MOLECULAR VARIATION IN COCONUT CADANG CADANG VIROID (CCCVd)

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Thesis submitted to the University of Adelaide in fulfillment of the requirement for the degree of Doctor of Philosophy

March, 1993

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SUMMARY

Molecular hybridization with ^{32}P-labelled cRNA and cDNA probes in dot-blots and electroblots and the use of two-dimensional polyacrylamide gel electrophoresis (2-D PAGE) demonstrated the presence of multiple coconut cadang-cadang viroid (CCCVd)-like nucleic acids in 11 understorey plant species in coconut plantations. These nucleic acids seemed to be at a lower concentration in their hosts than CCCVd is in coconut. Of the multiple bands found in each plant species, those which were approximately the same size as CCCVd usually had high nucleotide sequence homology with the probe. In contrast, the bands outside the CCCVd region had low homology with the probe. The use of the polymerase chain reaction (PCR) with some of the isolates purified by 2-D PAGE failed to amplify CCCVd-specific nucleic acids under the conditions developed for successful amplification of CCCVd.

The 11 plant species with CCCVd-like sequences are the monocotyledonous hosts *Maranta arundinacea, Alpinia* sp., *Zingiber officinale, Canna flaccida, Commelina diffusa, Commelina benghalensis, Paspalum conjugatum, Brachiaria distachya, Imperata cylindrica, Bambusa blumeana* and the dicotyledonous host *Urena lobata.* The samples were collected at 11 separate sites in five provinces of southern Luzon, Philippines. None showed symptoms which could be associated with the presence of the viroid-like nucleic acids. The incidence in these species was independent of the level of cadang-cadang disease in adjacent coconut plantations, and it is suggested that these plants could act as a reservoir for CCCVd-related nucleic acids in the Philippines.

An unusually severe type of symptom was observed in approximately 12% of 1,787 palms infected by artificial inoculation with preparations of CCCVd. The symptom was first observed in palms 3-7 years after inoculation and is characterized by a loss of leaf lamina, and is associated with severe stunting of the palm and occasional premature death. This symptom has been termed "brooming". PAGE and molecular analyses showed that all palms with brooming had patterns of CCCVd-related nucleic acids which differed from the patterns observed with the common form of cadang-cadang disease. Thus, four bands representing viroids with 246, 247, 296 and 297 nucleotides are observed in extracts from palms with

common cadang-cadang disease. Palms with "brooming" show additional or replacement bands which have electrophoretic mobilities distinct from the above forms of viroid. Bi-directional PAGE was used to separate the brooming-associated nucleic acids from other host nucleic acids. These nucleic acids were eluted as a mixture of the various electrophoretic forms. Three distinct primers were used for reverse transcription of the nucleic acids. The products were amplified by PCR with three pairs of primers. Products with the expected sizes were inserted into the Bluescript plasmid vector by the use of single base A:T overlaps. Twenty-eight full-length clones and 19 half-length clones were obtained, 29 of which were selected and sequenced by fluorescent dye primer cycle sequencing. A number of mutations were observed in the clones, and they fell into the following classes: (i) substitution of C at position 197 of the central conserved domain with either AU, UG, or UU; (ii) substitution of U with A at position 216 in the P domain; (iii) addition of A at position 86 or 87 so that AA became AAA at the boundary of the central conserved region and the V domain; (iv) mutants of varying length resulting from partial sequence duplications of the V and T2 domains commencing from varying positions in the right-hand end of the viroid molecule; and (v) mutations at the boundaries of duplications.

Models were proposed showing optimal secondary structures. These showed that the substitution of C at position 197 of the central conserved domain with either AU or UG could lead to the replacement of a U-loop with a single base pair and that mutations at the boundaries of partial sequence duplications could cause structural changes in the V and T2 domains. The other mutations appeared not to affect secondary structure.

As a mutation at position 197 in the central conserved domain has been previously reported for CCCVd, it is concluded that this site may be important for the control of pathogenicity of CCCVd.

A one-tube, reverse transcription-PCR reaction system was developed for use with CCCVd.