INTRODUCTION

Geminiviruses are a large family of insect-transmitted plant viruses with circular, single-stranded (ss) DNA genomes packaged within geminiviruses particles which infect a wide range of plants causing devastating crop diseases. From among these diseases, Tomato yellow leaf curl disease (TYLCSV), is one of the most important threats to tomato crops worldwide. One of the causal agents of TYLCSV is Tomato yellow leaf curl Sarawak virus (TYCSV), a member of the genus Begomovirus belonging to the family Geminiviridae. TYCSV 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 genes 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 defense 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.

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 (2R 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 Y3, 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.

RESULTS AND DISCUSSION

Silencing of viral mRNAs and endogenous GDI

Figure 3. Nicotiana benthamiana 2R-GFP plants. A) Constructs of 2R-GFP plants; B) Episomal region formation in 2R plants infected with TYLCSV; C) GFP expression in 2R-GFP plants under UV light at 15 dpi.

Figure 4. A) Geometric organization of TRV vectors. BM1K contains a multiple cloning site (MCS) where the genes of interest (GOI) have been cloned and, therefore, will be coexpressed with the viral mRNAs in 2R benthamiana plants. B) Coexpression of TRV does not affect TYLCSV infection.

Figure 5. Strategy used to identify genes required for TYLCSV infection in Nicotiana benthamiana 2R-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 infiltration with Agrobacterium containing TYLCSV. C) Plants are observed under UV light to see the effect of the expression of GOIs during the 7 to 15 dpi post-infection.

Figure 6. Selected genes for TRV silencing and their role in different pathways of vesicular transport. Several genes of each pathway were selected: X2 and X7 were chosen to interfere with both intracellular-Golgi transport and retrograde transport, Y2 and Y5 to interrupt anterograde transport, and W5, Y2, 27 and 28 for transport from the trans-Golgi network to the late endosomal compartment, and also from the plasma membrane to the early endosomes (adapted from Liu et al., 2009).

Figure 7. GFP expression in the last 2 leaves of 2R plants silenced with the different GOIs and infected with TYLCSV at 15 days.

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

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REFERENCES


For full details and relevant citations, see the provided references at the end of the document.