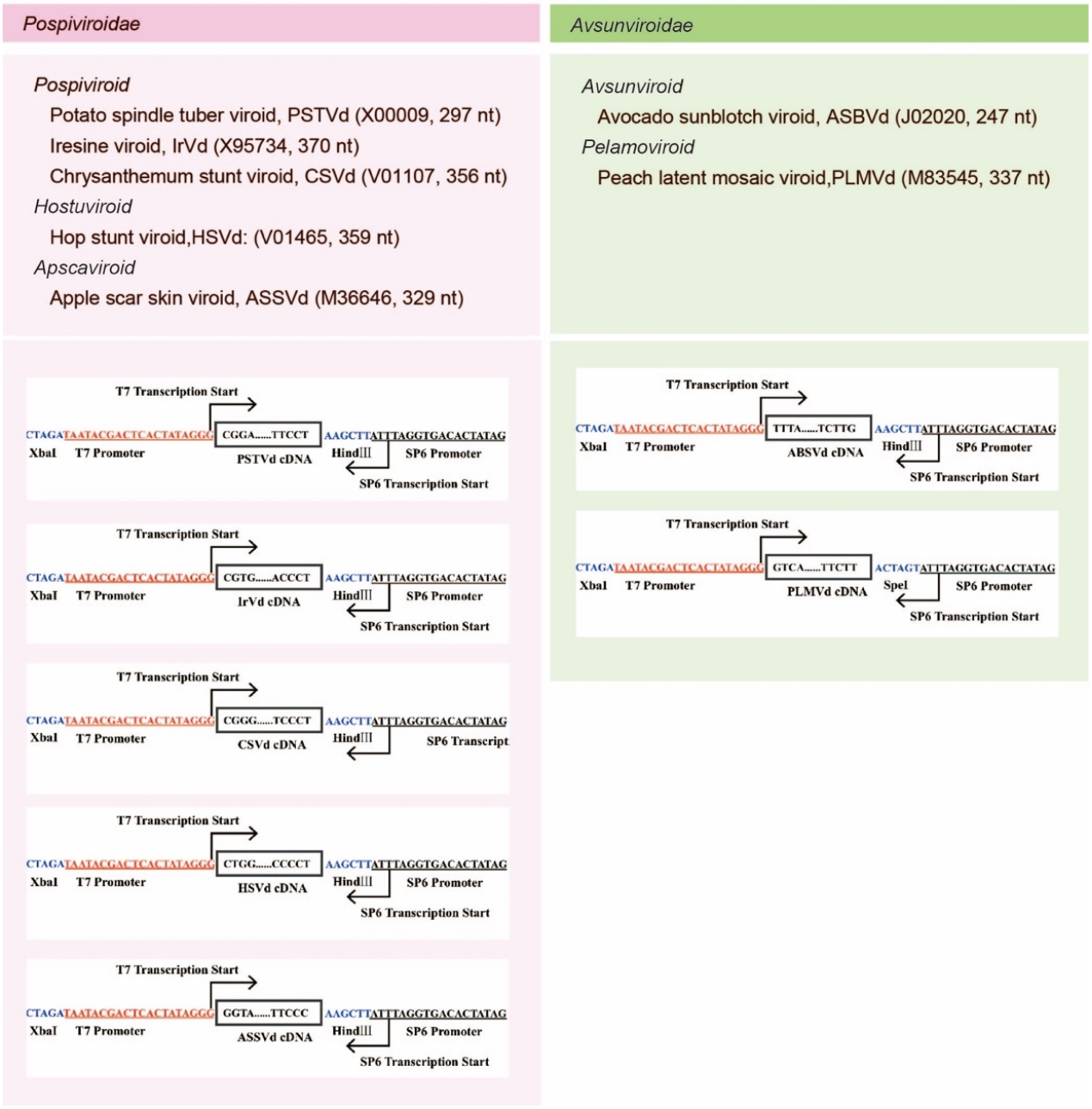
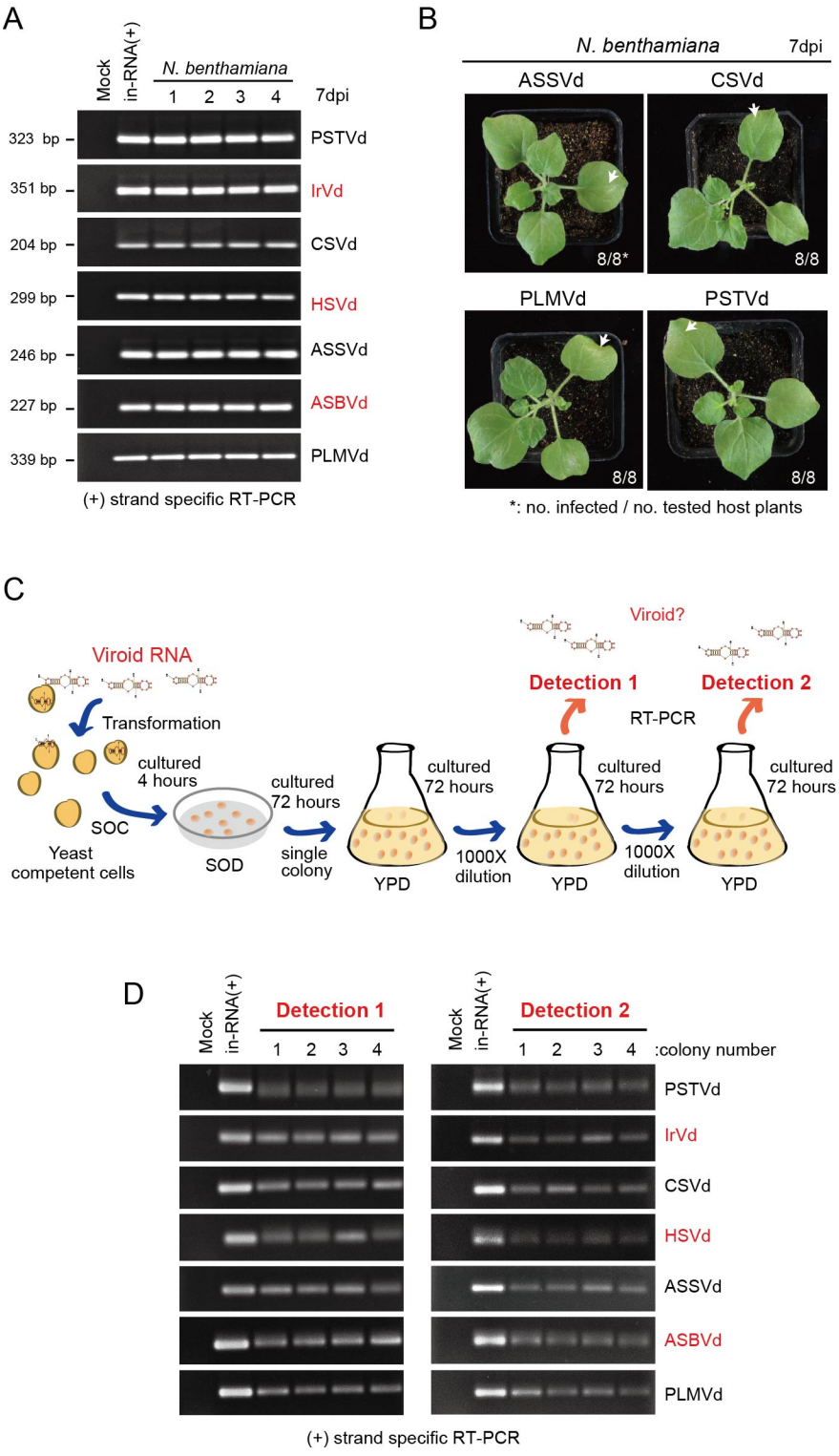
Supplementary information

Supplementary figures

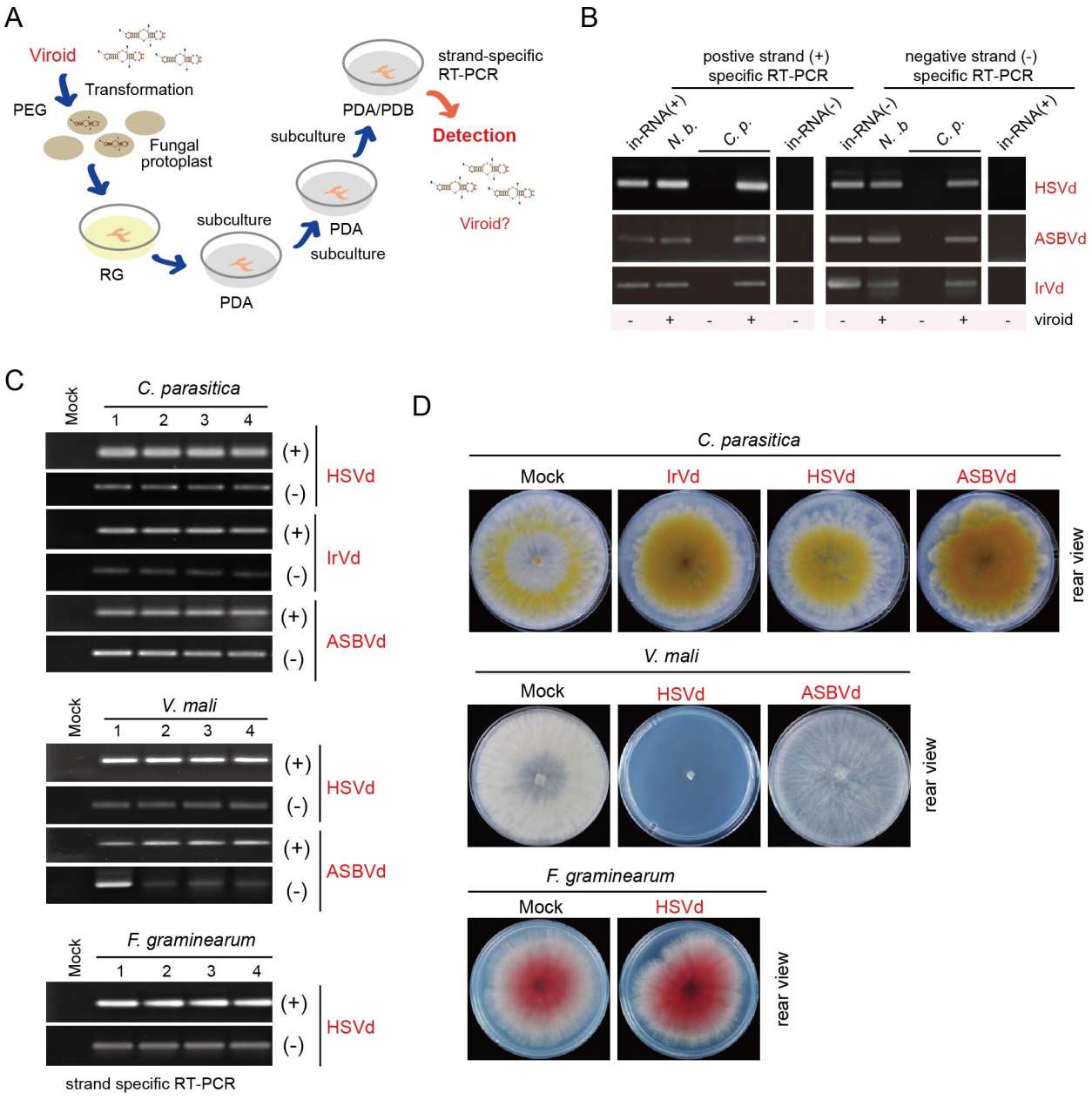


Supp. Figure S1. Sequence constructs for generating plus (+) strand *in-vitro* transcripts of viroid cDNA clones.



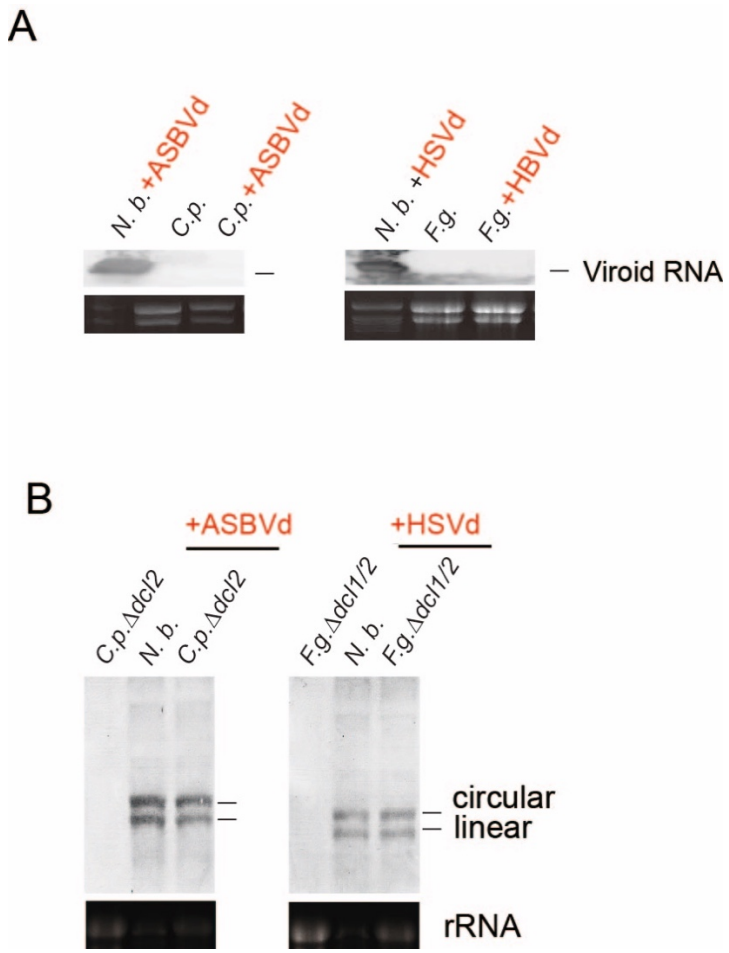
Supp. Figure S2. Viroid infection in plants and yeast. (A) RT-PCR detection of viroid RNA accumulation in systemic uninoculated leaves of *N. benthamiana* plants mechanically inoculated

with *in-vitro* transcripts of viroid cDNA clones. (B) *N. benthamiana* plants infected with viroids. (C) Experimental procedure for inoculating *in-vitro* transcripts of viroid cDNA clones to yeast. (D) RT-PCR detection of viroid RNA accumulation in yeast. Each lane represents RNA sample derived from single independent yeast colony. In-RNA, in vitro RNA transcripts of viroid cDNA clones.

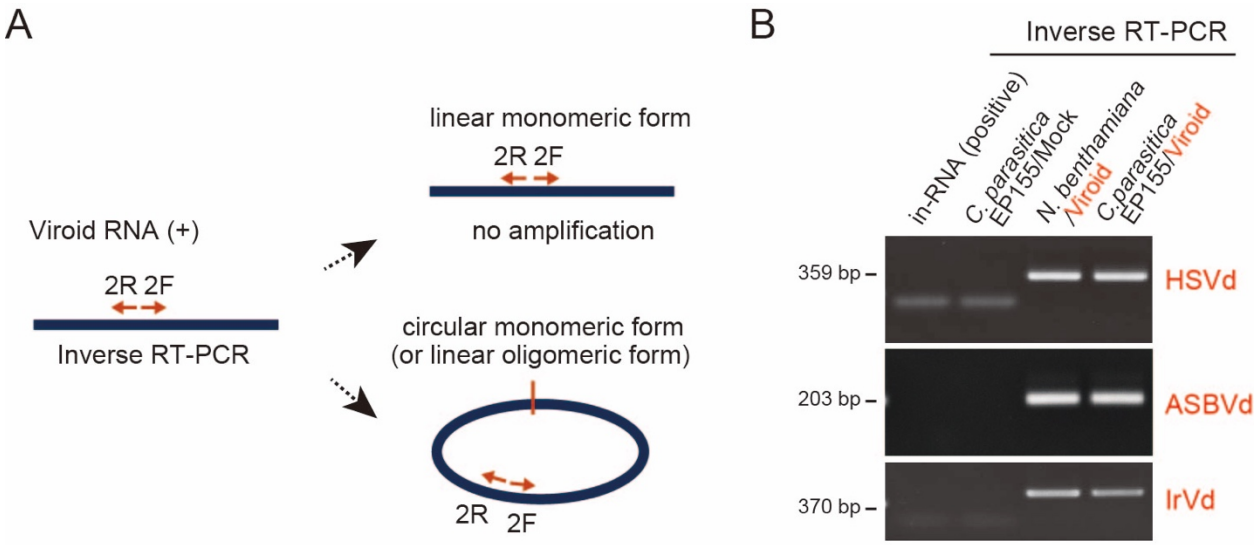


Supp. Figure S3. Viroid infection in filamentous fungi. (A) Experimental procedure for inoculating *in-vitro* transcripts of viroid cDNA clones to filamentous fungi. (B) Confirmation of strand specificity of RT-PCR detection. In-RNA: plus (+) or minus (-) strand *in vitro* transcripts of viroid cDNAs were included to confirm strand-specific amplification. (C) RT-PCR detection of viroid RNA accumulation in fungal isolates regenerated from fungal spheroplasts that have been transfected with *in-vitro* transcripts of viroid cDNA clones. (D) Phenotypic growth of fungi

infected with viroids. Fungi were grown on PDA medium (10 cm plate) for 3 (*V. mali* and *F. graminearum*) or 5 (*C. parasitica*) days and photographed.

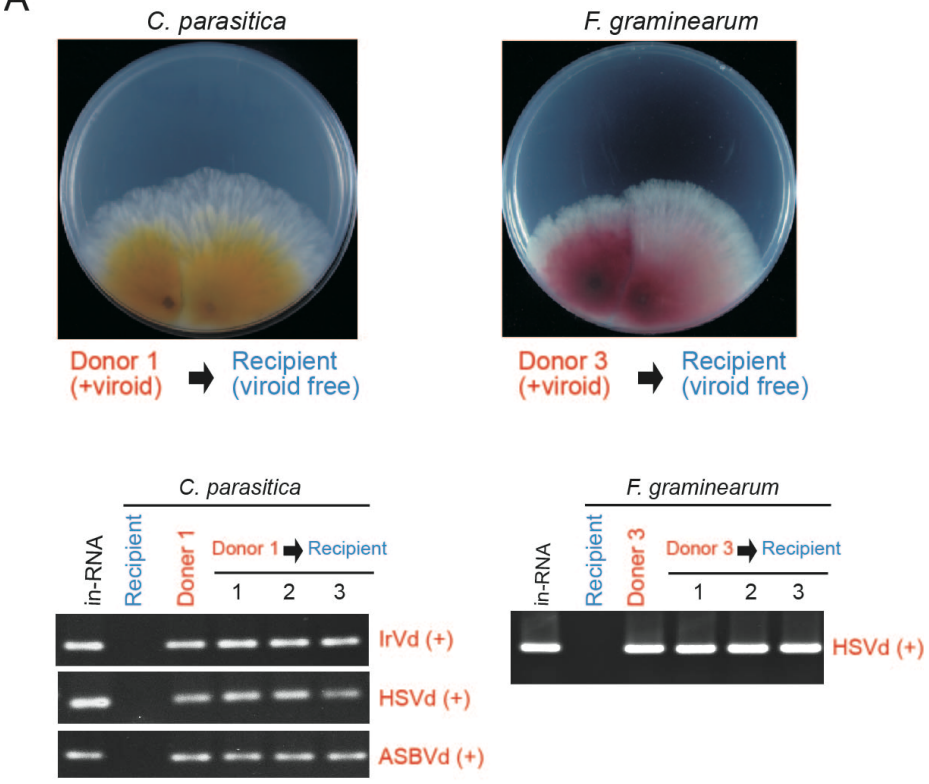


Supp. Figure S4. Detection of viroid RNAs in plants and fungi by via RNA blotting. (A) Total RNAs were separated on denaturing agarose gel (2%). (B) Total RNAs were separated on denaturing PAGE (7.5%). Viroid RNAs were detected via Dig-labelled DNA probes.



Supp. Figure S5. Detection of the circular (and/or linear oligomeric) form of (+)-genomic viroid RNAs via inverse RT-PCR. (A) Diagram of inverse RT-PCR, which can detect circular (and/or linear oligomeric) viroid RNAs. (B) inverse RT-PCR detection of circular (and/or linear oligomer) form of viroid RNAs in plant and fungal samples.

A

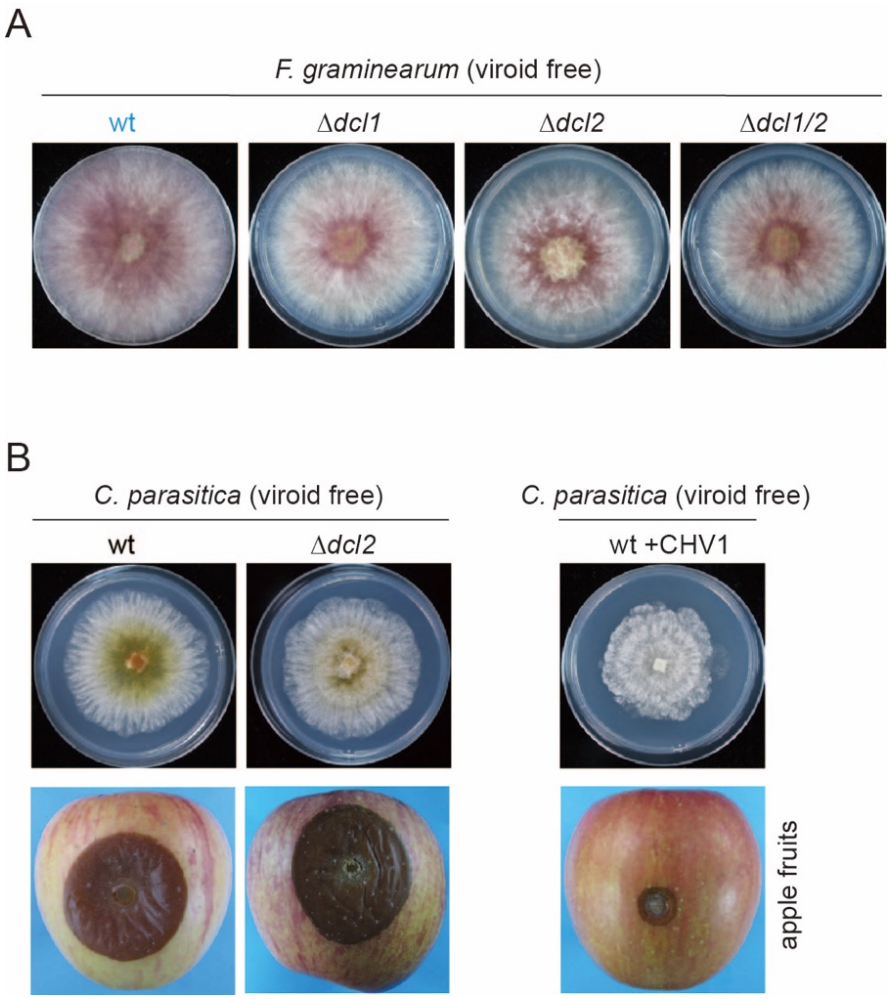


B

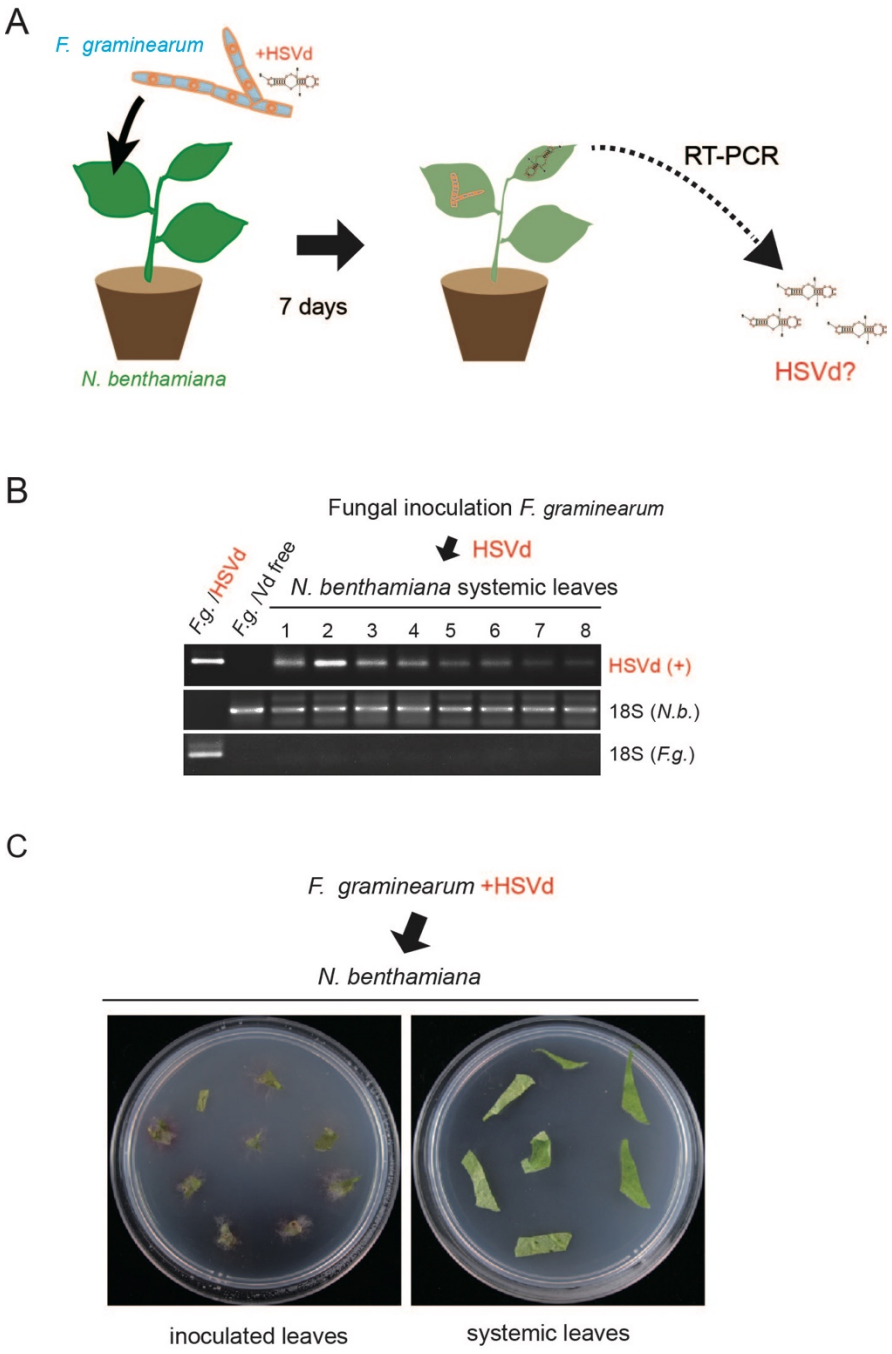
Efficiency of viroid transmission through conidia

viroid	host strain	no. infected/ no. tested spore germlins	total	% (mean ±SD)
ASBVd	*C. p.*	8/8, 7/8, 6/8,	21/24	87.5% ±0.10
	C. p. Δ*dcl2*	8/8, 8/8, 7/8	23/24	95.8% ±0.06
HSVd	*C. p.*	7/8, 5/8, 5/8	17/24	70.8% ±0.12
	C. p. Δ*dcl2*	5/8, 6/8, 6/8	17/24	70.8% ±0.06
IrVd	*C. p.*	6/8, 5/8, 6/8	17/24	70.8% ±0.06
ASBVd	*V. m.*	8/8, 7/8, 6/8	21/24	87.5% ±0.10
HSVd	*F. g.*	5/10, 6/10 , 5/10	16/30	53.3% ±0.03
	F. g. Δ*dcl1*	8/10, 7/10, 7/10	22/30	73.3% ±0.04
	F. g. Δ*dcl2*	8/10, 8/10, 5/10	21/30	70.0% ±0.04
	F. g. Δ*dcl1/2*	13/15, 11/15, 13/15	37/45	82.2% ±0.04

Supp. Figure S6. Horizontal and vertical transmission of viroids in filamentous fungi. (A) RT-PCR detection confirmed horizontal transmission of viroids through hypal anastomosis. (B) Efficiency of viroid transmission through conidia.

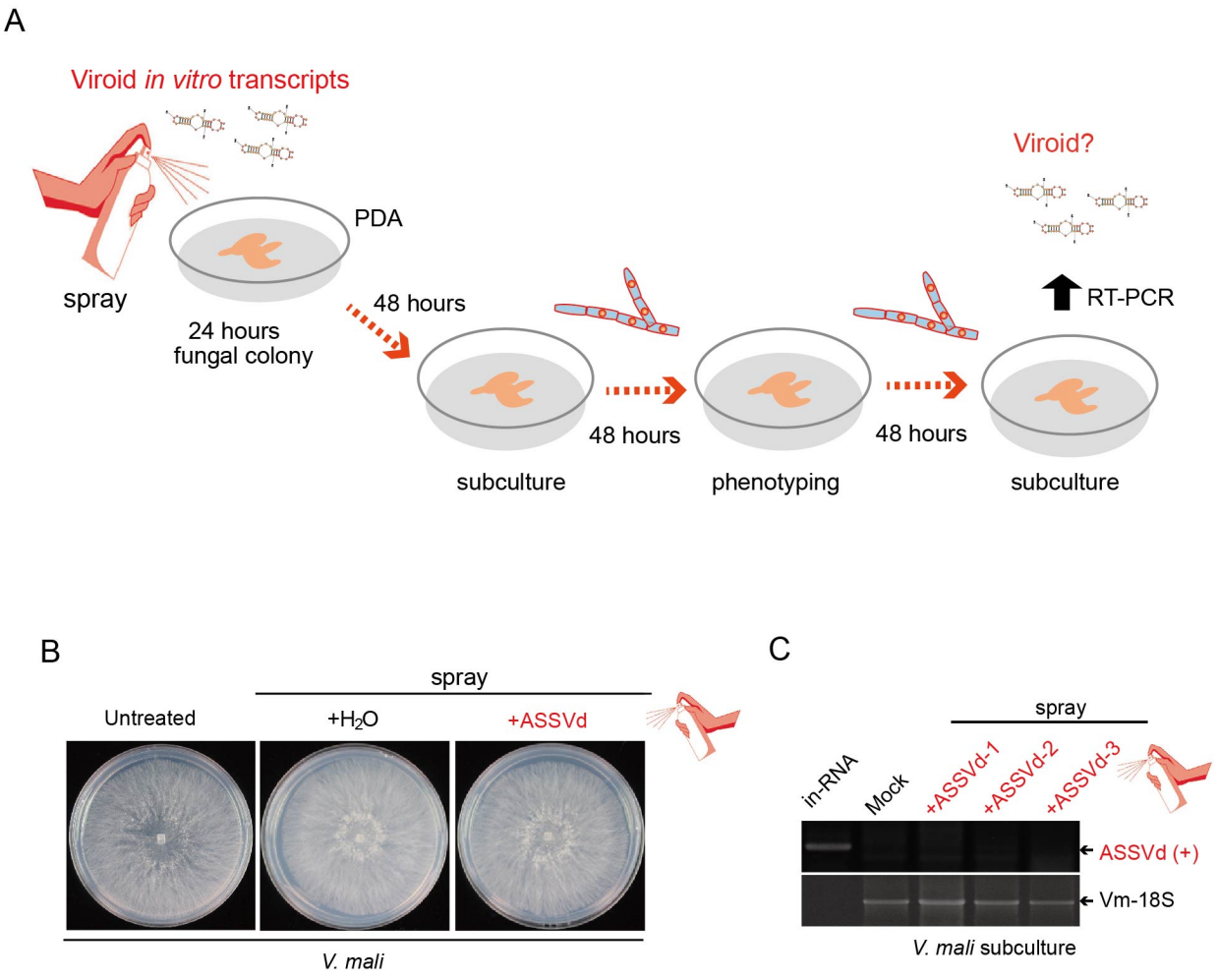


Supp. Figure S7. Phenotypic growth and virulence of viroid-free wild-type and *dcl2* mutants of fungi. (A) Phenotypic growth of viroid-free wild-type and *dcl2* mutants of *F. graminearum*. (B) Phenotypic growth and virulence on apple fruits of viroid-free wild-type and *dcl2* mutants of *C. parasitica*.



Supp. Figure S8. Transmission of HSVd from *F. graminearum* to the plant. (A) Experimental procedure for viroid transmission from *F. graminearum* to the plants. (B) RT-PCR detection of HSVd in uninoculated, systemic leaves of *N. benthamiana* plants inoculated with *F. graminearum* carrying HSVd. (C) The portions of systemic upper leaves were placed on medium to confirm the absence of *F. graminearum*.

Fig. S9



Supp. Figure S9. Exogenous inoculation of fungal mycelia with viroid RNAs. (A) Experimental procedure for direct application (spray) of *in-vitro* transcripts of viroid cDNA clones to fungal mycelia. (B) Phenotypic growth of *V. mali* treated with ASSVd RNAs (incompatible host). (C) RT-PCR analysis confirmed the absence of ASSVd RNA in treated fungi shown in B (three independent fungal subcultures).

Table S1. A list of primers used in this study.

Primer Name	Oligonucleotide sequence (5′- 3′)	Usage
107-T7-F	gtattgggatggaacggtaccTCTAGATAATACGACTCACTATAGGGCGGGACTTACTTGTGGTTCCTG	For CSVd infectious clone (Acc.no. V01107.1) (398 bp)
107-SP6-R	ccattgggatggaacggtaccCTATAGTGTCACCTAAATAAGCTTAGGGAACAATACTAAGGTTCCAAGG	
646-T7-F	gtattgggatggaacggtaccTCTAGATAATACGACTCACTATAGGGGGTAAACACCGTGCGGTTCC	For ASSVd infectious clone (Acc.no. M36646.1) (371 bp)
646-SP6-R	gtattgggatggaacggtaccTCTAGATAATACGACTCACTATAGGGGGTAAACACCGTGCGGTTCC	
09-T7-F	gtattgggatggaacggtaccTCTAGATAATACGACTCACTATAGGGCTGGGGAATTCTCGAGTTGCC	For HSVd infectious clone (Acc.no. X00009.1) (401 bp)
09-SP6-R	ccattgggatggaacggtaccCTATAGTGTCACCTAAATAAGCTTAGGGGCTCAAGAGAGGATCCG	
734-T7-F	gtattgggatggaacggtaccTCTAGATAATACGACTCACTATAGGGCGTGGTTCCAATGGTGCACC	For IrVd infectious clone (Acc.no. X95734.1) (412 bp)
734-SP6-R	ccattgggatggaacggtaccCTATAGTGTCACCTAAATAAGCTTAGGGTTTCCTTTAGAAGCCCAC	
465-T7-F	gtattgggatggaacggtaccTCTAGATAATACGACTCACTATAGGGCGGAACTAAACTCGTGGTTCCT	For PSTVd infectious clone (Acc.no. V01465.1) (339 bp)
465-SP6-R	ccattgggatggaacggtaccCTATAGTGTCACCTAAATAAGCTTAGGAACCAACTGCGGTTCCA	
020-T7-F	gtattgggatggaacggtaccTCTAGATAATACGACTCACTATAGGGTTTATTAGAACAAGAAGTGAGGATATGATT	For ASBVd infectious clone (Acc.no. J02020.1) (289 bp)
020-SP6-R	ccattgggatggaacggtaccCTATAGTGTCACCTAAATAAGCTTCAAGATTTTGTAAAAAAACAATGAAGATA	
545-T7-F	gtattgggatggaacggtaccTCTAGATAATACGACTCACTATAGGGGTCATAAGTTTCGTCGCATTTCAG	For PLMVd infectious clone (Acc.no. X95734.1) (379 bp)
545-SP6-R	ccattgggatggaacggtaccCTATAGTGTCACCTAAATACTAGTAAGAAGAGTTTCGTCTCATCTCAGAG	
ASBVd-6F	AGAACAAGAAGTGAGGATATGATTAAAC	For ASBVd RT-PCR (227 bp)
ASBVd-233R	ACAATGAAGATAGAGGAGTAAACC	
HSVd-2F	TGGGGAATTCTCGAGTTGCCGC	For HSVd RT-PCR (299 bp)
HSVd-301R	AGGGGCTCAAGAGAGGATCCGC	
PSTVd-5F	ACTAAACTCGTGGTTCCTGTGGTTC	For PSTVd RT-PCR (356 bp)
PSTVd-361R	AACCAACTGCGGTTCCAAGGGC	
PLMVd-2F	TCATAAGTTTCGTCGCATTTCAGC	For PLMVd RT-PCR (339 bp)
PLMVd-341R	AAGAAGAGTTTCGTCTCATCTCAGAG	
IRVd-3F	TGGTTCCAATGGTGCACCCCTGACC	For IRVd RT-PCR (351 bp)
IRVd-354R	ACCCTCGCCGCGCAGCAGAAGAATG	
CSVd-99F	AACCTGGAGGAAGTCCGACGAGA	For CSVd RT-PCR (204 bp)
CSVd-303R	GGTTGGAAAAAAAGGCGTTGAAG	
ASSVd-29F	GCCCCGCCAACGCAGAAGATAAA	For ASSVd RT-PCR (246 bp)
ASSVd-275R	AAAAGAGGGTGAGAGAACAGGGG	
IRVd-177F	AACAGGAGCTCGACTCCTTCCTTTC	For IRVd inverse RT-PCR (370 bp)
IRVd-177R	TCTTCCGCCGCGAGGAGTTCTCC	
HSVd-143F	TAGAGGCTCTGCCTTCGAAACACCATC	For HSVd inverse RT-PCR (359 bp)
HSVd-142R	CTCCAGAGCACCGCGGCCCTCTCTC	
ASBVd-115F	TGAGAGAAGGAGGAGTCGTGGTGAAC	For ASBVd inverse RT-PCR (203 bp)
ASBVd-71R	TCGGAAAGTCGGAACAGACCTGGTTTC	
Fg18S-F	AGGCAATAACAGGTCTGTGATGCC	

Fg-18S-R	TGAGCCATTCAATCGGTAGTAGCG	For *F.g.* 18s rRNA (Acc. no.AB250414) (201 bp)
Cp-18S-F	ATAACAGGTCTGTGATGCCCTTAG	For *C.p.* 18s rRNA (Acc. no.l42441.1) (187 bp)
Cp-18S-R	CTCGCTGGCTCTGTCAGTGTAG	
Vm-actin-F	GGTGGTACCACCATGTACCCTGG	For *V.m.* actin RNA (Acc. no.KC248177.1) (211 bp)
Vm-actin-R	AGAAGCACTTGCGGTGGACAATG	
Nb-18S-F	GCAAGACCGAAACTCAAAGG	For *N.b.* 18s rRNA (Acc. no. TC23401) (216 bp)
Nb-18S-R	TGTTCATATGTCAAGGGCTGG	
CpH4F	ACAGAACAGCAGAGACCACCATCC	For C.p. histone H4 RNA (Acc. no. TC23401) 510bp
CpH4R	AGGCATGCGCAGTTCCTGTAAGAG	
CpRsm22F	AGAACTTGCCCCAGCAGCCACTG	For C.p. CPNAD(P) RNA (Acc. no. 81184) 700bp
CpRsm22R	ACGAGCCGAAGCCGACGTCCTCTG	
CHV1-496F	ATGGCTCAATTAAGAAAACCCAGTC	For CHV1 *g*RNA (Acc. no. ATZ76099.1) 749 bp
CHV1-1245R	TCGGCCGCCAATCCGGGCAAG	