Study of the PTGS suppressor activity of V2 protein from geminivirus *Beet curly top virus*

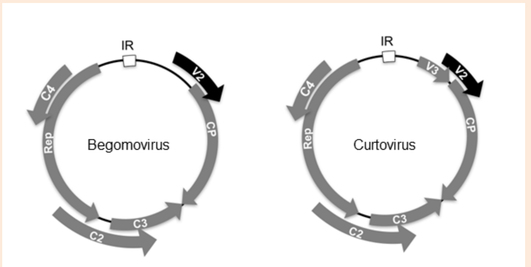
ANA P. LUNA*, EDGAR A. RODRÍGUEZ-NEGRETE, GABRIEL MORILLA, LIPING WANG, ROSA LOZANO-DURÁN, ARACELI G. CASTILLO, AND EDUARDO R. BEJARANO

**For correspondence (Ana P. Luna. Instituto de Hortofruticultura Subtropical y Mediterránea "La Mayora" (IHSM-UMA-CSIC), Área de Genética, Facultad de Ciencias, Universidad de Málaga, Campus de Teatinos s/n, E-29071 Málaga, Spain. Tel: +34 952131677; fax: +34 952132000; e-mail analuna@uma.es.*

INTRODUCTION

Geminiviruses constitute a group of plant viruses with circular, single-stranded DNA genomes packaged within geminate 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 to infect dicotyledonous plants. Monopartite begomovirus and curtovirus possess similar genome structures, encoding six and seven multifunctional proteins, respectively[2]. In both cases, the virion sense strand contains two open reading frames (ORFs) (V2 and coat protein, CP), and a third one (V3) is present only in curtovirus; four ORFs are present in the complementary sense strand (Rep, C2, C3 and C4).

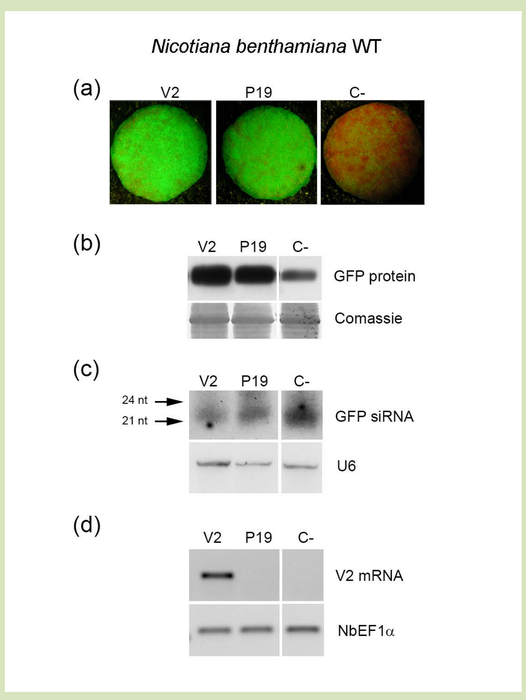
In plants, RNA silencing is an important antiviral mechanism. All plant viruses examined to date encode at least one protein that suppresses antiviral silencing (VSR) [3,4]. Geminiviruses must confront both transcriptional (TGS) and Post-Transcriptional Gene Silencing (PTGS) to achieve successful infections[5,6]. V2 from of Old Word begomoviruses has been described as a PTGS and TGS suppressor[7,8,9,10,11,12,13,14]. Besides begomovirus V2 is also involved in viral movement, it is required for full infection and elicits hypersensitive response (HR)-like cell death when expressed from a *Potato virus X* (PVX)-derived vector[9,10,14,15,16]. Less is known about the function of curtovirus V2. Although begomovirus and curtovirus V2 ORFs seem to be orthologous based on genome location and length, their homology at the protein level, which is highly conserved within each genus, is extremely poor.



References: 1. Zerbini *et al*., 2017; 2. Fondong, 2013; 3. Csorba *et al*., 2015; 4. Pumplin & Voinnet, 2013; 5. Hanley-Bowdoin *et al.*, 2013; 6. Pooggin, 2013; 7. Amin *et al.*, 2011; 8. Chowda-Reddy *et al.*, 2008; 9. Luna *et al.*, 2012; 10. Sharma & Ikegami, 2010; 11. Sharma *et al.*, 2010; 12. Zhang *et al.*, 2012; 13. Zrachya *et al.*, 2007;14. Wang *et al.,* 2014; 15. Hak *et al.*, 2015; 16. Iqbal *et al.*, 2012; 17 Mubin *et al.*, 2010.

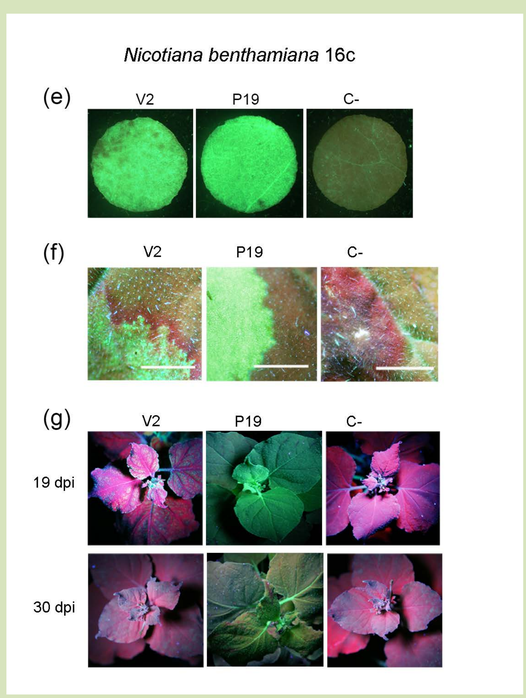
RESULTS

BCTV V2 is a local PTGS suppressor



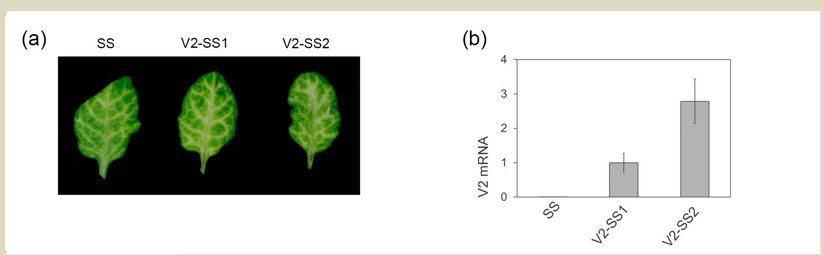
(a) ***N. benthamiana* wild-type** leaf discs infiltrated with a mixture of two *A. tumefaciens* cultures expressing GFP and BCTV V2 under UV light at 4 dpi. P19 and the empty vector (C-) were used as controls. (b) Western to detect GFP protein. (c) northern blot analysis to detect GFP siRNAs and sRNA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

BCTV V2 produces a delay in the spread of systemic silencing, but without impacting local cell-to-cell silencing movement



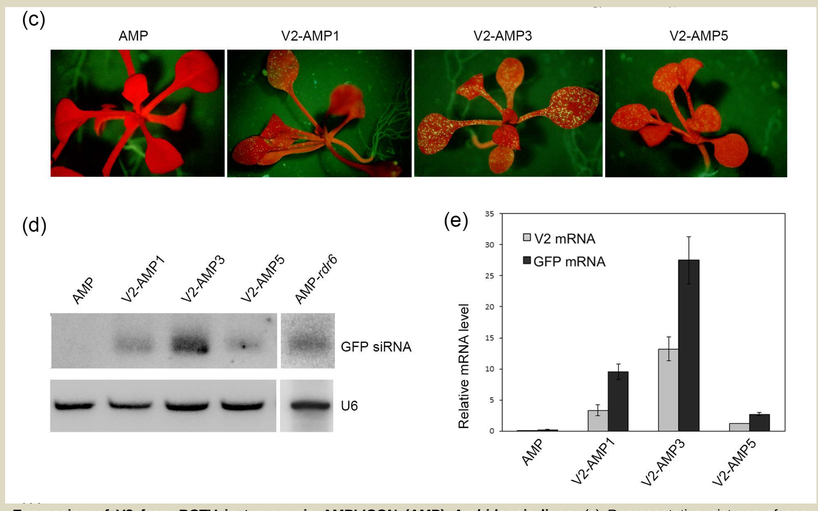
(e) ***Nicotiana benthamiana* 16c** leaf discs infiltrated with two *A. tumefaciens* cultures expressing GFP and BCTV V2 under UV light at 4 dpi. P19 and the empty vector (C-) were used as controls. (f) GFP expression in the cells surrounding the agroinfiltrated area at 5 dpi. Bar, 2 mm. (g) Agroinfiltrated 16c plants under UV light at 19 (top panel) and 30 dpi (bottom panel).

BCTV V2 does not affect the RDR6 independent silencing of the endogenous SUL gene

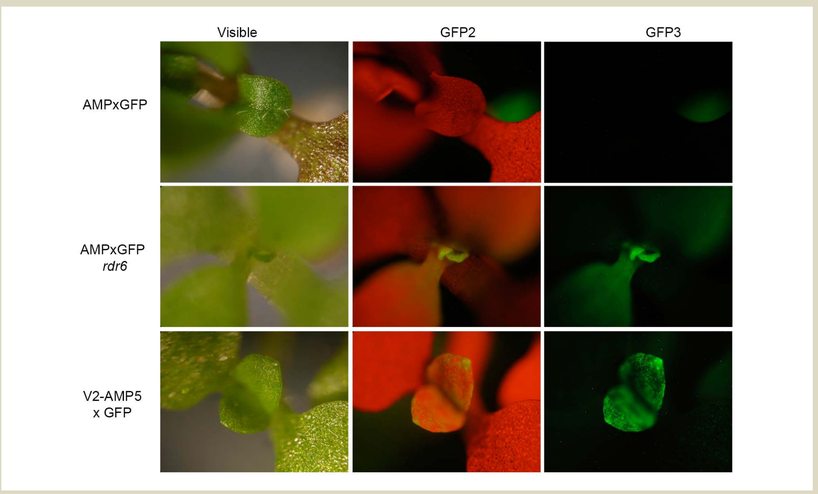


Expression of V2 from BCTV in transgenic SUC-SUL (SS) *Arabidopsis* lines. (a) Representative pictures of non-transformed (SS) and T2 kanamycin-resistant plants from the transgenic lines V2-SS1 and V2-SS2 (b) RT-qPCR to measure V2 mRNA levels that were normalized to actin

BCTV V2 phenocopies *rdr6* mutation in AMPLICON (AMP) and AMPxGFP lines.

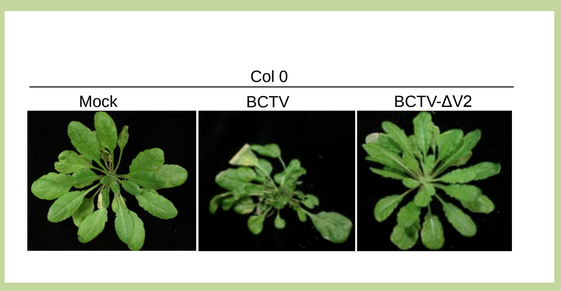


Expression of V2 from BCTV in transgenic AMPLICON (AMP) *Arabidopsis* lines. (c) Representative pictures of non-transformed AMPLICON plants (AMP) and T2 kanamycin-resistant plants from the transgenic lines V2-AMP1, V2-AMP3 and V2-AMP5. (d) northern blot analysis to detect GFP siRNAs (siGFP) and sRNA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

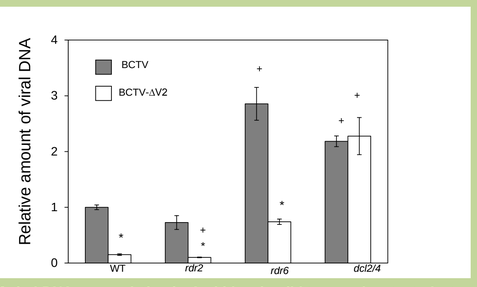


Representative F1 plants resulting from the crosses of AMPLICON and GFP line, V2-AMP5 and GFP line (V2-AMP5XGFP) or a homozygous plant AMPxGFP containing the *rdr6-15* mutation (AMPxGFP *rdr6*). Pictures were taken from plants under visible (left column) or UV light using either the GFP2 filter (allows the chlorophyll autofluorescence, middle column), or the GFP3 filter (only shows GFP fluorescence, right column).

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



Symptoms induced in *Arabidopsis* Col-0 plants agroinoculated with BCTV wild-type or V2 mutant (BCTV-ΔV2) at 28 days after inoculation (dpi). As a negative control, plants were agroinoculated with the empty vector (mock).



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 was extracted from five to six plants in each condition at 28 dpi and quantified by qPCR using actin as reference gene and represented as the relative level compared to Col-0 plants infected with the wild-type BCTV (set to 1). Bars represent the mean +/- the standard error from three technical replicates obtained from DNA extracted from these five to six plants. Asterisks (*) indicate the BCTV-ΔV2-infected sample that is statistically different from the BCTV-infected sample on each Arabidopsis background (*P<0.05), as determined by the Student's t-test. Plus sign (+) indicate the infected samples that are statistically different from Col-0 plants infected with BCTV (+P<0.05).

CONCLUSIONS

In spite of limited sequence homology, BCTV V2, as its begomovirus counterpart:

- is required for a systemic infection.
- is a strong suppressor of intracellular PTGS by impairing the RDR6/SGS3 pathway.
- does not impact local cell-to-cell silencing movement, but produces a delay in the spread of systemic silencing.