Study of the functional domains of the PTGS suppressor V2 from geminivirus *Beet curly top virus* (BCTV)

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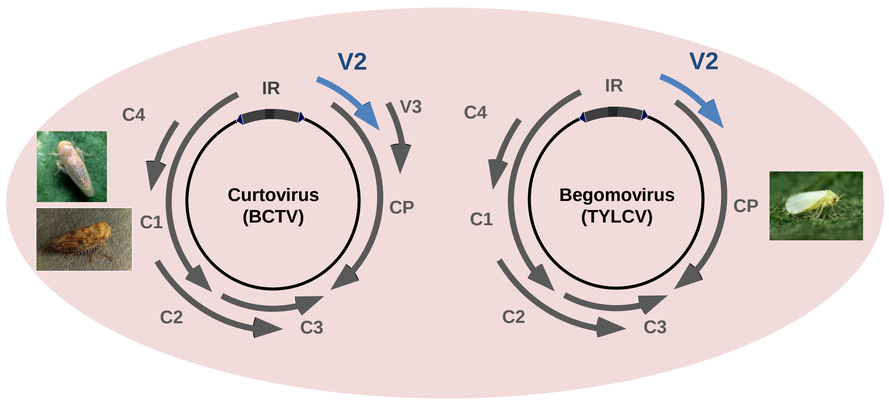
Geminiviruses constitute a group of plant viruses with circular, single-stranded DNA genomes packaged within geminated particles that infect a wide range of plants[1]. Among the *Geminiviridae* family, the genus *Mastrevirus*, *Begomovirus* and *Curtovirus* comprise most of the viral species capable of infecting dicotyledonous plants. Monopartite begomovirus and curtovirus possess similar genome structures[2].

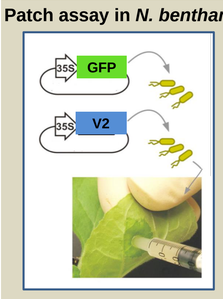


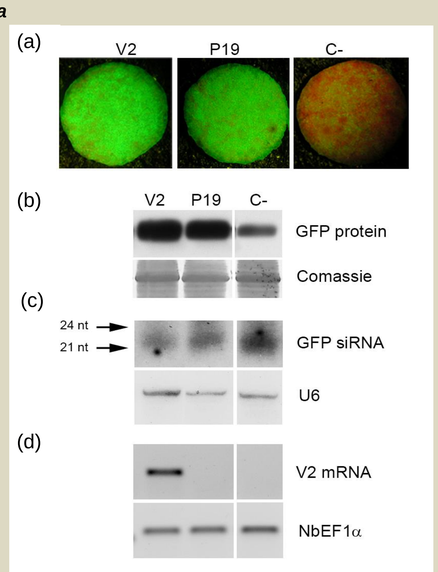
In plants, **RNA silencing** is an important antiviral mechanism. Geminiviruses must confront both transcriptional (TGS) and Post-Transcriptional Gene Silencing (PTGS) to achieve successful infections[3,4]. V2 from of Old Word begomoviruses and from curtovirus BCTV has been described as a PTGS and TGS suppressor[5,6,7,8,9,10,11,12,13].

V2 proteins from curtovirus and begomovirus have similar functions

1. Strong local PTGS suppressors

Patch assay in *N. benthamiana*

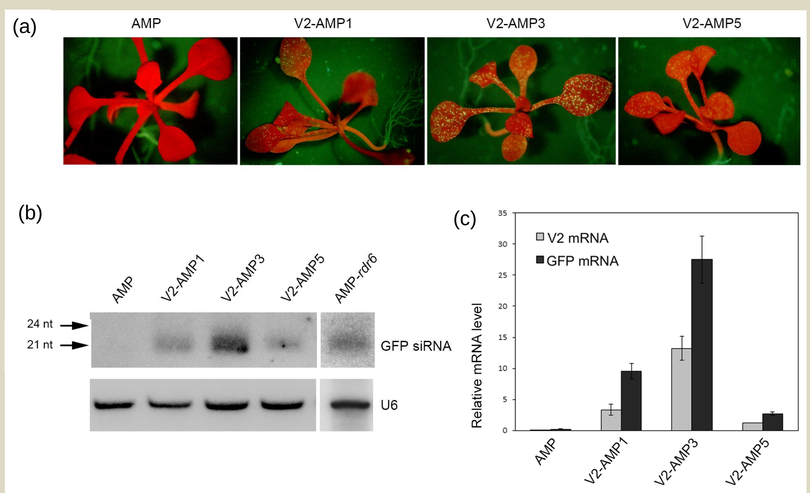




(a) *N. benthamiana* wild-type leaf discs infiltrated with a mixture of two *A. tumefaciens* cultures expressing GFP and BCTV V2 under UV light at 4 dpi. P19 and the empty vector (C-) were used as controls. (b) Western to detect GFP protein. (c) northern blot analysis to detect GFP siRNAs and sRNA U6 as loading control. (d) RT-semi-quantitative PCR.

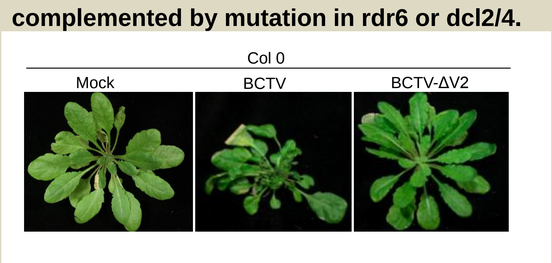
2. Act on RDR6/SGS3 silencing pathways

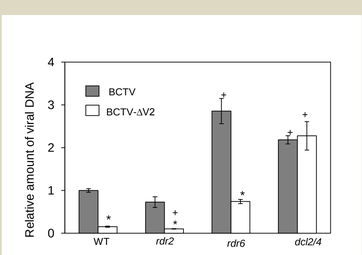
BCTV V2 phenocopies rdr6 mutation in AMPLICON (AMP) line



(a) Representative pictures of non-transformed AMPLICON plants (AMP) and T2 kanamycin-resistant plants from the transgenic lines V2-AMP1, V2-AMP3 and V2-AMP5. (b) northern blot analysis to detect GFP siRNAs (siGFP) and sRNA U6 as loading control (c) RT-qPCR to measure V2 and GFP mRNA levels that were normalized to actin.

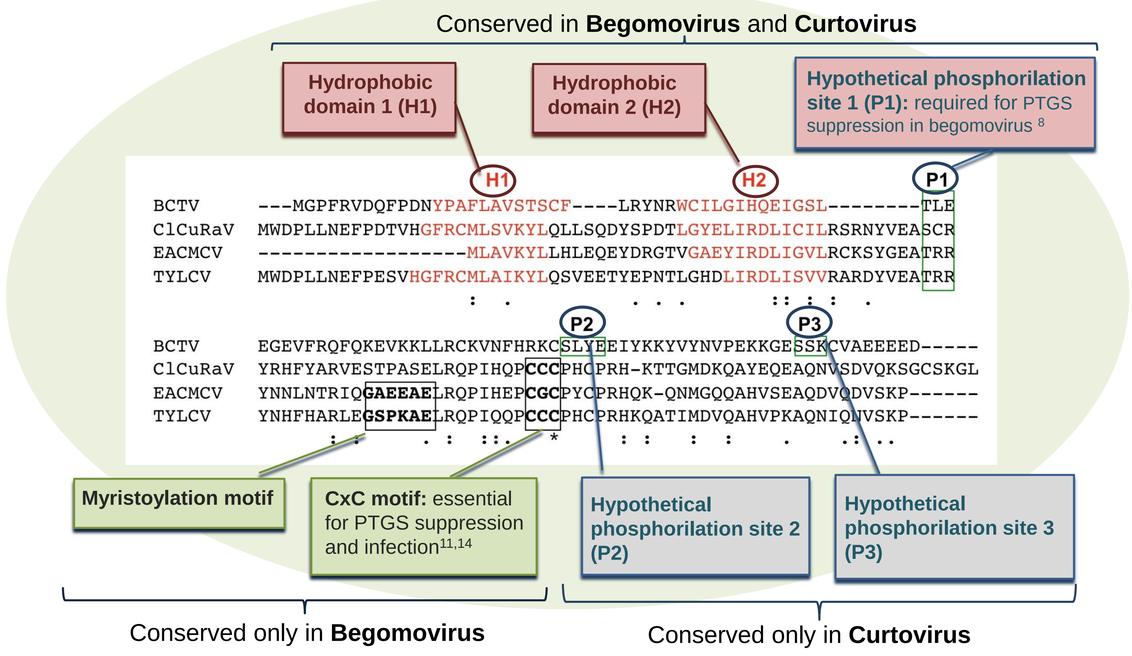
BCTV V2 is essential for infection and can be complemented by mutation in rdr6 or dcl2/4.



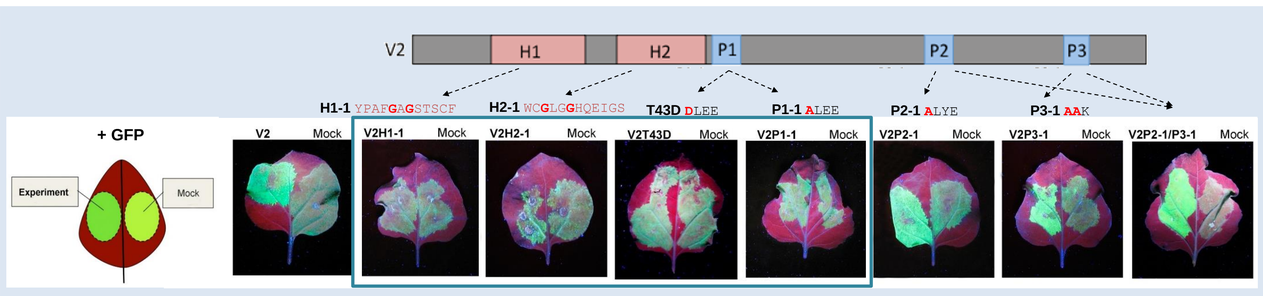


Analysis of viral DNA accumulation in *Arabidopsis* wild-type and mutant plants *rdr2-1*, *rdr6-15* and *dcl2/4* infected with BCTV or BCTV-ΔV2. DNA extracted from five to six plants. (*) the BCTV-ΔV2-infected sample that is statistically different from the BCTV-infected sample on each Arabidopsis background (*P<0.05). (+) infected samples that are statistically different from Col-0 plants infected with BCTV (+P<0.05).

In spite of the low homology, V2 proteins contain some conserved domains

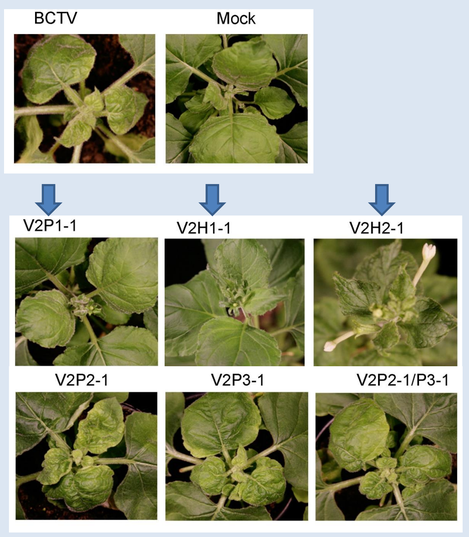


H1 and H2 domains and the phosphorylation site P1 are required for PTGS suppression activity

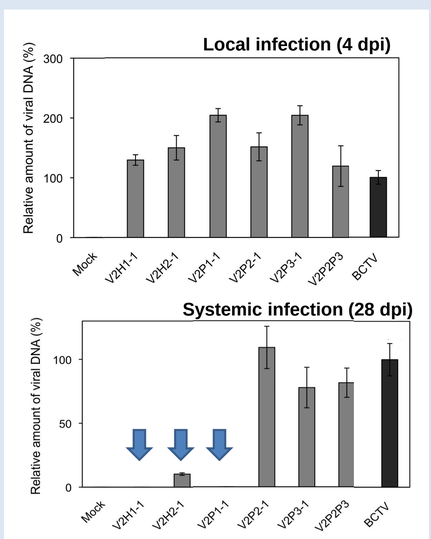


Leaves from *N. benthamiana* plants infiltrated with a mixture of two *A. tumefaciens* cultures expressing GFP and the desired V2 mutant and photographed under UV light at 5 dpi. Wild type V2 protein and the empty vector (mock) were used as a positive and negative control respectively.

H1, H2 and P1 are required for systemic but not for local BCTV infection

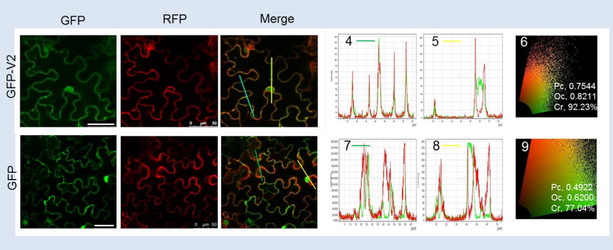


Symptoms observed at 21 dpi in *N. benthamiana* plants agroinoculated with wild-type and BCTV viruses containing mutations in V2.



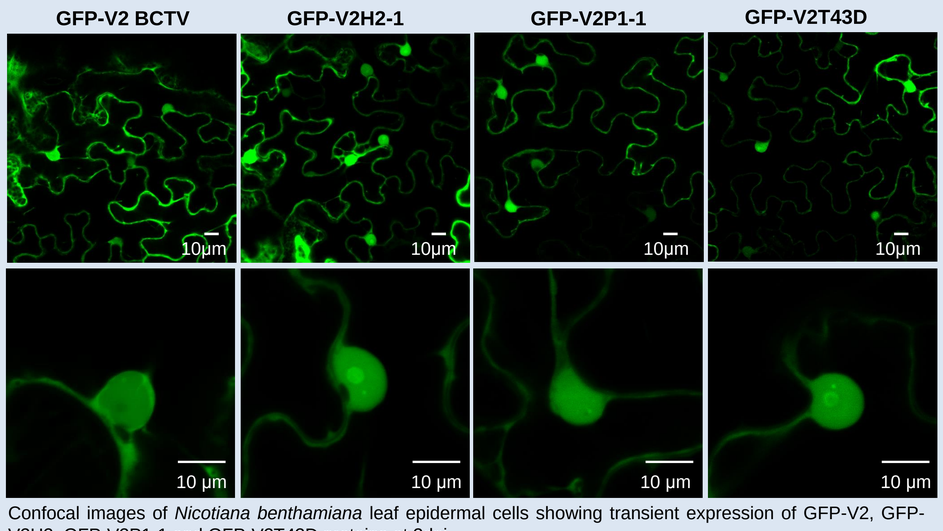
Relative viral DNA accumulation in leaf patches at 4 dpi (local infection) and in systemic infected leaves at 28 dpi. (systemic infection. BCTV DNA levels leaves were quantified by quantitative real-time PCR using the 25S ribosomal DNA interspacer (ITS) as the internal control. All data were refered to the the viral amount obtained in plants infected the wild-type (BCTV). Data represent means ± SD for three independent biological replicates.

BCTV V2 (as TYLCV V2) is localized in nucleus and ER



Confocal images of transiently transformed *N. benthamiana* epidermal cells co-expressing an ER marker (RFP-HDEL) and GFP-V2, or free GFP. White bars represent 50 µm. Plots 4, 5, 7 and 8 show fluorescence intensity plots corresponding to the green or yellow lines in the images. Images 6 and 9 show scatter plots of green and red pixels. Pc, Pearson's correlation. Oc, Overlap coefficient. Cr, colocalization rate.

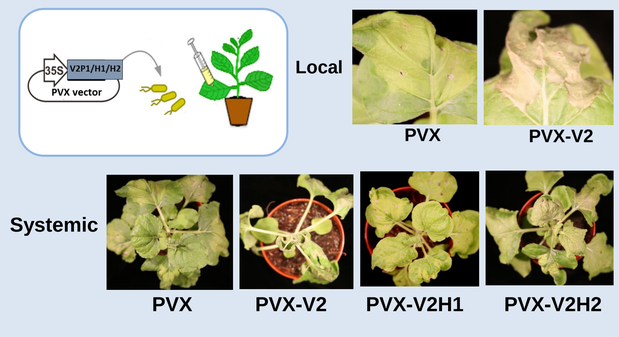
Mutations in H2 or P1 do not change subcellular localization of BCTV V2



Confocal images of *Nicotiana benthamiana* leaf epidermal cells showing transient expression of GFP-V2, GFP-V2H2, GFP-V2P1-1 and GFP-V2T43D proteins at 3dpi.

Mutations in H1, H2 and P1 cause a reduction in the symptoms produced by BCTV V2 expression from PVX

Construct	Local	Systemic 8 dpi	Systemic 11 dpi	Systemic 16 dpi
PVX	Y	C	C	C
PVX-V2P1	N	C+ and N	N	N
PVX-V2H1	Y	C+	C++	C+
PVX-V2H2	N	C+ and N	C++ and N	N
PVX-V2	N	N	N	N



References: 1. Zerbini *et al*., 2017; 2. Fondong, 2013; ; 3. Hanley-Bowdoin et al., 2013; 4. Pooggin, 2013; 5. Amin *et al.*, 2011; 6. Chowda-Reddy *et al.*, 2008; 7. Luna *et al.*, 2012; 8. Sharma & Ikegami, 2010; 9 Sharma *et al.*, 2010; 10. Zhang *et al.*, 2012; 11. Zrachya *et al.*, 2007;12. Wang *et al.*, 2014; 13. Luna *et al*., 2018; 14, Padidam *et al.*, 1996