ABSTRACT

Distribution and diversity of *Citrus tristeza virus* isolates in Jamaica and development of transgenic citrus materials

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Citrus tristeza virus (CTV) belongs to the *Closteroviridae* family and has a monopartite, positive sense single-stranded RNA (ssRNA) genome of 19,296 to 19,302 nucleotides. It is the most destructive and economical viral pathogen affecting citrus in all parts of the world. The predominant usage of the sour orange rootstock, the presence of CTV and its vector, the brown citrus aphid, have significantly impacted the Jamaican citrus industry. The study analyzes the present distribution, biological and genetic variations of indigenous CTV strains and initiates the development of transgenic materials for the management of the disease in Jamaica.

The distribution and incidence of CTV and the severe strains in Jamaica were determined through an island-wide survey. A total of 1,885 samples were

collected from 71 farms over 13 locations and analyzed by double antibody sandwich enzyme-linked immunosorbent assays (DAS-ELISA) for detection of CTV, including severe strains. CTV was detected throughout the island, with viral incidence ranging from 7 to 100%, while severe CTV strains were limited to 9 of 13 locations, with incidences ranging from 2 to 33%. Symptom expression typical of CTV, noted on the host trees at the time of sample collection, was not a good indication of viral incidence, when compared to DAS-ELISA findings. Overall, incidence of the virus has increased from 29 to 65% over a five year period.

Reverse transcription polymerase chain reaction (RT-PCR) and dot blot hybridization were also used to test samples for CTV, with the intention of identifying a fast and reliable detection method. DAS-ELISA gave the highest detection levels of CTV 40 to 100%. Lower levels of CTV in single or mixed infections with multiple strains, were obtained with RT-PCR, but this depended on the primers used. CTV was not consistently detected by dot blot hybridization. A combination of ELISA and RT-CPR detected most of the Jamaican CTV isolates.

CTV isolates from the six main citrus growing regions were subsequently biologically characterized for the four main CTV syndromes on four different citrus indicator hosts and one scion-rootstock combination. Based on the range in symptom intensity, three defined groups of strains (mild, decline and

stem pitting), were identified among the samples from the six main citrus growing regions. The seedling yellows strain was not identified in any of the samples collected.

To understand the genetic structure of the Jamaican CTV strains, the coat protein gene (*cp*) was molecularly characterized using single strand conformation polymorphism (SSCP), restriction fragment length polymorphism (RFLP) and nucleotide sequencing. SSCP produced 24 SSCP different patterns and was not able to differentiate between isolates based on their geographical origin or strain type. RFLP analysis with *Hinf*I, differentiated isolates into groups consistent with their biological properties and isolates belonging to groups associated with mild strain cross protection (MSCP) and stem pitting were also identified. Nucleotide sequences of 37 Jamaican CTV *cp* strains indicated high similarities (90 to 100%) among the isolates. Phylogenetic analysis revealed the clustering of Jamaican isolates, based on biology, with 62 CTV isolates in 7 distinct groups. These data suggest different introductions of CTV into Jamaica or an introduction from a location in which CTV is relatively diverse.

Transgenic plants were developed for testing the feasibility of pathogen-derived resistance (PDR) as a means of managing CTV in Jamaica. Using three genes namely *cp*, *p20* and *p23* from both a mild and severe Jamaican CTV sequences, four CTV chimeric constructs were designed in a direct repeat and an inverted repeat orientation. *Agrobacterium* strain Agl 1 carrying

either pCAMBIA2201 or pCAMBIA2202 plasmids, containing three of the constructs were subsequently used to transform cotyledon and epicotyl segments of sweet orange (*Citrus sinensis* cv 'Pineapple'), grapefruit (*C. paradisi* cv 'Duncan') and 'Key' lime (*C. aurantifolia*). Sweet orange shoots expressing the reporter *uidA* or *gfp* genes were recovered at lower frequencies (5 to 10%) than 'Key' lime shoots expressing *uidA* (10 to 33%), however, transgenic shoots were not recovered with grapefruit explants. Two transgenic sweet orange plantlets were obtained with two of the three chimeric constructs. Transgene sequences were confirmed present by histochemical assay of β-glucuronidase and CTV PCR.

Keywords: *Citrus tristeza virus,* coat protein gene, pathogen-derived resistance, transgenic.