Homology-dependent gene silencing associated with infection by *Tomato leaf curl virus-Australia* (*Begomovirus: Geminiviridae*)

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ABSTRACT

Tomato leaf curl virus-Australia (TLCV) promoters drive both constitutive and tissue-specific expression in tobacco. This study describes the silencing of tobacco transgenes carrying TLCV promoters following TLCV infection.

In a previous study to investigate TLCV promoter activity *in planta*, four complementary-sense (C1:GUS, C2:GUS, C3:GUS and C4:GUS) and two virion-sense (V1:GUSΔC and V2:GUSΔC) TLCV promoter:GUS transgenes were stably transformed into tobacco. Following systemic infection of the TLCV promoter:GUS plants with TLCV, transgene expression driven by all six TLCV promoters was silenced. Transgene silencing occurred in the vascular, mesophyll and floral tissues of V2:GUSΔC plants. Transgene silencing occurred with the continued replication of TLCV and was restricted to plants carrying TLCV-derived sequences, however infection of V2:GUSΔC plants by heterologous geminiviruses did not result in silencing. Thus, transgene silencing following TLCV infection was sequence-specific, requiring sequence homology between both the virus and the transgene.

Nuclear run-on assays to detect transcription from the V2:GUSΔC transgene in silenced plants indicated that silencing occurred at the level of transcription. The level of cytosine methylation of the V2:GUSΔC transgene in silenced tissue was assessed by bisulfite modification and sequencing. Following silencing, hypermethylation of cytosines in the TLCV-derived sequences of the transgene was observed. In contrast, hypomethylation of cytosines in the GUS sequences of the transgenes occurred in silenced tissue. The sequence-specific hypermethylation and transcriptional silencing of the V2:GUSΔC transgene

following TLCV infection represents the first case of virus-induced transcriptional gene silencing (VITGS) associated with a geminivirus infection.

Transgene expression was analysed in the virus-free progeny of silenced and non-silenced TLCV promoter:GUS plants. The silenced phenotype of infected V2:GUSΔC plants was inherited in progeny seedlings, however spontaneous partial restoration of transgene activity was observed with further growth. The heritable, yet reversible nature of TLCV-mediated VITGS was therefore a type of epimutation. The silenced phenotype was also inherited in V1:GUSΔC progeny from an infected parent. However, the silenced phenotype of the complementary-sense promoter:GUS plants was either partially (C1:GUS and C4:GUS) or completely (C3:GUS) reset in progeny. Interference with inherited cytosine methylation patterns and chromatin structures in C1:GUS and V2:GUSΔC progeny from infected parents indicated a role for both cytosine methylation and non-hypoacetylated heterochromatin formation in the inheritance of VITGS.

A component of the conserved antiviral RNA silencing pathway, short interfering RNAs (siRNAs), are reported to direct sequence-specific cytosine methylation in plants. siRNAs specific to transcribed TLCV sequences were detected during TLCV infection of four solanaceous host species, leading to the conclusion that TLCV infection induces the RNA silencing pathway. siRNAs homologous to the TLCV-derived V2:GUSΔC transgene sequences which became hypermethylated following VITGS were detected in tobacco following TLCV infection. Thus, TLCV-specific siRNAs were a candidate for the mechanism directing sequence-specific methylation of the TLCV promoter:GUS transgenes. siRNAs homologous to untranslated TLCV intergenic region sequences were detected in non-transgenic tobacco. This result may suggest previously uncharacterised transcription from the TLCV genome and/or the involvement of host RNA-directed RNA polymerases during the induction of RNA silencing by TLCV infection.