

Table S1: Primers used in this study.

Primer Name	Sequence	Note
F1-DCL1	AAAAACTAGTCTGGGCCCCGT	Dcl1 KO
F1-DCL1-nested	GGCTGGAGCATTTCACATTGG	Dcl1 KO
F2-DCL1	ACCCAATTCGCCCTATAGTGAGTCGTATGAACAGACGATGGCGGAC	Dcl1 KO
F3-DCL1	AAGCCTACAGGACACACATTCATCGTAGGTATTATACCACACCGGGAGAAGC	Dcl1 KO
F4-DCL1	GTGGTGGGGGAATCAGTTGT	Dcl1 KO
F4-DCL1-nested	CAAACCACCGGAGAATGCG	Dcl1 KO
F1-DCL2	GGCATGCCCCGTTTGTATTT	Dcl2 KO
F1-DCL2-nested	GGGGCCCCCTTTATTGTTCA	Dcl2 KO
F2-DCL2	ACCCAATTCGCCCTATAGTGAGTCGTTTCCGGGTGCAGTTATCCAT	Dcl2 KO
F3-DCL2	AAGCCTACAGGACACACATTCATCGTAGGTAGTTACTGGATATATATATCA	Dcl2 KO
F4-DCL2	TTCGGCTTGTA CTGTCCACC	Dcl2 KO
YG-F	CGTTGCAAGACCTGCCTGAA	All KO
YG-F-nested	CGATTGCTGATCCCCATGTG	All KO
P <sub>trpC</sub> -F	ACGACTCACTATAGGGCGAATTGGGT	All KO
T <sub>trpC</sub> -R	TACCTACGATGAATGTGTGTCCTGTAGGCTT	All KO
HY-R	GGATGCCTCCGCTCGAAGTA	All KO
HY-R-nested	GATGTTGGCGACCTCGTATT	All KO
F2-G418-DCL1	GAAGGGCGAATTCCACAGTGATGAACAGACGATGGCGGAC	Double DCL KO

F3-G418-DCL1	ACTGGCCGTCGTTTTACAACCTTATACCACACCCGGGAGAAGC	Double DCL KO
1F	CACATTCGAATTCAGACTAGTTCTGGTATA	SsHADV1 cloning
1R	CCCCCAGGGGGCCCCTC	SsHADV1 cloning
2F	CCATGTGGGGAGGGGCC	SsHADV1 cloning
2R	GAGTGGGAACTCGGCCGTCT	SsHADV1 cloning
3F	CCCCTTAGAATCAGACGGC	SsHADV1 cloning
3R	GGGTATACCAGAACTAGTCTGAATTC	SsHADV1 cloning, inverse PCR
2.2merRev	CTATCATTACGTCAGCGTCG	SsHADV1 inverse PCR
33F	CTGGTTTCATCGCAGGGTAT	SsHADV1 cloning- second copy
SV2F	GGACTAGTTCTGGTATACCCTGGTTTCATCG	SsHADV1 cloning- second copy
3R'-NotI	GAGCGGCCCGCCGTAGCCGTGTGAGAAGACA	SsHADV1 cloning- second copy
F1-Not1-Dcl2	TAACGAGCGGCCGCGGCAGACGGAAGATTATGGA	Dcl2 complementation
F4-SmaI-Dcl2	GAATCACCCGGGTTTCGGCTTGTACTGTCCAC	Dcl2 complementation
F1-SacI-Dcl1	TACTCAGAGCTCCATGTCTTCCGAACCACTT	Dcl1 complementation
F4-Not1-Dcl1	TTACTGCGGCCGCTTGCCCTAAATCTGCAATCC	Dcl1 complementation

Table S2: Primers and expected sizes of nested PCR reactions performed to confirm monokaryotic deletion of dicer genes with the corresponding gel image in Figure S1.

<b>Lane</b>	<b>Primers</b>	<b>Sample</b>	<b>Expected Size</b>
<b>1.</b>	<b>F1-qPCR_R(DCL1)</b>	<b>Wtdk3- PCR 1</b>	<b>1.3kb</b>
2.	F1 DCL1-HY	Wtdk3- PCR 1	No Amp (1.8kb)
3.	YG-F4 DCL1	Wtdk3- PCR 1	No Amp (1.7kb)
<b>4.</b>	<b>F1-qPCR_R(DCL1)</b>	<b><math>\Delta</math>Dcl1- PCR 2</b>	<b>No Amp (1.3kb)</b>
5.	F1 DCL1-HY	$\Delta$ Dcl1- PCR 2	1.8kb
6.	YG-F4 DCL1	$\Delta$ Dcl1- PCR 2	1.7kb
<b>7.</b>	<b>F1-qPCR_R(DCL2)</b>	<b>Wtdk3- PCR 3</b>	<b>3.4kb</b>
8.	F1 DCL2-HY	Wtdk3- PCR 3	No Amp (1.9kb)
9.	YG-F4 DCL2	Wtdk3- PCR 3	No Amp (2.1kb)
<b>10.</b>	<b>F1-qPCR_R(DCL2)</b>	<b><math>\Delta</math>Dcl2- PCR 4</b>	<b>No Amp (3.4kb)</b>
11.	F1 DCL1-HY	$\Delta$ Dcl2- PCR 4	1.9kb
12.	YG-F4 DCL1	$\Delta$ Dcl2- PCR 4	2.1kb
<b>13.</b>	<b>F1-qPCR_R(DCL1)</b>	<b><math>\Delta</math>Dcl1/2- PCR 5</b>	<b>No Amp (1.3kb)</b>
14.	F1 DCL1-PtrpCRev_G418	$\Delta$ Dcl1/2- PCR 5	741kb
15.	F4 DCL1-TtrpCF_G418	$\Delta$ Dcl1/2- PCR 5	996kb
<b>16.</b>	<b>F1-qPCR_R(DCL2)</b>	<b><math>\Delta</math>Dcl1/2- PCR 6</b>	<b>No Amp (3.4kb)</b>
17.	F1 DCL2-HY	$\Delta$ Dcl1/2- PCR 6	1.9kb
18.	YG-F4 DCL2	$\Delta$ Dcl1/2- PCR 6	2.1kb

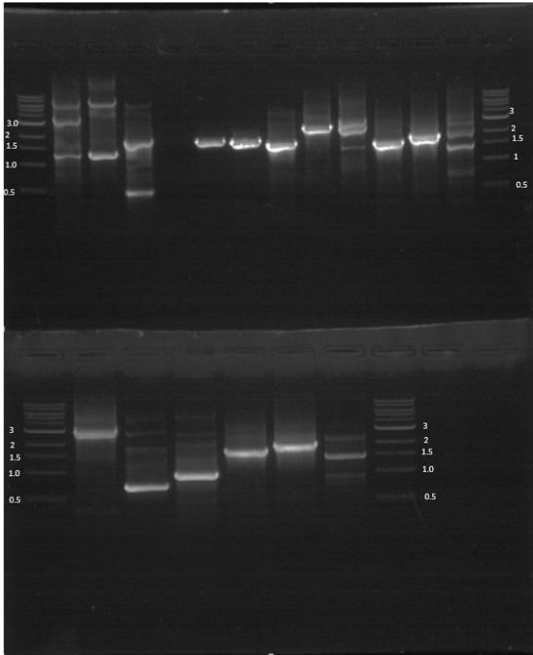


Figure S1: Electrophoresis gel image to confirm monoclonal deletion of dicer genes by nested PCR. Lanes as indicated in table S1. Lane 1~12 from right to left on the upper half of the agarose gel image. Lane 13~18 from right to left on the lower half of the agarose gel image. 1 kb ladders are shown on the farthest right and left lanes with corresponding size labelled.

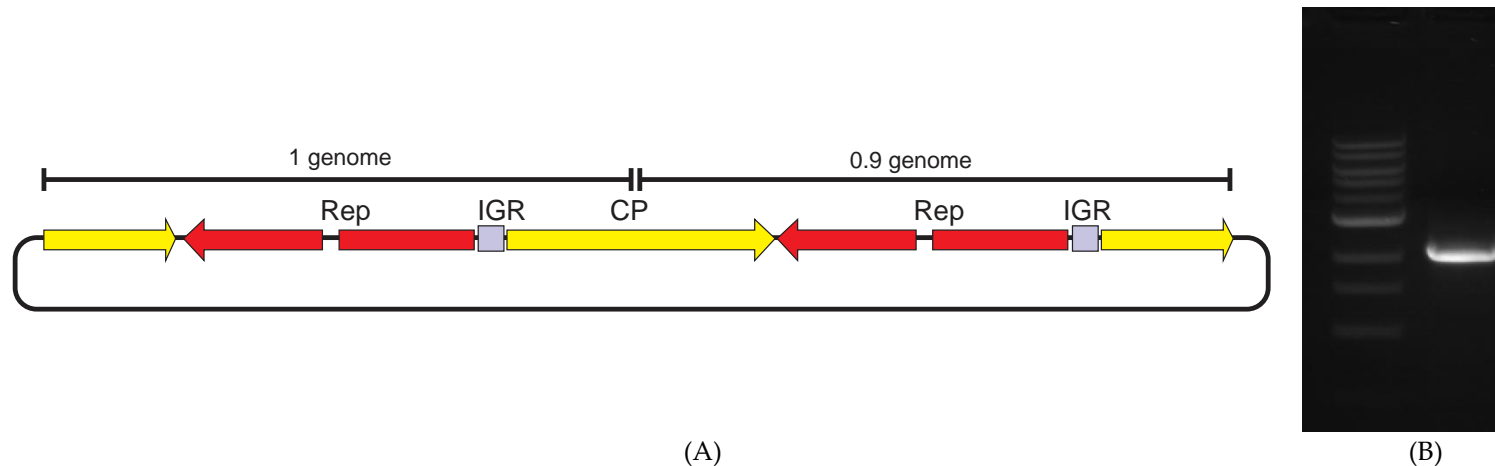


Figure S2: (A) Plasmid construct to test the infectivity of SsHADV-1; (B) Inverse PCR product amplified using SsHADV-1-specific primers to confirm the infectivity of the infectious clone from a >6 times transferred culture. A 2.2 kb band was amplified and confirmed by Sanger sequencing. The product shows the dimer clone has recombined circular template of SsHADV-1 genome and demonstrates the infectivity of the infectious clone assembled from synthetic DNA. Left Lane: 1kb ladder. Right Lane: 2.2 kb amplicon.

### **Procedure used in this study to make a 1.9mer clone of SsHADV01:**

To make a 1.9mer clone of *Sclerotinia sclerotiorum* hypovirulence-associated DNA virus 1 (SsHADV-1):

Start with a 1mer clone, sequenced as laid out in the first fasta file.

Use a primer set of 33F and 3R to amplify the genome by PCR using SV1 PCR product.

Clone it to pJET1.2 to make construct 1 – Sanger sequencing to confirm the orientation.

Amplify a second PCR product with SV2F and 3R'-NotI, and digest with SpeI and NotI.

Digest construct 1 with SpeI and NotI (NotI site is on the vector pJET1.2).

Ligate digested construct 1 and digested PCR product to make construct 2 – Sanger sequencing to confirm the orientation.

Primers used:

33F: CTGGTTTCATCGCAGGGTAT

3R: GGGTATAACCAGAACTAGTCTGAATTC

SV2F: GGACTAGTTCTGGTATAACCCTGGTTTCATCG

3R'-NotI: GAGCGGCCGCGTAGCCGTGTGAGAAGACA

>SsHADV-1\_1 mer\_clone

AGTTCTGGTATACCCTGGTTTCATCGCAGGGTATGTTTTACTTGGAAAGGTTTAGGGCCTTTCAACGTTATGGCAACAGAGTCTCCAACCTTCTGAT  
TTCCCTTGGTTACCTTATCAGGACTCCAGTAATGGAGTTCAACGTCTCTTTTTAATGAGAACGTCAACAACATGCCTCAGACAATCAACCGCCA  
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> SsHADV-1\_1.9mer

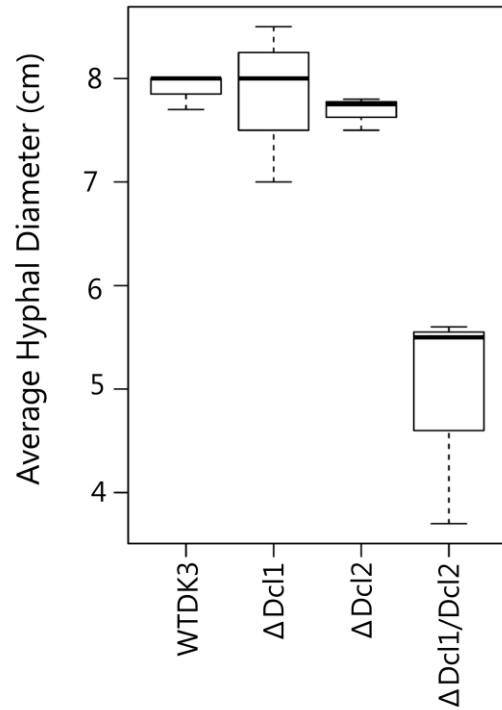
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CCTCAATGTCAAAGCGGAGCATCCTCAACACGACATCCCGAAAAAAACGTAATGGAATGCTTACGTTCTCCAACACTACCAACAGTGGGGCTAG  
TACAGCTCCAGCCGCTGCTCCCGTTTACGTAAATGCAGTTCTTGGCGGCACATTTATTTGGTGCCCTACTGCAATGAACC



**Statistics associated with the growth and leaf assay on Figure 2.**

**Figure 2A:** Average hyphal diameter 72h post inoculation



Bio.Reps	WTDK3	ΔDcl1	ΔDcl2	ΔDcl1/Dcl2
1.	8	8.5	7.75	3.7
2.	7.7	7	7.5	5.5
3.	8	8	7.8	5.6
<b>Average</b>	<b>7.9</b>	<b>7.8</b>	<b>7.7</b>	<b>4.9</b>

```
> t.test(WTDK3, Dcl2)
```

```
Welch Two Sample t-test
```

```
data: WTDK3 and Dcl2
```

```
t = 1.5882, df = 3.9778, p-value = 0.1878
```

```
alternative hypothesis: true difference in means is not equal to 0
```

```
95 percent confidence interval:
```

```
-0.1629364 0.5962697
```

```
sample estimates:  
mean of x mean of y  
7.900000 7.683333
```

```
> t.test(WTDK3, Dc11)
```

```
Welch Two Sample t-test
```

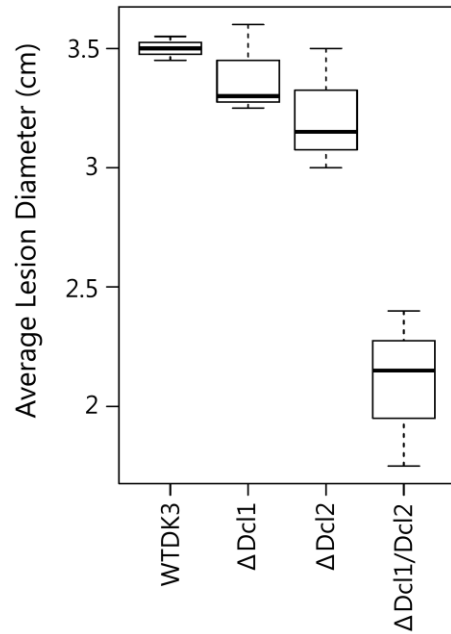
```
data: WTDK3 and Dc11  
t = 0.14744, df = 2.2052, p-value = 0.8952  
alternative hypothesis: true difference in means is not equal to 0  
95 percent confidence interval:  
-1.715233 1.848567  
sample estimates:  
mean of x mean of y  
7.900000 7.833333
```

```
> t.test(WTDK3, `Dc11/Dc12`)
```

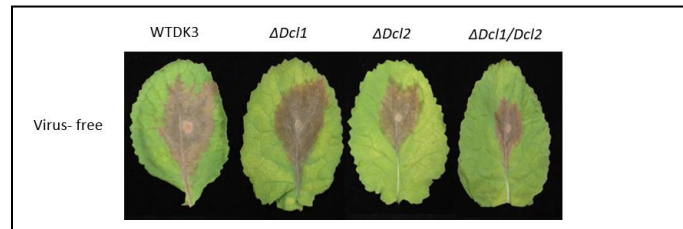
```
Welch Two Sample t-test
```

```
data: WTDK3 and Dc11/Dc12  
t = 4.7437, df = 2.1049, p-value = 0.0377  
alternative hypothesis: true difference in means is not equal to 0  
95 percent confidence interval:  
0.4003276 5.5330057  
sample estimates:  
mean of x mean of y  
7.900000 4.933333
```

**Figure 2B:** Average lesion diameter 72h post inoculation on CANOLA



Bio.Reps	WTDK3	$\Delta Dcl1$	$\Delta Dcl2$	$\Delta Dcl1/Dcl2$
<b>1.</b>	3.5	3.25	3.15	2.4
<b>2.</b>	3.45	3.6	3	1.75
<b>3.</b>	3.55	3.3	3.5	2.15
<b>Average</b>	<b>3.50</b>	<b>3.38</b>	<b>3.22</b>	<b>2.10</b>



```
> t.test(WTDK3, Dcl2)
```

```
Welch Two Sample t-test
```

```
data: WTDK3 and Dcl2
```

```
t = 1.8773, df = 2.1517, p-value = 0.1922
```

```
alternative hypothesis: true difference in means is not equal to 0
```

```
95 percent confidence interval:
```

```
-0.3240827 0.8907493  
sample estimates:  
mean of x mean of y  
3.500000 3.216667
```

```
> t.test(WTDK3, Dc11)
```

```
welch Two Sample t-test
```

```
data: WTDK3 and Dc11  
t = 1.0321, df = 2.2777, p-value = 0.399  
alternative hypothesis: true difference in means is not equal to 0  
95 percent confidence interval:  
-0.3170668 0.5504002  
sample estimates:  
mean of x mean of y  
3.500000 3.383333
```

```
> t.test(WTDK3, `Dc11/Dc12`)
```

```
welch Two Sample t-test
```

```
data: WTDK3 and Dc11/Dc12  
t = 7.3113, df = 2.093, p-value = 0.01605  
alternative hypothesis: true difference in means is not equal to 0  
95 percent confidence interval:  
0.610202 2.189798  
sample estimates:  
mean of x mean of y  
3.5 2.1
```