

Supplementary methods

Fungal pathogen assay and disease quantification

B. cinerea strain B05.10 and *V. dahliae* strain JR2 were cultured on Malt extract agar (2% malt extract, 1% Bacto peptone) and potato dextrose agar (PDA), respectively. The *B. cinerea* spores were diluted in 1% Sabouraud Maltose Broth buffer to a final concentration of 10^4 spores/ml for spray inoculation on tomato leaves and 10^5 spores/ml for drop inoculation on the other plant materials¹. A 10 μ l spore suspension was used for drop inoculation of all plant materials used, except tomato fruits, in which 20 μ l was used. *V. dahliae* soil-inoculation assay was performed as described previously². For *Arabidopsis* liquid root culture inoculation, 2-week-old *Arabidopsis* plants were grown in root culture (0.32% Murashige and Skoog salt, 2% sucrose, 0.1% MES, pH adjusted to 5.8 using KOH), and *V. dahliae* spores were added into the culture to a final concentration of 10^6 spores/ml. After 5 min inoculation, the root culture was replaced with fresh sterile medium solution. Fungal biomass quantification was performed as described previously³. The p values were calculated using Student's t-test for the comparison of two samples, and using one-way ANOVA for the comparison of multiple samples.

Fungal *DCL* gene transcript quantification

Infected plant tissues were collected 3 days after *B. cinerea* inoculation and 2–3 weeks after *V. dahliae* inoculation. The collected samples were subjected to RNA extraction using the Fisher BioReagents™ SurePrep™ Plant/Fungi Total RNA Purification Kit (Fisher scientific, Waltham, MA), cDNA synthesis using SuperScript III Reverse Transcriptase (Invitrogen Carlsbad, CA), and quantitative RT-PCR quantification⁴. The expression of *Bc-DCL1* and *Bc-DCL2* in *B. cinerea* after treatment of synthesized *Bc-DCL1/2*-sRNAs and -dsRNAs was measured as described previously⁴.

sRNA detection methods

sRNA detection was mostly carried out by Northern blot analysis, except for Fig. 3e, in which sRNA stem-loop RT-PCR was used due to very limited amount of *B. cinerea* protoplasts that were purified from the infected tissue. The Northern blot analysis was performed as described⁵⁻⁸, and sRNA stem-loop RT-PCR was done as described in Chen et al.⁹.

RNA treatment using varying concentrations

The RNAs were diluted to a final concentration of 1, 5, and 10 ng/μl. 20 μl of the diluted RNAs, as well as water, were applied on the surface of rose petals and tomato fruits immediately prior to *B. cinerea* infection. The pictures were taken at 3 dpi for the rose petals, and 5 dpi for the tomato fruits.

RNA treatment of varying incubation times

20 μl of 20 ng/μl YFP-dsRNAs and -sRNAs, *Bc-DCL1/2*-dsRNAs and sRNAs, and water were applied on the surface of rose petals and tomato fruits, which were then incubated in a box for 1, 3 and 5 days before *B. cinerea* infection, respectively. The pictures were taken at 3 dpi for the rose petals, and 5 dpi for the tomato fruits.

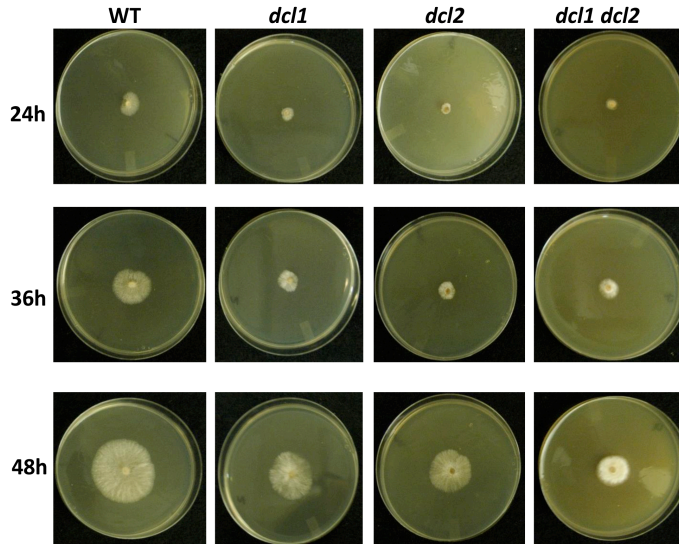
Cloning and data analysis of *Arabidopsis* AGO1- and AGO2-associated sRNAs

V. dahliae infected *Arabidopsis* prepared in liquid root culture were collected at 2 and 4 dpi. *At*-AGO1 and *At*-AGO2 immunoprecipitations were performed in parallel as previously described¹⁰. *At*-AGO1- and *At*-AGO2-associated RNAs were extracted and used for sRNA library construction and Illumina HiSeq 2000 deep sequencing¹¹. The read number of *Vd*-sRNAs in *At*-

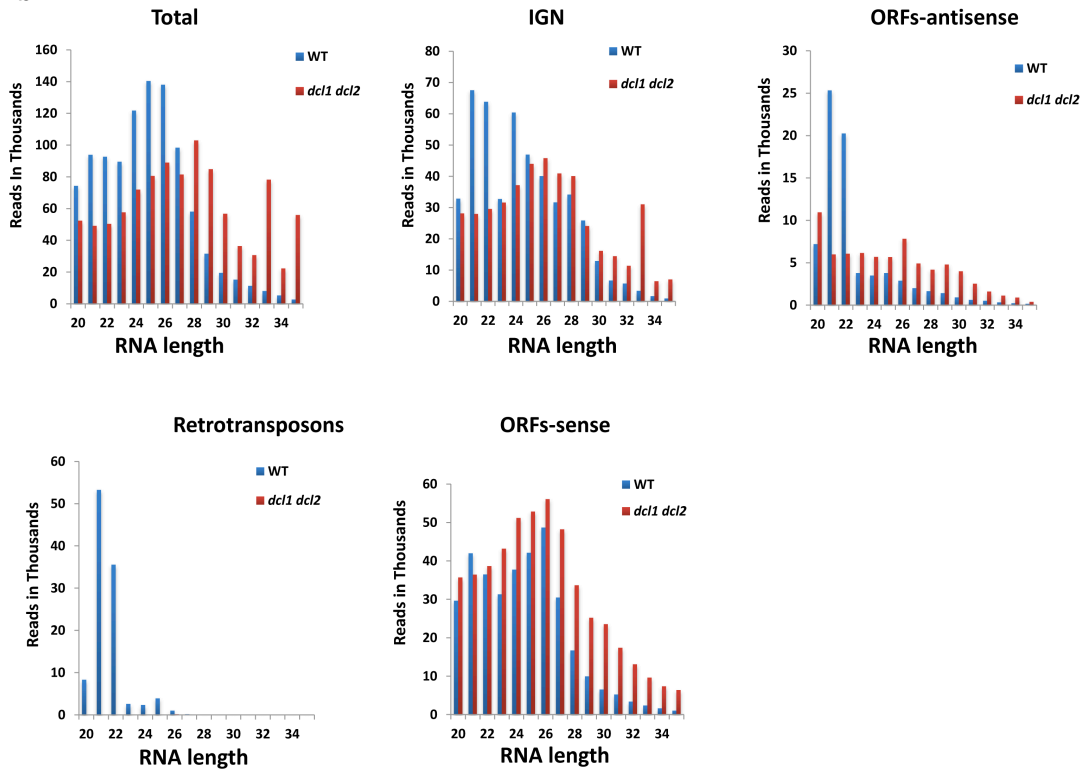
AGO1 and *At*-AGO2 IP libraries were normalized with total *V. dahliae* sRNAs after removing tRNAs, rRNAs, snoRNAs, and snRNAs, etc. The *Vd*-sRNAs that had a higher read number than 100 RPM after normalization and also had host target genes in *Arabidopsis* were selected. *At*-AGO1-enriched *Vd*-sRNA effectors were defined as the selected *Vd*-sRNAs with a 10 times greater read number in the *At*-AGO1 IP library compared to the *At*-AGO2 IP library. *At*-AGO2 enriched *Vd*-sRNAs were defined as the selected *Vd*-sRNAs with a 10 times greater read number in the *At*-AGO2 IP library compared to the *At*-AGO1 IP library.

Supplementary Figures

a



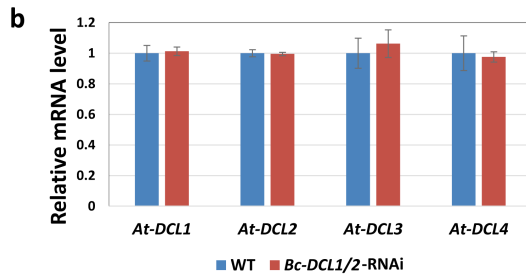
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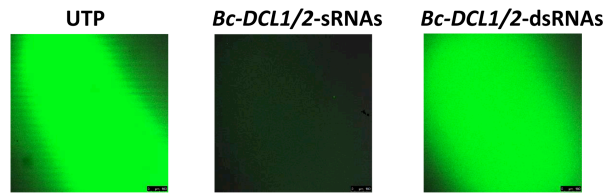
Supplementary Fig 1. *B. cinerea* *dcl1* and *dcl2* single mutants and the *dcl1 dcl2* double mutant attenuate fungal growth and development, and most of the retrotransposon-derived Bc-sRNAs were dependent on both Bc-DCL1 and Bc-DCL2. (a) *B. cinerea* WT, *dcl1* and *dcl2* single mutants, and the *dcl1 dcl2* double mutant were grown in ME medium, and pictures were taken after 24, 36, and 48 hours. (b) sRNA libraries were constructed from WT *B. cinerea* and the *dcl1 dcl2* double mutant and sequenced using Illumina deep sequencing. The normalized Bc-sRNAs were mapped to the whole genome (total), IGN, ORFs-antisense, retrotransposons, and ORFs-sense. The production of Bc-sRNAs from retrotransposon regions, as well as the ORFs-antisense and IGN regions, was largely impaired in the *dcl1 dcl2* double mutant.

a Alignment of selected *Bc-DCL1* and *Bc-DCL2* RNAi fragments with the four *Arabidopsis DCLs*

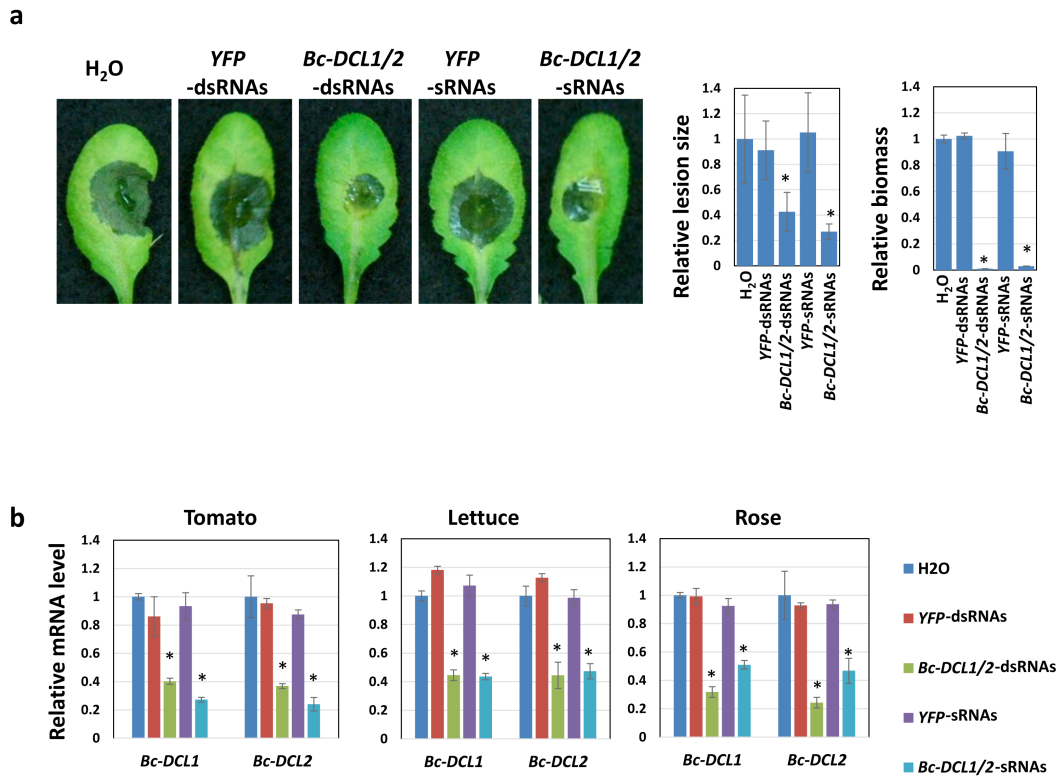
Bc-DCL1	----A--CCG-----GTAGATGCTAGAGATAATGTCAAGAAAGTGC ^{CGAAGAAC}	Bc-DCL2	^{GGATGCCATTTGCTGCACGCCAAAAATACATCGAG-----CAGATCTCGCCTTCGAG}
At-DCL3	-----CACCATTGGGGTGTATTTGGCCCAAGATG	At-DCL3	GGACTCAAAGATTTTTAATCCTGAAGGCGTGAAGGAGTGGAAAGTTTGTACAACGGT
At-DCL1	AATGTGAGCTCTGGAAAGCGCTGCTGCTGAAAAGTCGGGCGGAAGTTGGCAACCAG	At-DCL1	GGATTCACGGTTTGTACTATAAAAGATCGAAAAGAAATAGAGAAACATGTGCTATGCC
At-DCL2	---T-----GATGCTTCACAGGAGTTTTGGCTGAGATACCTCAAGGTG	At-DCL2	GAATCAAAGGCTATACCTGTGAGAATGAGTCTGTGCTGGCTGGGTTTGTCCCTTTTC
At-DCL4	---T-----ATGGCTGCTGAGGCCCTCTCTCTGGTGTCTCAAAGATG	At-DCL4	CAATGCCAAGGTTTATTCAGTGGAAAGCAATGTCAGGATGGATGGTTTTGTTTCATCTCC
			* * * * *
Bc-DCL1	^{TTGAAGGTTTGCTACACAGTCAAATATGTACTGCAGAAGATCCAGCTTGTGCACTAGT}	Bc-DCL2	^{TAAAGTACCACCTCTATCTATCTACTATACCCAGAGTCAAATATCATCGTG----}
At-DCL3	ATAAG-----AGTCTGGT	At-DCL3	TAAAGAAGG---TCCAATATGTATAACCCATCACCAT-CCTGTAGTTTGGAAATG----
At-DCL1	AAAAATGGTAATGC-ACATGACGAGATGGAGGAGGAGAGCTCCCT-GATGATCCTGTGGT	At-DCL1	TTCAGAGAT---AGTCGTCGAGTATGACAAAGCTGCTACTATGTGGTCTCTCATGAGAC
At-DCL2	TTAATTGGAGTGT-TGCAACACATAAATGGAAATG-----	At-DCL2	TACACCAAG---CTTCAAGTATTACCAGCAC-ATAAAAATCCAAGTCCCAAAAC-----
At-DCL4	AGAATGCATCTGA-CCTCTCAGCTTAGCGGCGT-----	At-DCL4	TTTAGTCAA---AGTATATATATATCGGTCAAGCTTAAAGTGTGATGCATCTCAATC----
	*		* * ** *
Bc-DCL1	^{CAATCAAAGGTAACCTGAG-----ACTCTTGCTACTATGATCCCTTGGGCCGAAA}	Bc-DCL2	^{-----ACGAAAACCTGTGGCGAGCTGAGAAAGATTGTGCAA}
At-DCL3	AGATTTGCAGCAAAA-----CCATCTGTGACAGTAATTTCTGGGCATGTATC	At-DCL3	----AAAGAAAAGTTAGAACTTCACAC-----CTCAAGTTTGTGCTCTCTTAGAA
At-DCL1	CTCGGAGGGGAGCACGTTGATGAAGTAAATAGCGCCGACGTGGCTGATGGGAAAGTTAC	At-DCL1	AATAAAGCAAATGATTGACGCTGTTGAAGAAGCGCCACAAGCAAGTTCAAGGAAAAGCAA
At-DCL2	-----CGAGGACAGGTCCTCTAATC	At-DCL2	---GAGC-----AAGCTTGGTAGAGAACCTAGAA
At-DCL4	-----TGAAGGAACCATATTCTC	At-DCL4	---GACCATCAGATA-----TGAAAACATGCTGGAGACATCAAAA
			* * *
Bc-DCL1	TTCAACTCTCTTTTCTCAATGCTCCGGCTTCT-AAAAGACAATCTCATTTTGG	Bc-DCL2	^{AGTCTCAACATTTTCGAAGACCCCTACGTTTTGACACTAAAAGGAGTGTAGCGAAAAA}
At-DCL3	TCCAAGCTAAAAGAACTCTTCCATCTATTGGATTCCTTTAGAGGTTGACAAGCAAAAGCA	At-DCL3	GCCTCAAGAGTTGGGAAAAGAC---AGTTTTC-----TGAATATGGA
At-DCL1	TCCAAGATACAATCATTGATCAAACTACTCTCAAATATCAGCACACAGCTGATTTTTCG	At-DCL1	GTGGCAATTTATGGGGCTAGGG---ATGCTGGAGCAAAAGGATGAATGAGACAGGTTTA
At-DCL2	TTTAAAAACTGTCTGCCATTGAGACTCTCTTGGTTATAGCTCCTTGGAGAACATACG	At-DCL2	AGACTAAGGATAA---AGCA---TCGCTTATCCCTTGGAACT---TGGATCTC-A
At-DCL4	TAGAAAGCTAGTTCAATTGATTAAGATCCTTTGCGTATTCAAGCTAGAGCTACACATGAA	At-DCL4	CAGCG--GTGCTT-----GGCA---TCACTTAAGCTGCTGATTGA---TACTCATCAA
	* * * * *		* * * * *
Bc-DCL1	^{GAAGCCATTTGTATTTG---GGACAGAAGCAGTAGAACTCTAGGATCTTGGTGTGTTGA}	Bc-DCL2	^{AGTCAACGTGAGCTGGCGAAAGTACTCAAGAGTTTAAAGACATATAGTCAAACCCAATTA}
At-DCL3	GTGCTTATTTAGTTGAGAGAATTATAACTGCGAAAGTGTGCAAGAGATCGTAAGAA	At-DCL3	T-----AATAAGTTT-GAGACATATCAAAGA--G-----ATTGTC
At-DCL1	AGCTATTGTTTGTGTTGAGAGGTTGCTGCTGTTGTTCTCTCAAGGTTTTGGCGA	At-DCL1	T-----GGCTCTCTGAAGAACGGAGAGCGA--TGGTGTGCTCAATTTG
At-DCL2	GTGCATCATTTTGTGGATAGGTTGATAACAGCCATGTTCTGGAATCCCTTTTGGCTGA	At-DCL2	A-----CTCCTCTACTGTGATTCTGTAGAGA--AG-----AGACTG
At-DCL4	ATGTATAATATTTGTCAATCGGATTGTGACTGCAAGAACATGTCTATGCTACTAAATAA	At-DCL4	A-----CACAAACCTCTAAGTATGAAAAG---G-----CTTCTC
	* * * * *		* * * * *
Bc-DCL1	^{CCAGATCTGGAC} TTTCTGTCTTCAAGAAGAAGAGTCTAAGAACTACAAGCAAGGACGGA		
At-DCL3	AGAA-GCCTCTTGGCTTACCTTAATGCTTGTATTTAA---CCGAAAACAACCCCTCC		
At-DCL1	GCTG-CCTT---CGCTTAGTTTTTACGCTGTGTCAGCATGATTGGACACAATAACAGC		
At-DCL2	GATT-CTTCCAACTGTAA-TAAGTGGAAAACCAAGTACGTTGACAGAAATAACTCTGGT		
At-DCL4	CT-----TGAAGTCTACGGCTTGGAAAGTGTGATTTCTTGTGGACTTAGTTCTGGAA		
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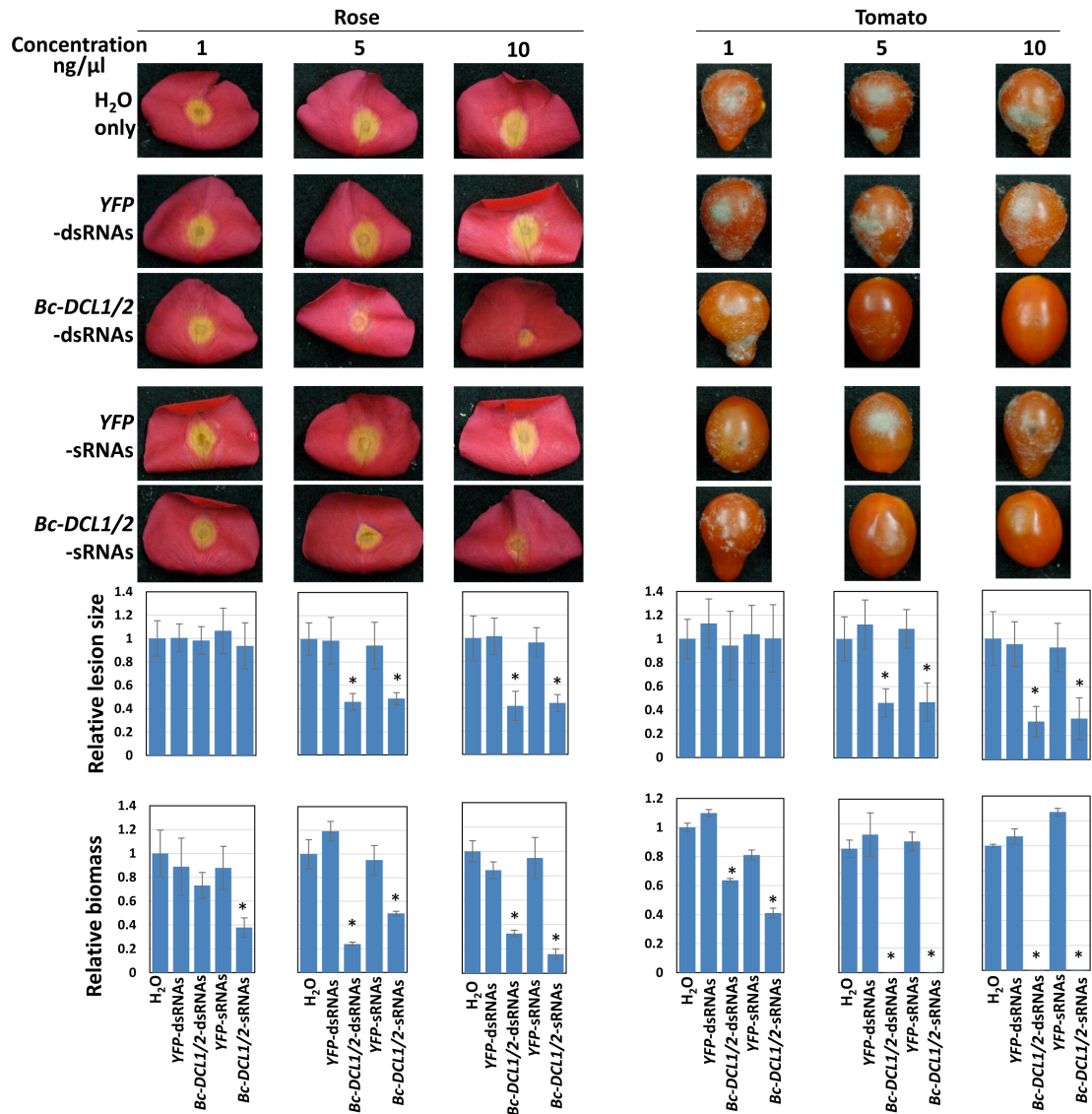
Supplementary Fig 2. Selected *Bc-DCL1* and *Bc-DCL2* RNAi fragments did not target *Arabidopsis DCLs*. (a) The selected RNAi fragments from *Bc-DCL1* (252 bp) and *Bc-DCL2* (238 bp) were aligned with 4 *Arabidopsis DCL* genes. The selected RNAi fragments are highlighted (yellow), and asterisks represent the conserved area among these genes. (b) The relative expression levels of four *Arabidopsis DCL* genes (*At-DCL1*, *At-DCL2*, *At-DCL3*, and *At-DCL4*) were measured by quantitative RT-PCR in *Arabidopsis Bc-DCL1/2*-RNAi plants. *Arabidopsis* WT was used as a control.



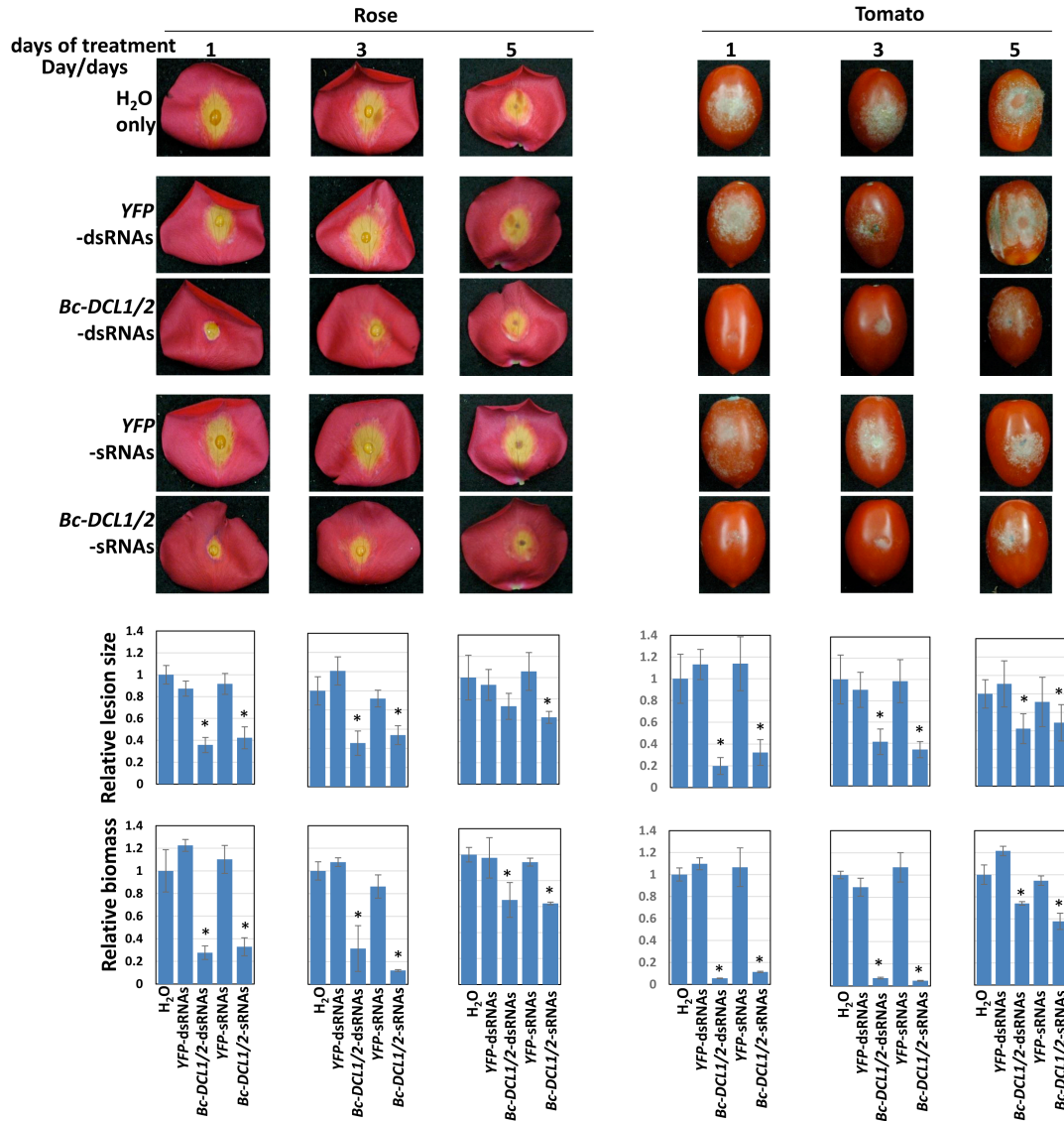
Supplementary Fig 3. The fluorescence intensity of *in vitro* transcribed *Bc-DCL1/2*-dsRNAs is stronger than that of *Bc-DCL1/2*-sRNAs. 300 ng of fluorescent *Bc-DCL1/2*-sRNAs and -dsRNAs, as well as the same amount of Fluorescein-12-UTP, were applied on microscope glass slides, and the fluorescence intensity were examined using a confocal microscope.



Supplementary Fig 4. *Bc-DCL1/2*-sRNAs and -dsRNAs treatment attenuated gray mold disease on *Arabidopsis* leaves, and silenced both *Bc-DCL1* and *Bc-DCL2* in various plants (a) *Arabidopsis* leaves were treated with *Bc-DCL1/2*-sRNAs and -dsRNAs, and the leaves were more resistant to *B. cinerea*, when compared with water, *YFP*-sRNAs and -dsRNAs treatments (controls). The relative lesion sizes were measured using imageJ, and the error bars indicate the standard deviation (SD) of 10 plant samples. The fungal relative DNA content (relative biomass) was measured using quantitative PCR, and error bars represent the SD of three technical replicates. (b) Both *Bc-DCL1* and *Bc-DCL2* were suppressed in the *B. cinerea* infected plant samples (tomato fruit, lettuce, and rose petal) that were pre-treated with *Bc-DCL1/2*-sRNAs and -dsRNAs, when compared with pre-treatment with water, *YFP*-sRNAs and -dsRNAs (controls). Asterisks in (a) and (b) indicate statistically significant differences ($P < 0.01$).



Supplementary Fig 5. *Bc-DCL1/2*-sRNAs and -dsRNAs remained functional when the concentration was as low as 5 ng/μl, but not at 1 ng/μl. The tomato fruits and rose petals were treated with various concentrations of *Bc-DCL1/2* and *YFP*-RNAs (1, 5 and, 10 ng/μl), and the protection against *B. cinerea* was observed with the 5 and 10 ng/μl, but not the 1 ng/μl treatment. Pictures were taken at 3 dpi for rose petals and 5 dpi for tomato fruits. The relative lesion sizes were measured using imageJ, and the error bars indicate the SD of 10 plant samples. The fungal relative biomass was measured by quantitative PCR, and error bars represent the SD of three technical replicates. Asterisks indicate statistically significant differences (P < 0.01).



Supplementary Fig 6. Treatment with *Bc-DCL1/2*-sRNAs and -dsRNAs protected plant samples from *B. cinerea* up to 8–10 days. The tomato fruits and rose petals were pre-treated with *Bc-DCL1/2*-sRNAs and -dsRNAs, YFP-sRNAs and -dsRNAs, and water for 1, 3, and 5 days, respectively, before *B. cinerea* infection. The pictures were taken at 3 dpi (up to 6–8 days after RNA pre-treatment) for rose petals and 5 dpi (up to 8–10 days after RNA pre-treatment) for tomato fruits. The relative lesion sizes were measured using software imageJ, and error bars indicate the SD of 10 plant samples. The relative biomass was measured by quantitative PCR, and error bars represent SD of three technical replicates. Asterisks indicate statistically significant differences ($P < 0.01$).



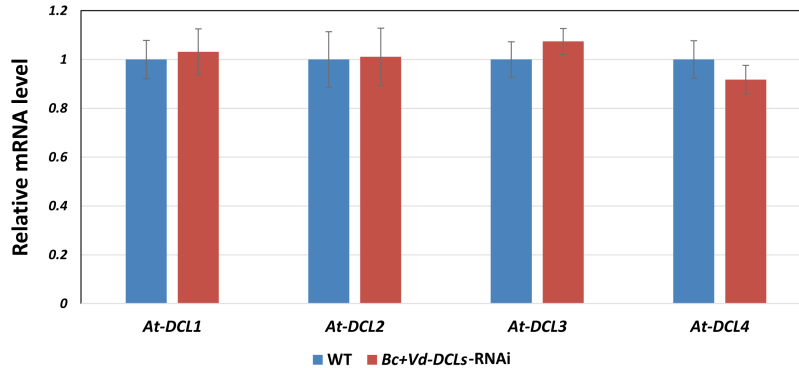
Supplementary Fig 7. A large amount of *Bc-DCL1/2*-sRNAs were produced in *N. benthamiana* plants transiently expressing the *Bc-DCL1/2*-RNAi construct. The level of *Bc-DCL1/2*-sRNA in 4 μ g (half of the amount applied in Fig. 5) and 400 ng of total RNAs from *N. benthamiana* plants expressing the *Bc-DCL1/2*-RNAi construct were examined by Northern blot analysis. 5 ng, 20 ng, 100 ng, and 400 ng (the amount applied in Fig. 4) of *in vitro* synthesized *Bc-DCL1/2*-sRNAs were used as controls.

a

Alignment of selected *Vd-DCL1* and *Vd-DCL2* RNAi fragments with the four *Arabidopsis DCLs*

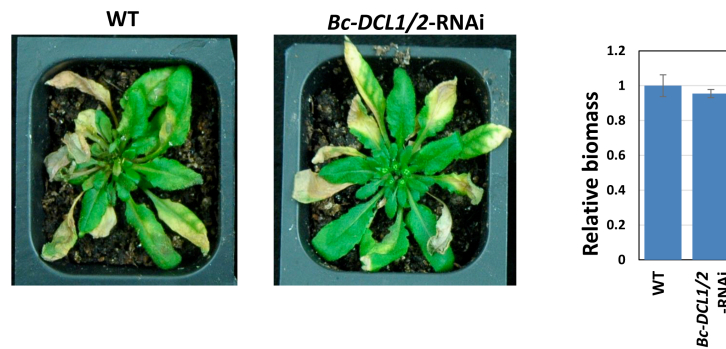
Vd-DCL1	TGCTCATGACCACAGTC-ATGAGTTCTCACCGAATGAAGACCCAGGGTCACTGATCGACT	Vd-DCL2	AGA--AGGTCAACG-----ATCTACTGCCACAGGCGACAACANGAAGCCATATGC
At-DCL3	TAGCTTCGAGTGCACAAATTGATTGGTCAGCT-ATCAA--CTCCT--GTGCCTCAATAGT	At-DCL3	AAAAATGACTATCAAAATAGGCAAGTCTTGGCAGATGGGTCTATAGA-TGGGTAGTTTCA
At-DCL1	TGCTTTCA-AGGGATCACTTGATTTACAGAA-AACCA--GCTATCATCTCTAAAAAAGT	At-DCL1	AAATTTGATTAGTT-----AT-----ATTTCCACATCA
At-DCL2	TGGATTGCATCTCAGAG-ATGGGGTGGCAC-----TTGAT-----TATCTACTAGT	At-DCL2	ATACGTTGCTAGTA-----GGAGGA-----ATCTGAAAGCG
At-DCL4	TCCTTGGAACTGCAAGACTCTCTAGAACAA-GCAAA--TCCACCTTCACTCTCTCT	At-DCL4	ATACTGGTGAATC-----AGATGTAG--CAAAGGCCATCAT-TGGTTGTACAAG
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Vd-DCL1	GGAGCCATCTGCTGTCGACCAAGAGGTTGAGTACTTGCCCTGGGATGAAGATCACAGTC	Vd-DCL2	TAGGGTTTATCCGGCAC-----CGTGGCGAAAAGTGCACACGATTCCATTAGATCATGCT
At-DCL3	TGAGTTCTGAAGAAAAATCTCTTTGATCTTCGGGATAGTGATGG--G-AATCAGTG	At-DCL3	AAATCTGTATCAGATTGCG--CTGAGGCCCTGATTGGTCCATTATGTAAAGCGGTGGA
At-DCL1	TTTCAT--GTGAGGTTAATGAGTAT--CGTGTGGATGTTGATGTTG--A--ACCCCTC	At-DCL1	AAGATTCTTGAAGCCTTGACTGCCGCTCTGTCAGGAAAGCTTCTGCTACGAGGAGCT
At-DCL2	TCATCCCACTCATTACATGAACA---TCTCTTATTGATTGGGAAGT--GATCA-GATC	At-DCL2	AAGAGTGTGGCCGATGTTG---TAGAATCATTAAATGGAGCATATCTCAGCAGGGAGGT
At-DCL4	TCCACTCTGCTGCATGATGGAGAA---AGTGTATATCTGTAGATTG--GGTGACTATC	At-DCL4	AAAAAATTGCTGATGTTGG---TTGAGGCTCTGTGGAGCTTCTTATGTTGACAGTGGC
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Vd-DCL1	CCAGCTT-----CTATCAAAGCAAGT-----TTGT	Vd-DCL2	TACTT-----TGGGGCTTGTCCCTTTCATTTCACTTCA-CATTG
At-DCL3	CAATACCTCATCCGGTCAGGAAGTCTTACTAGACGATAAAATGGAAAGCAAGTCTGAT	At-DCL3	TTGTCTGCTTCTCTCATATGATGAATGGCTGGTATTGACGTGGATTTTGGCCAAAC
At-DCL1	CACGACA-----CCATGGGA-TCCCTGCAAGGCCCTACCT--GTTTGT-----	At-DCL1	GAGCTTTAGGAGATGCGTATCTAAAATGGGTGATGCTGTTTCTGTTTCTCAAGTAT
At-DCL2	CGTGAATCTAACTTCTCATGAGGTTTTGGAAAAACACGAAAA-----	At-DCL2	GAACCTGCGCTTTGATGTTCTGAAATGGGTGGAAATAAAGTGCATTTACA--ACT
At-DCL4	AGAAACT--GCTGTATCACCATCTTTAAGACTCCATCTGTTTTAGTGGAA---GA	At-DCL4	TTCAAAGTGCTGTGAAATTTCTGAAAGTGGATTGGTGAATGTTGATTTGAA--TCC
	* * * * *		*** * * * *
Vd-DCL1	GATTGATCCATACAGGGATCGCGCAAG-----CTGTTTCTCAGAGGTATT	Vd-DCL2	TCG--AGGTTGCACTGGTTGCAGAACAGC-----TTTCTCGAGC-----
At-DCL3	TCATTTTGCCAATGCTCTGCTGATAAAA--ATAGTCTCGAAGAAGTGTGG-----	At-DCL3	CTAGTCGTTGA--AGCCATCAATAGAGT---TTCTACGGTGTGTACATTTCTAAAGAA
At-DCL1	CCCTGTACTGACAATACGTC---TATGGAAACCCATAAAAGGGATCAACTGGGAATTGGT	At-DCL1	CCTCAAAGCACAGGGGTGAGTTCAGAGGATGAGGCAACAATGGTTAGTAAATGTT
At-DCL2	TTGTTTACCAACGGTCTCTCGCATTCTACACACAAAAGACGGCTTGTTT-----	At-DCL2	ACGAAGATCCAGAGAGATTCCCAATACA-----AGCAGAGAAGCTGTGAATGAGGT
At-DCL4	TATATTTCTCTCTCGGGCTCTCATTAAAGCTAGCAA--ATGGCTGCTGG-----	At-DCL4	TTGCAAGTCAAGATGCTGTTGATTGCAAGCAGGGGCTACTTCCCTCTCACTCTGCAAT
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b

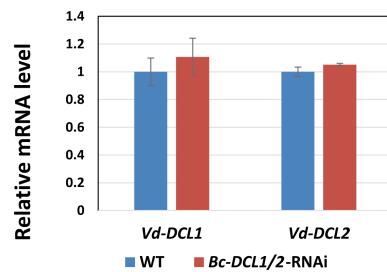


Supplementary Fig 8. Selected *Vd-DCL1* and *Vd-DCL2* RNAi fragments did not target any *Arabidopsis DCLs*. (a) The selected RNAi fragments from *Vd-DCL1* (156 bp) and *Vd-DCL2* (156 bp) were aligned against the four *Arabidopsis DCL* genes. The selected RNAi fragments were marked (yellow), and asterisks represent the selected area among these 5 genes. (b) The relative expression levels of *Arabidopsis DCL* genes (*At-DCL1*, *At-DCL2*, *At-DCL3*, and *At-DCL4*) were measured by quantitative RT-PCR in *Arabidopsis Bc+Vd-DCLs-RNAi* plants. *Arabidopsis* WT was used as a control.

a



b



Supplementary Fig 9. *Arabidopsis* Bc-DCL1/2-RNAi plants were not resistant to *V. dahlia*. (a) 2-week-old *Arabidopsis* Bc-DCL1/2-RNAi transgenic plants and WT plants were infected with *V. dahliae*, and the disease symptoms were recorded 3-week post-inoculation. The relative biomass was measured by quantitative PCR, and error bars indicate the standard deviation SD of three technical replicates. (b) The relative mRNA levels of *Vd-DCL1* and *Vd-DCL2* in the *V. dahliae* infected Bc-DCL1/2-RNAi plants and WT plants were measured by quantitative RT-PCR.

Supplementary Table legends:

Supplementary Table 1: The normalized read counts of previously predicted Bc-sRNA effector candidates in *B. cinerea* WT and *dcl1 dcl2* strains.

Supplementary Table 2: At-AGO1-associated *Vd*-sRNA effector candidates and their targets.

Supplementary Table 3: At-AGO2-associated *Vd*-sRNAs and their targets.

Supplementary Table 4: The list of primers and oligoes used in the manuscript.

References

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