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Interrelationships among coconut (*Cocos nucifera* L.) accessions using RAPD technique

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Abstract

The genetic relationships among 33 coconut germplasm accessions were analyzed using RAPD markers. The germplasm accessions were collected from various coconut growing regions viz. South Asia (SA), South East Asia (SEA), South Pacific (SP), Atantic and America, and Africa. Forty-five random primers produced a total of 399 polymorphic markers. The Polymorphism Information Content (PIC) ranged from 0.031 to 0.392 and the Marker Index (MI) ranged from 6.28 to 0.031 among the primers. Based on the MI a set of 5, 10 and 15 informative and reproducible primers were identified. The mantel matrix correlation was calculated to compare the similarity matrices of a set of reproducible informative primers and global primers. There was significant correlation among the similarity matrices ($r \geq 0.50$). The similarity matrix based on 399 polymorphic markers was used to construct the dendrogram to show the genetic relationship among the accessions. Similarity values ranged between 0.573 and 0.846. There was less genetic similarity (based on Jaccard's coefficient) among South Pacific and South East Asian accessions. The clustering pattern obtained in the present study was in agreement with the earlier reports based on RFLP, SSRs and AFLPs.

Introduction

Coconut (*Cocos nucifera* L.) is a perennial oil yielding tree crop of tropics. Its food and commercial products, such as tender nut, copra, coconut oil, and desiccated coconut play an important role in the economies of coconut producing countries like India, Sri Lanka, Malaysia, Indonesia, the Philippines, Côte d' Ivoire, Seychelles, South Pacific Islands, Nigeria, Brazil, Jamaica etc. Characterization of coconut germplasm accessions are essential for its preservation and maintenance. The CGIAR (Consultative Group on International Agricultural Research) has recognized coconut as 'the oil crop most in need of international research.' In 1993, Coconut Genetic Resources Network (COGENT) was established as part of International Plant Genetics Resources Institute's (IPGRI) program. Under the COGENT, International Coconut Genebank for South Asia (ICG-SA) was established at Central Plantation Crops Research Institute (CPCRI), Kidu, India, where a large collection of coconut germplasm accessions are being maintained.

Coconut (*Cocos nucifera* L.) is botanically classified in to two major groups based on its stature as Tall palms and as Dwarf palms. The Talls can also be referred to as var. Typica (Nav) and the Dwarfs as var. Nana (Griff) (Santos et al. 1996). Further, intermediate forms of coconut referred to as Aurantiaca are also available. 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 population (Perera et al. 2000). They are slow to mature, flowering 6–10 years after planting and has 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 intermediate type Niuleka has both the Tall and Dwarf characteristics.

Different molecular markers were employed in characterization of coconut populations in the past. Ashburner et al. (1997) used RAPD for analyzing South Pacific (SP) coconuts; Lebrun et al. (1998) analyzed genetic diversity in set of ten Talls and seven Dwarfs ecotypes using RFLPs; Perera et al. (1998) analyzed genetic diversity of Sri Lankan coconuts with AFLPs; SSR markers had also been used successfully in coconuts (Perera et al. 1999, 2000; Teulat et al. 2000; Meerow et al. 2003). So far, characterization and evaluation of coconut germplasm accessions held at CPCRI were done with the use of morphological traits (Kumaran et al. 2000; Ratnambal et al. 2002) and isozyme patterns (Geethalakshmi et al. 2004). In the present study, RAPD markers were used to derive genetic relationships among a subset of coconut germplasm accessions.

Materials and methods

Materials

Thirty-three coconut accessions (Table 1) collected from different geographical regions viz. South East Asia (SEA), South Asia (SA), South Pacific (SP), Atlantic and America (AA) and Africa were used for this study. These accessions are part of the germplasm collection maintained by ICG-SA, CPCRI, India and the RAPD analysis was carried out on these accessions at the Molecular Marker Assisted Selection laboratory, Dept. of Plant Molecular biology and Biotechnology, Tamil Nadu Agricultural University, Coimbatore, India.

DNA extraction

DNA was extracted from sprouting leaflets (pale yellow color). Two gram of leaf material was frozen in liquid nitrogen and ground to powder in a pestle and mortar. 0.50 g of Poly Vinyl Poly Pyrrolidone (PVPP, MW 40,000) was added to the ground powder, mixed well and transferred to a centrifuge tube containing 10 mL of extraction buffer (100 mM Tris (pH 8.0), 20 mM EDTA, 1.4 M NaCl, 1% SDS (Sodium Dodecyl Sulphate) and 10 mM β-Mercaptoethanol). The mixture was incubated at 65 °C for 1 h with intermittent mixing. To this, same volume of Chloroform: Iso Amly Alcohol (24:1) was added and homogenized by gentle inversion for 15 min. It was centrifuged at 12500 rpm for 15 min. Then the supernatant was transferred into equal volume of ice-cold Isopropanol. The DNA spool was collected in 1.50 mL micro tubes, air-dried and resuspended in 500 µL Tris EDTA (TE) buffer and incubated in 25 ng/µL of RNase at 37 °C for 1 h. Equal volume of Phenol:Chloroform:IAA was added, mixed well and centrifuged at 14,000 rpm for 15 min. The supernatant was transferred to equal volume of ice-cold absolute Ethanol and 1/10 volume of 3 M Sodium Acetate (pH 5.2). It was incubated at 4 °C for 1 h and centrifuged at 14,000 rpm for 10 min. Precipitate was washed with 70% Ethanol, air dried and resuspended in 100 µL TE. DNA concentration was measured in a spectrophotometer and the intactness was checked in 0.8% agarose gel. DNA was diluted to 15 ng/µL.

RAPD analysis

Amplification reactions were carried out in 10 µL volume containing 30 ng of template DNA, 200 µM of each dNTPs, 0.45 U of Taq DNA polymerase (Bangalore Genei Pvt. Ltd., India) and 25 pmol of random primer (Operon technologies, USA). Amplifications were performed in a Thermo Cycler (MJ Research Inc., USA) programed for an initial denaturation at 94 °C for 2 min., 40 cycles of 1 min denaturation at 94 °C, 1 min annealing at

Table 1. Accessions, international code, plant stature (type), place of collection, and geographic region of the coconut accessions used in the study.

No.	Accession	Code	Type	Country of origin	Geographic region
1	Kong Thienyong Tall	KTYT	Tall	Borneo	Southeast Asia
2	Straight Settlement Green Tall	SSGT	Tall	Malaysia	Southeast Asia
3	Straight Settlement Apricot Tall	SSAT	Tall	Malaysia	Southeast Asia
4	Philippines Kalambahim Tall	PKBT	Tall	Philippines	Southeast Asia
5	Laguna Tall	LAGT	Tall	Philippines	Southeast Asia
6	Philippines Palawan Tall	PPWT	Tall	Philippines	Southeast Asia
7	Philippines Dalig Tall	PDLT	Tall	Philippines	Southeast Asia
8	San Roman Tall	SNRT	Tall	Philippines	Southeast Asia
9	Markham Valley Tall	MVT	Tall	Papua New Guinea	South Pacific
10	Nufella Tall	NUFT	Tall	New Caledonia	South Pacific
11	Nugili Tall	NUGT	Tall	New Caledonia	South Pacific
12	Nuwallis Tall	NUWT	Tall	New Caledonia	South Pacific
13	Nu Quamen Tall	NUQT	Tall	New Caledonia	South Pacific
14	Kupien Tall	NHKT	Tall	New Caledonia	South Pacific
15	Nuwehnug Tall	NWHT	Tall	New Caledonia	South Pacific
16	Lifou Tall	LFT	Tall	Guam Islands	South Pacific
17	British Solomon Island Tall	BSIT	Tall	Solomon Islands	South Pacific
18	Jamaica Tall	JMT	Tall	Jamaica	Atlantic
19	Saint Vincent Tall	STVT	Tall	Trinidad	Atlantic
20	Panama Tall	PNT	Tall	Panama	America
21	Nigerian Tall	NIT	Tall	Nigeria	Africa
22	Kaithathali Tall	KAIT	Tall	Kerala, India	South Asia
23	Indian Spicata	WCT01	Tall	Kerala, India	South Asia
24	Indian East Coast Tall	ECT	Tall	Tamil Nadu, India	South Asia
25	Verrikobbari Tall	VKBT	Tall	Andra Pradesh, India	South Asia
26	Nadora Tall	NDRT	Tall	South West India	South Asia
27	Nicobar Tall	NICT01	Tall	Andaman and Nicobar Islands, India	South Asia
28	Hazari Tall	HZT	Tall	North East India	South Asia
29	Navassi Tall	NAVT	Tall	South India	South Asia
30	Niuleka Dwarf	NLAD	Intermediate	Fiji	South Pacific
31	King coconut	RTB04	Intermediate	Sri Lanka	South Asia
32	Laccadive Dwarf	LCOD	Dwarf	Lakshedweep Islands, India	South Asia
33	Chowghat Orange Dwarf	COD	Dwarf	Kerala, India	South Asia

37 °C and 2 min extension at 72 °C and a final extension of 7 min at 72 °C. The amplification products were subjected to electrophoresis through a 1.80% agarose gel using 1× TBE buffer at 90 V for 2 h in Bio-Rad submarine electrophoresis unit. The ethidium bromide stained gels were documented using the Alpha Imager^{TM} 1200 – Documentation and Analysis system (Alpha Innotech Corporation, USA). Amplification reaction was done twice for reproducible bands.

Data analysis

Only the clear, unambiguous and reproducible bands were considered for scoring. Each band was considered to be a single locus. Data were scored as '1' for the presence and '0' for the absence of a DNA band of each accession. DNA band size was estimated by comparing the DNA bands with a 1 Kb DNA ladder or lamda DNA *Eco* RI/*Hind*III double digest (MBI Fermentas, India).

The binary data matrices were entered into the NTSYS pc package (Exeter Software, USA). The data were analyzed using Qualitative routine to generate Jaccard's similarity coefficient. Similarity coefficients were used to construct a dendrogram using UPGMA (Unweighted Pair Group Method with arithmetic Average) and SHAN (Sequential Hierarchical and nested clustering) routine. The principal coordinate analysis (PcoA) was conducted with NTSYS pc.

Matrix comparison

Similarity matrix produced by 45 primers (global primers) and three sets of 15, 10 and 5 informative

primers were compared by the MXCOMP routine of NTSYS pc. The normalized Mantel statistic Z (Mantel 1967) was used to determine the level of association between the matrices.

The average Polymorphism Information Content (PIC) and Marker Index (MI) were calculated by applying the formula given by Powell et al. (1996) and Smith et al. (1997)

$$\mathrm{PIC} = 1 - \sum_{i=1-n} fi^2$$

where $f\,i$ is the frequency of the ith allele. The number of alleles refers to the number of scored bands. The frequency of an allele was obtained by dividing the number of accessions where it was found by the total number of accessions. The PIC value provides an estimate of the discriminating power of a marker. Marker indices were calculated for each primer as the product of PIC and the number of polymorphic bands. Based on the MI, the primers were ranked and according to the rank first 5, 10 and 15 primers were selected and regarded as informative primers and 45 primers regarded as global primers.

Results

Level of polymorphism

Forty-five random primers used in this study detected a total of 538 markers in 33 accessions, out of which 399 were polymorphic. The number of markers for each primer varied between 1 (OPE 1) and 18 (OPBE 6) with an average of 8.9 markers per primer. The amplification product size ranged from 117 to 3103 bp (Table 2)

The PIC values ranged from 0.031 to 0.392 and the marker index among the primers ranged from 6.28 to 0.031. Based on the high marker index 5, 10 and 15 informative primers sets were identified (Table 2) and these primers produced a total of 80, 144 and 210 polymorphic markers, respectively.

Genetic similarity among the accessions

Jaccard's coefficient of similarity between all the accessions ranged from 0.573 to 0.846 with a mean of 0.716. Most of the pair-wise similarity fell in to the range of 0.701–0.750. The accessions; Saint Vincent Tall (STVT) and Nigerian Tall (NIT) were the closest in this study with a genetic similarity percentage of 84.6 followed by Indian East Coast Tall (ECT) and Saint Vincent Tall (STVT) with a genetic similarity percentage of 83.9. The average pair-wise similarity among the South East Asian accessions was 0.735, while it was 0.722 among SP accessions and 0.746 for the South Asian accessions. The similarity between the intermediate types; Niuleka Dwarf (NLAD) and King coconut (RTB04) was 0.737 and between the Dwarf accessions Laccadive Dwarf (LCOD) and Chowghat Orange Dwarf (COD) was 0.738.

Cluster and principal coordinate analysis

The cluster analysis revealed four major clusters and three sub clusters (Figure,1). The cluster I consisted more of accessions belonging to South East Asia; cluster II consisted of South Asian and American accessions; cluster III comprised three sub clusters and included SP accessions, Dwarf accessions, accessions of intermediate types (NLAD and RTB04) and some of the South East Asian accessions; Cluster IV consisted of accessions of SP and SA in origin (Table 3).

The Dwarf accession, Laccadive Dwarf (LCOD) found closest with the South East Asian accession namely Straight Settlement Green Tall (SSGT). Panama Tall, an American accession was grouped along with South Asian accession (HZT). Two of the Caledonian accessions namely Nuwehnug Tall (NWHT) and Nufella Tall (NUFT) and a South Asian accession, Verrikobbari Tall (VKBT) have formed a separate cluster. Generally a grouping of accessions based on the geographic origin was observed, except that Indian Spicata (WCT01) which was clustered with the South East Asian accession and that Philippines Dalig Tall (PDLT) which was clustered with the South Asian accessions (Figure 1).

Three major groups of the accessions were distinguished well by the scatter plot (Figure not shown). The principal coordinate 1, 2 and 3 encompassed 71.7, 3.9 and 1.9% of total varia-

Table 2. Random (Operon) primers, polymorphic bands (POL), Polymorphism Information Content (PIC), Marker Index (MI), and product size obtained in this study.

	Primer	Polymorphic bands (POL)	PIC	MI	Product size (bp)
1	OPAG 18	5	0.061	0.310	910–205
2	OPAL 11[c]	8	0.344	2.750	1564–335
3	OPAG 13	8	0.112	0.896	1918–318
4	OPB 15	7	0.238	1.660	1460–174
5	OPBE 01	3	0.035	0.105	1030–549
6	OPBE 12	5	0.187	0.935	2753–213
7	OPBE 13	8	0.196	1.570	2087–198
8	OPBE 14	10	0.259	2.590	2051–415
9	OPBE 15	4	0.149	0.596	1022–260
10	OPBE16	6	0.306	1.836	1544–380
11	OPBE18	3	0.090	0.270	1779–240
12	OPBE 3[b]	11	0.332	3.652	1609–230
13	OPBE2[b]	14	0.296	4.140	1846–295
14	OPBE 4	4	0.255	1.020	1225–284
15	OPBE 5[b]	14	0.302	4.230	2008–220
16	OPBE 6[a]	18	0.349	6.280	1565–270
17	OPBE 7	6	0.205	1.230	1260–490
18	OPBE 8[b]	11	0.314	3.450	1808–343
19	OPC 10	6	0.176	1.060	1854–429
20	OPC 12	8	0.212	1.700	1762–424
21	OPC 13 [a]	17	0.329	5.590	1849–306
22	OPC 15	6	0.283	1.700	1641–463
23	OPC 3[c]	14	0.186	2.600	1949–317
24	OPC 4	12	0.179	2.150	1876–341
25	OPC 7[c]	11	0.286	3.150	1622–317
26	OPC 8	4	0.178	0.710	1286–787
27	OPC 9	5	0.189	0.945	1408–213
28	OPE 1	1	0.031	0.031	1612–342
29	OPE 11	12	0.217	2.600	1745–215
30	OPE 12	6	0.214	1.280	1267–171
31	OPE 16	10	0.222	2.220	1802–377
32	OPE 18	10	0.144	1.440	2278–220
33	OPE 2[c]	12	0.278	3.340	3103–312
34	OPF 14[a]	15	0.326	4.890	1857–377
35	OPG 8	9	0.249	2.240	1699–231
36	OPG 9	9	0.202	1.820	1827–464
37	OPM 10	5	0.175	0.875	1575–459
38	OPM 2[a]	17	0.329	5.590	1939–255
39	OPM 11	4	0.155	0.619	1434–117
40	OPM 17[a]	13	0.392	5.100	2552–351
41	OPM 20	10	0.302	3.020	1590–295
42	OPM 4[c]	11	0.298	3.280	2106–628
43	OPM 6[b]	14	0.275	3.850	1784–471
44	OPM 7	7	0.161	1.130	2012–508
45	OPM 9	6	0.216	1.300	2168–392

[a] First 5 informative primers (5 primers); [b] Second 5 informative primers; [c] third 5 informative primers.

tion respectively. The UPGMA and PcoA produced similar genetic clusters.

Matrix comparison

The Mantel test for comparison of the similarity matrices of the subset of primers having high marker index (the informative primers) and global primers revealed high correlation between them (Table 4). The correlation between the global primers with 5 informative primers was 0.827 which is statistically found to be significant. Absolute correlation was found between 10 and 5 primers ('*r*' value = 1.000).

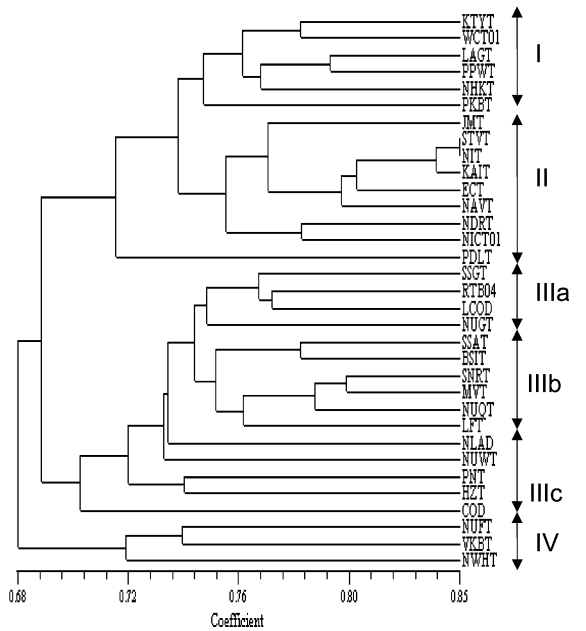

Figure 1. Dendrogram showing genetic relationships among coconut accessions used in this study. The accessions code was given in Table 1. Cluster numbers are indicated on the side.

Table 3. Distribution of accessions in the dendrogram classified by geographic origin.

Group	SEA	SA	SP	AA	Africa	Total
I	4	1	1	–	–	6
II	1	5	–	2	1	9
III a	1	2	1	–	–	4
IIIb	2	–	4	–	–	6
IIIc	–	2	2	1		5
IV	–	2	1	–	–	3
Total	8	12	9	3	1	33

SEA – South East Asia; SA – South Asia; SP – South Pacific; AA – Atlantic and America.

Table 4. Correlation coefficient between global primers and informative primers.

r	45	15	10	5
45	1.000			
15	0.901	1.000		
10	0.827	0.941	1.000	
5	0.827	0.941	1.000	1.000

Values are given for *r*, the product-moment correlation. Values greater than 0.5 are statistically significant at 1% level.

Discussion

Genetic similarity

High frequency of pair wise similarity in the range of 0.701–0.750 suggested the existence of moderate diversity among the accessions studied. The commonly employed parents for developing hybrid in coconut are Indian East Coast Tall (ECT) and Chowghat Orange Dwarf (COD), but based on the genetic similarity obtained in this study, Chowghat Orange Dwarf (COD) shared least similarity with Navassi Tall (NAVT); hence in future the palms of Navassi Tall (NAVT) and Chowghat Orange Dwarf (COD) may be considered for the crop improvement program. The average similarity for South Asian accessions was higher than the South Pacific and South East Asian accessions, reflects the existence of less diversity among South Asian accessions. Earlier, Upadhyay et al. (2004) reported less genetic diversity among the indigenous accessions of India (South Asian accessions) compared to exotic accessions. The genetic similarity among the SP accessions was found to be less compared to South East Asian accessions. High variation noticed among SP accessions was mainly due to the inclusion of New Caledonian accessions in this study, which is evident from Figure 1 that the New Caledonian accessions did not go into a single group. Earlier, Ashburner et al. (1997) analyzed SP coconuts and reported the existence of high variation between coconut populations.

Informative primers

The informative primer sets identified based on the high marker index (Table 2) has grouped all the accessions effectively as global primers. The first 5 informative primer set (OPBE6, OPC13, OPM2, OPM17 and OPF14) produced a total of 80 polymorphic markers. The dendrogram based on these 80 markers (Figure not shown) was comparable to the 399 markers of global primers. The correlation coefficient between the similarity matrices of informative primer sets (5, 10 and 15 primers) and the global primers indicated that informative primer sets were sufficient to identify the main tendencies in the structuration of the accessions. Hence it was noted that the 15

informative primer set was enough to construct the dendrogram of coconut accessions. So far no work has been reported on coconut that describes the saturation point for constructing stable dendrogram or developing core collections with least number of primers; however the work has been reported in wheat (Zhang et al. 2002).

Interrelationship among the accessions

In the dendrogram the Dwarf accession namely Laccadive Dwarf (LCOD) clustered along with the Talls of SEA and SP. Perera et al. (2000) and Lebrum et al. (1998) have also observed the same relationship and reported that Dwarfs sampled from Sri Lanka clustered with SEA coconuts and that African Dwarfs separated from African Talls and clustered with the Talls of South Pacific coconuts respectively. The grouping of King coconut (RTB04) with the Dwarf accessions was in agreement with the studies of Dasanayake et al. (2003) using SSR markers and reported that all the King coconut types fell within Dwarf coconut forms, and placed them towards Dwarfs.

Based on Jaccard's similarity coefficient, Saint Vincent Tall (STVT) and Nigerian Tall (NIT) both shared highest similarity, followed by Kaithathali Tall (KAIT) and Saint Vincent Tall (STVT). The dendrogram also showed clear and distinct clustering of African and Atlantic accessions with the Indian accessions. This pattern of clustering was in agreement with the studies of Perera et al. (2000) and Teulat et al. (2000) using SSRs.

The coconut accessions from New Caledonia belonging to SP region found to be highly diverse as these accessions did not group in to a single cluster and instead found taking place in all branches of the dendrogram. Two of the SP accessions; Nufella Tall (NUFT) and Nuwehnug Tall (NWHT) clustered with Verrikobbari Tall (VKBT), a SA accession. This was in agreement with the fruit component analysis of Ratnambal et al. (2002) who reported that the Caledonian accessions are intermediate between Talls and Dwarfs and nearer to Verrikobbari Tall. Harries (1978) reported that each group of South Pacific Islands appears to have one or two intermediate forms and one or both of extreme types of 'Niu kafa,' the wild coconuts and 'Niu Vai,' the domesticated coconuts.

References

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

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.

Geethalakshmi P., Niral V. and Parthasarathy V.A. 2004. Allozyme variation in population of dwarf coconut cultivars. J. Plantation Crops 32: 13–15.

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

Kumaran P.M., Koshy P.K., Arunachalam V., Niral V. and Parthasarathy V.A. 2000. Biometric clustering of coconut population of three Indian Ocean Islands. In: Muraleedharan N. and Rajkumar R. (eds), Proc. PLACROSYM XIII-Recent Advances in Plantation Crops Research. Allied Publishers, New Delhi, India, pp. 16–18.

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.

Mantel N. 1967. The detection of disease clustering and a generalized regression approach. Cancer Res. 27: 209–220.

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.

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.

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.

Powell W., Morgante M., Andre C., Hanafey M., Vogel J., Tingey S. and Rafalski A. 1996. The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. Mol. Breed. 2: 225–238.

Ratnambal M.J., Kumaran P.M. and Krishnan M. 2002. New Caledonian coconut cultivars. Indian Coconut J. 33: 3–6.

Santos G.A., Batugal P.A., Othman A., Baudouin L. and Labouisse J.P. 1996. Manual on Standardized Techniques in Coconut Breeding. IPGRI-COGENT Publication. Stamford Press, Singapore.

Smith J.S.C., Chin E.C.L., Shu H., Smith O.S., Wall S.J., Senior M.L., Mitchell S.E., Kresovich S. and Ziegle J. 1997. An evaluation of SSR loci as molecular markers in maize (*Zea mays* L.): Comparison with data from RFLPs and pedigree. Theor. Appl. Genet. 95: 163–173.

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.

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

Zhang X.Y., Li C.W., Wang L.F., Wang H.M., You G.X. and Dong Y.S. 2002. An estimation of the minimum number of SSR alleles needed to reveal genetic relationships in wheat varieties. 1. Information from large-scale planted varieties and corner stone breeding parents in Chinese wheat improvement and production. Theor. Appl. Genet. 106: 112–117.