8

MOLECULAR MARKERS FOR ASSESSING GENETIC DIVERSITY WITH SPECIAL REFERENCE TO COCONUT PALM (*COCOS NUCIFERA* L.)

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INTRODUCTION

The coconut palm, *Cocos nucifera* Linn. is a perennial oil yielding tree crop of tropics. It belongs to the family Arecaceae under the class monocotyledons. With its tall, slender and uniformly thick stem and massive crown with large number of leaves, bearing bunches of nuts in their axils is one of the most beautiful and useful trees in the world. It perhaps yields more products of use to mankind than any other tree. Coconut has been given different names such as Kalpa Vriksha (tree of Heaven), the consols of East, Mankind's Greatest Provider in the Tropics, Tree of Life, Tree of abundance, Tree of plenty etc.

Each and every part of the coconut palm is useful to man in one way or another. The most important products are tendernut, copra, oil, oil cake, desiccated coconut and fiber. The genus Cocos, formerly included besides *Cocos nucifera* Linn. over thirty species mostly confined to Central and South America. It is, however, now usual to regard one of them which is cosmopolitan in the tropics as monotypic genus containing *Cocos nucifera* L. the coconut, other species having been assigned to several other new genera. Coconut has a pantropic distribution mainly in coastal regions at 20 Ú either side of the equator. Grown in 11.6 million hectares in 86 countries of tropics including India. The area and production of coconut in India (2003-04) are 1.82 M ha and 9.5 M nuts respectively (FAO STAT, 2004).

Origin and distribution

The original home of coconut is still uncertain, but the region comprising Malaysia and Melanesia considered as the probable home of the coconut. According to Merril (1936), the Malaysia comprised the two continental regions (Asian Banks and Australian Banks) and an intermediate group of islands.

The Melanesian region extended from the equator Southwards to the Tropic of Capricon and between 145 Ú to 189 Ú E longitude roughly extending from New Guinea to Fiji Islands. It is in this region that the coconut had attained its highest development, and known by the maximum number of names. The weight of evidence is, therefore in favour of the original home of the coconut being located somewhere in Far East of Malayan region (Menon and Pandalai, 1958). Still there is much debate on this subject.

Dispersal of coconut

Having originated at some place in Far East, the coconut has moved Eastward towards the Pacific and further into America. Towards west it moved to India and Madagascar over the calm tropical water. The dispersal of coconut might have been effected through humans or through ocean currents (Purseglove, 1972; Child, 1974). RFLP study of Lebrun *et al.* (1998) confirms the movement of coconut from South East Asia/ Pacific to the west coast of America and India.

Genetic variability and varieties

The preliminary classification of coconut (*Cocos nucifera* L) based on stature was made by Narayana and John (1949) and Menon and Padalai (1958). Narayana and John (1949) recognized the palms as tall palms and as dwarf palms. Based on the observations of several morphological traits, coconut varieties were classified as

Spicata—un branched inflorescence or with one or two spikes only

Typica—tall palm having both male and female flowers

Androgena—having only male flowers

Javanica—intermediate between talls and dwarfs

Nana—dwarf palms

Tall palms are widely planted and they can grow upto 20-30 m height. They are normally cross pollinating and therefore considered to be heterozygous. Talls constitute the polymorphic populations (Perera *et al.*, 2000). They are slow to mature, flowering 6-10 years after planting and have economic life of 65-75 years. Dwarf palms grow to a height of 10-15 m, flowering 3-4 years after planting and they are self pollinating and considered to be homozygous and has economic life of 30-40 years. The dwarfs are presumed to have originated from talls either by mutation (Menon and Pandalai, 1958) or by inbreeding (Swaminathan and Nambiar, 1961; Lebrun *et al.*, 1998).

Harries (1978) classified talls as 'Niu Kafa' and 'Niu vai'. 'Niu kafa' type represents the coconut which evolved by natural selection on uninhabited coral atolls and newly emerged volcanic islands. The nuts are long, angular shaped, thick husked and has slow germination. These characters favour its survival under natural conditions in the absence of man and has advantage to float on water. In the natural state the 'Niu kafa'has a very restricted habitat. The palms infringe the shore and grow just above the high water mark where they are shaded by forest tree nor choked by undergrowth. The 'Niu vai' type has sweet nut water. It was selected for sweet uncontaminated drink by the first human being to know the coconut palm. Selection increased the volume of liquid endosperm in the immature fruit at the expense of other components, principally the husk. The reduction in husk and increase in volume of the nut cavity give the fruit a more spherical appearance, they are early to germinate and has vigorous growth.

'Niu Kafa' and 'Niu vai' types remain quite distinct where they are geographically isolated. When there are no barriers to hybridization and repeated opportunities for cross pollination at every generation allow intermediate forms to develop (introgression). These show characteristics of both types in proportions related to which type originally predominated. Some characteristics may be intermediate and the situation is modified by mutation, genetic drift, isolation and further selection. The wide range of variability produced in this way accounts for the many named varieties found today.

GENETIC DIVERSITY

The basic character of life is its unlimited diversity (Narain, 2000). No two individuals in sexually reproducing population are same. The underlying factor in this diversity is genetic. The estimation of genetic diversity between different genotypes is the first and foremost process in any plant breeding programme. For a plant breeder, reliable knowledge of the genetic diversity of his breeding material is important in order to select parents for a new breeding cycle. Genetic diversity is desirable for long term crop improvement and reduction to vulnerability to important crop pest and pathogens (Liu *et al.*, 2000).

The assessment of genetic diversity or genetic variation that may exist among a set or sets of germplasm reveals genetic and evolutionary relationships. Classical methods of estimating the genetic diversity among groups of plants have relied upon morphological characters. However, these characters can be influenced by environmental factors. Molecular markers avoid many of these complications by looking directly at the genetic material itself. Molecular markers, therefore, represent a powerful and potentially rapid method for characterizing and managing plant germplasm, both *in situ* and *ex situ* (Virk *et al.*, 1995). These markers are appropriate for identifying useful genes within germplasm collections. In coconut, molecular markers can be used to rationalize germplasm collections by reducing the duplications and maximizing the genetic diversity, to priorities collection and conservation activities. Lynch (1988) proposed that the technique of DNA fingerprinting could be employed for the estimation of relatedness. Coconut being perennial in nature the molecular markers offer advantages in choosing correct parental combination for crossing program for enhancing heterosis.

TOOLS FOR ASSESSING GENETIC DIVERSITY

Morphological markers

The assessment of genetic diversity and discrimination of individual genotypes is the basic objective of any breeding program. Till recently, only morphological markers were extensively used for this purpose. Polymorphic and highly heritable morphological traits were some of the earliest genetic markers employed in scientific investigations (De Vries, 1912).

The main advantage of the morphological marker system is that these assays generally require neither sophisticated equipments nor preparatory procedures, so monogenic and oligogenic morphological traits are generally simple, rapid and inexpensive to score, even from preserved specimens. Until recently scientific plant classification was based nearly exclusively on morphological assays (Stuessy, 1990). Some of which served as genetic markers (Gottlieb, 1984) suitable for plant germplasm management. The major limitation of this marker system is that, the morphological characters especially quantitative traits are subjected to environmental influences and to selective pressure during domestication and improvement (Leakey, 1988). But still, in coconut the morphological markers serve as an important maker system.

Harries (1981) identified coconut varieties originated from different region using fruit components *viz.*, thickness of endosperm, husk and shell, quantity of nut water, seednut germination, fruit colour, floral biology, precocity of flowering and fruiting, nut production and number of female flowers.

Fernando *et al.* (1993) distinguished illegitimate dwarf seedlings from the hybrids (Dwarf X Tall) based on the seedling characters viz., height, girth, no of leaves, split leaves, length of petiole, length of leaf and width of leaf.

The variation for floral and vegetative traits in 195 coconut palms as observed by Sugimura *et al.* (1997) showed significant differences for sizes of the outer and inner spathe, ovary diameter, leaf scar interval, precocity, bole size etc. and they classified the coconut accessions into three major groups –typica, nana and javanica. Nana was found to be an aggregate group, which is far distant from typica. But javanica was found to be an intermediate type.

The diversity of fruit characters in 29 South Pacific populations was studied (Ashburner *et al.*, 1997a) and a continuous variation in fruit morphology with geographic affinity was observed. Zizumbo and Garciamarin (2001) studied phenotypic plasticity in 18 Mexican coconut populations. Nallathambi *et al.* (1986) reported GBD X ECT hybrid with 7.1 % heterosis for oil content as against 13.4 % in its reciprocal cross hybrid.

Coconut accessions showed significant differences for the floral biology characters and important characters contributing to divergence were the length of male phase and nut setting percentage (Ratnambal *et al.*, 2003).

Arunachalam *et al.* (2006) used seven traits relevant to wind tolerance, dry matter production, and taxonomic discrimination to study diversity in coconut germplasm. They have used 206 individuals representing 30 accessions. Their results support the presence of low diversity in dwarf coconut due to its autogamous behaviour.

Protein markers (Isozyme markers)

Isozymes are the most widely used protein markers. Isozymes are enzymes with the same catalytic activity but with different molecular forms. Different genetic loci code for these multiple molecular forms of the same enzyme and hence isozyme markers are used as tools to distinguish between varieties. These are commonly separated on starch, polyacrylamide or cellulose acetate media and stained using enzyme specific reaction mixtures. Isozymes of a given enzyme are the products of different alleles at a specific locus.

The advantage of these markers are their cheapness compared with DNA methods, the short running period (30 min.) on prepared gels (Wynne *et al.*, 1992), the long shelf life of reaction materials and the use of simple apparatus (Thompson *et al.*, 1989). The limits include the low detectable variability of many enzyme loci and the requirement of fresh or deep frozen biological material (<-25 °C), also the protein expression is influenced by environmental conditions and that the number of markers are limited (Weeden, 1984).

In coconut, the work on isozyme analysis at I.R.H.O (Institut de Recherches pour les Huiles et Oléagineux) began in 1978 with a view to understand the structure of genetic diversity. Meunier (1992) concluded that isozyme analysis can be used for cultivar identification and progeny legitimacy.

Fernando and Gajanayake (1997) established protocols for the detection of isozyme polymorphism in coconut leaf tissues. Using a combination of all genotypes and principal component analysis they 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.

Electrophoretic pattern of leaf peroxidase (PRX), endopepdidase (END) and Coomassie Blue stained proteins were analysed in four cultivars (West African Tall, Rennell Tall, Malayan Yellow Dwarf, Cameroon Red Dwarf) of coconut and in the hybrids PB121 (Malayan Yellow Dwarf x West African Tall) and PB111 (Cameroon Red Dwarf x West African Tall). The data helped in coconut cultivar identification, testing progeny legitimacy, pollen contamination and studying breeding systems (Cardena *et al.*, 1998).

Low genetic diversity was reported by Zizumbo and Garciamarin (2001) by analyzing tall populations of Mexica, and Ivory Coast with fifteen enzymatic systems. Geethalakshmi (2003) analysed 'within population' diversity in coconut populations using isozymes. Parthasarathy *et al.* (2004) estimated genetic diversity among coconut cultivars and reported high diversity among tall accessions compared to dwarf accessions.

DNA based markers

Markers based on differences in DNA sequence between individuals generally detect more polymorphism than morphological and protein based markers (Tanksley *et al.*, 1989). DNA markers because of their heritable nature were found to act versatile tools in the fields like taxonomy, physiology, embryology, genetic engineering etc. Major applications of these

DNA markers in the field of genetics and plant breeding are in (i) diversity analysis and phylogenetic studies (ii) mapping genes and (iii) marker assisted selection (MAS).

DNA based molecular marker techniques have been proven powerful in genetic diversity estimations. Among them, RFLP was the first and is still the most commonly used in the estimation of genetic diversity of eukaryotic species. The recently developed PCR based multiple loci marker techniques which include random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), inter simple sequence repeat (ISSR), and more recently simple sequence repeat (SSR) or microsatellites (Staub *et al.*, 1996; Gupta and Varshne - 2000) are playing increasingly important roles in gene identification and genetic mapping (Seah *et al.*, 2004). The markers at DNA level include short DNA sequences, whole genes or even long sequences of DNA. These markers can be grouped into two major classes based on their number and distribution (i) single locus markers (ii) multi locus markers.

A. Single locus marker system

Genetic markers having unique locations on a genome are defined as single locus markers. These markers are considered to be good for genome mapping, mapping a gene to its corresponding position on a chromosome and inheritance studies. The following marker types are considered as single locus markers: restriction fragment length polymorphism (RFLP), sequence tagged sites (STS), sequence characterized amplified region (SCAR), expressed sequence tags (EST), simple sequence length polymorphism (SSLP), cleaved amplified polymorphic sequences (CAPS), simple sequence repeats (SSR) or short tandem repeats (STR), single nucleotide polymorphism (SNP), dynamic allele specific hybridization (DASH), DNA chip and DNA sequencing. Single locus markers can be more useful in some instance since single loci can be identified and codominance can be noted.

a. Restriction fragment length polymorphism (RFLP)

The first and the most common DNA polymorphic marker method used for genetic mapping and the estimation of genetic diversity in eukaryotic species is RFLP analysis (Botstein *et al.*, 1980;Tanksley *et al.*, 1989). In DNA strands, RFLP represent changes in the length of DNA between specific enzyme cutting sites brought about by sequence changes. DNA can be cut using one or more restriction enzyme that recognizes sites on the DNA template. The recognition sites are four to six bases in length. The cut DNA fragments may be visualized under UV light using ethidium bromide stained agarose gels or with more resolution in six per cent PAGE gels. RFLP analysis using genomic single copy probes has been generally used to characterize variation among wild and cultivated species (Jarret *et al.*, 1992). RFLP are codominant markers, inherited in simple Mendelian fashion (Botstein *et al.*, 1980; Droogenbroeck *et al.*, 2004).

RFLP can be of various types: single copy sequences, multiple copy sequences and repeated sequences. Among these, single copy sequences are highly amenable for linkage map construction and to some extent, the multiple copy sequences. RFLP markers have been successfully used for detecting genetic diversity of coconut accessions and throws light in coconut origin and dispersal (Lebrun *et al.*, 1999). Genetic diversity structuring of 17 populations from various geographic region was obtained by Lebrun *et al.* (1998). They used cDNA, mitochondria and genomic clones as probes. Their results revealed classification of populations into two major groups, one comprising populations from Far East and South Pacific, the second one comprised populations from India, Sri Lanka, and Eastern Africa. (Lebrun *et al.*, 1998). RFLP markers linked to a yield characters have also been identified (Lebrun *et al.*, 2001).

RFLP based technique yield codominant, non-epistatic, Mendelian markers, which provide great genetic resolution because of the large number of restriction probe combination available. The limits of this marker are that the whole process is specialized undertaking, expensive, time consuming and hazardous.

b. Simple sequence repeats (SSR)

Microsatellites or simple sequence repeat markers are short tandem repetitive DNA sequences with a repeat length of a few (1-5) base pairs (Litt and Luty, 1989). Microsatellites are one of the two types of variable number tandem repeats (VNTRs), the other one is being the minisatellites. Together both of them constitute the hyper variable regions of DNA. The sequences are abundant and dispersed throughout the genome (Akkaya *et al.*, 1992). Markers based on simple sequence repeats (SSR) have been shown to be highly polymorphic even between closely related individuals within a species (Edwards *et al.*, 1996) and tend to show more polymorphism than many alternative marker systems (Doldi *et al.*, 1997). Microsatellites have been increasingly used to assess genetic diversity and population structure among plants (Li *et al.*, 2006; Kuroda *et al.*, 2006).

Until recently, microsatellites have not been widely used in plants because of the high cost of their isolation, but the introduction of procedures such as automated sequencing and library enrichments has rendered the process quicker and more efficient and has placed these markers within the reach of plant geneticists. Moreover microsatellite DNA often has flanking regions that are highly conserved in related species and this renders primer pairs designed in one species useful for amplification of the same DNA regions in related genomes (Peakall *et al.*, 1998; Huang *et al.*, 1998; Zhebentyayeva *et al.*, 2003).

SSR marker has high information content and high rate of polymorphism, now provide a valuable tool kit for genetic resource management as they can be used in the laboratories of the different countries to identify populations with different alleles or heterozygosity, and also other practical problems such as detecting pollinations from non-parental types in breeding programmes (Karp, 2002).

Microsatellite enriched library constructions was described for tropical crops (Billotte *et al.*, 1999). In coconut, SSR markers are developed only at three laboratories (Perera *et al.*, 1999; Baudouin and Lebrun, 2002; Rivera *et al.*, 1999) and used by few others.

Rivera *et al.* (1999) constructed genomic DNA of cv. Tagnanan Tall enriched with microsatellites. Sequencing 197 clones revealed microsatellites are dinuclotides (GA/CT, CA/GT and GC/CG) (64 %), trinucleotide (6 %) and compound repeats (30 %). The primers were designed for the flanking region and 38 SSR primers were developed. These primer pairs are designated by prefixing CNZ. The work was carried out at IACR- Long Ashton Research station, University of Bristol, Bristol, UK. It produced 5.2 alleles per microsatellite in 20 coconut cultivars. Gene diversity values range from 0.141 to 0.809.

Perera *et al.* (1999) identified SSR using a pre-cloning enrichment procedure (White and Powell, 1997). They designed the primer pairs flanking SSR region. The primers are named by prefixing CAC. This work was carried out in Scottish Crop Research Institute (SCRI), Scotland, UK.

Baudouin and Lebrun (2002) identified a total of 83 coconut microsatellite primer pairs at CIRAD (Centre de Coopération Internationale en Recherche Agronomique pour le Développement). The most discriminating ones were selected through a screening with highly diverse accessions. Based on this, 14 highly polymorphic markers were identified with sufficient discriminating power for practical identification of accessions. The primer pairs are designated as CnCir. They also developed dedicated software 'Geneclass' 2 facilitating the management of coconut genetic resources. It aims at allowing the user to identify the most likely origin of a collection accession, or of a population found during a survey ie "unknown population". The origin is to be sought within a predetermined set of reference populations assumed to satisfactorily represent the genetic diversity of the species.

Using CAC primer pairs the genetic diversity and population genetic structure was analyzed. The results showed 6.4 alleles per locus. The mean alleles per locus was 6.3 and 3.3 for talls and dwarfs respectively and revealed high diversity values for talls compared to dwarfs (Perera *et al.*, 2000). Using CAC primers, the genetic diversity among the coconut accessions of different origin was revealed by Teulat *et al.* (2000). They obtained clustering of coconut accessions based on geographic origin.

Genetic diversity was estimated in *typica* types by Perera et al. (2001). The genetic diversity in *ex situ* conserved coconut germplasm in field gene bank of Coconut Research Institute (CRI) Srilanka was analyzed by Dasanayake *et al.* (2003) with CAC primer pairs and reported narrow genetic distances among dwarf accessions. The genetic variation within germplasm collections of Florida and parentage of progeny Fiji dwarf was analyzed with 15 SSR markers (Meerow *et al.*, 2003). Manimekalai *et al* (2005a) used SSR markers for detecting variation among the seedlings of Chowghat Green Dwarf(GB) and Laccadive tall (LCT) and reported more genetic variation among LCT than GBD

The extent of genetic diversity and genetic relationships among 94 coconut accessios was analysed (Perera *et al.*, 2003). In the phenogram, for talls two groups were observed One group comprising the talls from South East Asia, Pacific and Panama and the second groups comprising the talls from India, Srilanka and Africa. Partitioning of genetic diversity between different populations of Srilankan accessions was done by the procedure AMOVA by employing SSR markers (Perera *et al.*, 2001).

B. Multilocus marker system

DNA markers with multiple loci on a genome are known as multiloci markers. These markers because of their whole genome coverage are considered good for genetic diversity analysis and phylogenetic analysis. The following comes under multilocus marker system: random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) and inter simple sequence repeat (ISSR).

a. Random amplified polymorphic DNA (RAPD)

Among the different types of molecular markers used for genetic diversity analysis, random amplified polymorphic DNA (RAPD) analysis is fast and these markers are easily generated by PCR (Williams *et al.*, 1990). In RAPD, banding profiles are created using small oligo nucleotide primers (around 10 bp in length) of arbitrary sequence. These primers bind to homologous sequences along the genome and PCR amplification occurs when the regions between the opposing primer sites are within amplifiable distances. Bands are visualized using ethidium bromide. Within a population sample, mutations influence the base sequence of primer binding sites, allowing polymorphism to be detected (Williams *et al.*, 1990).

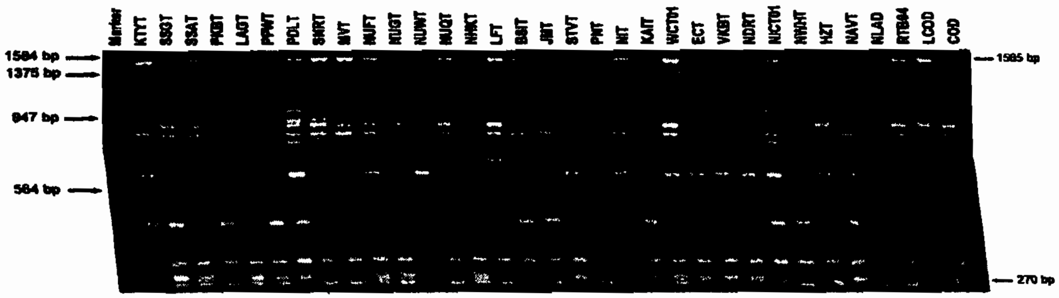
RAPD method is relatively quick when compared with other markers, reveals great genetic variability due to the regions in which amplification takes place and useful in differentiating closely related individuals. There are numerous commercially available primer kits (Operon Tech., USA) which can be used to screen populations. However the limits of the technique, they often reveal continuous variation between sample population, primer libraries and need screening to identify suitable primers and stable genetic polymorphism both of which are time consuming and costly. The dominant nature of the markers makes it impossible to distinguish between homozygous and heterozygous alleles. A review of work done based on RAPD makers in coconut is given below.

RAPD was the first marker used for the analysis of coconut ecotypes in Philippines and Tanzania (Rodriguez *et al.*, 1997; Duran *et al.*, 1997). Rodriguez *et al.* (1997) analyzed germplasm of Philippines Coconut authority (PCA) and reported clustering of talls and dwarfs separately. The analysis of diversity in South Pacific coconut palms showed continuous variation among the populations and suggested *ex situ* collection and conservation strategies for coconut palm in the South Pacific region (Ashburner *et al.*, 1997b).

Wadt *et al.* (1999) analyzed *ex situ* germplasm bank (BAG-coco) of Brazil (tall accessions namely Brazil Tall, East African Tall, Rennell Island Tall). Their results revealed greater levels of polymorphism among Brazil Talls compared to West African Talls and Rennell Island Talls. The phylogenetic relationship among 14 coconut accessions was established and the dendrogram revealed clustering of dwarf accessions originated from different regions (Upadhyay *et al.*, 2002).

Cardena *et al.* (2003) identified RAPD markers associated with resistance to lethal yellowing diseases of coconut palms. They analyzed the resistant (Malayan Yellow Dwarf and Atlantic Tall) and susceptible (West African Tall) populations. They identified RAPD markers appeared at frequencies ≥ 0.85 or < 0.15 in Malayan Yellow Dwarf populations.

Parthasarathy *et al.* (2005) used RAPD and Isozyme markers to study the diversity among two different ecotypes, Assam Tall (AST) and West Coast Tall (ECT). They reported less variation between WCT and AST, even though both the types are geographically separated. But, Cluster analysis showed separate cluster for WCT and AST. Manimekalai and Nagarajan (2006a) evaluated RPAD markers based on Marker Index and Polymorphism Information content. Reproducible and informative primers were identified (OPBE 06, OPC 13, OPF 14, OPM 02 and OPM 17), which are sufficient for large scale coconut germplasm screening and to establish core collection (Fig 1).



Marker: λ DNA / *EcoRI+HindIII* double digest

Fig. 1 : RAPD profile of coconut germplasm accessions produced by the primer OPBE 06.

Genetic relationship and diversity among Indian coconut accessions showed high genetic diversity for tall accessions (0.215) and less diversity for dwarf accessions (0.178). While comparing the diversity between exotic and indigenous accessions, the later showed less diversity (Upadhyay *et al.*, 2004). Manimekalai and Nagarajan (2006a) used RAPD technique for assessing genetic relationship among world wide collection of coconut germplasm accessions. Manimekalai *et al.* (2004) utilized RAPD markers in combination with ISSR markers for fingerprinting drought tolerant Gangabondam Dwarf and Laccadive Tall.

b. Inter simple sequence repeats (ISSR)

Inter simple sequence repeats (ISSR) technique is a PCR based method, which involves amplification of DNA segment present at an amplifiable distance inbetween two identical microsatellite repeat regions oriented in opposite direction (Zietkiewicz *et al.*, 1994). The technique uses microsatellite, usually 16-25 bp long as primers in a single primer PCR reaction targeting multiple genomic loci to amplify mainly the inter-SSR sequences of different sizes. The microsatellite repeats used as primer can be di-nucleotide, tri-nucleotide, tetra-nucleotide or penta nucleotide.

ISSR markers are highly polymorphic and are used in studies on genetic diversity, phylogeny, gene tagging, genome mapping and evolutionary biology (Reddy *et al.*, 2002). ISSR PCR is a technique which overcomes the problems like low reproducibility of RAPD, high cost of AFLP, the need to know the flanking sequences to develop species specific primers for SSR polymorphism (Goodwin *et al.*, 1997). ISSR segregate mostly as dominant markers following simple Mendelian inheritance (Wang *et al.*, 1998). However they have also been shown to segregate as codominant markers in some cases thus enabling distinction between homozygote and heterozygote (Sankar and Moore, 2001). ISSR amplification is a relatively recent technique which can differentiate closely related genotypes and helps to estimate genetic diversity both at inter and intra species level.

The technique combines most of the benefits of AFLP and microsatellite analysis with the universality of RAPD. ISSR have high reproducibility possibly due to the use of longer primers (16 - 25 mers) as compared to RAPD primers (10 mers) which permits the subsequent use of annealing temperature (45 - 60 °C) leading to higher stringency. The limitation of this marker is that the fragments with the same mobility originate from non-homologous regions, which can contribute to some distortion in the estimates of genetic similarities In coconut Herran *et al.* (2000) used ISSR markers for linkage map construction. Diversity among South East Asian coconut germplasm accessions were studied by Manimekalai *et al.* (2005b). They reported high diversity in Philippines Dalig Tall accession in terms of number of observed alleles, Shannon's index and number of polymorphic markers. Dendrogram revealed separate position for this accession. Manimekalai and Nagarajan (2006b) utilized ISSR markers for estimating genetic diversity among coconut germplasm accessions. Majority of the tall accessions had grouped into three clusters, each comprising talls of South East Asia, South Pacific and South Asia. For increased heterosis, the talls from different groups could be used for hybridization. As more divergence was observed between dwarfs and talls, hence, higher heterosis can be expected by crossing the dwarf and the talls belonging to different clusters. Dwarfs and intermediate accessions clustered along with South Pacific talls revealing the origin of dwarfs from the South Pacific talls.

High genetic diversity was present in South Pacific accessions confirming the Far East / South Pacific as the primary centre of diversity for coconut which was evident from the presence of South Pacific accessions at all the branches of dendrogram.

Manimekalai (2005) identified a set of informative primers (UBC 855, UBC 854, UBC 815, UBC 808 and UBC 810) for large scale germplasm analysis. ISSR primers were evaluated based on Polymorphism Information Content and marker index (Fig 2).

Fig. 2 : ISSR profile of coconut germplasm accessions produced by the primer UBC 889.

RAPD, ISSR and SSR markers were utilized to analyze the genetic diversity among the progeny lines derived from LCT X GBGD, GBGD XLCT, LCT and GBGD. Leaf water potential was measured among the progeny lines. The hybrids of LCT X GBGD exhibited more variation than the GBGD X LCT. When the dwarfs are used as mother palms the hybrids show less variation among themselves and the F_1s were similar to the mother palm. But the F_1s from the LCT X GBGD showed variation. GBGD and GBGD X LCT had almost similar banding pattern. There was less genetic variation among individuals of GBGD X LCT and GBGD when compared to LCT X GBGD and LCT (Manimekalai *et al.* 2004 ; Manimekalai *et al.* 2005a).

c. Amplified fragment length polymorphism (AFLP)

Amplified Fragment Length Polymorphism (AFLP) is a recently developed multilocus polymorphic marker technique. It is a technique which combines both classical restriction based and recent PCR based approaches. This PCR based technique permits inspection of polymorphism at a large number of loci within a very short period of time and requires very small amounts of DNA. The reproducibility of AFLP is ensured by using restriction site specific adapters and adapter specific primers with a variable number of selective nucleotide under stringent amplification conditions (Zabeau and Vos 1993; Vos *et al.*, 1995). The large number of AFLP markers potentially available makes them an attractive choice for fine scale mapping (Thomas *et al.*, 1995). Since polymorphism is detected as the presence or absence of amplified restriction fragments, AFLP are usually considered dominant markers (Mackill, 1996). The procedure to generate AFLP is also called as selected restriction fragment amplification (SRFA) and is applied to a portion of the genomic DNA digest using PCR (Zabeau and Vos 1993; Vos *et al.*, 1995). AFLP is a more promising tool in genetic mapping and diversity analysis compared to other molecular methods as the numbers of markers produced are very high in most and these can be manipulated by choosing the right kind of primers and changing their selective bases (McGregor *et al.*, 2000). A method which combines AFLP technique with SSR is called as microsatellite-AFLP; here one of the two AFLP amplification primers is replaced by a compound SSR in the PCR, so the polymorphism detected are presumed to be contributed by SSR (Vogel, 1995). The AFLP technique has been used in genetic diversity analysis (Adin *et al.*, 2004; Grati Kamouin, *et al.*, 2006; Bao et al. 2006). Microsatellite AFLP would also be a good candidate for genetic diversity studies.

The first report of AFLP marker for use in coconut was from Perera *et al.* (1998). They evaluated genetic relationship between indigenous coconut accessions of Sri Lanka and reported that most of the variation was present between the population rather than within population in dwarfs. In contrast, the out breeding talls exhibited as much variation within as between populations. Teulat *et al.* (2000) used AFLP markers in combination with SSR markers to analyze genetic diversity of 14 populations. AFLP markers also utilized for coconut linkage map construction (Herran *et al.*, 2000).

d. ISTR (Inverse Sequence-Tagged Repeat) markers

ISTR marker was developed for coconut (Rohde *et al.*, 1995) based on copia like elements in the genome. PCR amplification of spacer regions for a subset of tandemly arranged repeats detected polymorphisms, which allowed the analysis of biodiversity within coconut populations. This method was later extended into a generally applicable, multilocus/single allele strategy for plant, human, and animal genome analysis.

MOLECULAR MARKERS FOR LINKAGE MAPPING IN COCONUT

The applications of molecular markers for genetic mapping in coconut found only in two reports. Herran *et al.* (2000) constructed linkage map in coconut using AFLP, ISTR, ISSR and RAPD. They derived mapping population from Malayan Yellow dwarf X Laguna Tall and identified QTLs for early germination, early flowering and yield. Lebrun *et al.* (2001) constructed linkage map of Rennell Island Tall and detected QTLs for number of bunches and yield characters.

MOLECULAR MARKERS IN COCONUT CULTIVAR IDENTIFICATION

ISSR and SSR markers detected population specific markers. The locus CnCirA3 differentiated dwarfs and talls. The SSR marker CnCirA3 $_{234}$ was specific to dwarfs. The ISSR markers UBC 855$_{2155}$ was unique to dwarfs and two of the South Pacific populations Nufella Tall (NUFT) and Nuwallis Tall (NUWT). The marker UBC 815$_{1673}$ was specific to Panama Tall (PNT) and Saint Vincent Tall (STVT). These markers are useful for cultivar identification purposes (Manimekalai, 2006).

CONCLUSION

Molecular markers are versatile and used in a number of applications viz. genetic diversity estimation, phylogenetic relationship and linkage mapping in coconut. Being perennial in nature, application of molecular markers yields fruitful results for the improvement of coconut.

REFERENCES

Adin, A., Weber, J. C., Sotelo Montes, C., Vidaurre, H., Vosman, B. and Smulders, M. J. M. 2004. Genetic differentiation and trade among populations of peach palm (*Bactris gasipaes* Kunth.) in the Peruvian Amazon-implications for genetic resource management. *Theor. Appl. Genet.*, **108**: 1564-1573.

Akkaya, M. A., Bhagwat, A. A. and Cregan, P. B. 1992. Length polymorphism of simple sequence repeat DNA in soybean. *Genetics,* **132**: 1131-1139.

Arunachalam, V., Jerard, B. A., Damodaram, V., Ratnambal, M. J. and Kumaran, P. M. 2006. Phenotypic diversity of foliar traits in coconut germplasm. *Genet. Resour Crop Evol.*

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

Ashburner, G. R., Thompson, W. K., Halloran, G. M. and Foale, M. A. 1997a. Fruit component analysis of South Pacific coconut palm populations. *Genet. Resour. Crop Evol.*, **44**: 327-335.

Bao, J., Corke, H. and Sun, M. 2006. Analysis of genetic diversity and relationship in waxy rice (*Oryza sativa* L.) using AFLP and ISSR makers. *Genet. Resour. Crop Evol.*, **53**: 323-330.

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

Billotte, N., Lagoda, P. J. L., Risterucci, A. M. and Bauvens, F. A. 1999. Microsatellite enriched libraries: applied methodology for the development of SSR markers in tropical crops. *Fruits*, **54**: 277-288.

Botstein, D., White, R. L., Skolnick, M. H. and Davies, R. W. 1980. Construction of a genetic map in man using restriction fragment length polymorphism. *Am. J. Hum. Genet.*, **32**: 314-331.

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

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 Hort.*, **98**: 257-263.

Child, R. 1974. *Coconuts*. (Second Edition), Longman Group Ltd., London, pp. 335.

Dasanayake P. N., Everard, J. M. D. T., Karunanayake, E. H. and Nandadasa, H. G. 2003. Characterization of coconut germplasm by microsatellite markers. *Trop. Agrl Res.* **15**: 51-60.

De Vries, H. 1912. *Species and varieties, their origin by mutation*. Open Court publ. Co., Chicago.

Doldi, M. L., Vollmann, J. and Lelly, T. 1997. Genetic diversity in soybean as determined by RAPD and microsatellite analysis. *Plant Breed.*, **116**: 331-335.

Droogenbroeck, B., Kyndt, T., Maertens, I., Romeijn-Peeters, E., Scheldeman, X., Romero-Motochi, J. P., VanDamme, P., Goetghebeur, P. and Gheysen, G.. 2004. Phylogenetic analysis of the highland papayas (*Vasconcellea*) and allied genera (Caricaceae) using PCR-RFLP. *Theor. Appl. Genet.*, **108**: 1473-1486.

Duran, Y., Rohde, W., Kullaya, A., Goikoetxea, P. and Ritter, E. 1997. Molecular analysis of East African tall coconut genotypes by DNA marker technology. *J. Genet. Breed.*, **51**: 279-288.

Edwards, K .J., Barkers, J. H. A., Daly, A., Jones, C. and Karp, A. 1996. Microsatellite libraries enriched for several microsatellite sequences in plants. *Biotechniques*, **20**: 758-759.

FAO STAT Agriculture data. 2004. Available at http://apps./fao.org.

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. Develop.*, **4**: 256-261.

Fernando, W. M. U., Pieris, T. S. G. and Wickramaratne, M. R. T. 1993. Variation in seedling characters of three different coconut cultivars and their use in identification in nursery. *Cocos.*, **9**: 23-29.

Geethalakshmi, P. 2003. Allozyme variation in coconut hybrids and cultivars. PhD Thesis, Mangalore University, Mangalore, India.

Goodwin, I. D., Aitken, E. A. B., and Smith, L. W. 1997. Application of inter simple sequence repeat markers to plant genetics. *Electrophoresis*, **18**: 1524-1528.

Gottlieb, L. D. 1984. Electrophoretic evidence and plant populations. *Prog. Phytochem.*, **7**: 1-46.

Grati Kamouin, N., Mahmoud, L. F., Rebai, A., Gargauri, A., Panaud, O. and Saar, A. 2006. Genetic diversity of Tunisian olive treee (*Olea europaea* L.) cultivars assessed by AFLP markers. *Genet. Resour. Crop Evol.*, **53**: 265-275.

Gupta, P. K. and Varshney, R. K. 2000. The development and use of microsatellite markers for genetic analysis and plant breeding with emphasis on bread wheat. *Euphytica*, **113**: 163-185.

Harries, H. C. 1978. The evolution, dissemination and classification of *Cocos nucifera* L. *Bot. Rev.* **44**: 265-320.

Harries, H. C. 1981. Practical identification of coconut varieties. *Oleagineux*, **36**: 63-69.

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.). *Theor Appl. Genet.*, **101**: 292-300.

Huang, W. G., Cipriani, G., Morgante, M. and Testolin, R. 1998. Micorsatellite DNA in *Actinidia chinencis*; isolation, characterization and homology in related species. *Theor. Appl. Genet.*, **97**: 1269-1278.

Jarrat, R. L., Gawel, N. and Whittemore, A. 1992. Phylogenetic relationships of the sweet potato (*Ipomoea batatas* (L.) Lam.). *J. Am. Soc. Hort. Sci.*, **117**: 633-637.

Karp, A. 2002. The new genetic era: will it help us in managing genetic diversity In: Managing Plant Genetic Diversity. (eds) J. M. M. Engels, R. V. Ramanatha Rao, A.H.D. Brown and M.T. Jackson. CAB International, Wallingford, UK, pp. 43-56.

Kuroda, Y., Kaga, A., Tomooka, N., Vaughan, D. A. 2006. Population genetic structure of Japanese wild soybean (*Glycine soja*) based on microsatellite variation. *Mol. Ecol.* **15**: 957-974.

Leakey, C. L. A. 1988. Genotypic and phenotypic variation in common bean. In: Genetic resources of *Phaseolus* beans. (ed.), P. Gepts. Kluwer Academic Publishers, Dodrecht, The Netherlands, pp. 245-327.

Lebrun P., Grivet, L. and Baudouin, L. 1999. Use of RFLP markers to study the diversity of the coconut palm. In: C. Oropeza, J. L., Verdeil, G. R. Ashburner, R. Cardena and J. M. Santamaria (eds) Current plant science and biotechnology in agriculture- Current advances in coconut biotechnology. Kluwer Acadmic publishers, Dordrecht, The Netherlands, pp.73-87.

Lebrun, P., Baudouin, L., Bourdeix, J., Konan, 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 nucifer* L.) and QTL analysis for yield characters. *Genome*, **44**: 962-970.

Lebrun, P., Cho, Y. P. N., 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.

Li, W., Zhang, D. F., Wei, Y. M., Yan, Z. H., Zheng, Y. L. 2006. Genetic diversity of *Triticum turgidum* L. based on microsatellite markers. *Genetika*, **42**: 397-402.

Litt, M., and Luty, J.A. 1989. A hypervariable microsatellite revealed *in vitro* amplification of dinucleotide repeat within the cardiac muscle actin gene. *Am. J. Hum. Genet.*, **44**: 397-401.

Liu, S., Cantrell, R. G., McCarty, J. C. and Stewart, J. McD. 2000. SSR based assessment of genetic diversity in cotton race accessions. *Crop Sci.*, **40**: 1459-1469.

Lynch, M. 1988. Estimation of relatedness by DNA fingerprinting. *Mol. Biol. Evol.*, **5**: 584-499.

Mackill, D. J. 1996. Classifying Japonica rice cultivars with RAPD marker. *Crop Sci.*, **35**: 889-894.

Manimekalai, R. 2005. Informative Inter Simple Sequence Repeat (ISSR) primers for genetic analysis of coconut (*Cocos nucifera* L.) **CORD** **22**(2): 40-45.

Manimekalai, R., Nagarajan, P., Bharathi, M., Karun, A., Kumaran, P. M. and Parthasarathy, V. A. 2005b. Molecular diversity among South East Asian coconut germplasm accessions using ISSR markers. In proceeding of National Symposium on Biotechnological interventions for the improvement of Horticultural crops- issues and strategies. pp. 233-235.

Manimekalai, 2006. Cultivar identification and cluster analysis in coconut (*Cocos nucifera* L.) based on Inter Simple Sequence Repeat (ISSR) markers. Journal of Plant Breeding and Genetics.

Manimekalai, R. and Nagarajan, P. 2006b. Assessing genetic relationships among coconut (*Cocos nucifera* L.) accessions using Inter Simple Sequence Repeat Markers. *Scientia Hort.* **108**: 49-54.

Manimekalai, R. and Nagarajan, P. 2006a. Interrelationship among coconut (*Cocos nucifera* L) germplasm accessions using RAPD technique. *Genet. Resour. Crop Evol.* DOI: 10.1007/s10722-005-1303z (online first).

Manimekalai, R., Nagarajan, P., Bharathi, M., Karun, A., Kumar, S. N. and Kumaran, P. M. 2005a. Genetic variation among progeny lines of coconut (*Cocos nucifera* L.) based on SSR markers. *Trop. Agri. Res.*, **17**: 58-66.

Manimekalai, R., Nagarajan, P., Bharathi, M. and Naresh Kumar, S. 2004. DNA polymorphism among coconut (*Cocos nucifera*) cultivars and reciprocal cross derivatives. *Journal of Plant. Crops*, **32**: 117-124.

McGregor, C. E., Lambert, C. A., Greyling, M. M., Louw, J. H. and Warnich, L. 2000. A comparative assessment of DNA fingerprinting techniques (RAPD, ISSR, AFLP and SSR) in tetraploid potato (*Solanum tuberosum* L.) germplasm. *Euphytica*, **113**: 135-144.

Meerow 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. *Theor. Appl. Genet.*, **106**: 715-726.

Menon, K. P. V. and Pandalai, K. M. 1958. The coconut palm – A monograph. Indian Central Coconut Committee, Government Press, Madras, India.

Merril, E. D. 1936. Essays in geobotany in honour of William Albert Setchell, 47-61.

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,8, IPGRI, Rome, pp. 59-61.

Nallathambi, G., Raveendran, T. S., Ramanathan, T. and Vijayaraghavan, H. 1986. Exploitation of heterosis for oil content in coconut (*Cocos nucifera* L.). *Indian Coconut J.*, **17**: 1-2.

Narain, P. 2000. Genetic diversity- conservation and assessment. *Curr. Sci.*, **75**: 170-175.

Narayana G. V. and John, C. M. 1949. Varieties and forms of the coconut (*Cocos nucifera* Linn.). *Madras Agri. J.*, **8**: 349.368.

Parthasarathy, U., Parthasarathy, V. A., Manimekalai, R., Niral, V., Anuradha, U., Kumaran, P. M. 2005. Molecular diversity in coconut eco types of coastal and inland riverine ecosystems. *Indian J. Hort.* **62**: 315-318.

Parthasarathy, V. A., Geetha Lakshimi, P. and Niral, V. 2004. Analysis of coconut cultilvars and hybrids using isozyme polymorphism. *Acta Bot. Croat.* **63**: 69-74.

Peakall, R., Gilmore, S., Keys, W., Morgante, M. and Rafalski, A. 1998. Cross-species amplification of soybean (*Glycine max)* simple sequence repeats (SSR) within the genus and other legume genera. *Mol. Biol. Evol.*, **15**: 1275-1287.

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. *Mol. Ecol.*, **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.

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. 2003. Studying genetic relationships among coconut varieties/populations using microsatellite markers. *Euphytica*, **132**: 121-128.

Perera, L., Russell, J. R., Provan, J., McNicol, J. W. and Powell, W. 1998. Evaluating genetic relationships between indigenous coconut (*Cocos nucifera* L.) accessions from Sri Lanka by means of AFLP profiling. *Theor. Appl. Genet.*, **96**: 545-550.

Purseglove, J. W. 1972. Tropical Crops. Monocotyledons. Longman Group Ltd., pp. 607.

Ratnambal, M. J., Arunachalam, V. and Krishnan, M. 2003. Floral biology of some coconut accessions. *J. Plantation Crops*, **31**: 14-22.

Reddy, M. P., Sarla, N. and Siddiq, E. A. 2002. Inter simple sequence repeat (ISSR) polymorphism and its application in plant breeding. *Euphytica*, **128**: 9-17.

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. *Philipp. J. Coconut Stud.*, **22**: 1-7.

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. *J. Genet. Breed.*, **49**: 179-186.

Sankar, A. A. and Moore, G. A. 2001. Evaluation of inter simple sequence repeat analysis for mapping in citrus and extension of genetic linkage map. *Theor. Appl. Genet.*, **102**: 206-214.

Seah, S., Yaghoobi, J., Rossi, M., Gleason, C. A. and Williamson, V. M. 2004. The nematode-resistance gene, *Mi-1*, is associated with an inverted chromosomal segment in susceptible compared to resistant tomato. *Theor. Appl. Genet.*, **107**: 1635-1642 .

Staub, J. E., Serquen, F. C. and Gupta, M. 1996. Genetic markers, map construction and their application in plant breeding. *Hort Sci.*, **31**: 729-739.

Stuessy, T. 1990. Plant taxonomy. Columbia Unico Press, Newyork.

Sugimura, Y., Itano, U., Salid, C. D., Otsuji, K. and Yamaguchi, H. 1997. Biometric analysis of diversity of coconut palm, cultivar classification by botanical and agronomical traits. *Euphytica*, **98**: 29-35.

Swaminathan, M. S. and Nambiar, M. C. 1961. Cytology and origin of the dwarf coconut palm. *Nature*, **192**: 85-86.

Tanksley, S. D., Young, N. D., Paterson, A. H. and Bonierbale, M. W. 1989. RFLP mapping in plant breeding: New tools for an old science. *Biotechnology*, **7**: 257-264.

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*) population from across the geographical range using sequence tagged microsatellites (SSRs) and AFLPs. *Theor. Appl. Genet.*, **100**: 764-771.

Thomas, C. M., Vos, P., Zabeau, M., Jones, M. D. A. Norcott, K. A., Chadwick, B. P. and Jones, D. G. 1995. Identification of amplified fragment length polymorphism arkers linked to the tomato *cf9* gene for resistance to *Cladosporum fulvum*. *Plant J.*, **8**: 785-794.

Thompson, M. C., Davies, J. B. and Wilson, M. D. 1989. A portable allozyme electrophoresis kit used to identify members of the *Simullium damnosum Theobald* complex (Diptera: Simallidae) in the field. *Bullet Entomol Res.*, **79**: 685-691.

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

Upadhyay, A., Jose, J., Manimekalai, R. and Parthasarathy, V. A. 2002. Molecular analysis of phylogenetic relationship among coconut accessions. In: Managing Plant Genetic Resources (eds.) J. M. M Engels, A. H. D. Brown and M. T. Jackson CAB International, Wallingford, UK, pp. 61-66.

Virk, P., Newbury, H. J., Jackson, M. T. and Ford-Lloyd, B. V. 1995. The identification of duplicate accessions within a rice germplasm collection using RAPD analysis. *Theor. Appl. Genet.*, **90**: 1049-1055.

Vogel, J. M. 1995. A microsatellite based multiplexed genome assay (abstracts). **In:** Plant genome, Schargo International. New York, pp. 84.

Vos, P., Hogers, R., Blayer, M., Reijans, M., Tvande, Lee, Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M. and Zabeau, M. 1995. AFLP: A new technique for DNA fingerprinting. *Nucl. Acids Res.*, **23**: 4407-4414.

Wadt, L. H. O., Sakiyama, N. S., Pereiva, M. G., Tupinambra, E. A., Ribeiro, 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) C. Oropeza, J. L., Verdeil, G. R., Ashburner, R. Cardena and J. M. Santamaria. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 89-97.

Wang, G., Mahalingan, R. and Knap, H. T. 1998. (C-A) and (G-A) Anchored simple sequence repeats (ASSR) generated polymorphism in soybean *Glycine max* (L.) *Theor. Appl. Genet.*, **96**: 1086-1096.

Weeden, N. F. 1984. Distinguishing among white seeded bean cultivars by means of allozyme genotypes. *Euphytica*, **33**: 199-208.

White, G., and Powell, W. 1997. Isolation and characterisation of microsatellite loci in *Swietenia humilis* (Meliaceae): An endangered tropical hardwood species. *Mol. Ecol.* **6**: 841-850.

Williams, J. G. K., Kubelik, A. R., Livak, K. J., Rafalski, J. A., Tingey, S. V. 1990. DNA polymorphism amplified by arbitrary primers are useful as genetic markers. *Nucl. Acids Res.*, **18**: 6531-6535.

Wynne, I. R., Loxdale, H. D. and Brookes, C. P. 1992. Use of a cellulose acetate systems for allozyme electrophoresis. In: *Gene in ecology.* (eds) R .J. Berry, T. J. Crawford and G. M. Hewitt, Oxford, Blackwell Scientific Publications. pp. 49-99.

Zabeau, M. and Vos, P. 1993. Selective restriction fragment amplification: a general method for DNA fingerprinting. *European patent Application,* 92402629.7. (Publication No. 0534858A1).

Zhebentyayeva, T. N., Reighard, G. L., Gorina, V. M. and Abbott, A. K. 2003. Simple sequence repeat (SSR) analysis for assessment of genetic variability in apricot germplasm. *Theor. Appl. Genet.*, **106**: 435-444.

Zietkiewicz, E., Rafalski, A. and Labuda, D. 1994. Genome fingerprinting by simple sequence repeat (SSR) anchored polymerase chain reaction amplification. *Genomics*, **20**: 176-183.

Zizumbo, V. D. and Garciamarin, P. C. 2001. Morpho-physiological variation and phenotypic plasticity in Mexican populations of coconut (*Cocos nucifera* L.). *Genet. Resour. Crop Evol.*, **48**: 547-554.

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