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DISEASE NOTE

First report of citrus tristeza virus trifoliate resistance-breaking (RB) genotype in *Citrus grandis* in China

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Citrus tristeza virus (CTV) isolates have been classified into six genotypes: T30, T36, VT, T3, T68 and RB (Harper 2013). In 2002, a CTV isolate CT9 was collected from Taibeiyou pummelo (*Citrus grandis*) in Santai, Sichuan Province. Biological indexing indicated that CT9 produced very mild stem pitting on Mexican lime (*Citrus aurantifolia*), Duncan grapefruit (*C. paradisi*) and Symons sweet orange (*C. sinensis*), and vein clearing on Mexican lime (*C. aurantifolia*). CT9 did not produce decline on Daidai sour orange (*C. aurantium*). In previous studies, CT9 was used to protect Guanximiyou pummelo against a dominant severe stem-pitting CTV isolate CT3 in China. Furthermore, single strand conformation polymorphism (SSCP) analyses of the main coat protein gene of CT9 and its aphid transmission subisolates showed that CT9 contained two strains that could not be segregated by single aphid transmission using *Toxoptera citricida* (Zhou et al., 2005). To understand the composition of CT9 further, young leaf tissues were collected and sent to Beijing Genomics Institute for transcriptome sequencing. De novo assembly of 57.8 Mb sequenced reads was performed using CLC genomics Workbench (CLC bio v10.0, Denmark). Subsequent mapping enabled to identify two contigs similar to CTV. Sixteen primer pairs were designed according to the contigs (Table S1). RT-PCR products were cloned and sequenced and found to be the CTV genomic regions characterized from the contigs. The 5′ and 3′ termini were determined by rapid amplification of cDNA ends (RACE kit, Invitrogen). The two CTV full-length sequence variants, CN-RB-9 (MH558665) and CN-RB-L13 (MH558666) were respectively 19,270 nucleotides (nt) in length with a maximum identity of 99% to CTV isolate CA-RB-AT35 (KU358530) and 19,250 nt in length with a highest identity of 97% to HA18–9 (GQ454869). Phylogenetic analyses of full-genome sequence showed that CN-RB-9, CN-RB-L13 and other extant RB isolates available in NCBI fell into the same clade, indicating that the two variants here characterized were RB genotype. Three months after CT9 was graft-inoculated onto trifoliate orange (*Poncirus trifoliate* cv. 'Flying dragon') which is a true to type to *Poncirus trifoliate*, CT9 was detected by ELISA using the monoclonal antibody (Agdia, USA). The result indicated that CT9 could replicate in *P. trifoliate*, which has been extensively used as a rootstock in China over the past decades. This research provides information that may be relevant to control CTV by using cross protection.

References

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