**ABSTRACT**

*Citrus tristeza virus* (CTV) is the causal agent of one of the most devastating viral diseases of citrus trees in the world. CTV is phloem-restricted in natural citrus hosts, and has evolved three silencing suppressor proteins acting at intra- (p23 and p20) and inter-cellular level (p20 and p25) to overcome strong host antiviral defense in citrus. RNA interference (RNAi), an approach based on using dsRNA to trigger RNA silencing, has been widely used for generating transgenic plants resistant against viruses. Considering the important role of p23, p20 and p25 in CTV pathogenesis, we have transformed Mexican lime plants with an intron-hairpin vector carrying full untranslatable versions of genes *p25*, *p20*, *p23* and the 3'-UTR from the CTV strain T36, to attempt silencing their expression in CTV-infected cells. Complete resistance to viral infection was observed in three transgenic lines, with all their propagations remaining symptomless and virus-free after graft-inoculation with CTV-T36, either in the non-transgenic rootstock or directly in the transgenic scion. Accumulation of transgene-derived siRNAs was necessary but not sufficient for CTV resistance. Challenging immune transformants with a divergent CTV strain resulted in partial breakage of the resistance, stressing the importance of sequence identity in the underlying RNAi mechanism. This is the first evidence that it is possible to achieve full resistance to CTV in a highly sensitive citrus host by targeting simultaneously its three viral silencing suppressors through RNAi. The p23 protein encoded by the virus is additionally an important pathogenicity factor. Ectopic expression of p23 in

transgenic citrus plants induces developmental aberrations resembling CTV symptoms. To explore in more detail the role of p23 in CTV pathogenesis, the *p23* gene from CTV T36 and three truncated versions thereof under the control of the *Cauliflower mosaic virus* 35S promoter were used to transform Mexican lime. Only the truncated version expressing amino acids 1 to 157 (p23Δ158-209) elicited CTV-like symptoms, similar to, albeit milder than, those incited by expressing the whole p23 protein (209 amino acids), thus delimiting the region responsible for p23 pathogenesis in citrus to a 157 amino acid fragment including the Zn finger and flanking basic motifs of the protein. RNA silencing suppressor activity of p23 in *N. benthamiana* was abolished by all mutants tested, indicating that silencing suppression involves most p23 regions. To better define the role of p23 in CTV pathogenesis, we next restricted the expression of *p23*-derived transgenes to phloem-associated cells in Mexican lime plants by means of using the phloem-specific promoter from *Commelina yellow mottle virus* (CoYMV). Constructions carrying the complete gene *p23* from either the severe T36 or the mild T317 CTV strains, or a fragment comprising the zinc-finger and flanking basic motifs from the former, either under the control of the CoYMV promoter or the constitutive 35S promoter were used for genetic transformation of Mexican lime. Expression of these constructs in the phloem incited aberrations resembling CTV-specific symptoms, but not the unspecific symptoms observed when p23 was constitutively expressed. Moreover, appearance and intensity of the most notorious CTV-like phenotypic aberrations induced by the phloem-specific expression of

the *p23* gene were positively related with the aggressiveness of the source CTV strain used. Additionally, expression in phloem-tissues of the p23 fragment comprising the zinc-finger domain and flanking basic motifs was sufficient to induce CTV-like symptoms, corroborating that the N-terminal region (delimited by amino acids 1 and 157) determines, at least in part, CTV pathogenesis in Mexican lime.