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REGULAR ARTICLE

Genetic and phylogenetic relationships of coconut populations from Amini and Kadmat Islands, Lakshadweep (India)

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Abstract

A great deal of variability exists in coconut populations of the Lakshadweep group of islands in India, which is regarded as one of the likely centre's for coconut domestication. It is possible to gain insights into the evolution of coconut populations in these islands by undertaking detailed studies of these populations. In this study, the variability and phylogenetic relationships within populations of Laccadive Ordinary Tall (LCT) and Laccadive Micro Tall (LMT) from Amini and Kadmat Islands were studied using microsatellite markers. LCT collections from these two islands were earlier grouped, according to fruit shape, into three type's viz., elongated, round or oval shaped and pear shaped ones. Three accessions of LMT, one each from Amini and Kadmat, and a variant of LMT from Kadmat Island, characterized by the round shaped fruit and nut, were also used for the studies. Seedlings raised from these types were analysed using 20 highly polymorphic SSR markers. Elliptical type from Amini, which are described as 'Niu Kafa' type, emerged as distinct from other populations and was related to round or 'Niu Vai' type from Amini. Pear shaped type from both the islands, which was considered as the introgressed form, showed affinity and appear to have developed as a result of introgression between elliptical and round types. When the LMT palms were analyzed, the LMT palms from Amini and Kadmat clustered together, while the round variant of LMT from Kadmat Island was found to be distinct. A large extent of variations were also found among individual palms of these distinct types when analyzed using SSR markers, highlighting the importance of selection of LCT mother palms for hybrid seed production.

Key words: Lakshadweep islands, Coconut, Diversity, Microsatellites

Introduction

Coconut (*Cocos nucifera* L.) is an important palm grown in more than 93 countries in the tropical world providing livelihood to millions of people, besides sustaining the fragile island ecosystem in these areas. The palm provides food, fuel, fiber, shelter and tender nut water for human kind; additionally it supports numerous coconut-based industries.

Lakshadweep islands lie about 220 to 440 km off the Kerala State of the Indian main land, between 8° and 12° North Latitudes and 71° and 74° East Longitudes. The archipelago comprises of 36 main islands, many smaller islands, coral atolls and coral reefs. Out of 36 islands, only 10 islands are inhabited. Coconut constitutes the only economically important crop in Lakshadweep Islands and diverse forms of coconut have been reported from these islands. Wide variability and diversity has been reported earlier from coconut populations in these islands using morphological and molecular analysis (Krishnamoorthy and Jacob, 1982; Jacob, 1993; Samsudeen et al., 2006; Devakumar et al., 2010). Gunn et al. (2011) provided evidences suggesting that the region around the southern margins of the Indian sub-continent, comprising of Lakshadweep Islands, Sri Lanka and Maldives, represented a possible center of coconut domestication.

Traditionally, on the basis of fruit component analysis, coconuts have been classified as '*Niu kafa*' and '*Niu vai*'. The ancestral '*Niu kafa*' forms are characterized by a higher proportion of husk in nut and a low proportion of endosperm, traits which would have aided in its natural dissemination via oceanic currents to newer regions where they got established. In contrast, the '*Niu vai*' type, characterized by a low proportion of husk and high proportion of water in nut, were possibly selections

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made by humans (Harries, 1978). Coconut types in Lakshadweep islands have been described by earlier workers as mainly belonging to '*Niu kafa'* type (Krishnamoorthy and Jacob, 1982). The occurrence of '*Niu vai*' types and also introgressed forms, which developed as a result of crossing between '*Niu kafa*' and '*Niu vai*' types, have also been reported (Samsudeen et al., 2006). Laccadive Ordinary Tall (LCT) was considered as a form under 'typica' with medium sized nuts, good quality and quantity of copra and an annual yield of 100 nuts/ palm (Narayana and John, 1949). Earlier studies based on fruit component analysis considered LCT as an introgressed form between '*Niu kafa'* and '*Niu vai'* types (Bhaskara Rao and Vasudevan Pillai, 1982). But, a later study of coconut types of Lakshadweep classified Laccadive Micro, Laccadive Small, Laccadive Ordinary and Laccadive Dwarf as '*Niu kafa'* types (Krishnamoorthy and Jacob, 1982).

In an expedition to Amini and Kadmat islands (Samsudeen et al., 2006), significant variations were observed in Laccadive tall collections when grouped according to fruit shape and husk percentage *viz.*, elliptical (70% husk), round (55 % husk) and pear-shaped (58% husk). This exploration indicted that LCT is a mixed population and the earlier described Laccadive Ordinary types belong to the different forms of LCT identified now. These LCT populations were classified into '*Niu kafa'* (elliptical fruit type) type, '*Niu vai'* (round fruit type) and introgressed forms (pear shaped fruit types). The elliptical shaped nuts identified showed striking similarity to nuts from Seychelles Islands suggests that these nuts might have reached Lakshadweep islands from the Indian Ocean islands. During the same expedition, two accessions of LMT with elongated fruits (with 60% husk) were collected, one each from Amini and Kadmat Islands, in addition to round-shaped variant (with less than 49% husk) of LMT from Kadmat Island, similar to the LMT variant described earlier (Jacob, 1993).

Characterizing and deciphering the extent of genetic variations in these coconut populations can play a significant role in utilizing these populations for future coconut breeding programs as it will enable precise selection in these highly heterogeneous populations. Characterizing of coconut diversity, earlier undertaken through morphological traits, has now given way to the use of molecular markers, especially microsatellites, which are preferred because of their co-dominant and multi-allelic nature and their capacity to detect high polymorphism (Rivera et al., 1999; Perera et al., 2000; Rajesh et al., 2008a,b). Utilizing microsatellites, the present study aims to analyze the extent of variability and phylogenetic relationships within populations of Laccadive Ordinary Tall (LCT) and Laccadive Micro Tall (LMT) from two islands of the Lakshadweep archipelago *viz.* Amini and Kadmat. Conservation and evaluation of these diverse types will help in further selection of desirable traits and development of superior populations which can be used for future breeding programs, as Lakshadweep populations have been found to perform better under different agro-climatic zones (CPCRI, 1990).

Material and Methods

Plant materials

Nuts were collected from typical palms of nine accessions from Amini (11° 06' and 11° 08' N latitude and 72° 42' and 72° 45' E longitude, having a land area of 2.60 sq km) and Kadmat islands (11° 10' and 11° 16' N latitude and 72° 45' and 72° 48' E longitude, having a land area of 3.20 sq km). The variability in fruit shapes among LCT and LMT (Figures 1, 2) was reported earlier by Samsudeen et al. (2006). Nuts were transported and planted in the nursery at the International Coconut Gene Bank for South Asia (ICG-SA), Kidu, Karnataka State in the main land. Leaf samples were collected from 56 palms raised from these nuts, representing 5-8 palms per accession, the details of which are given in Table 1.

Table 1. Germplasm accessions from Amini and Kadmat Islands used in the present study

Sl. No.	Description	Place of collection	No. of samples	Expected Heterozygosity (He)	Observed Heterozygosity (Ho)	Fixation Index (f)
1.	LCT Elliptical	Amini	7	0.59	0.55	0.08
2.	LCT Pear	Amini	7	0.56	0.49	0.14
3.	LCT Round	Amini	6	0.57	0.51	0.11
4.	LCT Elliptical	Kadmat	7	0.60	0.56	0.06
5.	LCT Pear	Kadmat	6	0.58	0.45	0.08
6.	LCT Round	Kadmat	8	0.48	0.49	0.18
7.	LMT Elliptical	Amini	5	0.53	0.36	0.34
8.	LMT Elliptical	Kadmat	5	0.59	0.40	0.35
9.	LMT Round	Kadmat	5	0.54	0.37	0.34
	Mean			0.56	0.46	0.19

Figure 1(a). LCT nut types- Pear shaped, elliptical and round (from left to right).
(b). Longitudinal section of LCT nuts- elliptical, pear shaped and round (from left to right).

DNA extraction

Total DNA was extracted from the leaf samples of using a modified SDS method (Rajesh et al., 2013). About 1 g of fresh young leaves were crushed in liquid nitrogen and added to a tube containing 10 ml pre-heated extraction buffer (100 mM Tris- HCl, 50 mM NaCl; pH 8.0). To the above contents, 1 ml of 10 % SDS and 50 µl of β-mercaptoethanol were added. After incubation at 65 °C for one hour with intermittent mixing, an equal volume of chloroform: isoamyl alcohol mixture (24:1 V/V) was added and the contents homogenized by gentle inversion for 20 min and centrifuged at 10,000 rpm for 20 minutes at 4°C. The clear aqueous phase containing the nucleic acid was transferred to a fresh tube. Approximately 2/3rd volume ice-cold isopropanol was added to precipitate the DNA. After incubation at 4°C for 30 minutes, DNA spool was collected in Eppendorf tubes. The DNA was washed thrice with 70% alcohol. After discarding the article, the DNA pellet was air-dried completely and DNA pellet dissolved in 0.5 ml TE buffer (10 mM Tris-HCl, pH 8.0 and 1 mM EDTA). RNase (4 µl) was added to the tube and incubated at 37°C for 1 h. After RNase treatment, the supernatant containing the DNA was extracted with equal volume of chloroform: isoamyl alcohol (24:1) twice and the purified DNA was precipitated by adding double the volume of ethanol. The precipitated DNA was air- dried and dissolved in 0.75 ml TE buffer (10 mM Tris-HCl, pH 8.0 and 1 mM EDTA). Estimation of the quality and quantity of the extracted DNA was carried out using agarose gel electrophoresis (0.8%) and also using a spectrophotometer (A_{260}/A_{280} ratio). The isolated DNA was then diluted in TE buffer (10 mM Tris-HCl, pH 8.0 and 1 mM EDTA) for further analysis.

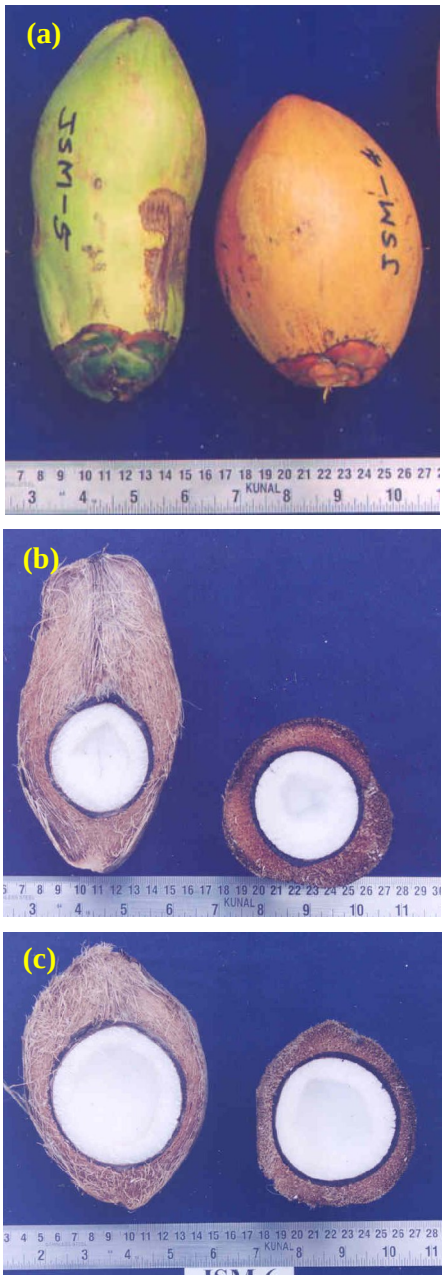

Figure 2(a). LMT nut types- Elliptical and round (from left to right)
(b). Longitudinal section of LCT and LMT nuts- elliptical type
(c). Longitudinal section of LCT and LMT nuts- round type

SSR analysis

A set of 20 hyperpolymorphic coconut SSR markers (Table 2), distributed in different coconut chromosomes, were used for the analysis. PCR reactions were conducted in volumes of 20 µl containing 35 ng genomic DNA, 0.2 µM each of forward and reverse primers, 50 µM of each dNTPs (M/s Bangalore Genei Pvt. Ltd., Bangalore), 1X buffer (10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$) and 0.3 Unit of *Taq* DNA polymerase (M/s Bangalore Genei Pvt. Ltd., Bangalore). PCR amplifications were performed on a Eppendorf gradient thermal cycler with a PCR profile of 94ºC for 5 min followed by 30 cycles of 1 min at 94ºC, 2 min at the different annealing temperatures standardized for the individual SSR locus, and 2 min at 72ºC with a final extension for 5 min at 72ºC. After amplification, a volume of 8 µl of loading buffer (98 per cent formamide, 10 mM EDTA, 0.005 per cent each of xylene cyanol and bromophenol blue as tracking dyes) was added to each of the amplified product and denatured at 94ºC for 5 min, snap cooled using ice and separated on 5 per cent denaturing polyacrylamide gels containing 7 M urea at a constant power of 100 W. The patterns of amplified products across the samples were resolved by silver staining.

Analysis of genetic diversity

The alleles were scored individually based on comparison with the molecular ladder. Observed number of alleles, effective number of alleles, Shannon's Information Index and F-Statistics were worked out for the 20 microsatellite loci using the software POPGENE (Yeh et al., 1999). We also estimated the degree of population structure over all loci available for each species with Wright's fixation indices (F_{ST}, F_{IT} and F_{IS}) (Wright, 1951). The expected and observed heterozygosity and the fixation index across the four coconut populations were worked out using the software GDA (Lewis and Zaykin, 2002). A cluster analysis was performed on the similarity matrix using the unweighted pair group method with arithmetic averages (UPGMA) and the resultant phenogram was constructed. We tested data sets for deviations from Hardy–Weinberg equilibrium (HWE) in GENEPOP v4.0 (Raymond and Rousset, 1995), using a Markov chain approximation to exact tests and likelihood-ratio tests, respectively.

Analysis of molecular variance

In order to estimate the variance between the groups of populations, pooled sample structuring was estimated using analysis of molecular variance (AMOVA) and 20,000 permutations implemented in Arlequin v 3.5.1.2 (Excoffier et al., 1992).

Table 2. Details of microsatellite loci, total number of alleles, number of effective alleles (N_e), Shannon's Information Index (I), Polymorphic Information Content (PIC), Wright's (1953) fixation indices (F_{ST}, F_{IT} and F_{IS}).

Sl. No.	Microsatellite loci	Total number of alleles	Number of effective alleles (N_e)	Shannon's Information Index (I)	Polymorphic Information Content (PIC)	F_{IS}	F_{IT}	F_{ST}	N_m
1.	CAC2	6	4.96	1.69	0.80	-0.10	0.14	0.15	1.38
2.	CNZ4	6	2.98	1.41	0.66	0.15	0.27	0.13	1.63
3.	CN1H2	6	3.59	1.45	0.72	0.18	0.30	0.15	0.36
4.	CnCirE4	7	4.85	1.70	0.79	-0.25	-0.07	0.15	0.47
5.	CnCirE1	3	1.72	0.75	0.42	0.19	0.26	0.09	2.47
6.	CNZ13	4	2.64	1.10	0.62	0.19	0.38	0.23	0.83
7.	CAC72	4	2.42	0.98	0.59	0.44	0.62	0.31	0.55
8.	CNZ17	4	1.56	0.70	0.36	0.16	0.27	0.13	0.60
9.	CnCir2	5	1.54	0.72	0.35	-0.12	-0.03	0.08	2.84
10.	CN1C6	3	1.33	0.49	0.25	-0.26	-0.12	0.11	2.02
11.	CAC71	3	2.95	1.09	0.66	-0.07	0.02	0.09	2.64
12.	CNZ31	5	4.06	1.50	0.75	0.13	0.24	0.12	1.74
13.	CnCirG4	3	1.14	0.28	0.12	-0.21	-0.05	0.13	1.62
14.	CnCir87	4	1.69	0.79	0.41	-0.21	-0.09	0.09	2.46
15.	CAC77	2	1.99	0.69	0.50	0.60	0.64	0.10	2.12
16.	CNZ26	3	2.86	1.07	0.65	0.24	0.32	0.10	2.23
17.	CNZ5	5	4.33	1.51	0.77	0.07	0.17	0.11	2.02
18.	CNZ10	4	3.80	1.36	0.74	0.28	0.39	0.15	1.43
19.	CnCirG11	8	5.81	1.85	0.83	0.16	0.25	0.10	2.21
20.	CnCirE11	7	5.84	1.83	0.83	-0.14	0.01	0.13	1.70
		Mean	3.10	1.15	0.59	0.09	0.21	0.14	1.58

Results and Discussion

Allele richness of SSR loci

The results of PCR amplification of SSR loci are summarized in Table 2. All the 20 SSR loci surveyed displayed polymorphism and a total of 92 alleles were detected among the nine accessions with a mean of 4.6 alleles per locus. The effective number of alleles per locus (N_e) ranged from 1.14 (CnCirG4) to 5.84 (CnCir E11) with a mean of 3.10. Shannon's Information Index ranged from 0.28 (CnCirG4) to 1.85 (CnCir E11) with a mean of 1.15. The PIC value, a measure of marker diversity, varied from 0.12 (CnCirG4) to 0.83 (CnCirE11 and CnCirG11) among the 20 microsatellite loci, the average being 0.59. Loci with a higher PIC value can be used to discriminate these accessions in future studies.

F_{IS} for 12 of the loci was greater than zero. Mean F_{IS} (0.09) and F_{IT} (0.21) were both positive and greater than zero indicating a heterozygote deficit within populations. The mean gene flow (N_m), based on mean F_{ST} (0.12), was 1.58 indicating a moderate gene flow among the coconut accessions. The absence of a strong genetic differentiation observed among these coconut populations could be attributed to factors like moderate gene flow (by wind/insect pollination within a specific area) and population history (recent colonization). Another factor could be adaptation to local conditions, which may result in the generation and maintenance of distinct populations.

Results of the likelihood ratio test for Hardy-Weinberg (HW) equilibrium across loci, considering heterozygote deficit as the alternative hypothesis, showed that 11 of the loci had significant ($p<0.001$) departures from HW proportions. The decreased level of heterozygosity and departures from HW proportions could be attributed to mating between closely related individuals within a specific, relatively small geographic area. According to Frankham (1997), a lower genetic diversity is generally observed in island populations of plants compared to main land populations. Some factors affecting the levels of genetic diversity and the pattern of the population's structure may be low recruitment of seed nuts and the population's colonization history under an island ecosystem.

Genetic diversity within populations

The fixation index ranged from 0.06 (LCT Elliptical Kadmat) to 0.35(LMT Kadmat) with a mean of 0.319, indicating highly variable levels of inbreeding in these populations (Table 3). Expected

heterozygosity ranged from 0.58 (LCT Round Kadmat) to 0.60 (LCT Elliptical Kadmat). The observed heterozygosity for all the accessions was less than expected indicating a tendency towards inbreeding within the population. High genetic diversity has been reported earlier in LCT populations using isozyme analysis (Parthasarathy et al., 2004) and microsatellite analysis (Devakumar et al., 2006; Devakumar et al., 2010).

Table 3. Analysis of molecular variance (AMOVA) partitioning genetic variability within and among populations.

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among populations	8	57.206	0.26622Va	6.28
Within populations	99	393.359	3.97332Vb	93.72
Total	107	450.565	4.23954	

When the LCT accessions were considered, elliptical type from Amini Islands, which are described as '*Niu Kafa'* type (presumed ancestral), emerged as distinct from other populations and was related to round or '*Niu Vai*' type (selected by humans) from Amini, as revealed by dendrogram generated from SSR data (Figure 3). This supports the earlier hypothesis that round type nuts evolved from ancestral elliptical types by human selection (Harries, 1978). Pear shaped type from both the islands, which was considered as the introgressed form, showed affinity and appear to have developed as a result of introgression between elliptical and round types. Affinity between seedlings of elliptical and round types from Kadmat Island, as revealed in this study, might be the result of the inter-crossing of different types in the island. Indian Ocean Island's might have first established in Amini and then transported to Kadmat, possibly by ocean currents and human dispersal. According to historical records, Amini was the one of the first inhabited island from where people migrated to other islands (Ellis, 1924; Forbes, 1979; Samsudeen et al., 2006).

When the LMT palms were analyzed, the LMT palms from Amini and Kadmat clustered together, while the round variant of LMT from Kadmat Island was found to be distinct (Figure 4). From the similarity matrix, it could be deduced that the LMT round variant from Kadmat Island showed more identity to LMT from Kadmat (0.87) compared to LMT from Amini (0.85), indicating that the round-type nuts could be a selection from the normal type.

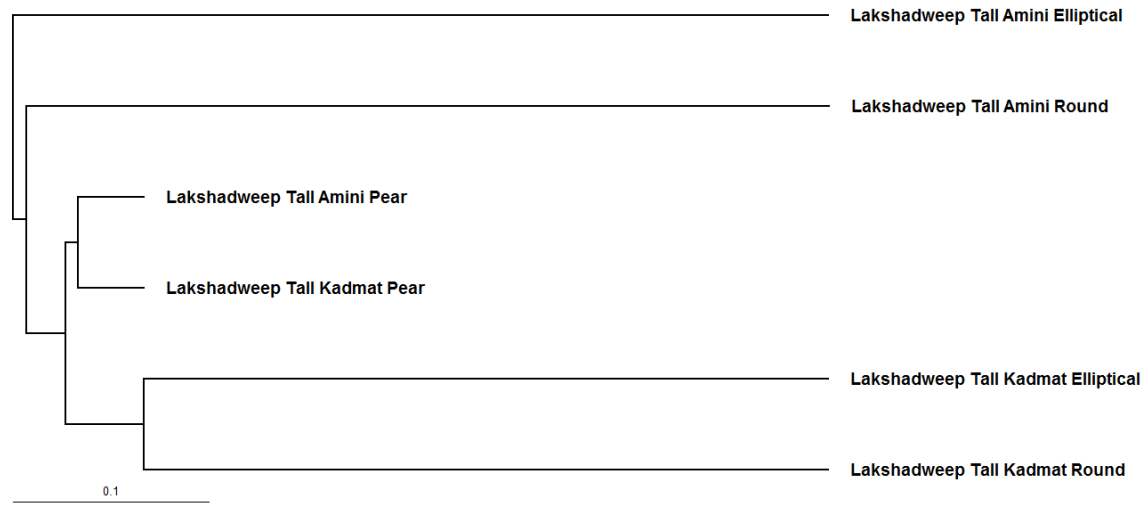

Figure 3. Genetic similarity between six Laccadive Ordinary Tall accessions from Amini and Kadmat Islands based on SSR data. Dendrogram was produced using UPGMA clustering of pair-wise similarities between the accessions.

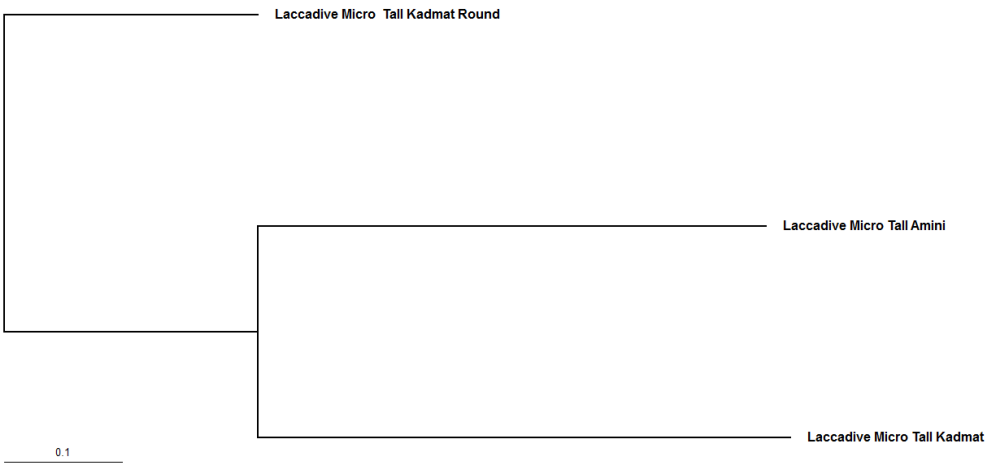

Figure 4. Genetic similarity between six Laccadive Micro Tall accessions from Amini and Kadmat Islands based on SSR data. Dendrogram was produced using UPGMA clustering of pair-wise similarities between the accessions.

Population structure

The locus by locus AMOVA analysis, performed considering among populations and within populations as sources of variation, is given in Table 3. The highest percentage of variation (93.72%) correspond to the within population component, while the among population component showed low magnitude (6.28%). This is expected in coconut, which is a perennial palm with an outcrossing breeding system (Perera et al., 1998; Rajesh et al., 2008a,b; Devakumar et al., 2010). Knowledge of how genetic diversity is apportioned within and among populations could be useful in formulating strategies for conservation of genetic diversity of the species under study.

Genetic variability within plant populations depends on a number of factors including the breeding system of the species and the species distribution area (Bartish et al., 1999). The large extent of variation detected within individual palms of these distinct accessions, when analyzed using SSR markers, has important inferences with respect to breeding for higher yield in coconut, specifically on selection of LCT mother palms for hybrid seed production. LCT has been earlier identified as a best general combiner in a study involving nine coconut cultivars (Nampoothiri et al., 1999) and two T x D (tall x dwarf) hybrids hybrids *viz*., 'Lakshaganga' (LCT x GBGD) and 'Chandralaksha' (LCT x COD) have been released for commercial cultivation In India (CPCRI, 1990). Selection of mother palms, therefore, would be highly crucial since the wide variability detected using microsatellite markers would mean that all coconut hybrids, derived using LCT as one of the parents, may not perform uniformly.

To conclude, the results of this study reveal the existence of different population structure of coconut palms in Amini and Kadmat Islands. From the dendrogram, it is evident that coconut accessions from Kadmat Islands were more advanced compared to Amini Islands. This is confirmatory to the earlier observations, based on fruit component analysis, of lower proportion of husk in the coconut accessions from Kadmat Islands, indicating an evolution from the ancestral '*Niu kafa*' types with higher proportion of husk. In Amini, frequency of elliptical types was more, whereas in Kadmat both elliptical and round types were in equal proportion. Between the islands, proportion of round type was more in Kadmat. It is possible that coconut coming from Indian Ocean Island's first established in Amini and seed nuts from selected palms were carried, by humans, to Kadmat from Amini to establish coconut population there. Further, human intervention could have resulted in the conversion of coconut populations into round types (Samsudeen et al., 2006). This supports the earlier hypothesis that round type nuts evolved from ancestral elliptical types by human selection. Affinity between palms of elliptical and round types from Kadmat Island, as revealed in this study, might be the result of the inter-crossing of different types in the island.

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