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Exogenous application of double-stranded RNA to reduce grapevine Pinot gris virus titre in *in vitro* grown *Vitis vinifera*

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Supplementary material

Table S1: Contents of initiation media to prepare grapevine tissue culture plantlets.

Ingredient	Amount
Murashige and Skoog (MS) Salts	2.2 g/L
Indole-3-butyric acid (IBA)	0.3 mg/L
saccharose	25 g/L
agar	7 g/L

Table S2: The GenBank accession numbers for amino acid (aa) sequences of the RNA-dependent RNA polymerase (RdRp), Movement Protein (MP) and Coat Protein (CP) for six exemplar species in the *Trichovirus* genus including *Grapevine Pinot virus, Apple chlorotic leaf spot virus, Apricot pseudo-chlorotic leaf spot virus, Cherry mottle leaf virus, Peach mosaic virus* and *Grapevine berry inner necrosis virus*.

Trichovirus species	RNA-dependent RNA polymerase (RdRp) GenBank accession number	Movement Protein (MP) GenBank accession number	Coat Protein (CP) GenBank accession number
Apple chlorotic leaf spot virus	NP_040551.1	NP_040552.1	NP_040553.1
Apricot pseudo-chlorotic leaf spot virus	YP_224130.1	YP_224131.1	YP_224132.1
Cherry mottle leaf virus	NP_062428.1	NP_062429.1	NP_062430.1
Peach mosaic virus	YP_002308565.1	YP_002308566.1	YP_002308567.1
Grapevine berry inner necrosis virus	YP_004293216.1	YP_004293217.1	YP_004293218.1
Grapevine Pinot gris virus	YP_004732978.2	YP_004732979.2	YP_004732980.2

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Table S3: The GenBank accession numbers for amino acid (aa) sequences of the RNA-dependent RNA polymerase (RdRp), Movement Protein (MP) and Coat Protein (CP) for 200 grapevine Pinot gris virus (GPGV) genome sequences and their respective country of origin.

Isolate	RdRp	MP	CP	Isolate	RdRp	MP	CP	Isolate	RdRp	MP	CP
ERR922628-GPGV	DAC84876	DAC84877	DAC84878	Beb3-GPGV2	QFG15452	QFG15453	QFG15454	CK1	WCZ55034	WCZ55035	WCZ55036
ERR922630-GPGV1	DAC84879	DAC84880	DAC84881	Beb8-GPGV	QFG15455	QFG15456	QFG15457	2.1	WCZ55037	WCZ55038	WCZ55039
ERR922630-GPGV2	DAC84882	DAC84883	DAC84884	Ma17-10-14-GPGV	QFG15458	QFG15459	QFG15460	2.12	WCZ55040	WCZ55041	WCZ55042
ERR922630-GPGV3	DAC84885	DAC84886	DAC84887	Ma17-10-18-GPGV1	QFG15461	QFG15462	QFG15463	2.17	WCZ55043	WCZ55044	WCZ55045
ERR922631-GPGV	DAC84888	DAC84889	DAC84890	Ma17-10-18-GPGV2	QFG15464	QFG15465	QFG15466	5.5	WCZ55046	WCZ55047	WCZ55048
ERR922632-GPGV1	DAC84891	DAC84892	DAC84893	Ma17-12-7-GPGV	QFG15467	QFG15468	QFG15469	5.6	WCZ55049	WCZ55050	WCZ55051
ERR922632-GPGV2	DAC84894	DAC84895	DAC84896	Ma17-2-27-GPGV	QFG15470	QFG15471	QFG15472	5.13	WCZ55052	WCZ55053	WCZ55054
ERR922633-GPGV1	DAC84897	DAC84898	DAC84899	Ma17-3-24-GPGV	QFG15473	QFG15474	QFG15475	5.14	WCZ55055	WCZ55056	WCZ55057
ERR922633-GPGV2	DAC84900	DAC84901	DAC84902	Ma17-3-26-GPGV1	QFG15476	QFG15477	QFG15478	5.17	WCZ55058	WCZ55059	WCZ55060
ERR923264-GPGV2	DAC84903	DAC84904	DAC84905	Ma17-3-26-GPGV2	QFG15479	QFG15480	QFG15481	5.21	WCZ55061	WCZ55062	WCZ55063
ERR923264-GPGV1	DAC84906	DAC84907	DAC84908	Ma17-3-27-GPGV1	QFG15482	QFG15483	QFG15484	5.22	WCZ55064	WCZ55065	WCZ55066
ERR926756-GPGV	DAC84909	DAC84910	DAC84911	Ma17-3-27-GPGV2	QFG15485	QFG15486	QFG15487	5.24	WCZ55067	WCZ55068	WCZ55069
SRR1631863-67-68-GPGV	DAC84912	DAC84913	DAC84914	Ma17-3-35-GPGV1	QFG15488	QFG15489	QFG15490	8.6	WCZ55070	WCZ55071	WCZ55072
SRR1658425-26-27-GPGV1	DAC84915	DAC84916	DAC84917	Ma17-5-36-GPGV1	QFG15491	QFG15492	QFG15493	8.7	WCZ55073	WCZ55074	WCZ55075
SRR1658425-26-27-GPGV2	DAC84918	DAC84919	DAC84920	Ma17-5-36-GPGV2	QFG15494	QFG15495	QFG15496	8.28	WCZ55076	WCZ55077	WCZ55078
SRR2120794-GPGV	DAC84921	DAC84922	DAC84923	Ma17-A2-37-GPGV	QFG15497	QFG15498	QFG15499	8.29	WCZ55079	WCZ55080	WCZ55081
SRR2845691-GPGV	DAC84924	DAC84925	DAC84926	Ma17-A4-24-GPGV1	QFG15500	QFG15501	QFG15502	8.33	WCZ55082	WCZ55083	WCZ55084
SRR3167555-GPGV	DAC84927	DAC84928	DAC84929	Ma17-A4-24-GPGV2	QFG15503	QFG15504	QFG15505	8.38	WCZ55085	WCZ55086	WCZ55087
SRR3190105-GPGV	DAC84930	DAC84931	DAC84932	Ma17-A4-25-GPGV	QFG15506	QFG15507	QFG15508	8.47	WCZ55088	WCZ55089	WCZ55090
SRR3288835-GPGV	DAC84933	DAC84934	DAC84935	Ma17-A4-25-GPGV	QFG15509	QFG15510	QFG15511	LT6	WCZ55091	WCZ55092	WCZ55093
SRR3288835-GPGV	DAC84936	DAC84937	DAC84938	Ma17-A8-12-GPGV	QFG15512	QFG15513	QFG15514	LT7	WCZ55094	WCZ55095	WCZ55096
SRR5227657-GPGV	DAC84939	DAC84940	DAC84941	Ma17-A8-20-GPGV	QFG15515	QFG15516	QFG15517	9.1	WCZ55097	WCZ55098	WCZ55099
SRR5332103-GPGV1	DAC84942	DAC84943	DAC84944	Ma17-A9-20-GPGV1	QFG15518	QFG15519	QFG15520	9.2	WCZ55100	WCZ55101	WCZ55102
SRR5332103-GPGV2	DAC84945	DAC84946	DAC84947	Ma17-A9-20-GPGV2	QFG15521	QFG15522	QFG15523	9.3	WCZ55103	WCZ55104	WCZ55105
SRR5332104-GPGV1	DAC84948	DAC84949	DAC84950	Ma17-A9-20-GPGV2	QFG15524	QFG15525	QFG15526	9.4	WCZ55106	WCZ55107	WCZ55108
SRR5332104-GPGV2	DAC84951	DAC84952	DAC84953	Ma17-A9-21-GPGV2	QFG15527	QFG15528	QFG15529	9.5	WCZ55109	WCZ55110	WCZ55111
SRR5332104-GPGV3	DAC84954	DAC84955	DAC84956	Ma17-A9-22-GPGV	QFG15530	QFG15531	QFG15532	9.6	WCZ55112	WCZ55113	WCZ55114
SRR5332107-GPGV	DAC84957	DAC84958	DAC84959	GPgV_MID38_ LYcovitis	QFG15533	QFG15534	QFG15535	9.9	WCZ55115	WCZ55116	WCZ55117
SRR5332108-GPGV	DAC84960	DAC84961	DAC84962	Pa10-GPGV	QFG15536	QFG15537	QFG15538	9.1	WCZ55118	WCZ55119	WCZ55120
SRR5457616-GPGV	DAC84963	DAC84964	DAC84965	IFV72_Vau_A224	QFG15539	QFG15540	QFG15541	9.11	WCZ55121	WCZ55122	WCZ55123
SRR5457630-GPGV	DAC84966	DAC84967	DAC84968	IP-C522-GPGV1	QFG15542	QFG15543	QFG15544	9.13	WCZ55124	WCZ55125	WCZ55126
SRR5457635-GPGV	DAC84969	DAC84970	DAC84971	IP-C522-GPGV2	QFG15545	QFG15546	QFG15547	9.14	WCZ55127	WCZ55128	WCZ55129

Table S3: Continued.

Isolate	RdRp	MP	CP	Isolate	RdRp	MP	CP	Isolate	RdRp	MP	CP
SRR5457636-GPGV	DAC84972	DAC84973	DAC84974	IV2_I19-12	QFG15548	QFG15549	QFG15550	Riesling 25-3	AOG16120	AOG16121	AOG16122
SRR5457637-GPGV	DAC84975	DAC84976	DAC84977	IV4_I68-2-11	QFG15551	QFG15552	QFG15553	PN	ASA45580	ASA45581	ASA45582
SRR5457659-GPGV	DAC84978	DAC84979	DAC84980	IV5_I76-1	QFG15554	QFG15555	QFG15556	SL13	AVD53915	AVD53916	AVD53917
SRR5457660-GPGV	DAC84981	DAC84982	DAC84983	IV6_I69-4-2	QFG15557	QFG15558	QFG15559	SL53	AVD53918	AVD53919	AVD53920
SRR5457661-GPGV	DAC84984	DAC84985	DAC84986	IV6_I70-5-3	QFG15560	QFG15561	QFG15562	H-JP1	BCR39156	BCR39157	BCR39158
SRR5457662-GPGV-1	DAC84987	DAC84988	DAC84989	IV6_I70-5-3	QFG15563	QFG15564	QFG15565	H-JP2	BCR39159	BCR39160	BCR39161
SRR5457662-GPGV-2	DAC84990	DAC84991	DAC84992	IV7_F81	QFG15566	QFG15567	QFG15568	fvg-Is1	AYN70038	AYN70039	AYN70040
SRR5457663-GPGV-1	DAC84993	DAC84994	DAC84995	IV7_I78-2	QFG15569	QFG15570	QFG15571	fvg-Is6	AYN70041		
SRR5457663-GPGV-2	DAC84996	DAC84997	DAC84998	IV8_F82	QFG15572	QFG15573	QFG15574	fvg-Is7	AYN70042		
SRR5457664-GPGV	DAC84999	DAC85000	DAC85001	IV8_F85	QFG15575	QFG15576	QFG15577	Fvg-Is8	AYN70043		
SRR7275242-GPGV	DAC85002	DAC85003	DAC85004	IV9_F83-2	QFG15578	QFG15579	QFG15580	Fvg-Is12	AYN70044	AYN70045	
SRR7275243-GPGV	DAC85005	DAC85006	DAC85007	W22-GPGV1	QFG15581	QFG15582	QFG15583	Fvg-Is13	AYN70046		
SRR8260939-GPGV	DAC85008	DAC85009	DAC85010	W22-GPGV2	QFG15584	QFG15585	QFG15586	Fvg-Is14	AYN70047		
SRR8260948-GPGV-1	DAC85011	DAC85012	DAC85013	W25-GPGV	QFG15587	QFG15588	QFG15589	Fvg-Is15	AYN70048	AYN70049	
SRR8260948-GPGV-2	DAC85014	DAC85015	DAC85016	GPGV-Gr-symptomatic	QUS52473	QUS52474	QUS52475	Fvg-Is17	AYN70050	AYN70051	
SRR8260950-GPGV	DAC85017	DAC85018	DAC85019	GPGV-Gr-Asymptomatic	QUS52490	QUS52491	QUS52492	12G1110	QBZ78363	QBZ78364	QBZ78365
SRR866574-GPGV	DAC85020	DAC85021	DAC85022	12G4110	UCJ00541	UCJ00542	UCJ00543	S103	QEQ49999	QEQ50000	QEQ50001
SRR866575-GPGV	DAC85023	DAC85024	DAC85025	12G4111	UCJ00544			S104	QEQ50002	QEQ50003	QEQ50004
SRR866575-GPGV	CCC20963	CCC20964	CCC20965	13C233	UCJ00556			S105	QEQ50005	QEQ50006	QEQ50007
SK30	AGV76021	AGV76022	AGV76023	Rk3	UVC46710	UVC46711	UVC46712	S106	QEQ50008	QEQ50009	QEQ50010
SK01	AGV76024	AGV76025	AGV76026	GPGV_CH_M	UTM04226	UTM04227	UTM04228	S123	QEQ50011	QEQ50012	QEQ50013
SK13	AGV76027	AGV76028	AGV76029	A1575h	UTH78988	UTH78989	UTH78990	S107	QEQ50014	QEQ50015	QEQ50016
SK30-1	AID59467	AID59468	AID59469	A1868h	UTH78991	UTH78992	UTH78993	S111	QEQ50017	QEQ50018	QEQ50019
Mer	AIY29054	AIY29055	AIY29056	A1873h	UTH78994	UTH78995	UTH78996	S115	QEQ50020	QEQ50021	QEQ50022
TI_21	AKP16327			A1542h	UTH78997	UTH78998	UTH78999	S142	QEQ50023	QEQ50024	QEQ50025
TI_25	AKP16328			A1872h	UTH79000	UTH79001	UTH79002	S145	QEQ50026	QEQ50027	QEQ50028
7_2_5	AKP16329			A1581h	UTH79003	UTH79004	UTH79005	S147	QEQ50029	QEQ50030	QEQ50031
20_5_3	AKP16330			A1584h	UTH79006	UTH79007	UTH79008	S148	QEQ50032	QEQ50033	QEQ50034
22_2_3	AKP16331			A1892h	UTH79011			GPGV-136-NS-1	QKV50564	QKV50565	QKV50566
GPGV-TN	AMQ49210	AMQ49211	AMQ49212	A1881h	UVC46658	UVC46659	UVC46660	Regent-BE	QIE48965	QIE48966	QIE48967

Table S3: Continued.

Isolate	**RdRp**	**MP**	**CP**	**Isolate**	**RdRp**	**MP**	**CP**	**Isolate**	**RdRp**	**MP**	**CP**
BC-1	AML83897	AML83898	AML83899	A1574h	UVC46661	UVC46662	UVC46663	Beb13-GPGV1	QFG15440	QFG15441	QFG15442
GPGV_FEM01	ANQ87152	ANQ87153	ANQ87154	A1588h	UVC46664	UVC46665		Beb13-GPGV2	QFG15443	QFG15444	QFG15445
Goldfinger	APT42884	APT42885	APT42886	A1889h	UVC46666	UVC46667	UVC46668	Beb16-GPGV	QFG15446	QFG15447	QFG15448
SK704	ANA05793	ANA05792	ANA05791	A1890h	UVC46669	UVC46670	UVC46671	Beb3-GPGV1	QFG15449	QFG15450	QFG15451

Table S4: The sample identity, cultivar, RT-qPCR cycle threshold value (Ct), number of raw reads generated from metagenomic high-throughput sequencing (HTS), number of reads after quality trimming, number of contigs generated by SPAdes, number of viral contigs, viral contig size, its percentage nucleotide identity to the closest matching isolate, average mapped read depth, and number of mapped reads calculated using Geneious in the grapevine sample.

Sample Identity	**Cultivar**	**Ct value**	**Total number of raw reads**	**Reads after trimming**	**No of viral contigs**	**Assembled GPGV contig size (nt)**	**Percentage nucleotide identity**	**Average mapped read depth**	**Number of mapped reads**
Grafted grapevine	Sauvignon blanc	18.6	2215609	2209873	10 (GPGV)	7275	99.4 (to NCBI GenBank isolate LT6)	22	785
					2 (GRSPaV*)	8681	99.7 (to NCBI GenBank isolate NC_001948.1)	54	2647

*GPGV: Grapevine Pinot gris virus
*GRSPaV: Grapevine rupestris stem pitting associated virus

Table S5: Conserved amino acid sequence motifs occurring in order as they are encoded along the grapevine Pinot gris virus (GPGV) genome, 5’ to 3’, in the RNA-dependent RNA polymerase (RdRp), Movement Protein (MP) and Coat Protein (CP) of the six exemplar species of the genus *Trichovirus* including *Grapevine Pinot virus, Apple chlorotic leaf spot virus, Apricot pseudo-chlorotic leaf spot virus, Cherry mottle leaf virus, Peach mosaic virus* and *Grapevine berry inner necrosis virus*.

ORF region Conserved *Trichovirus* motifs (function)	Conserved amino acid motifs in the exemplar *Trichovirus* species		
	RNA-dependent RNA polymerase (RdRp)	Movement Protein (MP)	Coat Protein (CP)
1	YRTP	PI	**NIAV***
2	EEL	SS	**GTS***
3	LSP	FR	FDFN
4	HSHP	NY	**IF***
5	CKTLEN	GALSISID	**RQ***
6	SIK	LF	**FA***
7	NRLV	GV	GL
8	**RYG***	RW	**PE***
9	HDE	LQ	
10	**VFP***	SPN	
11	EIL	DP	
12	**PDG***	LNF	
13	**FGPYD***	SVR	
14	DVG	FG	
15	IYVRVPI (GKVRVPI)		
16[#]	**FKKI***		
17[#]	**SLKKPD***		
18[#]	**AKLRQL***		
19[#]	**DDF***		
20[#]	**TLRV***		
21	KLLK		
22	TGI		
23[#]	**FGFAGSGKSH***		
24[#]	**TFESALK***		
25[#]	**GDPLQA***		
26	LVAS		
27	GESQGLTF		
28	**LSEE***		
29	**LCSD***		
30	TRF		
31	**WLK***		
32	REFK		
33	KKRLRF		
34	**RSD***		
35	**DW***		
36	KSQLCTKFEKRF		
37	AKAGQTLACF		
38	FSPWCRY		
39[#]	**HQRK***		
40[#]	**ESDY***		
41[#]	**AFDVSQDH***		
42[#]	**FEV***		

Table S5: Continued.

ORF region Conserved *Trichovirus* motifs (function)	Conserved amino acid motifs in the exemplar *Trichovirus* species		
	RNA-dependent RNA polymerase (RdRp)	Movement Protein (MP)	Coat Protein (CP)
43[#]	**LGC***		
44[#]	**FAIMRFTGEF***		
45[#]	**TFL***		
46[#]	**GDDMCAL***		
47[#]	**LKAKV***		
48	FCGWRL		
49	KEP		
50	ERL		
51	LEF		
52	AYKLG		
53	RFF		
54	HLL		
55	RGYSN		

* Conserved motifs in RdRp and CP of the GPGV genome in both aa sequence and position have been marked in bold.
# The three RdRp regions with five (FKKI, SLKKPD, AKLRQL, DDF and TLRV); three (FGFAGSGKS, TFESA, GDPLQA) and nine (HQRK, ESDY, AFDVSQDH, FEV, LGC, FAIMRFTGEF, TFL, GDDMCAL and LKAKV) conserved amino acid motifs, respectively, have been highlighted

Table S6: The cycle threshold (Ct) values with standard deviation before and after seven days post application of four treatments (nine replicates and three technical replicates for each) including a short synthetic grapevine Pinot gris virus (GPGV) targeted dsRNA, second PCR product, RNase-free water and no application to tissue culture (TC) grapevine shoot tips for 2 or 24 hours prior to reintroduction into TC or directly applying treatments to the leaves of plantlets in TC.

Application 1- Dipping excised shoot tips for 2 hrs and then introduce in TC plant culture vessels

	Before application			dip in dsRNA for 2 hrs			Difference in Ct		
	Technical replicate 1	Technical replicate 2	Technical replicate 3	Technical replicate 1	Technical replicate 2	Technical replicate 3	Technical replicate 1	Technical replicate 2	Technical replicate 3
replicate 1	20.9	21.2	21.2	23.5	23.5	23.7	2.6	2.3	2.5
replicate 2	20.9	21.2	21.2	23.8	24.3	24.2	2.9	3.1	2.9
replicate 3	20.9	21.2	21.2	23.3	23.5	23.8	2.4	2.3	2.6
replicate 4	20.9	21.2	21.2	24.5	24.8	25.0	3.5	3.6	3.8
replicate 5	20.9	21.2	21.2	24.0	23.6	23.9	3.1	2.4	2.7
replicate 6	20.9	21.2	21.2	23.9	23.3	23.1	3.0	2.1	1.8
replicate 7	20.9	21.2	21.2	22.7	22.8	22.3	1.8	1.6	1.0
replicate 8	20.9	21.2	21.2	23.6	23.6	24.0	2.6	2.4	2.8
replicate 9	20.9	21.2	21.2	25.5	26.1	25.7	4.6	4.9	4.4
	Average = 21.1	**Standard deviation = 0.1**		**Average = 23.9**	**Standard deviation = 0.9**		**Average = 2.8**	**Standard deviation = 0.9**	**Significance level (P) < 0.0001**

Table S6: Continued.

Application 1- Dipping excised shoot tips for 2 hrs and then introduce in TC plant culture vessels

	Before application			dip in the second PCR product for 2 hrs			Difference in Ct		
	Technical replicate 1	**Technical replicate 2**	**Technical replicate 3**	**Technical replicate 1**	**Technical replicate 2**	**Technical replicate 3**	**Technical replicate 1**	**Technical replicate 2**	**Technical replicate 3**
replicate 1	19.6	19.8	19.7	19.8	20.2	20.2	19.9	20.2	20.2
replicate 2	19.6	19.8	19.7	20.8	20.9	20.2	20.5	20.3	20.4
replicate 3	19.6	19.8	19.7	20.5	21.0	20.2	20.2	21.2	20.2
replicate 4	19.6	19.8	19.7	20.9	21.3	21.5	20.9	21.3	20.8
replicate 5	19.6	19.8	19.7	19.5	19.2	19.1	19.5	19.2	19.8
replicate 6	19.6	19.8	19.7	19.0	19.3	19.2	19.5	19.7	19.7
replicate 7	19.6	19.8	19.7	21.0	21.1	21.1	20.4	20.3	20.6
replicate 8	19.6	19.8	19.7	20.5	20.6	20.7	20.5	20.6	20.3
replicate 9	19.6	19.8	19.7	20.1	20.0	20.6	20.1	20.2	19.8
	Average = 19.7	**Standard deviation = 0.1**		**Average = 20.2**	**Standard deviation = 0.5**		**Average = 0.5**	**Standard deviation = 0.5**	**Significance level (P) 0.10 > P > 0.05**

	Before application			dip in buffer control (water) for 2 hrs			Difference in Ct		
	Technical replicate 1	**Technical replicate 2**	**Technical replicate 3**	**Technical replicate 1**	**Technical replicate 2**	**Technical replicate 3**	**Technical replicate 1**	**Technical replicate 2**	**Technical replicate 3**
replicate 1	20.9	20.7	20.8	21.5	21.7	22	0.6	1.0	1.2
replicate 2	20.9	20.7	20.8	21.2	21.6	21.4	0.3	0.9	0.6
replicate 3	20.9	20.7	20.8	21.1	21.4	21.7	0.2	0.7	0.9
replicate 4	20.9	20.7	20.8	21.0	21.3	21.9	0.1	0.6	1.1
replicate 5	20.9	20.7	20.8	21.6	21.5	22.0	0.7	0.8	1.2
replicate 6	20.9	20.7	20.8	21.0	21.6	20.6	0.1	0.9	-0.2
replicate 7	20.9	20.7	20.8	20.8	20.9	21.0	-0.1	0.2	0.2
replicate 8	20.9	20.7	20.8	21.1	20.8	20.7	0.2	0.1	-0.1
replicate 9	20.9	20.7	20.8	20.7	20.5	20.9	-0.2	-0.2	0.1
	Average = 20.8	**Standard deviation = 0.1**		**Average = 21.2**	**Standard deviation = 0.4**		**Average = 0.4**	**Standard deviation = 0.5**	**Significance level (P) 0.10 > P > 0.05**

	Before application			no treatment			Difference in Ct		
	Technical replicate 1	**Technical replicate 2**	**Technical replicate 3**	**Technical replicate 1**	**Technical replicate 2**	**Technical replicate 3**	**Technical replicate 1**	**Technical replicate 2**	**Technical replicate 3**
replicate 1	21.7	21.8	21.9	22.0	22.6	22.2	0.4	0.8	0.4
replicate 2	21.7	21.8	21.9	22.5	22.5	22.8	0.9	0.6	1.0
replicate 3	21.7	21.8	21.9	22.3	22.2	22.6	0.7	0.4	0.7
replicate 4	21.7	21.8	21.9	21.5	21.3	21.6	-0.2	-0.5	-0.3
replicate 5	21.7	21.8	21.9	20.6	20.6	20.8	-1.0	-1.2	-1.1
replicate 6	21.7	21.8	21.9	21.2	21.2	21.4	-0.5	-0.7	-0.4
replicate 7	21.7	21.8	21.9	20.6	20.3	20.7	-1.1	-1.5	-1.2
replicate 8	21.7	21.8	21.9	22.7	22.7	23.1	1.0	0.9	1.2
replicate 9	21.7	21.8	21.9	22.2	21.9	22.5	0.6	0.1	0.6
	Average = 21.8	**Standard deviation = 0.1**		**Average = 21.8**	**Standard deviation = 0.8**		**Average = -0.0**	**Standard deviation = 0.8**	**Significance level (P) > 0.10 (P= 1.0000)**

Table S6: Continued.

Application 2- Dipping excised shoot tips for 24 hrs and then introduce in TC plant culture vessels

	Before application			dip in dsRNA for 24 hrs			Difference in Ct		
	Technical replicate 1	**Technical replicate 2**	**Technical replicate 3**	**Technical replicate 1**	**Technical replicate 2**	**Technical replicate 3**	**Technical replicate 1**	**Technical replicate 2**	**Technical replicate 3**
replicate 1	19.6	19.8	20.0	24.1	23	23.4	4.5	3.2	3.4
replicate 2	19.6	19.8	20.0	25.5	25.7	25.8	6.0	5.9	5.8
replicate 3	19.6	19.8	20.0	26.2	26.8	27.1	6.7	7.0	7.1
replicate 4	19.6	19.8	20.0	24.1	23.7	25.0	4.5	4.0	5.0
replicate 5	19.6	19.8	20.0	24.1	24.7	25.0	4.5	5.0	5.0
replicate 6	19.6	19.8	20.0	23.3	23.7	23.9	3.7	3.9	3.9
replicate 7	19.6	19.8	20.0	24.9	24.7	24.9	5.3	5.0	5.0
replicate 8	19.6	19.8	20.0	26.1	26.4	26.8	6.5	6.7	6.9
replicate 9	19.6	19.8	20.0	24.2	24	24.4	4.6	4.2	4.4
	Average = 19.8	**Standard deviation = 0.1**		**Average = 24.9**	**Standard deviation = 1.2**		**Average = 5.1**	**Standard deviation = 1.2**	**Significance level (P) < 0.0001**

	Before application			dip in the second PCR product for 24 hrs			Difference in Ct		
	Technical replicate 1	**Technical replicate 2**	**Technical replicate 3**	**Technical replicate 1**	**Technical replicate 2**	**Technical replicate 3**	**Technical replicate 1**	**Technical replicate 2**	**Technical replicate 3**
replicate 1	21.2	21.6	21.6	21.7	21.9	22.1	0.5	0.4	0.5
replicate 2	21.2	21.6	21.6	21.8	21.1	22.2	0.6	-0.5	0.6
replicate 3	21.2	21.6	21.6	21.6	21.2	22.4	0.4	-0.4	0.8
replicate 4	21.2	21.6	21.6	21.5	22.2	22.4	0.3	0.6	0.7
replicate 5	21.2	21.6	21.6	22.0	21.8	21.9	0.8	0.3	0.2
replicate 6	21.2	21.6	21.6	20.8	20.3	21.7	-0.3	-1.3	0.1
replicate 7	21.2	21.6	21.6	21.5	21.4	22.6	0.3	-0.2	1.0
replicate 8	21.2	21.6	21.6	22.2	21.2	22.4	1.0	-0.3	0.8
replicate 9	21.2	21.6	21.6	20.7	20.9	21.0	-0.4	-0.7	-0.7
	Average = 21.5	**Standard deviation = 0.2**		**Average = 21.7**	**Standard deviation = 0.6**		**Average = 0.2**	**Standard deviation = 0.6**	**Significance level (P) > 0.10**

	Before application			dip in buffer control (water) for 24 hrs			Difference in Ct		
	Technical replicate 1	**Technical replicate 2**	**Technical replicate 3**	**Technical replicate 1**	**Technical replicate 2**	**Technical replicate 3**	**Technical replicate 1**	**Technical replicate 2**	**Technical replicate 3**
replicate 1	20.0	19.1	19.2	20.3	19.6	19.4	0.3	0.5	0.2
replicate 2	20.0	19.1	19.2	20.0	19.4	20.2	0.0	0.2	1.1
replicate 3	20.0	19.1	19.2	20.5	19.7	20.8	0.5	0.6	1.6
replicate 4	20.0	19.1	19.2	19.6	19.9	18.9	-0.4	0.8	-0.3
replicate 5	20.0	19.1	19.2	20.1	19.4	20.2	0.1	0.3	1.0
replicate 6	20.0	19.1	19.2	20.7	19.8	19.6	0.7	0.6	0.4
replicate 7	20.0	19.1	19.2	19.7	18.7	19.6	-0.2	-0.4	0.5
replicate 8	20.0	19.1	19.2	20.0	19.0	18.9	0.0	-0.1	-0.3
replicate 9	20.0	19.1	19.2	20.1	19.3	20.0	0.2	0.2	0.8
	Average = 19.4	**Standard deviation = 0.4**		**Average = 19.8**	**Standard deviation = 0.5**		**Average = 0.4**	**Standard deviation = 0.5**	**Significance level (P) 0.10 > P > 0.05**

Table S6: Continued.

Application 2- Dipping excised shoot tips for 24 hrs and then introduce in TC plant culture vessels

	Before application			no treatment			Difference in Ct		
	Technical replicate 1	Technical replicate 2	Technical replicate 3	Technical replicate 1	Technical replicate 2	Technical replicate 3	Technical replicate 1	Technical replicate 2	Technical replicate 3
replicate 1	20.6	20.8	21.0	21.1	21.5	22	0.5	0.6	1.0
replicate 2	20.6	20.8	21.0	20.7	21.2	21.6	0.1	0.4	0.6
replicate 3	20.6	20.8	21.0	21.1	20.5	21.6	0.5	-0.3	0.6
replicate 4	20.6	20.8	21.0	20.9	20.5	21.1	0.3	-0.3	0.2
replicate 5	20.6	20.8	21.0	20.6	21.0	21.9	0.0	0.2	0.9
replicate 6	20.6	20.8	21.0	20.4	20.7	20.4	-0.2	-0.2	-0.5
replicate 7	20.6	20.8	21.0	20.7	21.0	20.7	0.1	0.1	-0.2
replicate 8	20.6	20.8	21.0	21.5	20.5	21.2	0.9	-0.4	0.2
replicate 9	20.6	20.8	21.0	20.6	19.6	20.6	0.0	-1.3	-0.4
	Average = 20.8	**Standard deviation = 0.2**		**Average = 20.9**	**Standard deviation = 0.5**		**Average = 0.1**	**Standard deviation = 0.5**	**Significance level (P) > 0.10**

Application 3- Direct application of dsRNA in TC plant culture vessels

	Before application			apply dsRNA directly in the TC plant			Difference in Ct		
	Technical replicate 1	Technical replicate 2	Technical replicate 3	Technical replicate 1	Technical replicate 2	Technical replicate 3	Technical replicate 1	Technical replicate 2	Technical replicate 3
replicate 1	19.9	20.0	20.0	20.8	20.7	20.5	1.0	0.8	0.5
replicate 2	19.9	20.0	20.0	21.0	20.6	20.7	1.1	0.6	0.7
replicate 3	19.9	20.0	20.0	20.5	19.9	20.2	0.7	-0.1	0.2
replicate 4	19.9	20.0	20.0	22.7	21.7	22.4	2.9	1.7	2.4
replicate 5	19.9	20.0	20.0	23.4	22.4	23.2	3.5	2.4	3.2
replicate 6	19.9	20.0	20.0	21.2	21.4	21.2	1.3	1.4	1.2
replicate 7	19.9	20.0	20.0	20.4	19.9	20.3	0.5	-0.1	0.3
replicate 8	19.9	20.0	20.0	19.7	19.8	19.6	-0.2	-0.2	-0.4
replicate 9	19.9	20.0	20.0	20.6	20.9	20.1	0.7	0.9	0.1
	Average = 20.0	**Standard deviation = 0.1**		**Average = 21.0**	**Standard deviation = 1.1**		**Average = 1.0**	**Standard deviation = 1.1**	**Significance level (P) > 0.10**

	Before application			apply the second PCR product directly in the TC plant			Difference in Ct		
	Technical replicate 1	Technical replicate 2	Technical replicate 3	Technical replicate 1	Technical replicate 2	Technical replicate 3	Technical replicate 1	Technical replicate 2	Technical replicate 3
replicate 1	19.2	19.6	19.6	19.3	20.0	19.7	0.1	0.4	0.1
replicate 2	19.2	19.6	19.6	19.4	19.0	19.7	0.2	-0.6	0.1
replicate 3	19.2	19.6	19.6	19.3	19.7	20.6	0.1	0.2	1.0
replicate 4	19.2	19.6	19.6	19.2	19.9	19.7	0.0	0.3	0.1
replicate 5	19.2	19.6	19.6	19.6	19.0	20.0	0.5	-0.6	0.4
replicate 6	19.2	19.6	19.6	19.9	19.8	20.8	0.7	0.2	1.2
replicate 7	19.2	19.6	19.6	19.6	19.3	19.1	0.4	-0.3	-0.5
replicate 8	19.2	19.6	19.6	19.8	19.6	20.5	0.6	0.0	0.9
replicate 9	19.2	19.6	19.6	19.9	19.8	19.5	0.7	0.2	-0.2
	Average = 19.6	**Standard deviation = 0.2**		**Average = 19.7**	**Standard deviation = 0.4**		**Average = 0.1**	**Standard deviation = 0.4**	**Significance level (P) > 0.10**

Table S6: Continued.

	Before application			apply buffer control (water) directly in the TC plant			Difference in Ct		
	Technical replicate 1	**Technical replicate 2**	**Technical replicate 3**	**Technical replicate 1**	**Technical replicate 2**	**Technical replicate 3**	**Technical replicate 1**	**Technical replicate 2**	**Technical replicate 3**
replicate 1	20.5	20.1	20.2	20.1	20.8	20.8	-0.4	0.7	0.7
replicate 2	20.5	20.1	20.2	21.1	20.9	20.7	0.7	0.8	0.5
replicate 3	20.5	20.1	20.2	19.9	20.4	20.1	-0.5	0.2	-0.1
replicate 4	20.5	20.1	20.2	19.9	20.7	20.4	-0.6	0.6	0.2
replicate 5	20.5	20.1	20.2	21.0	20.7	20.6	0.5	0.6	0.4
replicate 6	20.5	20.1	20.2	20.6	20.3	20.2	0.2	0.2	0.1
replicate 7	20.5	20.1	20.2	20.3	20.2	19.9	-0.1	0.0	-0.3
replicate 8	20.5	20.1	20.2	20.5	20.3	20.5	0.0	0.2	0.3
replicate 9	20.5	20.1	20.2	21.3	22.3	22.0	0.9	2.2	1.8
	Average = 20.2	**Standard deviation = 0.2**		**Average = 20.6**	**Standard deviation = 0.6**		**Average = 0.4**	**Standard deviation = 0.6**	**Significance level (P) 0.10 > P > 0.05**

	Before application			no treatment			Difference in Ct		
	Technical replicate 1	**Technical replicate 2**	**Technical replicate 3**	**Technical replicate 1**	**Technical replicate 2**	**Technical replicate 3**	**Technical replicate 1**	**Technical replicate 2**	**Technical replicate 3**
replicate 1	19.6	19.8	20.0	19.4	19.2	20	-0.2	-0.6	0
replicate 2	19.6	19.8	20.0	20.0	20.4	20.5	0.4	0.5	0.5
replicate 3	19.6	19.8	20.0	19.7	20.6	20.3	0.1	0.7	0.3
replicate 4	19.6	19.8	20.0	19.9	20.3	20.6	0.3	0.5	0.7
replicate 5	19.6	19.8	20.0	20.1	19.9	20.3	0.5	0.1	0.4
replicate 6	19.6	19.8	20.0	19.2	20.1	20.1	-0.4	0.2	0.1
replicate 7	19.6	19.8	20.0	20.3	20.6	20.8	0.7	0.8	0.8
replicate 8	19.6	19.8	20.0	20.5	20.5	20.7	0.9	0.6	0.8
replicate 9	19.6	19.8	20.0	20.1	20.0	20.2	0.5	0.2	0.2
	Average = 19.8	**Standard deviation= 0.2**		**Average = 20.2**	**Standard deviation = 0.4**		**Average = 0.4**	**Standard deviation = 0.4**	**Significance level (P) > 0.10**

Table S7: The average cycle threshold (Ct) values with standard deviation comparing short synthetic grapevine pinot gris virus (GPGV) targeted dsRNA treatment with other controls by either dipping tissue culture (TC) grapevine shoot tips for 2 or 24 hours prior to reintroduction into tissue culture or directly applying treatments to the leaves of plantlets in tissue culture.

Treatment	Average	Standard deviation	Treatment	Average	Standard deviation	Difference in Ct	p-value
dip in dsRNA for 2 hrs	23.9	0.9	dip in the second PCR product for 2 hrs	20.2	0.5	-3.7	P < 0.0001
dip in dsRNA for 2 hrs	23.9	0.9	dip in buffer control (water) for 2 hrs	21.2	0.5	-2.7	P < 0.0001
dip in dsRNA for 2 hrs	23.9	0.9	no treatment	21.8	0.8	-2.1	P < 0.0001
dip in dsRNA for 24 hrs	24.9	1.2	dip in the second PCR product for 22 hrs	21.7	0.6	-3.2	P < 0.0001
dip in dsRNA for 24 hrs	24.9	1.2	dip in buffer control (water) for 24 hrs	19.8	0.5	-5.1	P < 0.0001
dip in dsRNA for 24 hrs	24.9	1.2	no treatment	20.9	0.5	-4.0	P < 0.0001
apply dsRNA directly in the TC plant	21.0	1.1	apply the second PCR product directly in the TC plant	19.7	0.4	-1.3	P < 0.0001
apply dsRNA directly in the TC plant	21.0	1.1	apply buffer control (water) directly in the TC plant	20.6	0.6	-0.4	P > 0.10
apply dsRNA directly in the TC plant	21.0	1.1	no treatment	20.2	0.4	-0.8	0.10 > P > 0.05

Table S8: The average cycle threshold (Ct) values with standard deviation comparing short synthetic grapevine pinot gris virus (GPGV) targeted dsRNA treatment with other controls by either dipping tissue culture (TC) grapevine shoot tips for 2 or 24 hours prior to reintroduction into tissue culture or directly applying treatments to the leaves of plantlets in tissue culture.

Treatment	Average	Standard deviation	Treatment	Average	Standard deviation	Difference in Ct	p-value
dip in dsRNA for 2 hrs	23.9	0.9	dip in dsRNA for 24 hrs	24.9	1.2	1.0	0.10 > P > 0.05
dip in dsRNA for 2 hrs	23.9	0.9	apply dsRNA directly in the TC plant	21.0	1.1	-2.9	P < 0.0001
dip in dsRNA for 24 hrs	24.9	1.2	apply dsRNA directly in the TC plant	21.0	1.1	-3.9	P < 0.0001

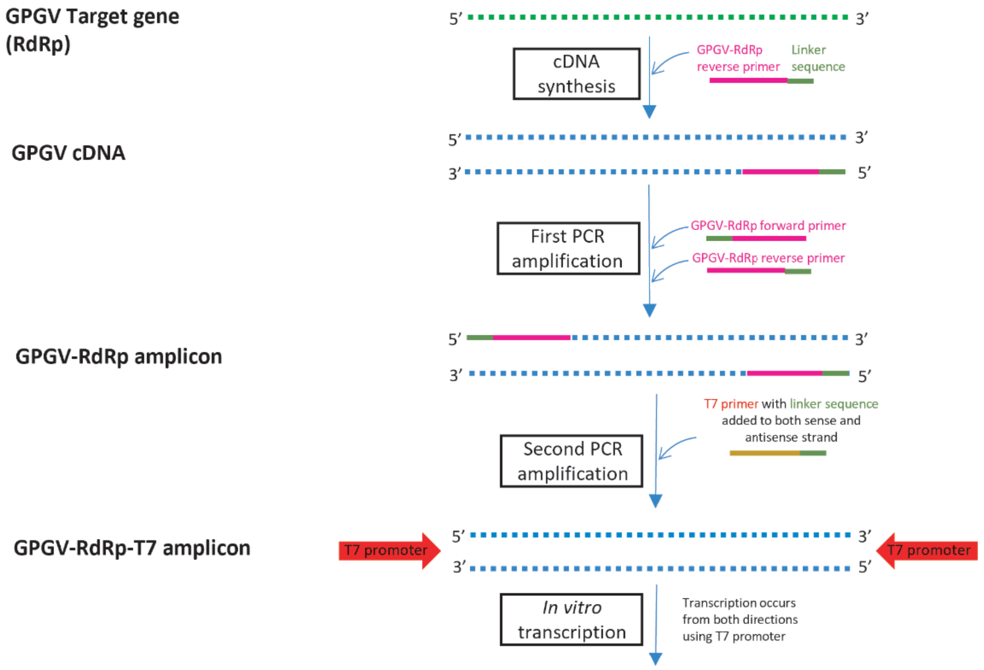

Figure S1: *In vitro* short synthetic grapevine Pinot gris virus (GPGV) double-stranded RNA (dsRNA) production using reverse transcription (RT) for cDNA synthesis and two-step PCR amplification of the GPGV RNA dependent RNA polymerase (RDRP) target sequence. A linker sequence is added to the reverse transcribed single-stranded copy DNA (cDNA) and to the 5′ ends of the double-stranded GPGV-RDRP amplicons in the first step of PCR amplification. The T7 promoter is added during the second step of PCR amplification. The derived GPGV-RdRp-T7 amplicon with the T7 prompter sequence is used for *in vitro* transcription to form the final short synthetic GPGV dsRNA.

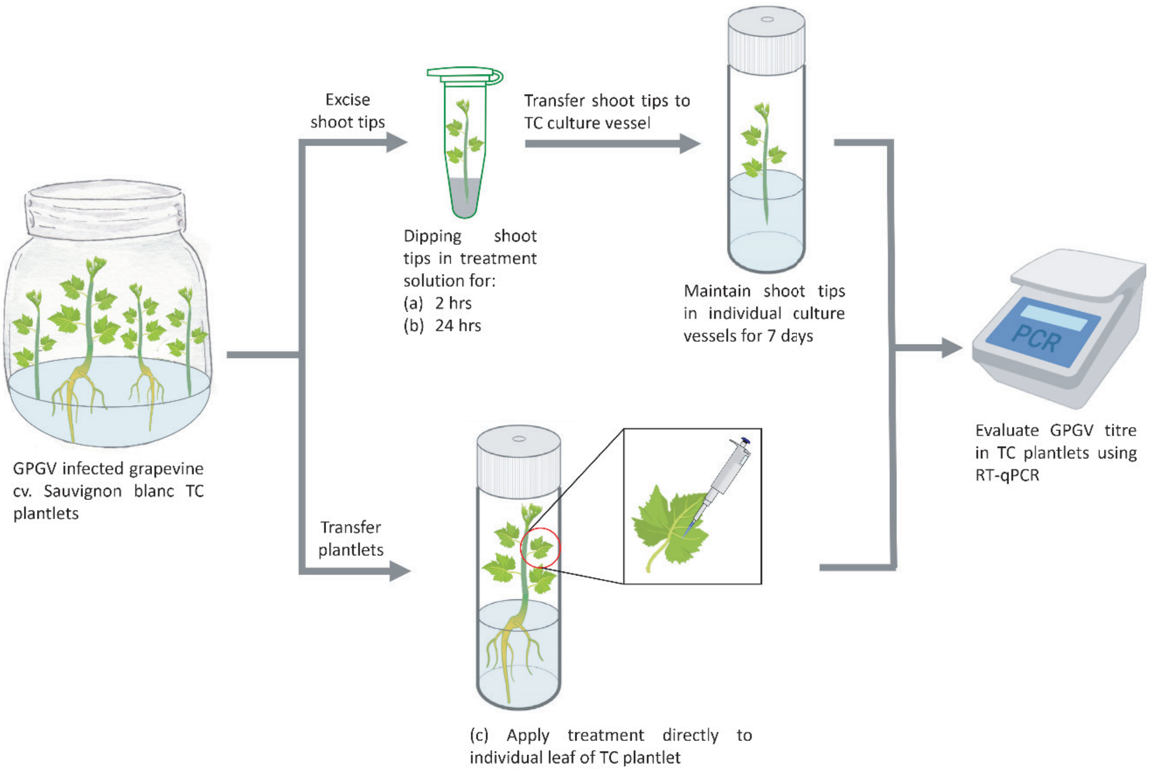

Figure S2: Exogenous application of short synthetic grapevine Pinot gris virus RNA dependent RNA polymerase double stranded RNA (GPGV-RdRp-dsRNA) or GPGV-RdRp-T7 amplicon or buffer control treatments to GPGV infected grapevine cv. Sauvignon Blanc tissue culture (TC) plantlet materials using three application methods: (a) dipping shoot tips excised from TC plants into GPGV-RdRp–dsRNA or control solutions for 2 hrs; (b) dipping shoot tips excised from TC plants into short synthetic GPGV dsRNA or control solutions for 24 hrs; and (c) exogenously applied GPGV-RdRp–dsRNA or control solutions to a single leaf of a TC plantlet in a culture vessel with media (Source: Biorender).

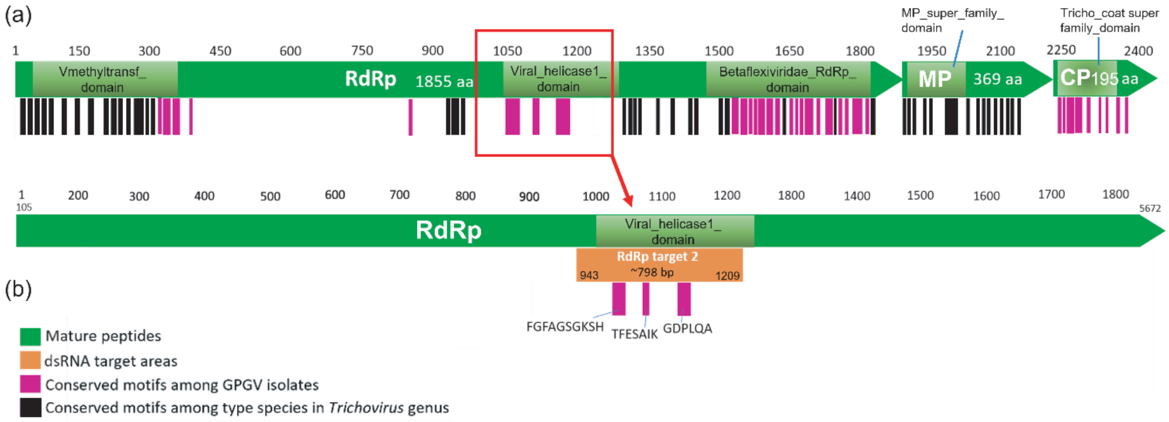

Figure S3: Schematic diagram indicating the conserved amino acid motif positions amongst a) Six exemplar species within the genus *Trichovirus* and their location on the amino acid sequences for the RNA-dependent RNA polymerase (RdRp; NCBI Reference Sequence: YP_004732978.2), movement protein (MP; NCBI Reference Sequence: YP_004732979.2) and coat protein (CP; NCBI Reference Sequence: YP_004732980.2) derived from the exemplar GPGV isolate (Refseq: NC_015782.2) and b) the enlarged view of the RdRp protein sequence illustrating the conserved amino acid motif positions amongst the 200 GPGV isolates and associated 798 bp target region to which the short synthetic GPGV dsRNA used to stimulate RNAi against GPGV was designed. The numbers indicate amino acid positions.

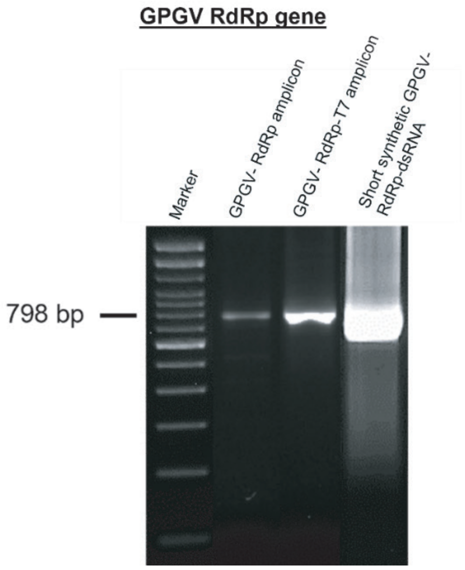

Figure S4. Visualization by agarose gel electrophoresis of the grapevine Pinot gris virus (GPGV) RNA-dependent RNA polymerase (RdRp) amplicons: GPGV-RdRp amplicon, GPGV-RdRp-T7 amplicon and the 798-base pair short synthetic GPGV-RdRp-double stranded RNA (GPGV-RdRp-dsRNA) molecule that was used to target the conserved 798 nucleotide region of the RdRp gene of GPGV to stimulate RNA interference and reduce GPGV titre. The polymerase chain reaction (PCR) amplicons and dsRNA molecules were produced after the two-step PCR method and *in-vitro* dsRNA transcription. Marker: 100 bp DNA ladder.