Coconut cadang-cadang viroid (CCCVd) mutants associated with severe disease vary in both the pathogenicity domain and the central conserved region

M.J.B.Rodriguez and J.W.Randles[1,*]

Philippine Coconut Authority, Albay Research Centre, Philippines and [1]Department of Crop Protection, Waite Agricultural Research Institute, University of Adelaide, Glen Osmond 5064, South Australia

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Coconut cadang-cadang viroid (CCCVd) causes a lethal disease of coconut palm in the Philippines (1). The minimal infectious viroid comprises 246 nucleotides. A single cytosine addition at position 197 to give a 247 nt molecule, and duplication of the right-hand terminus (V and T2 domains) to give molecules of 287 nts and larger are the only reported variations in the viroid structure (2, 3). Artificial passage of viroid inoculum in the Philippines has led to the appearance of a severe lamina-depleting symptom ('brooming') in about 12% of inoculated seedlings (1, 4). Electrophoretic analysis on 20% polyacrylamide gels has identified additional RNA bands with homology to CCCVd which are specifically associated with the 'brooming' (4).

Twenty-eight full length clones were obtained by reverse transcription and polymerase chain reaction (PCR) amplification of nucleic acids from palms with brooming. The PCR products were inserted in the Bluescript plasmid by the single A:T overlap method (5) and sequenced by fluorescent dye-primer cycle sequencing (Applied Biosystems Inc.). Figure 1 shows where mutations were observed in the clones. Two or more clones were obtained for each mutant. Position 197, in the lower strand of the central conserved region (CCR) (6) showed three addition and substitution mutations. A substitution was observed in the lower strand of the pathogenicity (P) (6) domain at position 216. An addition of A was also observed at the extreme right of the CCR. The clones obtained showed mutations at either one or two sites, but not at all three sites.

Therefore, CCCVd differs from the other viroids described in that mutations possibly associated with pathogenicity occur in the CCR. Other viroids of the potato spindle tuber viroid group (7, 8) show pathogenicity-associated variations in sequence outside the CCR.

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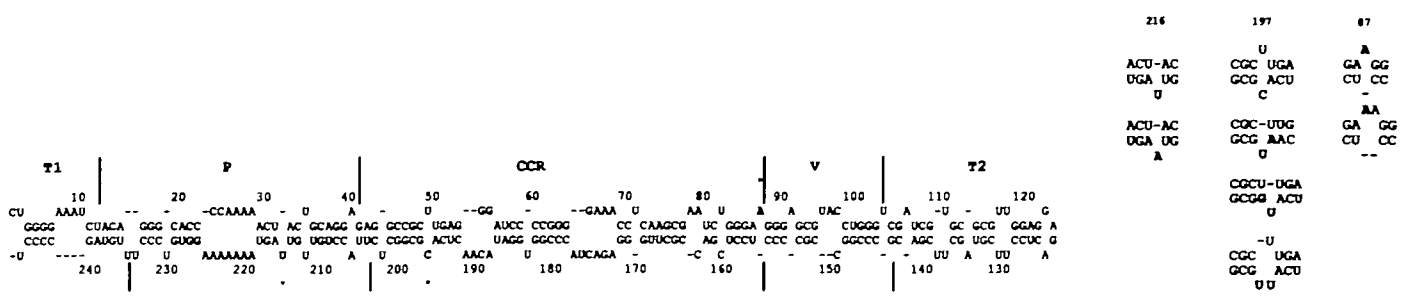

Figure 1. The minimum free energy secondary structure of $CCCVd_{246}$ showing the proposed T1, P, CCR, V and T2 domains (2, 3), and sites where mutations occur. The mutations at positions 87, 197 and 216 are also shown with their probable effects on secondary structure. The substituted or added nucleotides are indicated in bold letters.

* To whom correspondence should be addressed