13

Coconut

M.K. Rajesh, V. Niral, Anitha Karun and V.A. Parthasarathy

Introduction

The coconut palm (*Cocos nucifera* L.) is grown throughout the tropical world and virtually every part of the palm possesses some human use. It is a monotypic species in the genus *Cocos* and belongs to the Arecaceae family. The origins of the palm are still subjects of controversy with one group claiming a Melanesian origin, and the other group a South American origin. Regardless of its origin, the coconut has spread across the tropical regions of the globe mainly by natural dissemination - the fruit, being buoyant, is spread considerable distances by oceanic currents. Human selection and dissemination have also contributed significantly to the global dispersal of the palm (Harries *et al*., 2004). Presently, the palm grows in more than 86 countries, mainly between 26º N and 26º S (Harries, 2001). There are two main types in the coconut, talls and dwarfs. Palms belonging to tall varieties are mainly cross-pollinated while the dwarf varieties are generally self-pollinated.

Genetic improvement is very difficult and protracted in a perennial crop like coconut. The extended juvenile phase, the long interval between generations, heterozygous nature of the palm, the sizeable area required for planting experiments and the extensive period of experimentation required for obtaining results are the major reasons which hamper progress of crop improvement programmes in coconut.

Genetic diversity gives a species the ability to adapt to changing environments, including new pests, diseases and new climatic conditions. The main requirement in any breeding programme is knowledge of the genetic variability available in a particular crop. Molecular markers have brought to the fore an extremely efficient method of estimating the genetic diversity of germplasm collections of various crops.

With reference to coconut, the use of molecular marker techniques may enhance breeding

efficiency in various ways: characterization and management of germplasm, genetic diversity studies, linkage mapping and identification of QTLs for marker-assisted selection (MAS). Using molecular markers, it would be possible to organize accessions into genetic groups and also identify redundant collections. These markers would also aid in rationalizing the choice of crosses to be tested in a coconut hybrid breeding programme (Lebrun *et al.*, 1998). Identification of molecular markers linked to useful traits will strengthen and hasten breeding programmes, reduce costs, improve the efficiency and reduce the length of the selection cycles.

Molecular Profiling

A whole range of different techniques has been used to detect polymorphism and these includes proteins, isozymes, polyphenols, restriction fragment length polymorphism (RFLP), randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), inverse Sequence-Tagged Repeat (ISTR), simple sequence repeats (SSR) and inter simple sequence repeats (ISSR). The use of these molecular methods has opened up new possibilities for phylogenetic analysis and provides new tools for the efficient conservation and use of coconut genetic resources.

Biochemical Markers

Attempts to characterize coconut populations based on biochemical markers like isozymes, leaf proteins, polyphenols, and carotenoids have been reported from in many countries with different coconut populations. Various parts of the coconut palm like leaf, endosperm, pollen, inflorescence, etc. have been utilized for the study of isozymes.

Work on isozyme analysis in coconut began at IRHO, France in 1978 with a view to understanding the structure of genetic diversity. Studying diversity by enzyme electrophoresis came up against technical problems, as numerous systems proved monomorphic or not very active. Four systems revealed polymorphism and only two alleles per locus could be detected for each system (Benoit, 1979). Benoit and Ghesquiere (1984) obtained clearly readable patterns in coconut pollen, haustorium and embryo, while leaf, root and shoot tissues rapidly oxidized making extraction and interpretation difficult. Meunier (1992) analyzed six different Tall populations from different origins with 17 isozyme systems and concluded that isozyme analysis could be used for cultivar identification and progeny legitimacy. Although this method revealed initially a clear distinction between ecotypes from Southeast Asia, Africa and Pacific sources (Bourdeix *et al.*, 1993), only low polymorphism could be detected.

Carpio (1982) found a range of 12 to 16 bands in the electrophorograms of coconut meat proteins, with two bands, one at the upper and the second located nearly at the middle portion of the gel, consistent in all the populations. On the other hand, electrophoresis of pollen extracts yielded 22 to 29 protein bands.

White *et al.* (1987) and Moran (1991) analyzed six tall populations of Papua New Guinea and Solomon islands using 15 enzymatic systems. However, polymorphism was noticed in only four systems with low diversity in these studies.

Shivashankar (1988), observed that the pattern of *PPO* isozymes in West Coast Tall (WCT) and Chowghat Orange Dwarf (COD) leaves subjected to osmotic stress/air dehydration did not show any differences from the control, whereas two additional fast moving bands could be located in the case of stressed leaves of both COD × WCT and WCT × COD.

Canto-Canche *et al.* (1992) studied *PRX, ACP, EST* and *PGI* isozyme profiles in leaf pinnae and inflorescence extracts of four cultivars, Atlantic Tall, Malayan Green, Red and Yellow Dwarfs. Although the number of major bands detected varied in some cases across the tissues, the patterns of *MDH, ADH, EST* and *ACP* were identical in the four varieties analyzed. They also explored the possibility of using endosperm as isozyme source. They found that the pattern for the *PGI* activities were identical for the four varieties analyzed. They also analyzed the enzyme profiles at six stages of endosperm development (6-12 months) in nuts of two normal coconut trees and seven trees producing both normal and makapuno nuts. They concluded that no isozyme was unique to either type of endosperm with three anodic *PRX* isozymes being detected in both. However, the normal endosperm showed sharp changes in the intensity of the isoperoxidase bands as growth progressed whereas the bands were of low intensity in the makapuno endosperm.

Fernando *et al.* (1993) found that leaf tissues showed the maximum polymorphism and the pattern was clear than the haustorium tissues for all three enzyme systems *viz., ADH, EST* and *PRX* studied. Individuals of the Tall form showed polymorphism for the *EST* enzyme system with a maximum of five different alleles. Individuals with homozygous banding pattern (single bands) were low in frequency within the Tall form of coconut but were more frequently found in the Dwarf type. However, common bands appear for both Dwarf and Tall forms at this enzyme system with a differentiation possible only among the individuals. A difference in the frequency of alleles was reported among populations. Polymorphism among individuals of the Tall form was revealed for the *PRX* system with two different alleles. One allele was reported to be fixed in most individuals whilst only a few showed the other allele. There was no polymorphism among the individuals for the dehydrogenase enzyme systems. Among the three isozymes *EST, ADH* and *PRX, EST* showed the maximum polymorphism among individuals with five different alleles. The difference in the allelic frequency among various populations indicates the possibility of estimating the genetic diversity existing in the germplasm and also the possible linkage of certain allelic forms to important quantitative characters.

Bhattacharya *et al*. (1993) analyzed the *AMP, ACP, AO, EST, LAP* and *PRX* isozyme profiles in coconut pollen and recorded one, three, three, three, one and four bands, respectively.

Asmono *et al.* (1993) analyzed six enzymes by starch gel electrophoresis, of them five showed variation in the isozyme banding pattern namely *PRX, EST, ACP, END* and *GOT*; the number of banding patterns found for these enzymes being five, four, three, four and four, respectively. They found only one pattern for *CAT*. Many populations had more than one banding pattern. They recommended coconut palm leaves for isozyme analysis.

Hengky and Hartana (1994) assayed *EST, PRX* and *GOT* isozyme profiles in different organs and at different developmental stages to find the best organ/developmental stage and concluded that leaf enzyme extracts gave clear and readable patterns.

Fernando and Gajanayake (1997) established protocols for the detection of isozyme polymorphism in coconut leaf tissues. Out of six enzyme systems tested, only *EST* and *PRX* produced polymorphic banding patterns. The *EST* system produced a maximum of five bands for the Tall x Tall (CRIC60) and Plus Palm Tall populations. Dwarf populations showed only a limited number of bands at both loci, confirming their inbreeding nature. Using a combination of all genotypes, principal component analysis differentiated the two main groups, Dwarfs and Talls. The pattern of differentiation among Tall populations suggested that a relationship exists between the frequency of certain bands and palm yield potential which could be successfully used to screen coconut germplasm for desirable characters.

Cardena *et al*. (1998) studied the electrophoretic pattern of leaf *PRX*, *END* and Coomassie Blue stained proteins in four cultivars (West African Tall, Rennell Tall, Malayan Yellow Dwarf, Cameroon Red Dwarf) of coconut and in the hybrids PB121 (Malayan Yellow Dwarf × West African Tall) and PB111 (Cameroon Red Dwarf × West African Tall). Four distinctive genotypes were identified, one for each of the Tall cultivars, another for both of the Dwarf cultivars and one for both hybrids.

Jayalekshmy (1999) found variation at the biochemical and molecular level among the coconut cultivars. For biochemical studies, which included isozyme analysis and protein gel electrophoresis, eight cultivars of coconut were selected and three isozymes *viz.*, *PRX*, *EST* and *PPO* were analyzed. Two zones of activity were observed for PRX and EST, while only one zone of activity was observed for PPO.

Zizumbo-Villarreal *et al.* (2002) estimated the diversity in 28 Mexican and imported coconut populations using 15 enzymatic systems and reported low polymorphism. The fixation indices indicated low total heterozygosity and low heterozygosity within populations suggesting endogamy and genetic drift, and a high diversity among populations due to differentiation between Pacific and Gulf of Mexico coastal populations.

Geethalakshmi *et al*. (2004) undertook a study on isozyme polymorphism in six dwarf coconut cultivars. Among the cultivars, higher enzyme polymorphism was observed in Gudanjali Dwarf and least in Gangabondam Dwarf. Of the seven isozyme systems, *PPO* showed higher polymorphism followed by *EST*, *GOT*, *PER* and *MDH*. *ACP* and *ADH* did not show any intrapopulation variation.

Parthasarathy *et al.* (2004) took up the task of isozyme profiling for 11 isozyme systems in 40 different coconut cultivars and six hybrids. Cluster analysis indicated that the cultivars grouped into six main clusters. In case of hybrids and their parents, the hybrids clustered intermediate between parents.

Geethalakshmi *et al.* (2005a) analyzed the genetic diversity at the isozyme level of thirty coconut cultivars. They studied 10 enzyme systems *viz*., *ADH*, *GOT*, *G-6-PD*, *PRX*, *EST*, α-Amylase, Phosphorylase, *MDH*, *SOD* and *ACP* and observed 20 loci and 40 alleles, of which, 14 loci were polymorphic. Seven loci were heterozygous. They observed null alleles for *ACP*, *ADH* and *MDH*. They reported greater heterozygosity for Glucose-6 Phosphate Dehydrogenase and least for *SOD*. Among the cultivars, they found that the heterozygosity was highest in Nadora Tall and Calangute and least in Kulasekharam Green Dwarf.

Geethalakshmi *et al.* (2005b) undertook characterization of different coconut cultivars and hybrids by analyzing the banding profiles of native proteins. Intrapopulation variation was studied in six Dwarfs, eight Talls, two hybrids and their parents. Among Dwarfs, Malayan Orange Dwarf showed highest intrapopulation variation and Gudanjali Dwarf showed least polymorphism. Among Talls, Java Tall showed highest and Kappadam Tall showed least intrapopulation variation. While among the hybrids and their parents, WCT showed highest and Laccadive Ordinary Tall and Gangabondam Dwarf showed least variation. Interpopulation variation was studied in seven Dwarfs, 19 Talls and four hybrids. Allelic frequency was highest in New Guinea Tall, Benaulim Tall, while least in Fiji Tall.

Parthasarathy *et al.* (2005) studied the *EST* and *PER* profiles of West Coast Tall and Assam Tall coconut populations and detected very little differences at the isozyme level and concluded that both these populations are genetically similar, differing only with regard to their adaptation to different ecosystems.

Geethalakshmi *et al.* (2006a) used 11 isozyme systems *viz*., *EST, PRX, PPO, MDH, ACP, ADH, GOT, α-AMY, PHOS, G-6PDH, SOD* and PROT to study the interpopulation variation among 30 different coconut cultivars and hybrids. They reported highest mean allelic frequency in Andaman Giant and Ceylon Tall and least frequency in Kenthali Dwarf.

Geethalakshmi *et al.* (2006b) studied the banding pattern of spindle leaf isoperoxidases in six dwarf (Chowghat Orange Dwarf, Malayan Yellow Dwarf, Chowghat Green Dwarf, Malayan Orange Dwarf, Gudanjali Dwarf, Gangabondam Dwarf) and eight tall coconut varieties (West Coast Tall, Java Tall, Laccadive Ordinary Tall, Philippine Ordinary Tall, Laccadive Micro Tall, Kappadam Tall, Andaman Ordinary Tall, San Ramon Tall) and reported differences in banding patterns both within and between cultivars. They observed that Gudanjali Dwarf alone showed intrapopulation polymorphism among dwarfs while, Java Tall showed highest intrapopulation variation among talls.

Niral *et al.* (2007) reported intra-population allelomorphism in eight tall and six dwarf populations of coconut, based on total soluble proteins and seven isozyme systems (*EST, PRX, PPO, MDH, ACP, ADH* and *GOT*). Among the dwarfs, they observed highest enzyme polymorphism in Gudanjali Dwarf, and least in Gangabondam Dwarf showed. Amongst the Talls, Java Tall showed highest isozyme polymorphism, while least polymorphism was seen in San Ramon Tall. Differences in the allelic frequency were obtained even though there were no specific differences in banding pattern of varieties.

Other groups working with proteins (White *et al.*, 1987; Canto-Cache *et al.*, 1992) found that leaf extracts were easily oxidized, gave low enzyme activities, and inconsistent results.

Analysis of leaf polyphenol polymorphism was also undertaken and it provided a picture of variability that matched geographical origins (Jay *et al.*, 1989). Champakam and Ratnambal (1993) reported significant differences in the levels of leaf polyphenols in 36 cultivars from eight different geographical origins. But the sensitivity of the polyphenol banding patterns to ecological conditions limits its applications.

DNA Markers

Restriction Fragment Length Polymorphism (RFLP)

RFLP technique was used for the first time in coconut by Lebrun *et al.* (1998) to study the genetic diversity in 100 palms from diverse geographical locations, representing 10 Tall and seven dwarf coconut populations. Eleven cDNA probes from rice and wheat were hybridized on coconut DNA digested using four restriction enzymes. The study revealed two genetic groups- the first one included ecotypes from the Far East and from the South Pacific, whereas the other group comprised the ecotypes from India, Sri Lanka and Western Africa. The results from the RFLP studies tallied with reports of historical dispersion of coconut. Substantial diversity was recorded in ecotypes collected from Far East and Pacific regions, which are considered putative area of origin of the coconut palm. The West African ecotypes were related to the Indian and Sri Lankan ecotypes suggesting recent extension of the palm along the length of the Atlantic Coasts of Africa via nuts originating from the Indian Ocean. The tall ecotypes exhibited the higher polymorphism compared to dwarfs.

Randomly Amplified Polymorphic DNA (RAPD)

RAPD markers, which require less cost and infrastructure compared to other presently available markers, can be effectively used for the estimation of genetic distances among coconut collections in major coconut growing developing countries.

A moderate level of genetic diversity was detected in 17 distinct South Pacific populations analyzed by means of RAPD technique using 14 primers by Ashburner *et al.* (1997b). The study revealed occurrence of over 60 per cent of the observed variability within populations. Genetic drift and a possible bottleneck in the past of the species were suggested as reasons for the low inter-population diversity. A few RAPD markers unique to specific populations were identified.

Everard *et al.* (1996), Rodriguez *et al.* (1997) and Wadt *et al.* (1999) have reported use of RAPD technique to access the genetic diversity of coconut populations from Sri Lanka, the Philippines and Brazil respectively.

Hayati *et al.* (2000) used RAPD to analyze genetic diversity of four dwarf populations from East Java. They found that variability of coconut population grown outside East Java was higher than that at East Java since those coconut population collected from seeds of open pollinated plants.

Cardena *et al.* (2003) carried out studies to identify RAPDs associated with resistance to lethal yellowing disease of the coconut palm using three coconut populations *viz*. susceptible West African Tall, resistant Malayan Yellow Dwarf and a resistant population of Atlantic Tall palms. Markers potentially linked to lethal yellowing disease were detected.

Upadhyay *et al.* (2004) used RAPD method to analyze the genetic diversity among 15 Indian and five exotic accessions with eight polymorphic primers. On the whole, tall accessions were more heterozygous than dwarf accessions; they had higher proportions of polymorphic

bands and higher genetic diversity. Likewise, exotic accessions displayed higher variation. Dwarf accessions from geographically distant regions clustered together.

Ritto *et al.* (2008) analyze the genetic diversity and genetic relationship among three yellow Dwarf coconut accessions *viz.* Malayan Yellow Dwarf (MYD), Kulashekaram Yellow Dwarf (KYD) and Andaman Yellow Dwarf (AYD) using RAPD markers (Fig.13.1). Similarity matrix based on Jaccard's coefficient indicated a close association of the two Indian yellow dwarfs (AYD and KYD) with the exotic yellow Dwarf (MYD) (Fig.2) indicating the likelihood of AYD and KYD evolving from a common progenitor, the MYD.

Inverse Sequence-Tagged Repeat (ISTR)

Rohde *et al*. (1995) developed a novel PCR-based approach for detection of DNA polymorphisms, inverse sequence-tagged repeat (ISTR) analysis, in which primers complementary to repetitive, copia-like sequences in the coconut genome (Rohde *et al.*, 1992) were used to amplify a large number of genetic loci with an abundance of polymorphisms occurring among a set of selected coconut genotypes from various regions of the world. Duran *et al.* (1997) extended this technique to analyze East African Tall coconut populations. It was concluded that these molecular marker types represent powerful tool for genotype identification, analysis of germplasm variability and breeding purposes in coconut. These studies also provided evidence for the existence of truncated, copia-like repetitive sequences in the coconut genome indicating that retro-elements may have played a role in the generation of genetic diversity in coconut.

DNA Amplification Fingerprinting (DAF)

The DNA Amplification Fingerprinting (DAF) approach (Caetano- Anolles *et al*., 1992) is a variation of the RAPD technique, but is more informative due to the use of altered reaction conditions (He *et al.*, 1995), shorter primers and silver staining.

Nagaraju *et al.* (2003) utilized DAF technique to study the relationship among eight coconut accessions. In the same study, the DAF technique was compared with AFLP technique and the latter was found to be more efficient in detecting polymorphism. In DAF, out of 300 primers screened, 28 (9.33%) detected polymorphism, while in AFLP 55 (86%) primer combinations generated polymorphic bands (6.42). Cluster analysis revealed clustering of dwarf ecotypes into a single group and the tall ecotypes into three groups.

DAF technique was subsequently used by Jayadev *et al.* (2005) to identify molecular markers, which could differentiate between coconut root (wilt) disease resistant and susceptible palms. Out of the 16 primers screened, three primers *viz*. UBC 66, UBC 84 and UBC 729 could differentiate between field resistant and susceptible coconut palms.

Amplified Fragment Length Polymorphism (AFLP)

Amplified Fragment Length Polymorphism (AFLP) is a high-multiplex PCR-based method for DNA profiling (Vos *et al*., 1995) with the potential to generate a large number of polymorphic genetic loci and involved both restriction digestion and PCR.

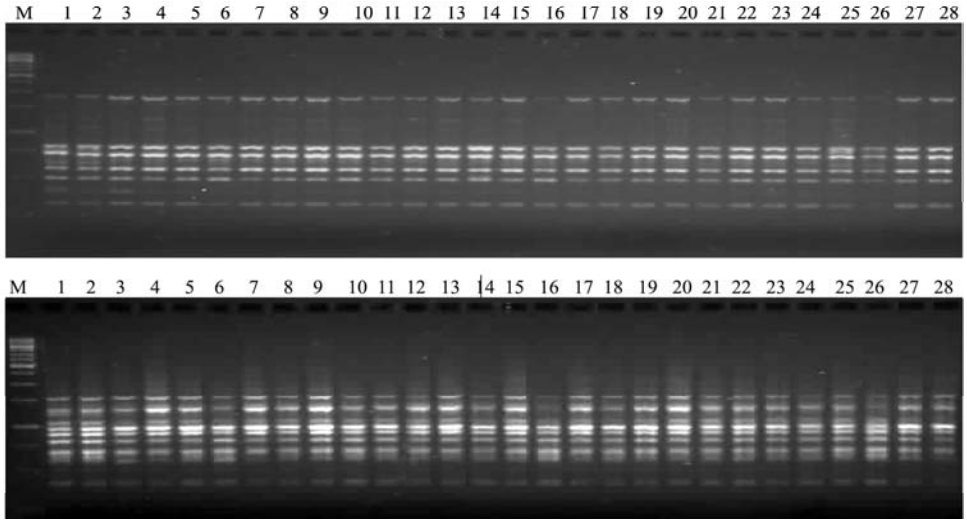

Fig 13.1. RAPD marker profile of yellow dwarf coconut accessions using the primers OP M4 (top) and OP AH 13 (bottom).
M: 1 Kb ladder
Lanes 1-16: KYD, 17-19: AYD, 20-28: MYD

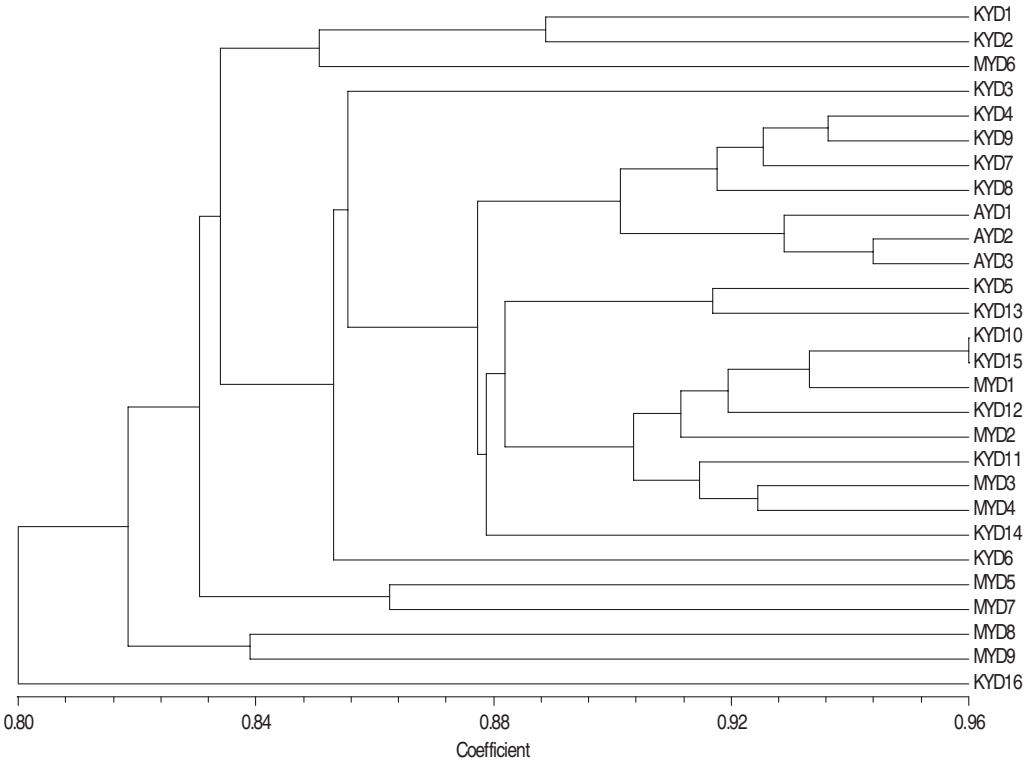

Fig 13.2. UPGMA dendrogram of yellow dwarf palms based on Jaccard's co-efficient using RAPD markers

Perera *et al.* (1998) conducted AFLP analysis of 42 genotypes indigenous to Sri Lanka using eight primer pairs. Overall, more variation was detected in tall forms (*typica),* rather than intermediate (*aurantiaca*) and dwarf (*nana*) forms. *Aurantiaca* group was more similar to the dwarf rather than the tall group. In addition, putative duplicate accessions were identified in the *Aurantiaca* group. A hierarchical analysis of molecular variance (AMOVA) was used to quantify and partition levels of variability into between and within form components. It was found that for the inbreeding dwarf and intermediate forms most variation was observed between, rather than within forms. In contrast, the outbreeding tall forms exhibited as much variation within as between forms.

Teulat *et al*. (2000) used AFLP markers in combination with SSR markers to analyze genetic diversity of 31 palms from 14 coconut populations from different ecological regions.

Inter-Simple Sequence Repeats (ISSR)

Thirty-three coconut accessions representing different geographical regions of the world, maintained at the International Gene Bank in India, were analyzed using 19 ISSR primers (Manimekalai and Nagarajan, 2006). A total of 199 ISSR markers were generated, out of which 154 were polymorphic. Least similarity was found between Nicobar Tall and Chowghat Orange Dwarf, both accessions from India. Coconut accessions from Southeast Asia, South Asia and South Pacific formed separate groups, which was generally in accordance with their origin and dispersal.

Simple Sequence Repeats (SSR)

The use of polymorphic microsatellites for assessing genetic diversity in coconut has been gaining popularity because of their high information content and co-dominant nature. Microsatellites, or simple sequence repeats (SSRs), are short tandemly repeated sequence motifs of approximately 1–8 bp in length, which are scattered throughout the genome and can vary between individuals in repeat length (Tautz and Renz, 1984). High frequencies of polymorphism have been described for SSRs in several plant species Primer pairs designed for the flanking sequences can be used in PCR reactions for site-specific amplification of the microsatellite, thereby producing sequence-tagged microsatellite markers (Powell *et al*., 1996).

Rivera *et al.* (1999) characterized 40 coconut samples from the Philippines using eight SSR primer pairs. Dwarfs grouped separately from talls and showed less genetic diversity.

Using a pre-cloning enrichment procedure, Perera *et al.* (1999) isolated eight coconut microsatellites. These eight microsatellites were used to study the levels and patterns of genetic diversity of Sri Lankan coconut populations. The results showed that the Sri Lankan tall coconuts exhibit higher levels of diversity than the dwarfs and intermediates, and the intermediate coconuts are more similar to dwarfs than talls. This was in agreement with the results obtained using AFLPs in the same set of genotypes in an earlier study (Perera *et al.*, 1998).

Perera *et al*. (2000) used eight pairs of SSR primers to analyze the genetic diversity in 130 individuals of coconut comprising 75 tall and 55 dwarf individuals representing 94 ecotypes from different geographical regions. A phenetic tree based on genetic distance clustered

individuals into five groups, each mainly composed of either talls or dwarfs. Thirty-three tall coconut populations from Sri Lanka were subjected to microsatellite assay with eight SSR primer pairs in order to study the levels and distribution of genetic variation for formulating future collection strategies and selecting parents for breeding programmes (Perera *et al*., 2001). A high level of genetic diversity was detected in all the populations.

A coconut microsatellite kit was developed by CIRAD in collaboration with COGENT and it consists of 14 microsatellite markers with sufficient discriminating power for practical identification of coconut cultivars (Baudouin and Lebrun, 2002). Standard protocols, without the use of radioactive probes, as well as a dedicated statistical software, Gene Class 2, were developed which could be adapted to use in developing countries.

Merrow *et al.* (2003) utilized 15 simple sequence repeat (SSR) microsatellite DNA loci to analyze genetic variation within coconut germplasm collections maintained at two locations in South Florida, representing eight cultivars. Parentage analysis of 'Fiji Dwarf' cultivar was also carried out using these loci. The Red Malayan Dwarfs were found to be genetically distinct from Green and Yellow ones. Also, genetic identity of 'Red Spicata' was found to be more to "Fiji Dwarf'.

Devakumar *et al*. (2006) carried out an assessment of genetic diversity of 21 Indian and 24 exotic coconut accessions using eight SSR primers. The eight coconut microsatellite loci distinguished a total of 48 alleles, with an average of 6 alleles per locus. Genetic diversity values were low for most dwarfs and high for the tall accessions, which is in accordance with their breeding behaviour. However, an indigenous dwarf, Kulasekharam Orange Dwarf, showed genetic diversity higher than many talls. Within population variation (58%) was found to be higher than among population variation (42%).

Microsatellite analysis of lethal yellowing disease tolerant genotypes (Vanuatu Tall and Sri Lankan Green Dwarf) and susceptible genotype (West African Tall) was carried out by Konan *et al*. (2007). A total of 58 alleles were detected by the 12 microsatellite loci analyzed. Genotypes of susceptible West African Tall cultivar were found to be less genetically clustered to the genotypes of the two tolerant cultivars. The fingerprinting based on microsatellites aided in identification of suitable parents to be used in crossing programmes for developing a segregating mapping population for marker-assisted selection of lethal yellowing resistant genes.

The Maypan, a hybrid of Malayan Yellow Dwarf (MYD) and Panama Tall coconut, earlier considered highly resistant, is presently being devastated by an outbreak of lethal yellowing disease in Jamaica. Lebrun *et al*. (2007) used 34 SSR markers to compare the MYD sampled from four locations in Jamaica along with a reference DNA of MYD collected from five different countries *viz*. Ghana, Malaysia, Philippines, Mexico and India to check whether the affected planting material in Jamaica was genetically similar to the material earlier shown to be resistant to lethal yellowing disease. The results revealed more variation at 34 simple sequence repeat loci in MYD samples from Jamaica than from the rest of the world. About 16% of alleles in Jamaican MYD samples did not match the usual typical MYD genotypes showing that the Jamaican MYD palms were only partially true to type and this heterogeneity may have some undesirable effect on its degree of resistance to lethal yellowing disease.

The extent of genetic diversity in 26 coconut accessions from the Andaman and Nicobar Islands, India was determined using 14 microsatellite markers by Rajesh *et al*. (2008). A total of 103 alleles were detected by the microsatellite markers with an average of 7.35 alleles per locus (Fig.13.3). The average observed and expected heterozygosity was 0.29 and 0.66 respectively. A mean fixation index (FST) of 0.49 was observed, indicating a high level of population differentiation among the coconut accessions. Majority of rare alleles were observed in tall accessions from the Nicobar Islands. This study using microsatellites confirms the rich genetic diversity of coconut accessions from these Islands.

Generation of Molecular Linkage Maps and Tagging of Useful Genes

An important step in genetic analysis is to produce genetic linkage maps. Such maps represent the relative order of genetic markers, and their relative genetic distances from one another, along each chromosome of an organism.

Herran *et al.* (2000) used AFLP, ISSR, ISTR and RAPD markers to construct linkage map in coconut for the two parents of the cross *Malayan Yellow Dwar*f x *Laguna Tall*. A total of 382 markers generated 16 linkage groups for each parent. QTLs for early germination were identified.

These QTLs correlate with early germination and yield, representing characters, which are important in coconut breeding. AFLP and SSR markers were used to construct a linkage map in the coconut type *Rennel Island Tall* (Lebrun *et al.*, 2001). A total of 227 markers were arranged

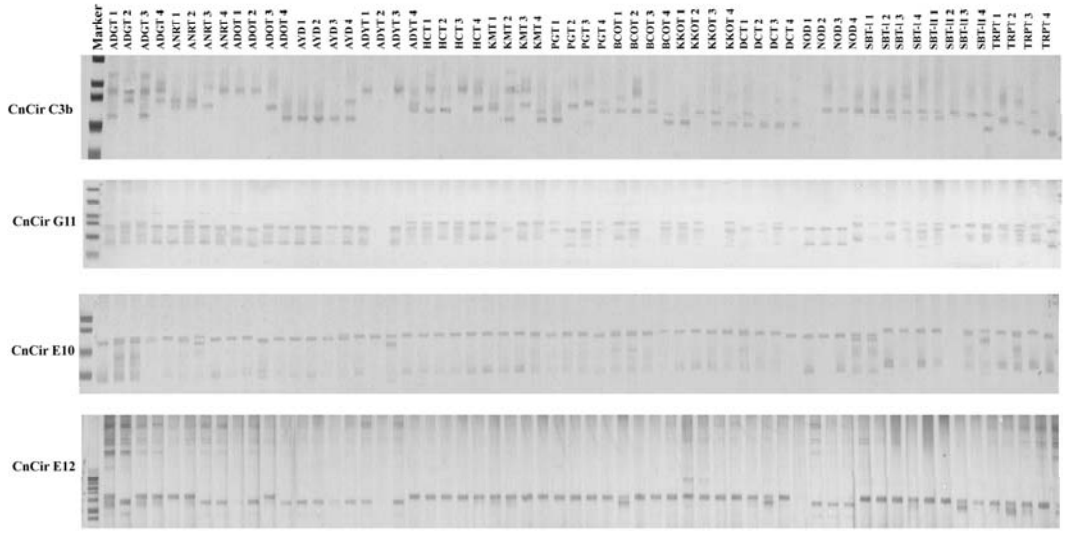

Fig 13.3. Electrophoretic pattern of coconut accesions from Andaman and Nicobar Isalands using the SSR primers CnCir C3b, CnCir GII, CnCir E10 and CnCir E12.

ADGT: Andaman Giant Tall; ANR: Andaman Ranguchan Tall; ADOT: Andaman Oridinary Tall; AYD: Andaman Yellow Dwarf; ADYT: Andaman Yellow Tall; HCT: Horned Cocos Tall; KMT: Katchal Micro Tall; PGT: Perka Green Tall; BCOT: Bachdera Ordinary Tall; KKOT: Kakana Oval Tall; DGT: Dugong Creek Tall; NOD: NIcobar Orange Dwarf; SBT-I: South Bay Tall: TRPT: Trinket Papaya Oval Tall; Marker: 100 bp ladder.

into 16 linkage groups. QTLs were detected for yield characters *viz.* number of bunches and number of nuts.

Baudouin *et al*. (2006) investigated the genetic factors, which controlled fruit components in coconut. QTL analyses was performed for fruit component weights and ratios in a segregating progeny of a Rennell Island Tall genotype, complemented by the linkage map constructed previously by Lebrun *et al*. (2001). Out of the 52 putative QTLs identified for the 11 traits studied, 34 grouped in six small clusters. Interestingly, the QTLs for fruit component weight, endosperm humidity and fruit production were found at different locations in the genome, which suggested the need for selection of QTLs for individual traits for efficient marker-assisted selection for yield.

Shalini *et al*. (2007) reported identification of molecular markers with mite resistance in coconut. Mite resistant and susceptible accessions were collected and analyzed using RAPD and SSR primers. Nine SSR and four RAPD primers were identified with mite resistance using single marker analysis. When step wise multiple regression analysis of RAPD and SSR data was done, a combination of five markers could account for 100% of the association with mite resistance.

Future Perspectives

The important advances in coconut biotechnological techniques, which have been made particularly during the last decade, could immensely benefit the coconut breeder in practice and open new perspectives for the development of new varieties. The molecular markers have revealed a high genetic diversity in coconut, specifically in the tall accessions. There is a great potential for the exploitation of untapped germplasm utilizing the tools of biotechnology. The integration of marker-assisted selection in coconut breeding promises to drastically increase the efficiency of breeding programs. With the advances in genomics, the acquisition of knowledge, with further development of tools for modifying and interrogating genomes, will be highly accelerated.

References

Ashburner, G.R., Thompson, W.K. and Halloran, G.M., 1997. RAPD analysis of South Pacific coconut palm populations. *Crop Science,* 37:992-997.

Asmono, D., Hartana, A., Guhardja, E. and Yahya, S., 1993. Genetic diversity and similarity of 35 coconut populations based on isoenzyme banding pattern analysis. (Diversitas dan kemiripan genetic 35 populasi kelapa berdasarkan analisis pola pita isozim). *Buletin Pusat Penelitian Kelapa Sawit* 1:39-54.

Baudouin, L. and Lebrun, P., 2002. The development of microsatellite kit and dedicated software for use with coconuts. *Burotrop Bulletin,* 17:16-20.

Baudouin, L., Lebrun, P., Konan, J.L., Ritter, E., Berger, A. and Billotte, N., 2006. QTL analysis of fruit components in the progeny of a Rennell Island Tall coconut (Cocos *nucifera* L.) individual. *Theoretical and Applied Genetics,* 112:258-268.

Benoit, H., 1979. Study of protein polymorphism in coconut. Proceedings of 5th Session of FAO Working Party on Coconut Production, Protection and Processing. 9p. Manila, Philippines.

Benoit, H. and Ghesquiere, M., 1984. Electrophorese compte-rendu cocotier. IV. *Determinisme genetique. Internal Report*, Paris, IRHO-CIRAD, pp. 11.

Bhattacharya, S., Das, S. and Mukherjee, K.K., 1993. Biochemical studies on palm pollen. *Grana,* 32:123-127.

Bourdeix, R., Sangare, A., Le Saint, J.P., Meunier, J., Gascon, J.P., Rognon, F. and Nuce de Lamothe, M., 1993. Coconut Breeding at IRHO and its Application in Seed production. In: Advances in Coconut Research and Development. (Eds. Nair, M. K., Khan, H.H., Gopalasundaram, P. and Bhaskara Rao, E.V.V.) pp. 79-94. Oxford and IBH Publishing Company, New Delhi, India.

Caetano- Anollès, G., Bassam, B.J. and Gresshoff, P.M., 1992. Primer-template interaction during DNA amplification fingerprinting with single arbitrary oligonucleotides. *Molecular and General Genetics,* 235:157-165.

Canto-Canche, B., Quintal-Salazar, E. and Villanueva, M.A., 1992. Biochemical markers of variety in *Cocos nucifera* L. from Yucatan. *Turrialba,* 42:375-381.

Cardena, R., Ashburner, G.R. and Oropeza, C., 2003. Identification of RAPDs associated with resistance to lethal yellowing of the coconut (*Cocos nucifera* L.) palm. *Scientia Horticulturae,* 98:257-263.

Cardena, R., Oropeza, C. and Zizumbo, D., 1998. Leaf proteins as markers useful in the genetic improvement of coconut palms. *Euphytica,* 102:81-86.

Chempakam, B. and Ratnambal, M.J., 1993. Variation for leaf polyphenols in coconut cultivars. In: Advances in Coconut Research and Development. (Eds. Nair, M. K., Khan, H.H., Gopalasundaram, P. and Bhaskara Rao, E.V.V.) pp. 51-53. Oxford and IBH Publishing Company, New Delhi, India.

Devakumar, K., Jayadev, K., Rajesh, M.K., Chandrasekhar, A., Manimekalai, R., Kumaran, P.M. and Parthasarathy, V.A., 2006. Assessment of genetic diversity of Indian coconut accessions and their relationship to other cultivars using microsatellite markers. *Plant Genetic Resource Newsletter,* 145:38-45.

Duran, Y., Rohde, W., Kullaya, A., Goikoetxea, P. and Ritter, E., 1997. Molecular analysis of East African Tall coconut genotypes by DNA marker technology. *Journal of Genetics and Breeding,* 51:249-288.

Everard, J.M.D.T., Katz, M. and Gregg, K., 1996. Inheritance of RAPD markers in the coconut palm, *Cocos nucifera* L. *Tropical Agricultural Research,* 8:124-135.

Fernando, W.M.U. and Gajanayake, G., 1997. Patterns of isozyme variations in coconut (*Cocos nucifera* L.) populations used for breeding improved varieties. *Plantations Recherche Development,* 4:256-261.

Fernando, W.M.U., Abeysekara, J.D.J. and Jayatilleke, R., 1993. Annual session of the Sri Lanka Association for Advancement of Science, pp. 82-84.

Geethalakshmi, P., Niral, V. and Parthasarathy, V.A., 2004. Allozyme variation in population of dwarf coconut cultivars. *Journal of Plantation Crops,* 32(2):13-15.

Geethalakshmi, P., Niral, V. and Parthasarathy, V.A., 2005b. Characterization of coconut germplasm based on protein polymorphism. *Indian Journal of Horticulture,* 62:118-121.

Geethalakshmi, P., Niral, V. and Parthasarathy, V.A., 2006a. Interpopulation variation in Coconut. *Indian Journal of Horticulture,* 63:439-445.

Geethalakshmi, P., Parthasarathy, V.A. and Niral, V., 2005a. Genetic diversity among coconut (*Cocos nucifera L.*) genotypes using isozymes. *Asian Journal of Plant Sciences,* 4:678-683.

Geethalakshmi, P., Parthasarathy, V.A. and Niral, V., 2006b. Polymorphism of isoperoxidases in Coconut cultivars. *Indian Journal of Horticulture,* 63:73-75.

Harries, H.C., 2001. The coconut palm (*Cocos nucifera*). In: Tree Crop Ecosystems. (Ed. F.T. Last) pp. 321-338. Elsevier, Netherlands.

Harries, H., Baudouin, L. and Cardena, R., 2004. Floating, boating and introgression: Molecular techniques and the ancestry of coconut palm populations on Pacific Islands. *Ethnobotany Research and Applications,* 2:37-53.

Hayati, D.P.K., Hartana, A., Suharsono and Aswidinnoor, H., 2000. Genetic diversity of Jombang coconut populations based on RAPD markers. *International Conference on Science and Technology For Managing Plant Genetic Diversity in the 21st Century*, 12 -16 June, 2000, Kuala Lumpur, Malaysia. p.11 (abst.).

He, G., Prakash, C.S. and Jarret, R.L., 1995. Analysis of genetic diversity in sweet potato (*Ipomoaea batatus*) germplasm collection using DNA amplification fingerprinting. *Genome,* 38:938-945.

Hengky, N. and Hartana, A., 1994. Perkembangan dan diffensiasi beberapa isozim pada tanaman kelapa. *Journal Biologi Indonesia,* 1:35-39.

Herran, A., Estioko, L., Becker, D., Rodriguez, M.J.B., Rhode, W. and Ritter, E., 2000. Linkage mapping and QTL analysis in coconut (*Coconut nucifera* L.). *Theoretical and Applied Genetics,* 101:292-300.

Jay, M., Bourdeix, P., Potier, F., Sanslaville, E., 1989. Initial results from the study of polymorphism of coconut leaf phenols. *Oleagineux,* 44:158-161.

Jayadev, K., Rajesh, M.K., Devakumar, K., Thomas, R.J., Nair, R.V. and Parthasarathy, V.A., 2005. DNA amplification fingerprinting in coconut: protocol optimization and analysis of resistance to root (wilt) disease. *CORD,* 21:39-49.

Jayalekshmy, V.G., 1999. Genetic fingerprinting in coconut cultivars. Proceedings of the Eleventh Kerala Science Congress, Kasaragod, February-March 1999. pp. 200-204.

Konan, J.N.K., Koffi, K.E., Konan, J.L., Lebrun, P., Dery, S.K. and Sangare, A., 2007. Microsatellite gene diversity in coconut (*Cocos nucifera* L.) accessions resistant to lethal yellowing disease. *African Journal of Biotechnology,* 6:341-347.

Lebrun, P., Baudouin, L., Myrie, W., Berger, A. and Dollet, M., 2007. Recent lethal yellowing outbreak: why is the Malayan Yellow Dwarf Coconut no longer resistant in Jamaica? *Tree Genetics and Genomes,* 4:125-131.

Lebrun, P., Baudouin, L., Bourdeix, R., Konan, J.L., Barker, J.H.A., Aldam, C., Herran, A. and Ritter, E., 2001. Construction of a linkage map of the Rennell Island Tall coconut type (*Cocos nucifera L.*) and QTL analysis for yield characters. *Genome,* 44:962-970.

Lebrun, P., N'Cho, Y.P., Seguin, M., Grivet, L. and Baudouin, L., 1998. Genetic diversity in coconut (*Cocos nucifera* L.) revealed by restriction fragment length polymorphism (RFLP) markers. *Euphytica,* 101:103-108.

Manimekalai, R. and Nagarajan, P., 2006. Assessing genetic relationships among coconut (*Cocos nucifera* L.) accessions using inter simple sequence repeat markers. *Scientia Horticulturae,* 108:49-54.

Merrow, A.W., Wisser, R.J., Brown, J.S., Kuhn, D.N., Schnell, R.J. and Broschat, T.K., 2003. Analysis of genetic diversity and population structure within Florida coconut (*Cocos nucifera* L.) using microsatellite DNA, with special emphasis on the Fiji Dwarf cultivar. *Theoretical and Applied Genetics,* 106:715-726.

Meunier, J., 1992. Genetic diversity in coconut on brief survey of IRHO's work. In: Papers of the IPGRI Workshop on Coconut Genetic Resources. International Crop Network Series VIII, pp. 59-61. IPGRI, Rome, Italy.

Moran, G., 1991. Report of isozyme research of coconuts. CSIRO. Division of Forest Research, Canberra, School of Botany, University of Melbourne, Australia.

Nagaraju, V., He, G., Parthasarathy, V.A. and Prakash, C.S., 2003. Fingerprinting of coconut (*Cocos nucifera L.)* accession using DNA markers. *Journal of Plantation Crops*, 31:8-13.

Niral, V. Geethalakshmi, P. and Parthasarathy, V.A., 2007. Intra population allelomorphisms in tall and dwarf population of coconut. *Acta Botanica Croatica,* 66(1):35-42.

Parthasarathy, U., Parthasarathy, V.A., Manimekalai, R., Niral, V., Anuradha, U. and Kumaran, P.M., 2005. Molecular diversity of coconut ecotypes of coastal and inland riverine ecosystem. *Indian Journal of Horticulture,* 62:315-318.

Parthasarathy, V.A., Geethalakshmi, P. and Niral, V., 2004. Analysis of coconut cultivars and hybrids using isozyme polymorphism. *Acta Botanica Croatica,* 63:69-74.

Perera, L., Russel, J.R., Provan, J., McNicol, J.W. and Powel, W., 1998. Evaluating genetic relationships between indigenous coconut (*Cocos nucifera* L.) accessions from Sri Lanka by means of AFLP profiling. *Theoretical and Applied Genetics,* 96:545-550.

Perera, L., Russell, J.R., Provan, J. and Powell, W., 2001. Levels and distribution of genetic diversity of coconut (*Cocos nucifera* L, var. *Typica* form *typica*) from Sri Lanka assessed by microsatellite markers. *Euphytica,* 122:381-389.

Perera, L., Russell, J.R., Provan, J. and Powell, W., 1999. Identification and characterization of microsatellites in coconut (*Cocos nucifera* L.) and the analysis of coconut population in Sri Lanka. *Molecular Ecology,* 8:344-346.

Perera, L., Russell, J.R., Provan, J. and Powell, W., 2000. Use of microsatellite DNA markers to investigate the level of genetic diversity and population genetic structure of coconut (*Cocos nucifera* L.) *Genome,* 43:15-21.

Powell, W., Machray, G.C. and Provan, J., 1996. Polymorphism revealed by simple sequence repeats. *Trends in Plant Science,* 1:215-222.

Rajesh, M.K., Nagarajan, P., Jerard, B.A., Arunachalam, V. and Dhanapal, R., 2008. Microsatellite variability of coconut accessions (*Cocos nucifera* L.) from Andaman and Nicobar Islands. *Current Science,* 94:1627-1631.

Ritto, P., Jiji, G., Rajesh M.K., Jerard, B.A. and Niral, V., 2008. Genetic diversity in yellow dwarf populations of coconut (*Cocos nucifera* L.) assessed using RAPD markers. *Indian Journal of Horticultural Research,* 65:60-64.

Rivera, R., Edwards, K.J., Barker, J.H.A., Arnold, G.M., Ayad, G., Hodgkin, T. and Karp, A., 1999. Isolation and characterization of polymorphic microsatellites in *Cocos nucifera L. Genome*, 42:668-675.

Rodriguez, M.J.B., Estioko, L.P., Namia, T.I. and Soniega, J.A., 1997. Analysis of genetic diversity in coconut by RAPD. *Philippine Journal of Coconut Studies,* 22:1-7.

Rohde , W., Salamani, F., Ashburner, R., and Randles, J.W., 1992. An *Eco* RI repetitive sequence family of the coconut palm (*Cocos nucifera* L.) shows sequence homology to copia-like elements. *Journal of Genetics and Breeding* 46:391-394.

Rohde, W., Kullaya, A., Rodriguez, J. and Ritter, E., 1995. Genome analysis of *Cocos nucifera* L. by PCR amplification of spacer sequences separating a subset of copia-like *Eco* RI repetitive elements. *Journal of Genetics and Breeding* 49:179-186.

Shalini, K.V., Manjunantha, S., Lebrun, P., Berger, A., Baudouin, L., Pirany, N., Ranganath, R.M. and Theertha Prasad, D., 2007. Identification of molecular markers associated with mite resistance in coconut (*Cocos nucifera* L.). *Genome,* 50:35-42.

Shivashankar, S., 1998. Polyphenol Oxidase isozymes in coconut Genotypes under water stress. *Plant Physiology and Biochemistry,* 15:87-92.

Tautz, D. and Renz, M., 1984. Simple sequences are ubiquitous repetitive components of eukaryote genomes. *Nucleic Acids Research,* 12:4127-4137.

Teulat B., Aldam, C., Trehin, R., Lebrun, P., Barker, J.H.A., Arnold, G.M., Karp, A., Baudouin, L. and Rognon, F., 2000. An analysis of genetic diversity in coconut (*Cocos nucifera L.*) population from across the geographical range using sequence tagged microsatellites (SSRs) and AFLPs. *Theoretical and Applied Genetics,* 106:411-422.

Upadhyay A., Jayadev, K., Manimekalai, R. and Parthasarathy, V.A., 2004. Genetic relationship and diversity in Indian coconut accessions based on RAPD markers. *Scientia Horticulturae,* 99:353-362.

Vos, M., Hogers, R., Bleeker, M., van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M. and Zabeau, M., 1995. AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research,* 23:4407-4414.

Wadt, L.H.O., Sakiyama, N.S., Pereira, M.G., Tupinambra, E.A., Riberio, F.E. and Aragao, W.M., 1999. RAPD markers in the genetic diversity study of the coconut palm. In: Current plant science and biotechnology in agriculture – Current advances in coconut biotechnology. (Eds. Oropeza, C., Verdeil, J.L., Ashburner, G.R., Cardena, R. and Santamaria, J.M) pp. 89-97. Kluwer Academic Publishers, Dordrecht, The Netherlands.

White, T., Moran, G. and Knox, R.B., 1987. *Estimation of genetic diversity in coconut: preliminary investigation report of a survey trip*. School of Botany, The University of Melbourne, Australia.

Zizumbo-Villarreal, D., Cardena-Lopez, R. and Pinero, D., 2002. Diversity and phylogenetic analysis in *Cocos nucifera* L. in Mexico. *Genetic Resources and Crop Evolution,* 49:237-245.