# Discerning the relationship between geminiviral infection and vesicle trafficking using virus induced gene silencing

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## INTRODUCTION

Geminiviruses are a large family of insect-transmitted plant viruses with circular, single-stranded (ss) DNA genomes packaged within geminated particles which infect a wide range of plants causing devastating crop diseases. From among these diseases, Tomato yellow leaf curl disease (TYLCD), is one of the most important threats to tomato crops worldwide. One of the causal agents of TYLCD is *Tomato yellow leaf curl Sardinian virus* (TYLCSV), a member of the genus *Begomovirus* belonging to the family *Geminiviridae*. TYLCSV has a monopartite genome, which encodes six proteins and contains an intergenic region (IR) comprising the origin of replication and viral promoters. Due to the few proteins encoded by the viral genome, they rely heavily on host cellular machineries and interact with a wide range of plant proteins to complete all processes required for infection, such as viral replication, movement, and suppression or evasion of plant defence mechanisms. While cell-to-cell movement has been described to occur through plasmodesmata (Zhou et al., 2011), the way in which geminiviruses move inside the host cells is yet unknown. Here we describe how vesicle trafficking is essential for viral movement inside host cells.



**Figure 1. Geographical distribution (A) and symptoms of TYLCD (B).** (taken from Navas-Castillo et al., 2011)

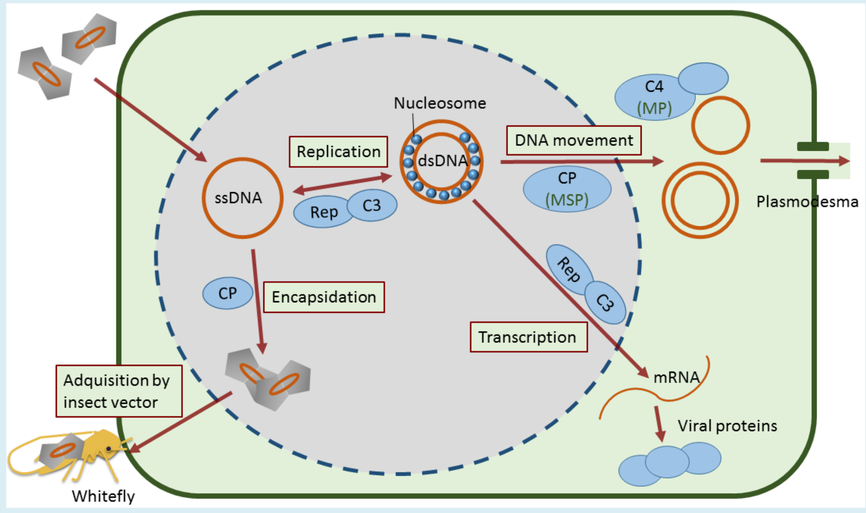


**Figure 2. The begomovirus life cycle.** (Adapted from Hanley-Bowdoin et al., 2013)

## MATERIAL AND METHODOLOGY

In our laboratory, transgenic *Nicotiana benthamiana* plants containing a green fluorescent protein (GFP) expression cassette flanked by two direct repeats of the intergenic region of TYLCSV have been constructed (2IR plants) (Morilla et al., 2006). When these plants are infected with TYLCSV, an overexpression of the reporter gene is observed in those cells where the virus replicates. These plants have been used together with virus induced gene silencing (VIGS) based on a TRV vector, in an effort to identify host genes involved in the infection process using a reverse genetics approach. As a result, two genes involved in vesicular trafficking were identified such as: X2 and X7. A set of genes involved in this process were later assayed in order to see their effect over infection (genes Z4, Z7, Z8, Y3, Y5 and W5). The identification of the host proteins involved in viral infection will be an important step towards the understanding of the mechanisms underlying this process.

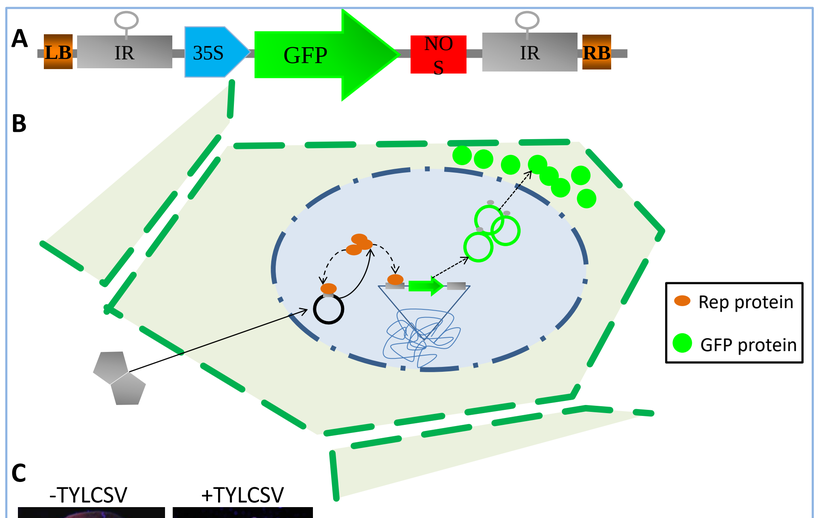


**Figure 3. *Nicotiana benthamiana* 2IR GFP plants**.
A) Construct of 2IR GFP plants. B) Episomal replicon formation in 2IR plants infected with TYLCSV. C) GFP expression in 2IR GFP plants under UV light at 15 dpi .

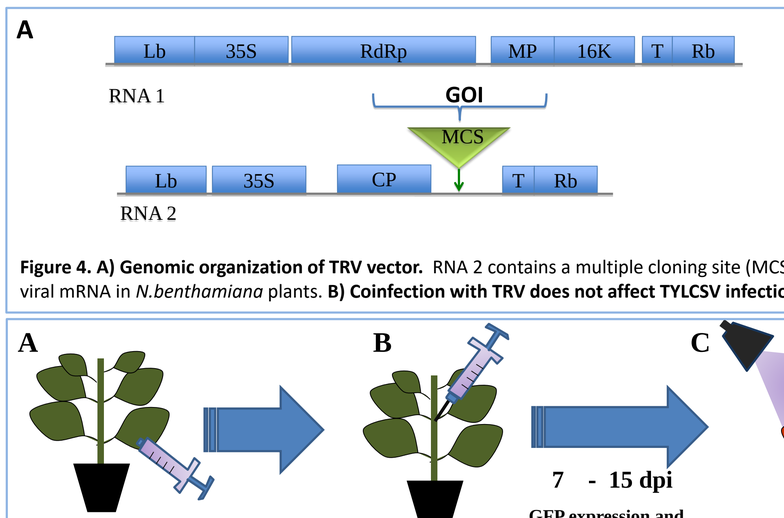


**Figure 4. A) Genomic organization of TRV vector.** RNA 2 contains a multiple cloning site (MCS) where the genes of interest (GOI) have been cloned and, therefore, will be silenced together with the viral mRNA in *N.benthamiana* plants. **B) Coinfection with TRV does not affect TYLCSV infection.**

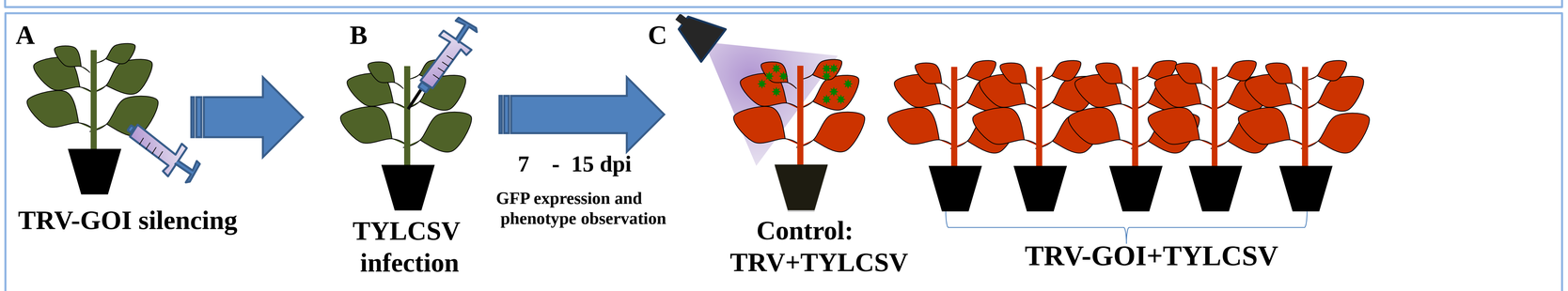


**Figure 5. Strategy used to identify genes required for TYLCSV infection in *Nicotiana benthamiana* 2IR GFP plants.** A) Silencing of the gene of interest (GOI) through infiltration with *Agrobacterium* containing a TRV- GOI construct. B) Immediately afterwards, an infection is carried out through injection with *Agrobacterium* containing TYLCSV. C) Plants are observed under UV light to see the evolution of GFP expression from days 7 to 15 post infection.

## RESULTS AND DISCUSSION

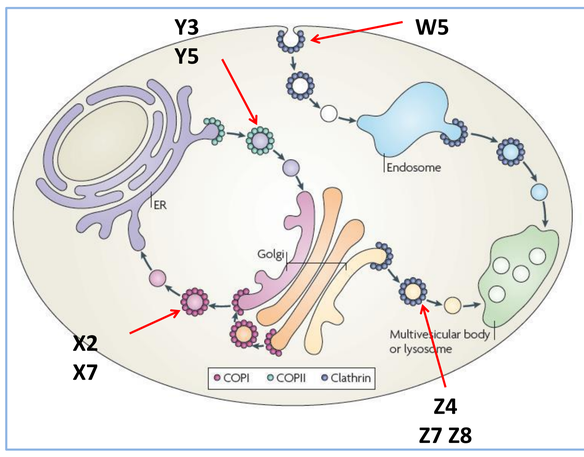


**Figure 6. Selected genes for TRV silencing and their place in the different pathways of vesicular transport.** Several genes of each pathway were selected: X2 and X7 were chosen to interrupt both intra-Golgi transport and retrograde transport, Y3 and Y5 to interrupt anterograde transport, and W5, Z4, Z7 and Z8 for transport from the *trans-Golgi network* to the later endosomal compartments, and also from the plasma membrane to the early endosomes (adapted from Hsu et al., 2009).

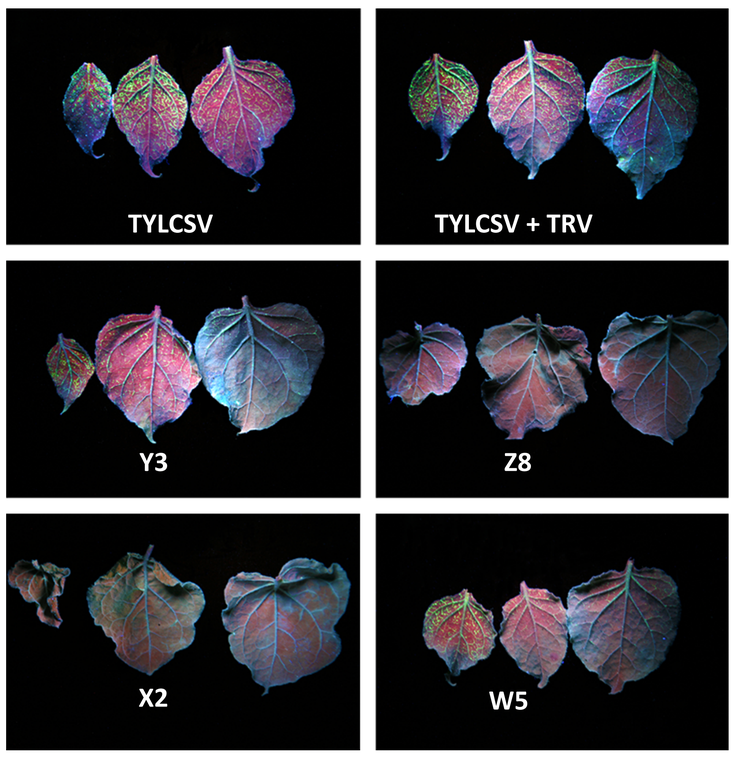


**Figure 7. GFP expression in the last 3 leaves of 2IR plants silenced with the different GOIs and infected with TYLCSV at 15dpi.**

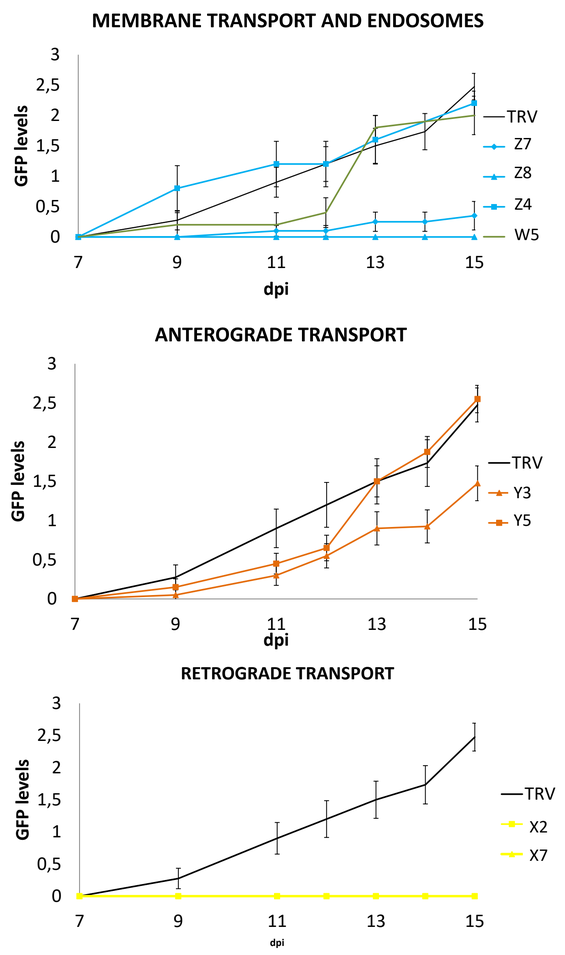


**Figure 8. Evolution of GFP expression levels in 2IR plants silenced with the different GOIs and infected with TYLCSV during days 7-15 post-infection.**

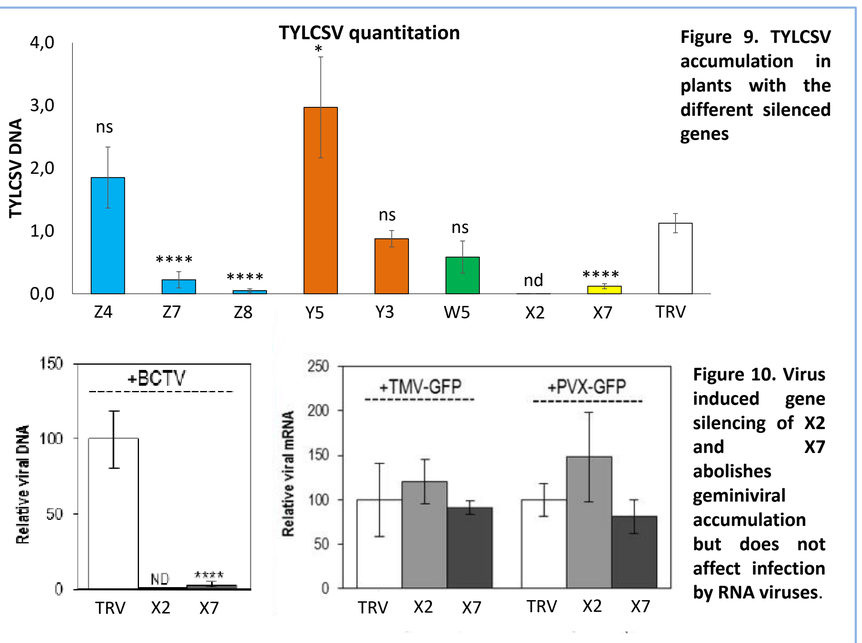


**Figure 9. TYLCSV accumulation in plants with the different silenced genes**

**Figure 10. Virus induced gene silencing of X2 and X7 abolishes geminiviral accumulation but does not affect infection by RNA viruses.**

Silencing of genes X2, X7, Z7 and Z8, produced a clear effect over TYLCSV infection, dropping the levels of GFP expression and viral DNA to 0 or almost 0. For plants which had genes X2 and X7 silenced different infection assays were carried. These plants were separately infected with TYLCSV (a begomovirus), BCTV (a curtovirus) and two different RNA viruses: PVX and TMV. Both geminiviruses presented almost null levels of DNA meanwhile both RNA viruses were not affected by the silencing. This data unveils a role of the COPI-dependent retrograde transport as an essential and specific pathway for geminivirus infection. For both Z7 and Z8, the measurement of relative amounts of TYLCSV DNA confirmed the preliminary results which seem to dramatically affect infection. The fact that these two genes and not Z4 and W5, involved in other parts of the membrane transport and endosomes pathway, affect infection remains a subject still to elucidate. Genes involved in anterograde transport did not notably affect infection, Y5 did promote a slightly higher accumulation of viral DNA.

**Acknowledgements:** This research was supported by a grant from the Spanish Ministerio de Ciencia y Tecnología (AGL2013-48913-C2-2-R).

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