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Genetic relationship and diversity in Indian coconut accessions based on RAPD markers

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Abstract

Randomly amplified polymorphic DNA (RAPD) markers were used to analyze genetic diversity and genetic relationship among coconut accessions. DNA from 81 palms representing 20 accessions, 15 Indian and 5 exotic, was used to amplify with 8 highly polymorphic primers. The 8 primers yielded 77 markers, with an average of 9.6 markers per primer. The within-accession genetic diversity ranged from 0.057 to 0.196. In general, tall accessions were more heterozygous as they had higher proportions of polymorphic bands and genetic diversity. The proportion of variation explained by within accession and between accession diversity was 0.58 and 0.42, respectively. Similarly exotic accessions exhibited more variation. Dwarfs from geographically distant regions did not cluster separately. Based on the similarity matrix, cluster and principal coordinate analysis was performed. A dendrogram of genetic relationship was obtained. The extent of genetic diversity and genetic relationship among accessions is discussed.

Keywords: *Cocos nucifera*; Genetic diversity; Molecular markers; RAPD

1. Introduction

Coconut (*Cocos nucifera* L.), a member of family Arecaceae (Palmaceae) is an important palm which sustains the livelihood of millions of families in coastal regions of

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the tropics. Grown in more than 80 countries, India is the leading country in area and production in the world. The area and production of coconut in the country is estimated at 1.84 million hectare and 12,597 million nuts, respectively (Anonymous, 2002). It forms an integral part of spiritual and social life of Indians. Vegetable oil is the most important product of coconut and occupies an important place in the international market because of high lauric acid content (Jones, 1991). However, factors like low productivity, several diseases and gradual decline in prices of coconut oil have forced the farmers, solely dependent on coconut, to look for other alternatives. This has resulted in the neglect of the coconut palms and further decline in coconut cultivation.

In view of these, at the Central Plantation Crops Research Institute (CPCRI), India, concerted efforts are being made to develop coconut cultivars and hybrids with high yield and tolerance to biotic and abiotic stresses using indigenous accessions with diverse traits. The success of such breeding programme is largely dependent on the availability of the broad diversity-based germplasm. A large coconut germplasm is being maintained for evaluation at the International Coconut Gene bank for South Asia (ICG-SA) under CPCRI. Under ICG-SA large scale collecting, conservation and cataloguing of indigenous and exotic coconut accessions is underway.

Traditionally, based on the growth habits, breeding behavior and other morphological characters, coconut is classified as talls, dwarfs and intermediate types. Morphological traits, mainly fruit component (Foale, 1987; Kumaran et al., 2000), isozymes (Carpio, 1982) and polyphenols (Jay et al., 1989; Chempakam and Ratnambal, 1993) have been used to assess variation in coconut from different regions. A rapid, reliable, unambiguous and cost-effective estimation of genetic diversity is a pre-requisite for utilization of germplasm in crop improvement. Morphological and biochemical markers have the disadvantage of being influenced by environment and are limited in number.

Molecular markers, which detect variation at the DNA level overcome most of the limitations of morphological and biochemical markers. As demonstrated by their use in various plant species, molecular markers are best suited for estimation of genetic diversity and varietal identification. Besides their unlimited numbers, molecular markers are not affected by environmental and developmental stage. Use of molecular markers gains further importance for perennial and recalcitrant crops like coconut, where progress in crop improvement is often hampered by its long generation period. Various molecular marker techniques like RFLPs (Lebrun et al., 1998), RAPD (Ashburner et al., 1997; Duran et al., 1997; Everard, 1999; Rodriguez et al., 1997; Upadhyay et al., 2002), AFLP (Perera et al., 1998; Teulat et al., 2000), ISTR (Rohde et al., 1995), SSRs (Perera et al., 1999; Rivera et al., 1999; Teulat et al., 2000) have been used for analysis of coconut biodiversity in different regions. Although newer techniques like AFLP and SSR are preferred due to their informativeness, RAPD is still method of choice for less advanced laboratories because of its simplicity, low cost and lower infrastructure requirement. Although a major coconut growing country, little information is available on the genetic diversity among Indian coconut populations. The present study was undertaken to estimate the genetic diversity and genetic relationship among some Indian coconut accessions using RAPD markers.

2. Materials and methods

2.1. Plant material

The plant material consisting of 81 palms representing 20 accessions (13 talls, 6 dwarfs and 1 intermediate) was used for the study. These accessions are maintained at CPCRI. Each accession was represented by three to six palms. Fifteen accessions were indigenous to India and five were exotic collections (Table 1). Indigenous accessions represented different parts of the country.

2.2. DNA extraction

The DNA was extracted from 1 g fresh leaf as per the protocol described earlier by Upadhyay et al. (1999).

2.3. RAPD analysis

Eight primers identified as highly polymorphic from previous work (Upadhyay et al., 2002), were used for PCR amplification of 81 DNA samples. The PCR parameters were essentially the same as given in Upadhyay et al. (2002). Amplified products were resolved by electrophoresis in a 1.2% agarose, 1× TAE (Tris:acetate:EDTA) at 60 V for 4 h. Bacteriophage λ DNA cut with *Eco*RI and *Hin*dIII was used as molecular weight marker. The gel

Table 1
List of accessions with abbreviations and place of collection

S. no.	Name	Abbreviation	Place of collection	No. of palms
1	West Coast Tall	WCT	Kerala	4
2	Kappadam Tall	KPDT	Kerala	6
3	Chowghat Orange Dwarf	COD	Kerala	4
4	Chowghat Green Dwarf	CGD	Kerala	4
5	Tiptur Tall	TPT	Karnataka	4
6	Kenthali Orange Dwarf	KTOD	Karnataka	4
7	Gangapani Tall	GPNT	Andhra Pradesh	3
8	Gangabondam Green Dwarf	GBGD	Andhra Pradesh	4
9	Ayiramkachi Tall	AYRT	Tamil Nadu	4
10	Kulasekharan Orange Dwarf	KOD	Tamil Nadu	4
11	Orissa Giant Tall	OGT	Orissa	4
12	Benaulim Tall	BENT	Goa	4
13	Andaman Ordinary Tall	ADOT	Andaman Islands	4
14	Laccadive Ordinary Tall	LCT	Lakshdweep	4
15	Laccadive Micro Tall	LMT	Lakshdweep	4
16	Philippine Ordinary Tall	PHOT	Philippine	4
17	Java Tall	JVT	Indonesia	4
18	San Ramon Tall	SNRT	Philippines	4
19	Malayan Orange Dwarf	MOD	Malaysia	4
20	Malayan Yellow Dwarf	MYD	Malaysia	4

was stained with ethidium bromide and viewed under UV light. Each band was considered as a RAPD marker and was identified by its molecular weight.

2.4. Data analysis

Each band was scored for the presence or absence across all the palms. Proportion of polymorphic bands for each accession as well as for exotic and indigenous accessions was calculated. Within-population genetic diversity for each accession, was estimated by using the Shannon information index (Chakraborty and Rao, 1991). The Shannon information index is defined as

$$H = -\sum_{I=1}^{k} P_i \log_e P_i$$

where H denotes the diversity of RAPD markers in a population, k denotes the number of RAPD markers and P_i denotes the frequency of the ith RAPD marker in a given accession. For each accession, H was averaged over all the primers to determine the within-accession diversity (H_{WA}). The average diversity of RAPD markers for all the accessions (H_M) was calculated as the mean of H_{WA}. Similarly, average diversity for tall accessions (H_{MT}) and dwarf accessions (H_{MD}) was calculated as the mean of H_{WA} based on talls and dwarfs, respectively. The genetic diversity for all the studied coconut palm (H_T) was calculated with a P_i based on all palms rather than on individual accession. Similarly, genetic diversity was calculated only for tall and dwarf accessions as well as indigenous and exotic accessions by considering P_i based on palms of respective types. The proportion of diversity that is found within-accession relative to total diversity, was derived by dividing H_M with H_T.

The presence or absence of data was entered into a binary data matrix and was used for calculating the similarity coefficient using Jaccard's coefficient (Jaccard, 1908). The mean similarity coefficient for each accession pair was calculated and used for cluster analysis using the UPGMA method and a dendrogram was constructed using the software package NTSYS-PC (Rohlf, 1993). The binary data for 81 palms was also subjected to principal coordinates analysis and scores for the first two components were plotted.

3. Results and discussion

3.1. Degree of polymorphism

Eight primers detected 77 polymorphic markers in 81 palms. The number of markers for each primer varied between 7 (OPA 4, OPB 1, OPC 5) and 15 (OPB 5) with an average of 9.6 markers per primer. The molecular weight of these markers ranged from 200 to 2750 bp, with an average molecular weight of 960 bp. A typical RAPD gel is shown in Fig. 1.

The number of markers present in an accession varied. The accession-wise details of number of bands present and the proportion of polymorphic bands are listed in Table 2. A perusal of the table revealed that the number of markers present in each accession varied between 46 (COD) and 71(KPDT). The average number of markers present in tall accessions was 61 while in dwarfs, on an average 52 markers were present. The proportion of polymorphic bands among tall accessions varied from 22% (GPNT) to 71% (KPDT) with an average of 49%, whereas among the dwarfs, it varied from 20% (KTOD) to 44% (MOD) with an average of 36%.

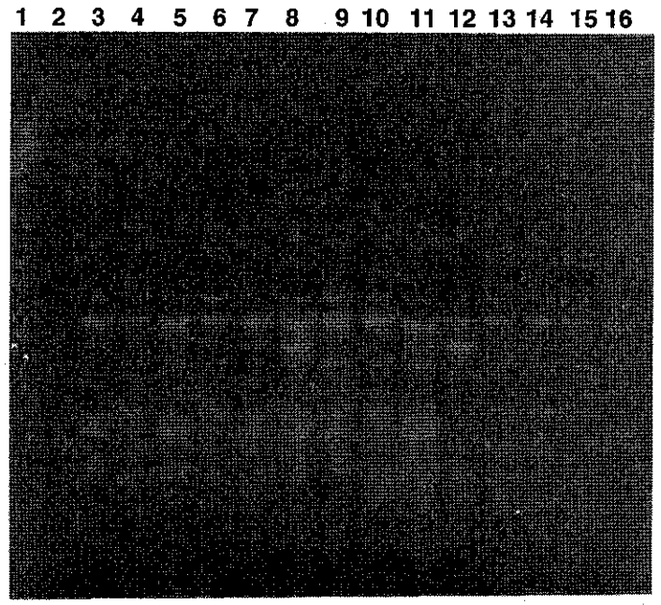

Fig. 1. RAPD analysis of coconut DNA. Lane 1: MW marker, lane 2: control, lanes 3–16: different coconut accessions.

As highly polymorphic primers, selected from a previous study (Upadhyay et al., 2002) were used for the analysis, a relatively large number of polymorphic markers were detected by these primers. The number of markers detected by each primer depends on primer sequence and the extent of variation is genotype specific. The number of markers varied in different accessions. In general, more markers were present in tall accessions than in dwarfs. The extent of variation in terms of proportion of polymorphic markers and genetic diversity was also found to be higher in talls than dwarfs. These results are in agreement with findings of earlier workers (Ashburner et al., 1997; Perera et al., 1999; Upadhyay et al., 2002) who observed more variability among talls in terms of number of polymorphic bands as well as genetic diversity. This is attributed to the differing breeding behavior of talls and dwarfs. Talls are precociously cross-pollinating where as dwarfs are self-pollinating.

3.2. Genetic diversity

The data on genetic diversity for different coconut types are given in Table 3. The genetic diversity for all the accessions (H_T) was 0.214. When considering only all tall accessions, the genetic diversity was 0.215, which was significantly higher than that for dwarf accessions

Table 2
Number of markers, proportion of polymorphic markers and within-population diversity in different coconut accessions

Accession	No. of present markers	Proportion of polymorphic markers (%)	H_{WA}
Tall			
WCT	64	53	0.147
PHOT	56	71	0.196
LCT	55	40	0.105
BENT	62	55	0.154
ADOT	64	56	0.166
JVT	61	39	0.117
TPT	64	30	0.086
AYRT	62	42	0.114
GPNT	63	22	0.071
SNRT	59	59	0.166
LMT	62	60	0.165
KPDT	71	72	0.182
OGT	60	43	0.117
H_{MT}			0.137
Dwarf			
GBGD[a]	52	38	0.101
COD	57	40	0.108
MOD	55	44	0.130
CGD	46	43	0.110
MYD	47	28	0.079
KOD	62	40	0.112
KTOD	49	20	0.057
H_{MD}			0.099

[a] GBGD is an intermediate type.

(0.178). The genetic diversity for indigenous accession was 0.209, which was significantly lower than that of exotic accession (0.225).

The average diversity (H_{WA}) for individual accession varied between 0.057 (KTOD) to 0.196 (PHOT) (Table 2). The mean within-accession (H_M) diversity was 0.124. The proportion of total diversity found within-population (H_M/H_T) was 0.58. Thus the proportion of between-population diversity was 0.42. The average within-accession diversity for talls (H_{MT}) and dwarfs (H_{MD}) was 0.137 and 0.099, respectively.

Table 3
Genetic diversity in different coconut types

Types	Genetic diversity value
Talls	0.214
Dwarfs	0.178
Total (H_T)	0.215
Indigenous	0.209
Exotic	0.225

The within-accession genetic diversity is the reflection of heterozygosity and it varied considerably for the studied accessions. The diversity was greater for tall accessions. Dwarfs like MYD and KTOD had very low diversity of 0.079 and 0.057, respectively, suggesting higher homozygosity in these accessions. Within-population variation was up to 58% of the total variation. Ashburner et al. (1997) while analyzing South Pacific coconut palm populations observed similar levels of within- and between-population diversity. Similarly, tall accessions contributed more to the total variation compared to the dwarfs, which is due to the highly cross-pollinating nature of talls. Less diversity was observed among Indian collections as compared to exotic collections.

3.3. Principal coordinate and cluster analysis

The first two components from principal coordinate analysis contributed 15 and 12.5% variation, respectively. The scatter plot of these two principal components (Fig. 2) reveals the extent of within- and between-accession variations. While the palms of dwarf accessions were distinctly clustered in two regions, tall showed largely dispersed and continuous

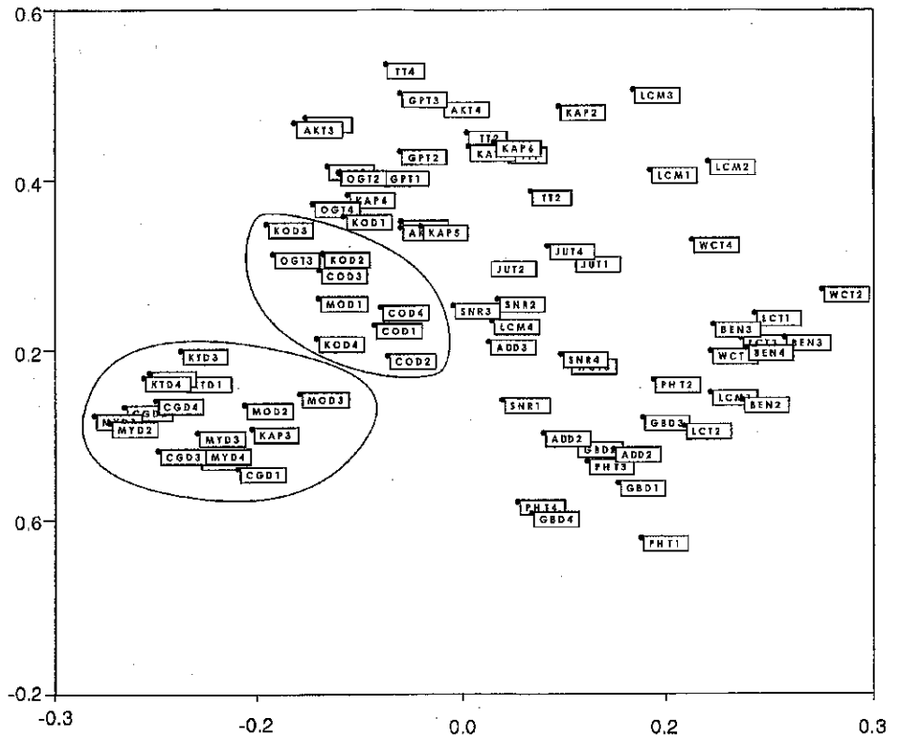

Fig. 2. Scatter plot of 81 palms based on first two components of principal coordinate analysis (the dwarfs clustered in two distinct clusters).

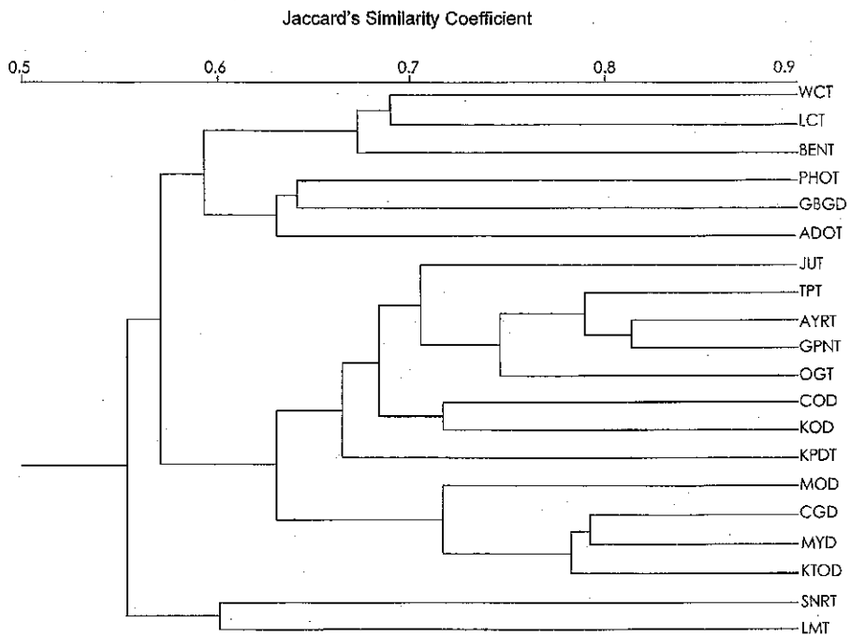

Fig. 3. Dendrogram of genetic relationship in 20 coconut accessions based on mean similarity matrix. Scale on top is Jaccard's similarity coefficient.

variation. Some of the dwarfs were clustered closer to tall accessions. MOD and MYD, the two exotic accessions, were clustered with indigenous dwarfs. Similarly, exotic tall accessions from Philippines (PHOT and SNRT) and Indonesia (JVT) were not separated and clustered with Indian tall accessions. GBGD an intermediate type was grouped with talls. The Dendrogram of mean similarity matrix revealed the genetic relationship among these accessions (Fig. 3). The clustering was in accordance with the individual palm clustering. LMT, an accession with small nuts was separated in PCO analysis, however in the dendrogram it clustered with SNRT, an accession with diverse nut character. The similarity among Indian accessions was more than 60% suggesting that although collected from different parts of the country, narrow diversity exists among these accessions.

The genetic relationships obtained by RAPD analysis are in accordance with previous results (Everard, 1999; Perera et al., 1998, 2000; Rivera et al., 1999; Upadhyay et al., 2002), wherein dwarf accessions grouped together and tall accessions show more variation. In a scatter plot, tall palms show more dispersed and continuous variation. Exotic accessions, viz. SNRT, PHOT and JVT were grouped with Indian accessions in the dendrogram. Similar results were found in the earlier studies (Perera et al., 1998; Teulat et al., 2000; Upadhyay et al., 2002), where accessions from geographically distant places clustered together. Two accessions with diverse nut characters, LMT (small nut) and SNRT (big nut) were clustered together with up to 60% similarity index. This suggests that estimation of genetic distance along with geographical and morphological data should be used for germplasm collecting.

Dwarfs from geographically distant regions did not occupy a distant position in the dendrogram. These results are in agreement with earlier findings (Ashburner et al., 1997; Rivera et al., 1999) and have been attributed to a common origin for all the dwarf forms and their subsequent isolation from local populations (Lebrun et al., 1998).

The present study revealed relatively narrow diversity in Indian coconut populations. This information is important for ongoing efforts to develop high yielding hybrids and varieties. Inclusion of more exotic collections with desirable traits will be a useful step towards achieving coconut breeding objectives. This study also demonstrated the efficacy of RAPD markers to analyze diversity and genetic relationship in the germplasm. RAPD markers revealed a genetic relationship pattern that was in agreement with the results obtained with AFLP and SSR markers. In view of this, 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.

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References

Anonymous, 2002. Agricultural Statistics at a Glance—2002. Directorate of Economics and Statistics, Ministry of Agriculture, Government of India.

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

Carpio, C.B., 1982. Biochemical studies of *Cocos nucifera* L. Kalikasan. Philipp. J. Biol. 11, 319–338.

Chakraborty, R., Rao, C.R., 1991. Measurements of genetic variation for evolutionary studies. In: Rao, C.R., Chakraborty, R. (Eds.), Handbook of Statistics, vol. 8. Elsevier, Amsterdam, pp. 271–316.

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

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

Everard, J.M.D.T., 1999. An investigation towards developing a molecular approach to improve the efficiency of coconut breeding by RAPD marker assisted selection. CORD XV (2), 115–130.

Foale, M.A., 1987. Coconut germplasm in the South Pacific Islands. ACIAR Technical Report No. 4. Australian Centre for International Agricultural Research, Canberra.

Jaccard, P., 1908. Wonvelles recherches sur la distribution florale. Bull. Soc. Vaud. Sci. Nat. 44, 223–270.

Jay, P., Bourdex, R., Potier, F., Sanlaville, C., 1989. Note on polymorphism of coconut leaf polyphenols. Oleagineux 44, 151–161.

Jones, L.H., 1991. Perennial vegetable oil crops. In: Persley, G.J. (Ed.), Agricultural Biotechnology: Opportunities for International Development. CAB International, Wallingford, UK, pp. 213–224.

Kumaran, P.M., Koshy, P.K., Arunachalam, V., Niral V., Parthasarathy, V.A., 2000. Biometric clustering of coconut population of three Indian Ocean Islands. In: Recent Advances in Plantation Crops Research. Allied Publishers, New Delhi, India, pp. 16–18.

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

Perera, L., Russell, J.R., Provan, J., NcNicol, J.W., Powell, W., 1998. Evaluating genetic relationship between indigenous coconut (*Cocos nucifera* L.) accessions from Sri Lanka by means of AFLP profiling. Theoret. Appl. Genet. 96, 545–550.

Perera, L., Russell, J.R., Provan, J., Powell, W., 1999. Identification and characterisation 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., 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.

Rivera, R., Edwards, K.J., Barker, J.H.A., Arnold, G.M., Ayad, G., Hodgkin, T., 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., Soniega, J.A., 1997. Analysis of genetic diversity in coconut by RAPD. Philipp. J. Coconut Stud. XXII, 1–7.

Rohde, W., Kullaya, A., Rodriguez, J., 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.

Rohlf, F.J., 1993. NTSYS-PC Numerical taxonomy and Multivariate Analysis System, Version 1.8. Exeter Publications, Setauket, New York.

Teulat, B., Aldam, C., Trehin, R., Lebrun, P., Barker, J.H.A., Arnold, G.M., Karp, A., Baudouin, L., 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. Theoret. Appl. Genet. 100, 764–771.

Upadhyay, A., Parthasarathy, V.A., Seema, G., Karun, A., 1999. An efficient method of DNA extraction from coconut. Agrotropica 11 (1), 35–38.

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