

Nucleotide sequence of a new viroid species, citrus bent leaf viroid (CBLVd) isolated from grapefruit in Israel

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Several citrus viroids (CVds) were found in 'Etrog' citron plants inoculated with the graft transmissible dwarfing agent (GTDA). GTDA isolate 225 # T has been shown to dwarf some grapefruit trees in Israel (1). One of CVds, citrus bent leaf viroid (CBLVd), previously designated as CV-Ib (2) was separated by graft transmission on avocado plants (3). In this study CBLVd was propagated and purified from avocado leaves, the circular RNA molecules separated, S1 treated, polyA tailed and reversely transcribed using a chimeric polylinker-oligo dT (P-dT) primer. The cDNA was polyadenylated and P-dT was used as a bidirectional PCR primer. The PCR generated cDNA was cloned in the BamHI site of pBluescript KS(+) vector (Stratagene). Plasmids containing inserts which hybridized positively with CBLVd were sequenced using the Sequenase® (USB) protocol. Based on sequencing information derived from three separate CBLVd inserts, two end-to-end 18-mer primers were synthesized and used respectively for cDNA and PCR synthesis of full length CBLVd cDNA molecules (4).

CBLVd consists of 318 nts which can be arranged in the viroid

specific rod-like structure (5). According to this model more than 66% of the bases are paired. The molecular size of CBLVd is slightly smaller than previously estimated (330 nts) by PAGE analysis (1, 3). CBLVd is a novel chimeric viroid species constituted from a part of the central conserved region (CCR) of the apple scar skin viroid (ASSVd) subgroup (6, 7), and part of the P region and the left terminal region of citrus exocortis viroid (CEVd) (5). CBLVd is the first member of the ASSVd subgroup infecting citrus plants to be characterized.

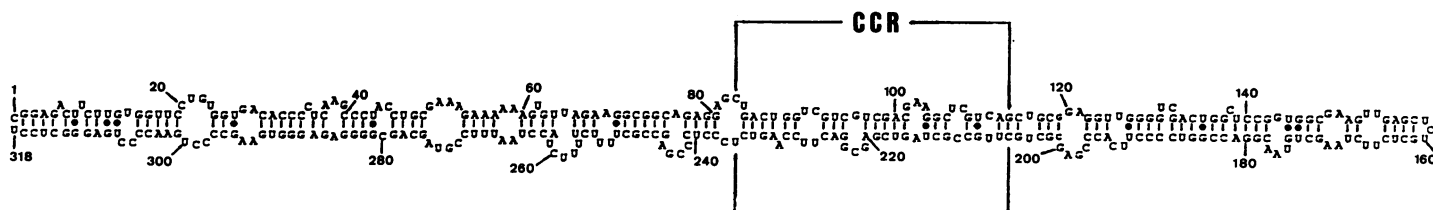
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1   CGGAGACUUC UUGUGGUUCC UGUGGUGACA CCCCUC AAGC CCUACCUGCG   50
51  AAAGAAAAAA GUGUUAGAAG GCGGCAGAGG AGCUGACUGG UCGUCGUCGA   100
101 CGAAGGCUCG UCAGCUGCGG AGGUUGGGGU CGACUGGCUC CGGUGGCGAA   150
151 GUUGAGCUCU GCUCUUCUAA GCUGUAACGG ACCGGUCCCC UUCACCCGAG   200
201 CGCUGCUUGC CGCUAGUGGA GCGGACUUC AAGUCUCCCU CCCGAGCCGC   250
251 UUUUCUUUC  UACCUAAUUU CCGUAGCAGC GGGGAGAGGG UGAAGCCCCU   300
301 GAACCCUGA  GGGCUCCU                                     318

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