

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

Ana P. Luna*, Edgar A. Rodríguez-Negrete⁺, Gabriel Morilla, Araceli G. Castillo,
Eduardo R. Bejarano

Instituto de Hortofruticultura Subtropical y Mediterránea "La Mayora" (IHSM-UMA-CSIC), Area de Genética, Facultad de Ciencias, Universidad de Málaga, Campus de Teatinos s/n, E-29071 Málaga, Spain. ⁺ Present address: Instituto Politécnico Nacional, CIIDIR-IPN, Unidad Sinaloa, Departamento de Biotecnología Agrícola, Blvd. Juan de Dios Bátiz Paredes No 250. Guasave, Sinaloa CP 81101, Mexico.

+ Present address: Instituto Politécnico Nacional, CIIDIR-IPN, Unidad Sinaloa, Departamento de Biotecnología Agrícola, Blvd. Juan de Dios Bátiz Paredes No 250. Guasave, Sinaloa CP 81101, Mexico.

Geminiviruses constitute a group of plant viruses that infect vegetable crops all over the world. Among the *Geminiviridae* family, the genera *Mastrevirus*, *Begomovirus* and *Curtovirus* are the most abundant.

Suppression of gene silencing is a key mechanism for viral infection in plants. In begomovirus, V2 is a strong posttranscriptional gene silencing suppressor. We recently showed that, despite the lack of sequence homology with V2 from begomovirus, V2 from curtovirus *Beet curly top virus* (BCTV) is also a PTGS suppressor by impairing the RDR6/SGS3 pathway.

In order to identify the domains involved the suppression activity and viral pathogenicity, we analysed the aminoacidic sequence, performing an alignment of several begomovirus and curtovirus V2 proteins. A protein kinase C (PKC) phosphorylation motif essential for suppression activity in begomovirus (P1) was founded in a similar position in all analysed sequences. Besides, other two putative CK2 and PKC phosphorylation motifs (P2 and P3) were also predicted only in BCTV V2. We also found similar hydrophobic profiles, consisting of two hydrophobic domains (H1 and H2) followed by a long hydrophilic domain. Then we generated BCTV V2 mutant proteins in each of them and performed transient assays in *Nicotiana benthamiana* plants to test their suppression activity. We also expressed them from a Potato virus X-derived vector to check the symptoms produced. Additionally, their subcellular localization was determined. Finally, we produced BCTV viruses mutated in the different domains and *N. benthamiana* plants were infected, analysing virus levels and symptoms produced. The results showed that P1, H1 and H2 are involved in the suppression activity and viral pathogenicity.