Primary and secondary structure of citrus viroid IV (CVd IV), a new chimeric viroid present in dwarfed grapefruit in Israel

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'Etrog' citron plants inoculated with the isolate no. 225-T of the graft-transmissible dwarfing agent (GTDA) used to dwarf grapefruit trees in Israel are known to contain several citrus viroid (CVd) species (1). One of those is citrus viroid IV (CVd IV) as previously designated by Duran-Villa *et al.* (2). To separate CVd IV from the other CVds, dilution endpoint experiments were performed and a pure culture of CVd IV was finally obtained in the wild cucurbit *Benincasa hispida* which does not exhibit any symptoms of disease. Total nucleic acids were phenol-extracted from the CVd IV-infected leaf material and salt-fractionated (3). The circular CVd IV RNA was purified from the 2 M NaCl-soluble fraction by three sequential steps of polyacrylamide gel electrophoresis, one performed under non-denaturing and two under denaturing conditions (4, 5), respectively. Northern blot hybridization experiments with *in vitro* synthesized viroid-specific (−) RNA transcripts (3) revealed that CVd IV must share longer sequence motifs with citrus exocortis viroid (CEVd) (6, 7), potato spindle tuber viroid (PSTVd) (8, 9) and hop stunt viroid (HSVd) (10, 11). Consequently the complete nucleotide sequence of CVd IV could be established at the level of its cDNA by reversely transcribing the circular viroid RNA starting with the 'universal primer' p15II for PSTVd-like viroids (3, 9, 11) and continuing with three CVd IV-specific DNA primers. From the resulting four overlapping primer-extended cDNAs sequenced chemically as previously described (3), the complete nucleotide sequence of CVd IV could be constructed. CVd IV consists of 284 nucleotides (nts) that can be arranged into the viroid-specific rod-like secondary structure model as depicted below in which 63 G:C, 32 A:U and 8 G:U pairs are present so that 71% of all its nts are base-paired. Sequence comparison revealed that CVd IV is a novel mosaic-type chimeric viroid, and that most of its right hand part and of its central region (CR) resembles CEVd, whereas the left terminal region is very similar to that of HSVd. It is noteworthy that these two parent-viroids of CVd IV are also members of the viroid complex of the GDTA. 'Etrog' citron plants inoculated with CVd IV alone displayed only transiently mild symptoms of leaf epinasty after hot summer weather.

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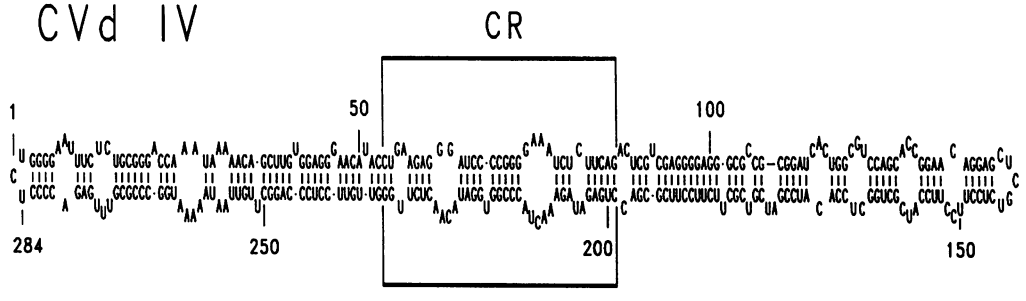

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