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Genetic Transformation of Citrus paradisi with Antisense and Untranslatable RNA-dependent RNA Polymerase Genes of Citrus tristeza closterovirus

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Abstract: Protein and RNA-mediated forms of pathogen-derived resistance (PDR) have been developed against many viruses in different plants. However, no resistance has been reported against Citrus tristeza virus (CTV), a closterovirus, in Citrus species transformed with coat protein genes or other sequences of CTV. The successful use of replication-associated genes in RNA-mediated resistance in other crops prompted the use of the RNA-dependent RNA polymerase (RdRp) gene of CTV for the development of RNA-mediated PDR in Citrus. The RdRP gene was amplified from CTV isolate DPI3800 from Florida and used to generate antisense (RdRp-AS) and untranslatable (RdRp-UT) constructs with point mutation consecutive stop codons in the 5’ end of the RdRp gene for use in plant transformation. A total of 3120 etiolated epicotyl segments of Duncan grapefruit (Citrus paradisi Macf. cv. Duncan) were transformed with these constructs using Agrobacterium tumefaciens-mediated transformation. From these segments 1040 kanamycin-resistant shoots were regenerated, and a total of 131 putative transgenic shoots were identified by fluorescent microscopy and histochemical b-glucuronidase (GUS) assays. One hundred GUS positive plants were rooted and 66 plants survived and were established on soil. A total of 41 plants were tested by polymerase chain reaction (PCR) for the presence of the GUS gene and for the transgenes. Eighteen GUS-positive and transgene-positive plants (8 with RdRp-AS, and 10 with RdRp-UT) were identified.

Key Words: Citrus tristeza virus, replicase and RNA-mediated resistance, plant transformation, RNA-dependent RNA polymerase

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