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Analysis of genetic diversity among Indian Ocean coconut accessions through microsatellite markers

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ABSTRACT

The extent of genetic diversity among nineteen coconut accessions comprising collections from the Indian Ocean Islands were characterized with eight polymorphic microsatellite primers. The fixation index (Fst) was found to be higher (0.78) between Laccadive Micro (LMT) and Chowghat Orange Dwarf (COD) population and the lowest Fst value (0.04) was found among the population Guelle Rose Tall (GLT) and Sri Lankan Tall (SLT). An average Fst value of 0.48 was observed for the accessions indicating higher level of population differentiation among the accessions. The maximum genetic distance (2.29) was observed between Laccadive Green Tall (LGT) and Chowghat Orange Dwarf (COD). The minimum genetic distance (0.04) observed between Laccadive Micro (LMT) and Srilankan Tall (SLT). Overall, the within population variation was found to be higher (67%) than among the population variation (33%) for these coconut accessions. The clustering pattern distinguished two main groups among the Indian Ocean Islands population. The control population COD formed the first group and the remaining populations form the second group. The clustering within the second group revealed the relationship among the accessions under study and the information on possible migration of coconut types within the region which could be useful for planning future collections as well the utilization of conserved types.

Key words: *Cocos nucifera* L., genetic diversity, microsatellite markers.

INTRODUCTION

Coconut (*Cocos nucifera* L.) is an important pan-tropical crop grown in the islands and in the countries lining the Atlantic, Pacific and Indian oceans. The Indian Ocean is the third largest ocean with islands such as Madagascar, Zanzibar, Pemba, Sri Lanka, Andaman and Nicobar, Comoros, Lakshadweep, Seychelles and Mauritius scattered over a vast surface area of 73 million km² with wide presence of coconut mostly as cultivated and as natural stand in few places. Coconut as one of the main cultivated crops in these islands, play a major role in livelihood security of large number of families in these countries. While the history of coconuts is said to be relatively recent in some of these islands, the coconuts were ought to have been grown naturally in many islands such as Seychelles even before human arrival, which needs to be confirmed through further investigations.

While the exact origin of coconut is unknown, the centre of domestic diversity is generally agreed to be South or South East Asia. It is believed to be endemic in the Indo-Malayan region and dispersed by floating on the ocean currents to sandy and tropical coasts of the Indian and Pacific oceans, where it naturally established (Harries, 4). These naturally disseminated coconuts have characteristics such as long angular fruit, high husk to nut ratio and slow germination that favour seed dispersal by floating. Human selection in the Malesian region between South East Asia and the Western Pacific is considered as the most likely region for coconut domestication (Harries, 5, 6). The ancient sea faring Austronesian were believed to have visited as far as Madagascar and spread domesticated coconuts in Indian Ocean islands where the wild type would be naturally established already. Introgression, or introgressive hybridisation, accounts for the two groups that have been identified by fruit component analysis (Harries, 4) and by molecular analyses by various workers. The recent claim for independent origins for Indian Ocean and Pacific centres of diversity (Gunn *et al*., 3) is also consistent with the hypothesis that introgressive hybridisation between the facultatively cross-pollinated palms from these two groups resulted in the phenotypic variability within and between modern cultivated populations (Harries, 4). The present study was undertaken to understand the extent of genetic variability and relationship of eighteen coconut populations belonging to eight groups of the Indian Ocean islands.

MATERIALS AND METHODS

The experimental material consisted of eighteen exotic collections made by ICAR-CPCRI, Kasaragod

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from islands of Indian Ocean region and one indigenous germplasm conserved at the national gene bank. All the nineteen accessions used in the present study are now being conserved at the International Coconut Gene bank for South Asia under CPCRI. The details of the accessions and their place of collection are given in the Table 1. DNA was extracted from the spear leaf of the palms following the procedure of Upadhyay *et al*. (11), whereby 5 g of spear leaf tissue was ground in liquid nitrogen and transferred to extraction buffer containing 10% sodium dodecyl sulfate **(**SDS). The contents were heated at 65°C, cooled and extracted with an equal volume of 24:1 chloroform: isoamyl alcohol mixture. The supernatant was transferred to a new tube and DNA was precipitated with 70% ethanol. For microsatellite analysis, 12.5 ng of DNA template, 200 µM deoxynucleotide triphosphate (dNTPS), 1 unit of *Taq* polymerase (Bangalore Genie, India) and 1 µM

Table 1. Details of Indian Ocean coconut accessions studied.

Sl. No.	Name of Accn. (Abbrv. and No.)	No of palm(s)/ population	Place of collection	% Polymorphic loci	Observed hetero-zygosity	Observed gene diversity	Inbreeding coefficient
1.	Sambava Tall - ST-(IND139)	3	Sambava, Madagascar	87.50	0.25	0.46	0.48
2.	Sambava Green Tall - SGT-(IND141)	4	Sambava, Madagascar	75.00	0.25	0.38	0.34
3.	Pemba Orange Dwarf - POD-(IND133)	1	Pemba Island, Zanzibar	37.50	0.25	0.13	0
4.	Pemba Red Tall - PRT-(IND 136)	2	Pemba Island, Zanzibar	25.00	0.13	0.13	0
5.	Comoros Tall - CT-(IND142)	2	Comoros	75.00	0.31	0.39	0.15
6.	Comoros Moheli Tall - CTT-(IND177)	3	Comoros	62.50	0.38	0.28	0.08
7.	Coco Belu Dwarf - CBD-(IND145)	3	Seychelles	75.00	0.21	0.37	0.33
8.	Sri Lankan Tall - SLT-(IND015)	5	Sri Lanka	100.00	0.58	0.48	-0.13
9.	Sri Lankan Yellow Dwarf SLYD-(IND063)	3	Sri Lanka	100.00	0.25	0.49	0.56
10.	King Coconut - KCD-(IND052)	3	Sri Lanka	25.00	0.17	0.13	-0.06
11.	Laccadive Dwarf - LD-(IND337)	3	Lakshadweep Islands	50.00	0.13	0.23	0.25
12.	Laccadive Micro Tall - LMT-(IND030)	4	Lakshadweep Islands	50.00	0.15	0.23	0.27
13.	Laccadive Green Tall - LGT-(IND336)	7	Lakshadweep Islands	75.00	0.22	0.36	0.43
14.	Gonthembili Tall II - GT-(IND 156)	7	Sri Lanka	87.50	0.38	0.38	*0.07*
15.	Andaman Ranguchan Tall - ART-(IND017)	7	Andaman	100.00	0.43	0.51	0.28
16.	West African Tall II - WAT-(IND140)	2	Madagascar	50.00	0.31	0.23	-0.06
17.	Guelle Rose Tall - GLT-(IND138)	4	Mauritius	100.00	0.38	0.53	0.30
18.	Zanzibar Tall - ZBT-(IND037)	3	Zanzibar	75.00	0.35	0.37	0.16
19.	Chowghat Orange Dwarf COD-(IND007) (Control)	2	Kerala	0.00	0.00	0.00	0.00
	Total	68	Mean	65.79		0.32	0.18

of each primer were used as PCR reaction mixture. The polymerase chain reaction (PCR) conditions were identical to those of Perera *et al.* (10) for CAC primers and 51°C annealing temperature was set for the CnCIRG11 primer. The amplified products were resolved in a 5% denaturing polyacrylamide gel and the bands were visualized by silver staining (Bassam and Caetano-Anollés, 1). The microsatellite bands were scored manually and the alleles were sized with reference to a 30-330 bp ladder (Gibco Brl).

The calculation of genetic diversity values and construction of the unweighted pair group method with arithmetic mean (UPGMA) dendrogram using Nei's genetic distance (Nei *et al.*, 8) was carried out using the POWERSSR v 1.2. Software (Liu, 7). The analysis of molecular variance was done using GENALEX software (Peakall and Smouse, 9) with a significance setting permutation value of 999.

RESULTS AND DISCUSSION

A total of 68 individual coconut palms from eighteen Indian Ocean coconut accessions and an indigenous dwarf coconut accession Chowghat Orange Dwarf (COD) from the Indian mainland were analyzed. The number of individuals varied from one for the Pemba Red dwarf (PRD) to seven each for Laccadive Green (LGT), Gonthembili (GLT) and Andaman Ranguchan (ART). A total of 31 alleles with an average of 3.9 alleles/ locus were detected across 19 Indian Ocean coconut accessions. The highest numbers of five alleles were detected for the CAC2 and CAC4 loci and lowest numbers of three alleles were detected for the CAC11, CAC13 and CAC 8 loci. The CAC2 loci showed the highest average of 2.68 alleles, while the CAC11 loci showed the lowest average of 1.21 among all the 19 accessions studied. The mean number of alleles detected for all the eight loci were highest (3.25) for the Gonthembili Tall (GLT), while it is lowest (1.13) for the Pemba Red Dwarf (PRD). The control sample COD showed a mean value of 0.75 for the alleles detected in all the loci. The highest percentage of polymorphic loci (100%) was observed for the Srilankan Yellow Dwarf (SLYD), Guelle Rose Tall (GLT), Andaman Ranguchan Tall (ART) and Sri Lankan Tall (SLT). The maximum mean observed heterozygosity value was found to be in SLT population and lowest mean observed heterozygosity were found in Pemba Red (PRD) and Laccadive Dwarf (LD) populations. The overall mean heterozygosity among all the 19 accessions for the eight loci studied was observed to be 0.27 (Table 1). The mean gene diversity value (Ho) ranged from 0.13 (Pemba Orange Dwarf (POD), Pemba Red (PRT) and King coconut (KCD)) to 0.53 (Guelle Rose Tall (GLT) among the 19 populations studied with an average of 0.32 (Table 1). Among the 8 loci studied, the primer CAC3 shows the highest gene diversity value of 0.73, while the primer CAC11 and CAC10 had a gene diversity value of 0.22.

The coefficient of inbreeding (F) is the probability that two alleles at a randomly chosen locus are identical by descent. This is an important measure since inbreeding depression results in lower performance and viability, reproductive fitness is particularly affected due to loss of dominance arising from increased homozygosity. The coefficient of inbreeding ranged from -0.13 to 0.56. The Sri Lanka Yellow Dwarf (SLYD) showed the highest inbreeding coefficient of 0.56. The Sri Lanka tall (SLT), King coconut Dwarf (KCD) and West African Tall (WAT) showed the lowest inbreeding coefficient of -0.13, -0.6 and -0.6, respectively among the populations studied. The only private allele of 172 bp was found in the CAC11 loci for the Sri Lankan Tall population (data not shown).

Wright's F-statistics was used to characterize population genetic structure. These statistics allow the partition of genetic diversity (~heterozygosity) within and among populations. Among the eight loci studied, CAC13 showed the maximum F_Is, while the minimum was observed at the CAC8 and CAC10 loci. The overall mean value was observed to be 0.19. The Fit value was found to be higher in CAC13 loci and lower in CAC10 loci. The mean value was observed to be 0.58. The Fst value was found to be higher in CAC11 loci and lower in CAC2 loci. The mean value was observed to be 0.48. The Nm value was higher in CAC2 loci and lower in CAC11 loci. The mean value was found to be 0.29. The results indicate the potential of the primers to elucidate the genetic diversity among the coconut accessions under study (Table 2). Among the 19 coconut populations studied, the Fst value was found to be higher (0.78) between Laccadive Micro (LMT) and Chowghat Orange Dwarf (COD) population and the lowest Fst value (0.04) was found among the population Gonthembili (GLT) and Sri Lanka Tall (SLT). The average Fst value of 0.46 indicates the higher level of population differentiation among the accessions (Table 3). The maximum genetic distance (2.50) was observed between Laccadive Micro Tall (LMT) and Chowghat Orange Dwarf (COD) followed by Laccadive Green (LGT) and COD (2.29). The minimum genetic distance (0.04) was observed between Laccadive Micro (LMT) and Sri Lanka Tall (SLT). Generally, the genetic distance between COD and other accessions was higher. The Laccadive Green tall (LGT) and Laccadive Micro Tall (LMT) are closely related (Table 4). The within population variation was found to be higher (67%) than between the population variation (33%) of 19

Table 2. F-Statistics and estimates of Nm over all populations for each locus.

All Popln.	Locus	Fis	Fit	Fst	Nm
	CAC2	0.38	0.57	0.31	0.57
	CAC3	0.18	0.60	0.52	0.23
	CAC4	0.26	0.66	0.54	0.21
	CAC6	0.16	0.56	0.48	0.27
	CAC8	-0.01	0.44	0.45	0.31
	CAC10	-0.08	0.37	0.42	0.35
	CAC11	0.10	0.61	0.58	0.19
	CAC13	0.54	0.80	0.56	0.20
	Mean	0.19	0.58	0.48	0.29
	SE	0.07	0.05	0.03	0.05

F_{IS} = Inbreeding coefficient, F_{IT} = Overall fixation index, F_{st} = Fixation index.

coconut population accessions from Indian Ocean islands (data not shown). This indicate the need to evaluate the accessions further for effecting selection for desirable traits.

The unweighted pair group mean arithmetic (UPGMA) dendrogram was drawn for nineteen coconut populations in Indian Ocean Islands using the Nei's genetic distance. The clustering pattern distinguished two main groups among the Indian Ocean Islands population studies. COD formed the first group and the remaining populations formed the second group. The second group was further clustered into two sub-divisions. West Africa Tall (WAT), Comoros Moheli Tall (CTT), Andaman Ranguchan (ART), Zanzibar Tall (ZBT), Sri Lanka Yellow Dwarf (SLYD), Guelle Rose Tall (GLT), Laccadive Green (LGT), Laccadive Micro Tall (LMT) and Sri Lanka Tall (SLT) formed the first division and Coco Belu (CBD), Pemba orange (POD), Pemba red (PRD), King coconut (KCD), Laccadive Dwarf (LD), Comoros Tall (CT), Sambava Green (SGT), Gonthembili Tall (GT) and Sambava Tall (ST) formed the second division (Fig. 1).

Among the dwarf accessions, the Sri Lankan Yellow Dwarf (SLYD) alone separated from the rest of the dwarfs and grouped with the first cluster of talls indicating the level of out crossing in the accession. Other dwarf accessions CBD, POD, KCD and LD were grouped together within the first division along with PRT. The accession PRT though it is clustered with dwarfs, the clustering pattern indicate that it may be an out crossed POD as both the collections were from the same location and it possesses the traits of POD. The low level of observed heterozygosity and low level of gene diversity (0.13) in PRT was also substantiating the conclusion. The dwarf populations *viz.*, Sri Lankan Yellow Dwarf, Laccadive Dwarf, Pemba Orange Dwarf, King Coconut Dwarf and Coco Belu Dwarf clustered with the Tall populations and this shows the long history of cultivation in their respective regions and intercrossing with the Talls. The information stress the need to purify these accessions before using them in breeding programmes which needs further evaluation and selection.

In the present study, the coconuts as far as from Andaman in the east to the Madagascar in the western Indian Ocean showed less differentiation. The low gene diversity and microsatellite polymorphism in dwarfs is in consistent with the previous findings and the autogamous nature of the dwarfs. The Sambava Tall showed a high inbreeding coefficient (0.48) indicating the presence of an increased number of homozygotes and thus inbreeding as compared to the other populations. The Sri lankan Tall exhibited 100% polymorphic loci and a low inbreeding coefficient in contrast to the Sri lankan Yellow Dwarf, which despite a high polymorphic loci exhibited a high inbreeding coefficient (0.56) (Table 1). This is because the Sri Lankan dwarf exhibits more homozygotes in all the loci, whereas in comparison the SLT shows more heterozygotes. Similarly, the ART and GT also show more number of homozygotes despite having a polymorphism in all the loci studied. In case of other dwarfs such as COD, PRT and POD, there is complete absence of heterozygotes and hence the

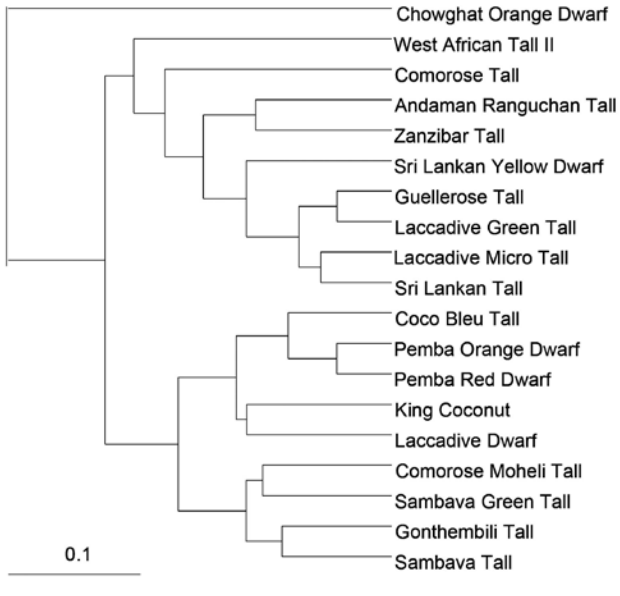

Fig. 1. UPGMA dendrogram showing the relationship between the 19 Indian Ocean coconut accessions.

Table 3. Pair-wise population Fst values of 19 coconut germplasm accessions.

ST	SGT	POD	PRT	GT	CT	CTT	CBD	WAT	KCD	LD	SLYD	LMT	LGT	GLT	ART	ZBT	COD	SLT	
0.00																			ST
0.17	0.00																		SGT
0.34	0.30	0.00																	POD
0.40	0.35	0.29	0.00																PRT
0.11	0.15	0.27	0.30	0.00															GT
0.13	0.15	0.34	0.38	0.22	0.00														CT
0.22	0.28	0.45	0.50	0.38	0.21	0.00													CTT
0.20	0.15	0.15	0.26	0.19	0.18	0.26	0.00												CBD
0.35	0.23	0.47	0.56	0.30	0.34	0.41	0.29	0.00											WAT
0.36	0.23	0.46	0.54	0.28	0.32	0.48	0.20	0.45	0.00										KCD
0.32	0.25	0.29	0.35	0.23	0.24	0.39	0.16	0.30	0.30	0.00									LD
0.15	0.19	0.37	0.42	0.25	0.15	0.18	0.25	0.33	0.41	0.31	0.00								SLYD
0.31	0.27	0.54	0.61	0.37	0.29	0.33	0.43	0.41	0.58	0.47	0.18	0.00							LMT
0.23	0.20	0.42	0.47	0.29	0.17	0.19	0.29	0.30	0.44	0.34	0.12	0.09	0.00						LGT
0.15	0.14	0.31	0.35	0.20	0.13	0.18	0.21	0.21	0.31	0.23	0.06	0.09	0.06	0.00					GLT
0.14	0.18	0.34	0.39	0.25	0.17	0.22	0.22	0.27	0.31	0.34	0.14	0.29	0.21	0.12	0.00				ART
0.16	0.27	0.44	0.49	0.32	0.16	0.19	0.29	0.37	0.43	0.34	0.12	0.24	0.16	0.10	0.12	0.00			ZBT
0.55	0.54	0.74	0.84	0.49	0.59	0.66	0.50	0.69	0.77	0.65	0.58	0.78	0.70	0.57	0.51	0.65	0.00		COD
0.16	0.15	0.30	0.36	0.19	0.16	0.19	0.22	0.24	0.33	0.27	0.10	0.07	0.08	0.04	0.17	0.15	0.59	0.00	SLT

Table 4. Pair-wise population matrix of Nei's Genetic Distance (1972) of 19 coconut germplasm accessions.

ST	SGT	POD	PRT	GT	CT	CTT	CBD	WAT	KCD	LD	SLYD	LMT	LGT	GLT	ART	ZBT	COD	SLT	
0.00																			ST
0.32	0.00																		SGT
0.59	0.41	0.00																	POD
0.56	0.36	0.09	0.00																PRT
0.22	0.24	0.38	0.30	0.00															GT
0.23	0.25	0.49	0.39	0.41	0.00														CT
0.38	0.48	0.72	0.71	0.91	0.35	0.00													CTT
0.38	0.23	0.13	0.18	0.32	0.32	0.42	0.00												CBD
0.77	0.34	0.55	0.55	0.56	0.56	0.66	0.46	0.00											WAT
0.60	0.24	0.40	0.29	0.35	0.39	0.76	0.17	0.45	0.00										KCD
0.62	0.36	0.30	0.20	0.33	0.34	0.66	0.20	0.32	0.22	0.00									LD
0.40	0.46	0.88	0.95	0.76	0.32	0.32	0.66	0.90	1.03	0.72	0.00								SLYD
0.58	0.43	0.94	0.89	0.71	0.46	0.43	0.89	0.52	0.90	0.72	0.25	0.00							LMT
0.51	0.39	0.85	0.84	0.70	0.34	0.28	0.74	0.51	0.86	0.70	0.18	0.07	0.00						LGT
0.43	0.33	0.81	0.78	0.55	0.30	0.40	0.65	0.40	0.67	0.51	0.16	0.08	0.07	0.00					GLT
0.38	0.40	0.72	0.70	0.73	0.40	0.45	0.53	0.57	0.52	0.83	0.47	0.67	0.51	0.40	0.00				ART
0.33	0.55	0.95	0.88	0.80	0.27	0.29	0.62	0.66	0.70	0.60	0.22	0.36	0.27	0.19	0.24	0.00			ZBT
1.22	0.94	1.03	0.88	0.74	1.18	1.37	0.68	0.99	0.57	0.60	1.45	2.50	2.29	1.69	1.07	1.55	0.00		COD
0.42	0.36	0.62	0.62	0.47	0.39	0.40	0.59	0.46	0.68	0.57	0.26	0.04	0.12	0.10	0.55	0.36	1.61	0.00	SLT

observed gene diversity and inbreeding coefficient is nil. The Coco Belu Dwarf exhibited all the values for tall indicating the possibility of this sample as being an out crossed one.

The accessions COD and PRT showed the maximum population differentiation measure (0.84) as compared to any other talls (0.49-0.78) included in this study. This may indicate that the PRT had an extensive out crossing in the past and selection towards more homozygosity. Moreover, PRD has been classified as belonging to the South pacific group of dwarfs in contrast to COD, which is classified with the South East Asian Dwarfs (Baudouin, and Lebrun, 2). The lowest Fst value (0.04) was observed for the SLT and the GLT followed by Yellow Dwarf and the GLT. The LMT showed low differentiation of 0.07 with SLT and 0.09 with both LGT and GLT. The genetic distance measure was the highest for the coconut populations of the Laccadive Islands (2.29-2.50), followed by the coconut populations of the western Indian Ocean (0.88-1.69) and the Srilanka Tall populations (0.74-1.61). The lone coconut population from the Seychelles showed the least genetic distance measure (0.68) and the Andaman population recorded a higher genetic measure as compared to it. The low genetic distance measure of the Seychelles can be attributed to the complete absence of the human intervention in these Islands till recently and possibly represents the naturally established coconuts that have floated from South East Asia during pre historic times. The Srilankan Tall populations are closer to the Lakshadweep and distantly related to the Andaman and Seychelles coconut population. Moreover, the cluster diagram of the Indian Ocean coconut populations did not reveal any region-wise grouping pattern.

Assessment of genetic diversity through morphological tools alone is insufficient as it is always influenced by environmental interaction. Application of DNA-based markers is complimentary to the morphological markers in identifying the genotypes in genetic resources management. The present study has revealed the interrelationships among the accession in terms of genetic diversity, which will be useful in planning new germplasm explorations for targeted traits for further enriching the gene pool.

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