanced in *vir1-1* mutant plants compared to Col-0 plants, *cr5* mutant plants or the two *VIR1*-complemented lines of *vir1-1* (Fig. 3d–f).
In this work, we searched for the natural variation among wild *A. thaliana* populations that is most significantly associated with quantitative resistance to an endemic RNA virus. We conducted independent GWAS screens with wild-type Q-CMV and the VSR-deficient CMV-Δ2b, respectively. Unlike Fny-CMV, from which CMV-Δ2b was derived, Q-CMV replicates to higher levels in *dcl2 dcl4* mutant plants than wild-type Col-0 plants, indicating incomplete suppression of antiviral RNAi by Q-CMV. Surprisingly, none of the SNPs significantly associated with quantitative virus resistance from both of our GWAS screens and an additional GWAS screen reported recently by others mapped to any of the known antiviral RNAi genes, including AGO2 shown recently to exhibit natural variation in non-host virus resistance. However, genetic studies show that both of the highest-ranked gene significantly associated with quantitative virus resistance identified from each of our GWAS screens...
function in antiviral RNAi. Further studies on the two identified genes evolved in Columbia-0 accession demonstrate opposing roles in antiviral RNAi. Our findings provide direct evidence to support antiviral RNAi as a dominant defense mechanism in virus-host coevolution, which is consistent with previous genetic studies that identify antiviral RNAi as an essential antiviral defense in plants1,2,5,6,8.

Most of the antiviral RNAi pathway genes characterized to date have been identified by their known activity in experimentally induced RNAi to target mRNAs transcribed in the nucleus2,7,8,18,19,45–49. Recently, mutant *Arabidopsis thaliana* and *Caenorhabditis elegans* defective in antiviral RNAi have been isolated by sensitized genetic screens using viruses or viral RNA replicons rendered inactive in RNAi suppression36,50. In this work, we showed that antiviral RNAi pathway genes can be identified by GWAS mapping of natural variation in quantitative virus resistance among wild-collected *A. thaliana* accessions. Our findings illustrated the technical feasibility of using GWAS to identify antiviral RNAi pathway genes that are active in the presence or absence of DCL4 in uninfected plants9,55.

Whereas VIR1 has not been characterized before this work, RDO5 has a known function to promote seed dormancy with the mechanism yet to be defined. Our mechanistic studies demonstrate that RDO5 specifically enhances antiviral RNAi by promoting amplification of the vsiRNAs in a pathway dependent on RDR6, but independent of RDR1. By contrast, VIR1 dampens antiviral RNAi by restricting production of the vsiRNAs and suppresses seed dormancy in wild-type Col-0 plants. Our results suggest that VIR1 may act by blocking viral induction of DCL4 in a manner similar to the dominant negative mutant version of AtTFIIS shown recently to modify the transcriptome in tfi1s mutant plants41. Interestingly, VIR1 is necessary for the transcriptional induction of DCL2 and the upregulation of DCL2-dependent antiviral RNAi by 22-nt vsiRNAs in the absence of DCL4, which may explain at least in part the dominant role of DCL4 over DCL2 in the biogenesis of vsiRNAs known since 20069–11,19,33. However, it is unknown whether VIR1 modulates the functional roles of DCL2 that are active in the presence or absence of DCL4 in uninfected plants9,35–58. Notably, we show that VIR1 inactivation by CRISPR/Cas9 genome editing confers resistance to CMV either active or defective in RNAi suppression, providing a strategy to generate transgene-free virus resistant plants. In summary, our findings indicate that RDO5 and VIR1
have opposing roles in both antiviral RNAi and seed dormancy. We propose that a shared mechanism is under natural selection to regulate antiviral RNAi and seed dormancy.

Methods

Viruses and plant materials. Mutant viruses CMV-Δ2b and CMV-2aTA2b were derived from the subgroup 1 strain Fny-CMV isolated and cloned in New York from a muskmelon farm75. In CMV-Δ2b, three A/G codons at the first (start) codon, 8th, and 18th positions of 2b ORF encoded by wild-type RNA2 of Fny-CMV were mutated to A so that the amino acids encoded in the +1 overlapping 2a ORF were not altered14. CMV-2aTA2b contained a 295-nt deletion in the 2b coding sequence, which also removed the C-terminal 80 amino acids of the viral RdRP 2a protein12,13. The 2aTA2b strain was isolated in Australia and molecularly cloned in 1995 following passages since 1964 in laboratory host plants61. In Q-CMV-Δ2b, the 2b coding sequence of RNA2 was deleted and replaced with CCCGGC, which also removed the C-terminal 68 amino acids of the 2a protein3,27. All viruses were purified after propagation in Nicotiana clevelandii. Virion concentration was measured using nanodrop after propaga

Characterization of virus infection. To characterize virus infection, upper systemically infected leaves from 16 plants were harvested at two weeks post-inoculation and pooled for total RNA and protein extraction72. Five and 20 μg total RNAs were loaded in each lane for Northern detection of the viral genomic and vsiRNAs by a–P– and y–P–labelled probes, respectively73. Hybridization signals were detected by phosphor imager Typhoon 9410 and analyzed by ImageQuant TL 7.0 (GE Healthcare). Ratios of vsiRNAs vs viral genomic RNAs (gRNAs) were calculated from Phosphor-imager readings of Northern hybridization signals19,40. With the primer pairs listed in Supplementary Data 2 and EFiα mRNA as the internal reference72, RT–qPCR was used to detect the accumulation of RDOS, VIR1, DCL1, DCL2, DCL3 or DCL4 mRNA in upper non-infected leaves of plants one week after mock or virus inoculation. For Western blotting, total proteins were separated on 12% polyacrylamide gel before transferred to 0.45 μm nitrocellulose membrane (GE Healthcare). Monoclonal antibody (1: 5000 dilution) specific to Fny-CMV CP66 was used for viral CP detection and probing with A. thaliana tubulin alpha chain specific antibody (AgriSera, AS20 4483, 1:5000 dilution) as loading control, Goat anti-mouse IgG (H+L) (Invitrogen, G-21040, 1:2000 dilution) was used as secondary antibody. All experiments were biologically repeated at least three times. We examined the expression pattern of VIR1 in A. thaliana from the Arabidopsis RNA-Seq Database (http://ipf.suestech.edu.cn/pub/atharna/)75 using 960 libraries generated from Col-0.

Seed dormancy assay. For seed dormancy assays76, 50 newly harvested seeds were sown on wet filter paper in 9 cm diameter culture dishes, and incubated in growth room with 16 h light – 8 h dark cycle at 23°C. Seed germination rates for each of the genotypes were determined 7 days after incubation with 4 independent repeats.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

Data supporting the findings of this work are available within the paper and its Supplementary Information files. A reporting summary for this Article is available as a Supplementary Information file. Source data are provided with this paper.

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Author contributions

S.L. designed and performed experiments, analyzed data, and wrote the paper. S.-W.D. conceived, designed, and supervised the study, and wrote the paper. Z.J. designed and supervised the GWAS screens and statistical analysis, and wrote the paper. M.C., R.L., W.-X.L. performed experiments or analyzed data. A.G.-O. provided intellectual input to the study. All authors revised and provided feedback for the final version of the paper.

Competing interests

The authors declare no competing interests.

Additional information

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