

SUPPLEMENTARY INFORMATION FOR

A nuclear-replicating viroid antagonizes infectivity and accumulation of a geminivirus by upregulating methylation-related genes and inducing hypermethylation of viral DNA

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Supplementary Table S1. Expression of the housekeeping gene Ubiquiting Conjugating enzyme in the different experimental conditions.

Mean Threshold Cycles (Ct)				Significativity
Non Infected	TYLCSV	PSTVd	TYLCSV/ PSTVd	
24.926	26.132	23.633	24.004	
25.083	26.574	25.191	23.984	0.076
25.050	24.541	24.295	24.263	

Analysis of variance (ANOVA) of the Mean Ct values obtained from three biological replicates per treatment, each with three technical replicates.

Supplementary Table S2. P-values of the fold changes of gene expression calculated for each experimental conditions.

Tomato gene	TYLCSV vs. mixed infection	TYLCSV vs. PSTVd	PSTVd vs. mixed infection
MET1	0.010	0.024	0.446
CMT3	0.006	0.019	0.488
DDM1	0.009	0.023	0.500
KYP	0.025	0.050	0.527
IBM1	0.044	0.063	0.476
DRM2	0.020	0.050	0.896
NRPD1	0.018	0.073	0.816
NRPE1	0.018	0.037	0.367
HEN1	0.018	0.048	0.211
AGO4a	0.019	0.038	0.767
ADK	0.026	0.060	0.633
SAHH	0.027	0.033	0.711
SAMS	0.035	0.050	0.581
SAMDC1	0.008	0.009	0.879
ROS1	0.015	0.021	0.910

Supplementary Table S3. P-values of the percentage of methylated cytosines in the TYLCSV region analyzed, calculated for the total cytosines and for each cytosine context.

Cytosine context	TYLCSV vs. mixed infection
Total	1.92 E-08
CHH	2.13 E-09
CHG	1.81 E-07
CG	2.42 E-06

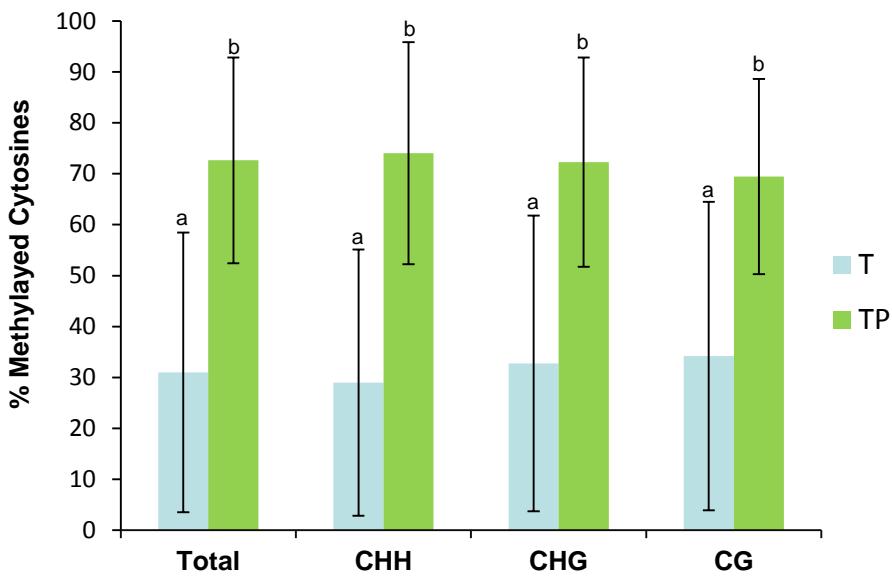
A

CCAAACGAGCTGGCGTAATGAAGTATCTGGGTAAGTGCAGCAGCACCGCTTAGTGGTCAGCACACCCCGAAGCTCTCTGGCATGGCTCTTCAACCCAGACCACCATGAA GTAAACATGGTAGGGAGATGAAACGCCACGCTGCCCTACCGAAGAGGTAGCTCTTGCAAGATGCCAGAGTCATAGCTGCAGCAGGAGCTACTCCGCTACCCCTGAGTTGAAACGC TTGGGGGAGGCGCTGGTGGATAGCCAACGGCTCTCTGACAGGGAGCTGGTCAACCAATAAGGGGGCTTTTCTGCTCGAGGGTGTGAAACAGGAGCTCCGAGGGCACCACAGATGATCAGGGTAGAGAAAAACTC CCCCCCGAACCTTGGAAACTCTTCCCACGGTGTGAAACCCGCTGATCAAAACACTGTTCAAAACCTAAGGGAGGTTGCTGAGAGTCTTACGTGAAAGAAAATAAGCAGTTAGTACGTCTCCCTGTCGAAAAGGGGGCTTCAGAAAATGGGTTACCTACGAGCCTAACATTGCTTAGACTCTCCAAACATGCCAGGATCTAACAGAAATTGTTGATGCTCATGTTGAGAGAAGAGTGTCTACGAGTTGCTGCCATGACCCCTCTCTGAGAGACCTCTGGACCGCAAGGATGCCAGCTGAAAGACGTTTAAAGGCCGAAAATTTGCAAGGCCGACCCAGCTCCCCGGTTACAACCAAAGAG TCCCGCTACACGTTGAATTAGGCCGATATCTCAAGAAATTCGAACACTCGGCCATCAGAACGCTGGACAAGATCTGGGAGGAAGACCGTGTGAAAGGGTACTCTGTTGAGATAGGGAAACATATCTCAAGGGCTGGGATTCATCCCTCTCCGGTTGCTCGGATTCGACATGAGCCGTTGACAGCACGTTAGTGTGCTCGCTCAATTGCAACACAGTTGTTACCTGCTGTTTCCGGAGACCGCATTTGG CGGAACCTTGTAGCTGAGAGTGGGTGAGATGTCGGAGACATGAAACACTGCACTGGGAACTGCTTATGGCTTGCT CATAACTAGGCAACCTCATGAAGGACCAATAATTGTCGA

B

C

Supplementary Figure S1. Methylation of the internal DNA control used in bisulfite conversion experiments. **(A)** cDNA fragment of *Pelargonium flower break virus* genomic RNA (Genbank Acc. No. DQ256073; nt 451-1700), added (50 ng) to the bisulfite reactions performed to establish TYLCSV DNA methylation in TYLCSV- and TYLCSV/PSTVd-infected tomato plants (see Figure 4). A bisulfite-converted DNA fragment (387 bp, nt 721-1108) was amplified using forward and reverse primers (underlined in **A**), cloned and sequenced. **B,C.** Individual clones of the bisulfite-converted controls of the results reported for TYLCSV DNA in Fig. 4B,C, respectively. Methylated cytosines (indicated with C) are grey, red or green, if in the CHH, CHG and CG contexts, respectively. Unmethylated cytosines are reported as T. Conversion efficiency was > 99% in each replicate.



Supplementary Figure S2. Bisulfite sequencing analysis of TYLCSV genome methylation in tomato plants infected by TYLCSV alone (T) or doubly infected by TYLCSV and PSTVd (TP). Columns represent mean percentage of methylated cytosines in the TYLCSV region analyzed independently from the DNA context (Total) or taking into consideration the CHH, CHG and CG contexts. Bars, standard deviation; letters, statistical significance ($P \leq 0.0001$; Student t-test).