USE OF MOLECULAR MARKERS FOR BREEDING OF THE COCONUT PALM *COCOS NUCIFERA*

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by

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DECLARATION

The studies presented in this thesis were completed by the author whilst a postgraduate student with the Institute of Biotechnology, Department of Molecular and Cellular Biology, University of New England, Armidale, NSW, Australia.

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I certify that the substance of this thesis has not already been submitted for any other degree and is not being currently submitted for any other degree.

I certify that any help received in preparing this thesis, and all sources used, have been acknowledged.

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ABSTRACT

Coconut palm, *Cocos nucifera*, is the most widely grown plantation crop in Sri Lanka and plays a major role as a perennial oil crop and as an important constituent of the Sri Lankan diet. Breeding of the coconut palm for characters such as high yield of copra, drought tolerance and pest and disease resistance is a high priority. Coconut breeding has progressed a long way over a period of about 75 years through mass selection and inter and intra-varietal hybridisations. However, the optimum utilisation of the natural variability by classical breeding is constrained by lack of vegetative propagation methods, long breeding cycle and the high cost and land requirement for breeding experiments.

Development of molecular markers to assist the coconut breeding programme was regarded as a timely move in planning future breeding strategies. Thus, this work represents the most probably the first attempt to study the genetic variation in the coconut palm population in Sri Lanka by RAPD and RFLP assay procedures.

Initially a protocol to extract DNA from fresh coconut leaves was developed by modifying the miniprep procedure of Dellaporte et al., (1983) for RAPD-PCRs and Southern blot studies. Thereafter, a pedigree consisting of two parent palms, one of *typica typica* (commercial tall type) and the other of *nana* (dwarf green type) and 18 F_2 individuals were assayed by RAPDs. Thirty eight primers were used and seven of them produced 16 bands segregating in the F_2 progeny. Twelve of the fragments distinguished the parents. Fifteen of these fragments segregated in the F_2 progeny in Mendelian fashion. The second RAPD study assayed the genetic diversity between 19 accessions of the coconut palm population in Sri Lanka. These accessions represented all varieties, forms within varieties and five germplasm accessions of the *typica typica*. Out of the 38 primers 18 detected polymorphisms among the accessions giving a total of 91 polymorphic bands. The RAPDs revealed genetic relationships between the accessions closely parallels with the morphology based classifications. The *typica* form San Ramon was an exception clustered

with *nana* forms in agreement with pollen protein based clustering in a previous study. This indicates that the genetic diversity of the coconut forms is not accurately reflected by the morphology based classification.

For detection of RFLPs, coconut DNA digested by six restriction enzymes, *Bam*HI, *Dra*I, *Eco*RI, *Eco*RV, *Hind*III and *Pst*I were hybridised to random coconut DNA sequences from a *Sau*3AI library in Southern blots. A total of 98 clones (72 high copy and 27 low copy) were used in combinations of two or three to screen restriction digested DNA of *typica typica*, *nana* green and *typica* San Ramon in 384 DNA template / probe /enzyme combinations. With *Bam*HI, *Hind*II and *Pst*I digests clones hybridised to only high molecular weight regions. With *Dra*I, *Eco*RI and *Eco*RV the probes detected clearly resolved bands. However, the majority of probes detected the same fragments in *Dra*I (1150 bp), *Eco*RI (2700 bp, 2500 bp, 1400 bp and 1300 bp) and *Eco*RV (1800 bp, 600 bp and 400 bp) digested DNAs. These banding positions are shown in ethidium bromide stained agarose gels as repetitive sequence sites in coconut DNA indicating that these high copy clones contain a sequence that hybridises to the large repetitive element in the coconut genome. The polymorphic fragment, (300 bp *Eco*RV fragment was detected by seven pairs of probes did not segregate in the F_2 population. The fragments detected by low copy clones appear promising although the results were not conclusive. The Southern blot hybridisations that probed coconut DNA digests by single strand M13 bacteriophage DNA and simple sequence repeats failed to resolve DNA fingerprints.

The potential of the RAPD technique for assessment of genetic diversity in the coconut palm is well evident. Development of RAPD markers to tag genes of interest is achievable provided that well defined segregating families are available. Bulk segregation analysis is suggested as a suitable method for tagging useful traits in coconut with RAPD markers. RAPDs can be immediately applied for evaluation of germplasm collections. The relative merits of RFLP and RAPD are discussed with a suggestion to use a methylation sensitive enzyme (eg. *Pst*I) to clone coconut DNA inserts as potential RFLP probes instead of using high copy sequences.