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The work described in this thesis was carried out by me under the supervision of Mr. J.M.D.T.Everard, Prof. H.G.Nandadasa and Prof. E.H. Karunanayake and a report on this has not been submitted to any University for another degree.

.....

(P.N.Dasanayake)

We certify that the above statement made by the candidate is true and this thesis is suitable for submission to the University for the purpose of evaluation.

.....	.....	.....
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Development of molecular markers for breeding and germplasm conservartion of *Cocos nucifera* L.

by

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Abbreviations

AFLP	Amplified Fragment Length Polymorphisms
bp	base pair
cDNA	complementary DNA
CGIAR	Consultative Group on International Agricultural Research
CGRD	Coconut Genetic Resource Database
COGENT	Coconut Genetic Resource Network
cM	centi Morgan
CRI	Coconut Research Institute
DAF	DNA Amplification Fingerprinting
DNA	Deoxyribo Nucleic Acid
dNTP	Deoxy Nucleotide Triphosphate
ISTR	Inverse Sequence Tagged Repeat
kb	kilo base
PCR	Polymerase Chain Reaction
RAPD	Randomly Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphisms
QTL	Quantitative Trait Loci
SCAR	Sequence Characterized Amplified Region
SNP	Single Nucleotide Polymorphism
SSRP	Simple Sequence Repeat Polymorphism

TAC	Technical Advisory Committee
UPGMA	Unweighted Paired Group Method of Arithmetic mean
UV	Ultra Violet

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I dedicate this thesis to
my parents and husband

Development of molecular markers

for breeding and germplasm conservation of *Cocos nucifera* L.

P.N.Dasanayake

ABSTRACT

Coconut Research Institute (CRI) of Sri Lanka has made a concerted effort with the Coconut Genetic Resources Network (COGENT) of the International Plant Genetic Resources Institute (IPGRI) to conserve coconut germplasm in the country and consequently, around 100 accessions were conserved *ex-situ* in CRI gene banks as rejuvenated populations from islandwide coconut collections. Understanding the true genetic variation of these accessions was an important requirement for effective management and utilization of coconut germplasm in the country. Due to high environmental dependence of morphological descriptors, powerful DNA techniques, randomly amplified polymorphic DNA (RADP), simple sequence repeat polymorphisms (SSRP) and amplified fragment length polymorphisms (AFLP) were applied to elucidate genetical relationships of coconut genetic resources in the country.

A sample of 43 coconut accessions comprising 19 distinct phenotypes [7 tall (*typica*), 9 dwarf (*nana*) and 3 thembili (*aurantiaca*) forms], 7 San-Ramon tall-like ecotypes and 17 Sri Lanka tall ecotypes were used in the assessment. For RAPD assessment, 20 Operon primers selected from 100 primers belonging to OPERON kits A, B, C, D and E were

used. The selected primers readily amplified coconut DNA generating 186 amplification products averaging 9.3 bands per primer. Among the bands, 166 exhibited polymorphisms (89.25%) averaging 8.3 polymorphic bands per primer. The Nei and Li pair-wise genetic distance matrix revealed a narrow genetic base in the coconut in Sri Lanka with distances ranging from 0.07-0.43 with an average distance of 0.24. RAPDs revealed genetic relationships of the 43 accessions to a certain degree but failed to explain the entire variation accurately.

Seventeen pairs of coconut specific micro-satellite primers were used for detection of simple sequence repeat polymorphisms in the 43 accessions. The primers detected 82 alleles with an average of 4.8 alleles per locus ranging from 2 to 10. All alleles were polymorphic within the 43 genotypes analyzed. Eight accession-specific-alleles were found in six accessions. Genetic distances among the accessions ranged from 0.13 to 1.0 with an average of 0.63. The SSR polymorphisms clearly revealed the genetical organization of the coconut in the country unveiling much important kinship that could be related to accepted theories of evolution and dissemination of coconut.

Amplified fragment length polymorphisms were also detected among the accessions using eight *Eco*RI and *Mse*1 selective primer combinations. A total of 221 fragments were obtained of which 163 (73.75%) exhibited polymorphism. The average number of scorable bands per primer combination was 27.6 with 34 maximum and 20 minimum bands per primer combination. The genetic distances ranged from 0.05 to 0.25 with an

average of 0.13. The AFLPs too failed to discriminate coconut germplasm accurately although aptly identified the relationship of a number of close groups.

The results of the three systems generated adequate information to shed light on understanding the underlying genetic variation in the coconut palm population in Sri Lanka well in par with the roots of coconut evolution and sources of probable introduction to the country superceding the current morphological-descriptor-based relationships. According DNA polymorphisms, coconut population in Sri Lanka predominantly consists of widely grown Sri Lanka tall resembling a genome similar to coconuts from Africa and India. Few other collections of coconut, dwarf and San Ramon and San-Ramon-like type coconut populations represent a South East Asian or Pacific genome. All the germplasm accessions of Sri Lanka tall share a similar genome indicating a narrow genetic variation within them probably resulted from genetic constriction due to domestication. The results led to readjustment of taxonomic status of two Sri Lanka tall coconut forms, Bodiri and Ran Thembili, as they appeared to have a different genome more similar to that of SEA or Pacific coconuts. The dwarf coconuts distinguished by colour of seed coat largely shared a genome common to all dwarf coconuts worldwide, which too is more close to coconuts in the Pacific and South East Asia where coconut was believed to have originated. King coconut in Sri Lanka currently considered as an intermediate between Sri Lanka Tall and Sri Lanka Dwarf coconuts is certain to be a dwarf and not a distinct group between tall and dwarf. Tall coconuts other than Sri Lanka Tall such as San Ramon (Clovis), Nipuni and Indian have

demonstrated genomes that are more common to coconuts from Pacific and South East Asia.

The results had important implications on effective conservation and utilization of coconut germplasm. It is clear that widening of gene banks by further random sampling of Sri Lanka Tall is futile, as the assessed ecotypes did not show much variation within themselves or any kind of genetic isolation. Therefore, searching for phenotypically distinct populations in the country appears a more sensible sampling approach for effective conservation of coconut in the country. The sampling size of *ex-situ* conserved coconut populations too can be minimized to reduce the cost of conservation, which is very high for coconut.

The prioritization of crosses for testing hybrid vigor based on genetic distance was another important implication although significant results have been already achieved in the coconut-breeding programme of the Coconut Research Institute by testing combinations of crosses between the most distant, Sri Lanka Tall, Sri Lanka Dwarf and San Ramon. Therefore the present data strongly suggest the need for germplasm enrichment by introducing more tall coconuts from diverse populations in the Pacific and South East Asian where coconut palm populations still appear to maintain wild genes, which were lost during domestication in the African region.

The preliminary study carried out towards developing a genome map of coconut surfaced following useful information. The potential of SSRs was promising and the

need for construction of at least another 50 pairs of coconut specific micro-satellite primers was a high research priority. The legitimacy of crosses and correct identification of progeny is also found to be a matter of concern because of high rate of contaminants in segregating populations available for study. Therefore, it is necessary to construct more populations giving emphasis on careful execution of pollination programs, accurate labeling of seedlings in the nursery and scoring of individuals in segregating families for phenotypic characters in carefully designed field experiments with minimum environment variability.