Microsatellite variability of coconut accessions (*Cocos nucifera* L.) from Andaman and Nicobar Islands

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The extent of genetic diversity in 26 coconut accessions from the Andaman and Nicobar (A&N) Islands was determined using 14 microsatellite markers. A total of 103 alleles were detected by the microsatellite markers with an average of 7.35 alleles per locus. The average observed and expected heterozygosity was 0.29 and 0.66 respectively. A mean fixation index (F_{ST}) of 0.49 was observed, indicating a high level of population differentiation among the coconut accessions. Heterozygosity was highest in tall coconut accessions. Majority of rare alleles were observed in tall accessions from the Nicobar Islands. The UPGMA dendrogram revealed clustering of majority of tall and dwarf accessions separately. This study using microsatellites confirms the rich genetic diversity of coconut accessions from A&N Islands.

Keywords: Alleles, coconut accessions, genetic diversity, microsatellite loci.

COCONUT is an important subsistence crop of the humid tropical zones and is a life-supporting species in fragile island and coastal ecosystems. Coconut is believed to have originated in the Indo-Malayan region (Indonesia, Malaysia and the Philippines) from where it was dispersed, mainly via oceanic currents, to sandy and coralline tropical coasts where it got established. Based on the morphological diversity of the coconut fruit, a diversification model has been proposed[1]. The wild-type coconut (*Niu kafa*-type), because of natural selection for improved floating ability, has long, angular fruit with thick husk and with little liquid endosperm. On the other hand, the domesticated coconut (*Niu vai*-type) has high content of liquid endosperm with low husk, which resulted because of human selection during the domestication process. Populations that display intermediate fruit characteristics probably resulted from introgression between the original wild-type coconut populations and the introduced domesticated coconut populations[1].

The archipelago of the Andaman and Nicobar (A&N) Islands stretches over 800 km in the Bay of Bengal, approximately 1220 km southeast of the coast of West Bengal and 1190 km east of Chennai. It comprises 572 islands, reefs and rocks, of which 38 islands are inhabited. A deep Ten Degree Channel, a wide gap of 155 km with heavy tidal flows, separates the A&N group of islands. These islands have a luxuriant evergreen tropical rainforest canopy. About 2100 varieties of plants have been recorded from these islands, out of which 11% is endemic and 1300 do not occur in mainland India[2]. A major part of the island flora is either of the Indo-Myanmarese–Thailand order or the Malaysian–Indonesian order[2].

Germplasm plays a key role in coconut breeding programmes, since it is a source of variability enabling a search for characters of adaptation to soil and climatic conditions as well as pests and diseases[3]. The diversity of coconut in the A&N Islands is exceptional[4]. Coconut accessions having horned fruits, beaked fruits, palms with persistent petiole and inflorescence, fused leaflets (plicata) and unbranched inflorescence (spicata) are found in these islands. These rare types may be potential sources of resistance to pests and diseases, and may prove invaluable in future coconut breeding programmes. Andaman Ordinary Tall, an accession from South Andaman, recorded low incidence of root (wilt) disease[5], *Pestalotia* leaf spot and is also found to possess relative drought tolerance[6]. Andaman Giant Tall, a large-fruited accession again from South Andaman region, is tolerant to burrowing nematodes[7].

Assessment of diversity using morphological tools alone is less precise, as it is influenced by both genetic and environmental factors. DNA-based markers possess the potential to significantly increase the efficiency of coconut genetic improvement programmes, especially in the areas of germplasm management, genotype identification and marker-assisted selection of economically important traits[8]. Although a wide range of morphometric variability exists in coconut populations native to the A&N Islands[9], no extensive study has been undertaken on harnessing molecular markers to assess the diversity of these populations. With the above background, the present study was undertaken to assess the genetic diversity among the coconut accessions collected from the A&N Islands using microsatellite markers.

One hundred palms representing 26 coconut accessions (3–4 palms per accession) native to these islands were subjected to diversity analysis. Details of the 26 accessions (23 tall and three dwarf accessions), the island of collection and the number of palms sampled are given in Table 1. These palms are being maintained in the International Coconut Gene-Bank for South Asia, Central Plantation Crops Research Institute (Regional Station), Kidu, Karnataka, India. DNA was extracted from spindle leaves of the palms using the standardized protocol[10].

A total of 14 highly polymorphic SSR primer pairs from the coconut microsatellite kit[11] were used in the present study. PCR reaction was conducted in volumes of 20 μl containing 35 ng genomic DNA, 0.2 μM each of

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forward and reverse primers, 50 μM of each dNTPs (Bangalore Genei Pvt Ltd, Bangalore), 1× buffer (10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$) and 0.3 units of *Taq* DNA polymerase (Bangalore Genei Pvt Ltd). PCR amplifications were performed on an Eppendorf gradient thermal cycler with a PCR profile of 94°C for 5 min, followed by 30 cycles of 1 min at 94°C, 2 min at the different annealing temperatures standardized for the individual SSR locus, and 2 min at 72°C with a final extension for 5 min at 72°C.

After amplification, a volume of 8 μl of loading buffer (98% formamide, 10 mM EDTA, 0.005% each of xylene cyanol and bromophenol blue as tracking dyes) was added to each of the amplified products and denatured at 94°C for 5 min, snap-cooled using ice and separated on 5% denaturing polyacrylamide gel containing 7 M urea at a constant power of 100 W. The patterns of amplified products across the samples were resolved by silver staining following the procedure of Panaud *et al.*[12].

The alleles were scored individually based on comparison with the molecular ladder and also the control samples (West African Tall and Malayan Yellow Dwarf). The observed number of alleles, the expected and observed heterozygosity and *F*-statistics[13] were worked out for the 14 microsatellite loci using the software[14] POPGENE version 1.31. The expected and observed heterozygosity, the inbreeding coefficient (F_{IS}) and the presence of rare alleles for the 26 coconut accessions were worked out using the software GDA[15]. A cluster analysis was performed on the similarity matrix using the unweighted pair group method with arithmetic averages (UPGMA) and the resultant phenogram constructed.

Table 1. Coconut populations used in the study, island of collection and number of palms sampled

Accession	Place of collection	No. of palms sampled
Nicobar Auck Chung Tall	Nicobar Islands	4
Andaman Giant Tall	South Andaman	3
Andaman Ranguchan Tall	South Andaman	4
Andaman Ordinary Tall	South Andaman	4
Andaman Yellow Dwarf	South Andaman	4
Andaman Yellow Tall	Champin Island	4
Horned Cocos Tall	South Andaman	4
Katchal Micro tall	Katchal Island	4
Perka Green Tall	Car Nicobar	4
Beachdera Ordinary Tall	Katchal, Car Nicobar	4
Katchal Oval Tall	Katchal, Car Nicobar	4
Dugong Creek Tall	Little Andaman	4
Nicobar Orange Dwarf	Car Nicobar	4
South Bay Tall I	Little Andaman	4
South Bay Tall II	Little Andaman	4
Trinket Papaya Tall	Trinket Island	3
Burmanella Brown Tall	South Andaman	4
Harminder Bay Giant Tall	Little Andaman	4
Carbin Brown Tall	South Andaman	4
Burmanella Giant Tall	South Andaman	4
Nicobar Micro Tall	Car Nicobar	4
Katchal Green Dwarf	Trinket Island	4
Nicobar Tall	Car Nicobar	4
Trinket Tall	Trinket Island	3
Kakana Long Tall	Katchal, Car Nicobar	3
Rounded Point Tall	Kamorta Island	4

A total of 103 alleles were detected by the 14 microsatellites with an average of 7.35 alleles per SSR locus (Table 2). The number of alleles observed at each locus in the dataset ranged from four (loci CnCir E12, CnCir A9, CnCir H4b and CnCir C12) to 17 (locus CnCir E2). Such a high number of alleles detected within a small geographic region further supports the high genetic diversity of coconut available in these islands. In earlier studies, 2–16 alleles were reported when coconut SSRs were tested on a global range of coconut germplasm[16,17].

The expected heterozygosity varied from 0.41 (CnCir A9) to 0.90 (CnCir E2), the average being 0.66 (Table 2). The observed heterozygosity ranged from 0.11 (CnCir H7) to 0.59 (CnCir C3b) with an average of 0.29. For all the accessions the observed heterozygosity was less than the expected heterozygosity, indicating a tendency towards inbreeding within the population.

Genetic structure of the populations was analysed through Wright's *F*-statistics. The F_{ST} varied among the loci from 0.34 (CnCir C3b) to 0.69 (CnCir A3) with a mean of 0.49, indicating a high level of population differentiation among the coconut accessions. While a higher level of population differentiation (F_{ST} = 0.36) was observed in Mexican coconut populations analysed using 15 enzymatic systems[18], a moderate level of population differentiation (F_{ST} = 0.054) was observed in Sri Lankan coconut populations[19] analysed using eight SSR primer pairs. Negative inbreeding coefficient (F_{IS}) was observed for the loci CnCir C3b, CnCir C7, CnCir F2, CnCir G11 and CnCir C12 indicating an excess of heterozygotes detected by these loci. The F_{IT} values ranged from 0.28 in CnCir C7 to 0.84 in CnCir H7. However, mean F_{IS} (0.10) and F_{IT} (0.54) were both positive and greater than zero, indicating a general heterozygote deficit within populations. The mean number of migrants per generation among populations (N_m), based on F_{ST} calculations was 0.27; it was maximum for the locus CnCir C3b (0.49) and minimum for CnCir A3 (0.11) (Table 2). When $N_m > 1$, gene flow is able to offset the differentiation among populations caused by isolation and genetic drift[20]. The low levels of gene flow (0.27) between coconut populations in the A&N Islands and occurrence of populations as isolated stands in different islands suggest that genetic drift may contribute to the high genetic differentiation among the coconut populations. During the early history of coconut evolution, the dispersal of nuts by floatation via oceanic currents probably might have led to the establishment of a few individuals in remote, geographically isolated regions and islands[21]. These populations could have been subjected to genetic restrictions due to the effect of genetic drift, which would explain the low levels of intra-population diversity found in the South Pacific

Table 2. Number of alleles detected, product size, expected and observed heterozygosity, and *F*-statistics for the 14 coconut microsatellite primers

Loci	No. of alleles	Allele sizes (bp)	Expected heterozygosity (H_e)	Observed heterozygosity (H_o)	F_{IS}	F_{IT}	F_{ST}	N_m
CnCir E12	4	162–174	0.42	0.13	0.36	0.69	0.51	0.23
CnCir A9	4	89–103	0.41	0.16	0.02	0.60	0.60	0.17
CnCir B12	13	135–179	0.85	0.45	0.06	0.44	0.40	0.37
CnCir C3b	12	176–210	0.88	0.59	–0.01	0.33	0.34	0.49
CnCir A3	5	228–240	0.59	0.17	0.09	0.72	0.69	0.11
CnCir C7	6	157–167	0.70	0.48	–0.13	0.29	0.37	0.41
CnCir H4b	4	218–232	0.52	0.22	0.24	0.56	0.43	0.33
CnCir E2	17	123–175	0.90	0.40	0.19	0.55	0.45	0.31
CnCir F2	7	193–211	0.55	0.29	–0.18	0.45	0.53	0.22
CnCir H7	6	129–141	0.75	0.11	0.63	0.84	0.57	0.19
CnCir B6	8	196–214	0.79	0.22	0.36	0.73	0.57	0.19
CnCir E10	6	232–246	0.72	0.24	0.28	0.64	0.50	0.24
CnCir G11	7	164–188	0.80	0.51	–0.16	0.33	0.42	0.34
CnCir C12	4	154–174	0.45	0.22	–0.25	0.49	0.59	0.17
Mean	7.35		0.66	0.29	0.10	0.54	0.49	0.27

Table 3. Expected and observed heterozygosity and inbreeding coefficient (F_{IS}) for the 26 coconut populations

Accession	H_e	H_o	F_{IS}
Nicobar Auck Chung Tall	0.48	0.38	0.21
Andaman Giant Tall	0.70	0.76	–0.11
Andaman Ranguchan Tall	0.54	0.49	0.10
Andaman Ordinary Tall	0.47	0.23	0.54
Andaman Yellow Dwarf	0.00	0.00	0.00
Andaman Yellow Tall	0.49	0.37	0.28
Horned Cocos Tall	0.25	0.19	0.25
Katchal Micro tall	0.23	0.19	0.15
Perka Green Tall	0.25	0.16	0.57
Beachdera Ordinary Tall	0.39	0.35	0.12
Katchal Oval Tall	0.30	0.21	0.34
Dugong Creek Tall	0.49	0.37	0.28
Nicobar Orange Dwarf	0.04	0.00	1.00
South Bay Tall I	0.32	0.31	0.04
South Bay Tall II	0.35	0.27	0.39
Trinket Papaya Tall	0.32	0.27	0.35
Burmanella Brown Tall	0.47	0.34	0.30
Harminder Bay Giant Tall	0.43	0.28	0.37
Carbin Brown Tall	0.55	0.49	0.12
Burmanella Giant Tall	0.62	0.50	0.22
Nicobar Micro Tall	0.38	0.30	0.22
Katchal Green Dwarf	0.41	0.25	0.43
Nicobar Tall	0.46	0.26	0.48
Trinket Tall	0.40	0.48	–0.25
Kakana Long Tall	0.45	0.36	0.27
Rounded Point Tall	0.38	0.19	0.54

region[22]. However, the populations could have achieved distinct genetic differentiation as a result of the reproductive isolation imposed. This might account for the high level of inter-population diversity and the fact that the coconut gene pool is mainly fragmented and dispersed in a large number of islands, atolls and remote tropical regions[23].

Among the accessions, the expected heterozygosity was highest in the tall accessions Andaman Giant Tall (0.7) and Burmanella Giant Tall (0.62) (Table 3). Out of the three dwarfs studied two, viz. Andaman Yellow Dwarf and Nicobar Orange Dwarf, exhibited very low diversity. The overall lower genetic diversity in dwarf coconuts when compared to tall ones is related to their breeding habits; tall coconuts are mainly allogamous and dwarf coconuts are mainly autogamous. However, Katchal Green Dwarf exhibited high level of heterozygosity (0.46), more than many talls. High frequencies of heterozygous loci were detected by microsatellites in some of the dwarf cultivars from the Philippines, which suggested the occurrence of outcrossing in the origin of these cultivars[24]. Katchal Green Dwarf formed a distinct accessions based on morphometric traits observed *in situ*[9]. Katchal Green Dwarf seems to have a genetic structure similar to that of Niu Leka Dwarf, an allogamous dwarf from Fiji, which exhibited high degree of heterozygosity when assessed using foliar traits[25], RFLP[26] and SSR loci[16,27]. Negative inbreeding coefficient was noticed only for two accessions, viz. Andaman Giant Tall and Trinket Tall, indicating an excess of heterozygotes (Table 3).

Fourteen rare alleles were detected across the 26 accessions, the details of which are given in Table 4. Interestingly, majority of the rare alleles were found in tall accessions from the Nicobar Islands, which also exhibited high foliar diversity[25].

The dendrogram showing the relationships among the coconut accessions from A&N Islands using microsatellites is given in Figure 1. Two major clusters could be visualized in the dendrogram – one cluster representing all tall accessions with a single dwarf and the other two dwarfs with a single tall. Distinct clustering of tall and dwarf accessions has been earlier reported using various marker systems[17,26,28].

Katchal Green Dwarf was the only dwarf which clustered with tall populations. This accession exhibited a high level of heterozygosity (0.46), like most of the tall coconut populations. Andaman Yellow Tall was the only tall

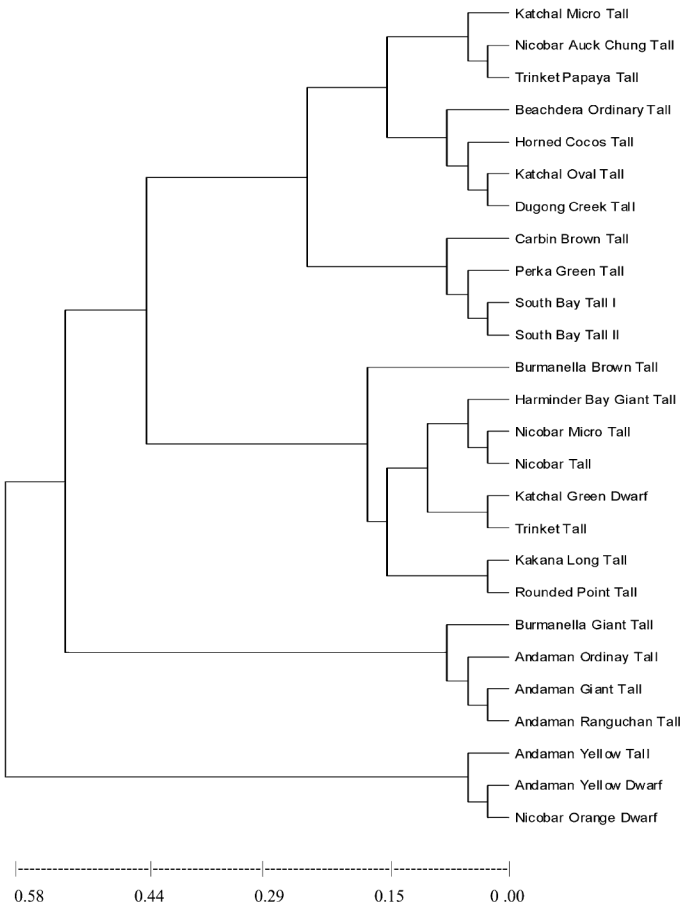

Figure 1. UPGMA dendrogram showing clustering pattern between the 26 coconut accessions from the A&N Islands.

Table 4. Rare alleles detected in coconut populations

Locus	Allele (bp)	Frequency	Found in
E12	162	0.16	Andaman Giant Tall
B12	179	0.25	Burmanella Giant Tall
B12	137	0.08	Nicobar Auck Chung Tall
C3b	190	0.25	Burmanella Brown tall
C3b	200	0.16	Nicobar Auck Chung Tall
C7	163	0.33	Andaman Giant Tall
C7	167	0.08	Nicobar Auck Chung Tall
E2	161	0.08	Nicobar Auck Chung Tall
E2	137	0.08	Nicobar Auck Chung Tall
F2	201	0.37	Dugong Creek Tall
F2	209	0.12	Katchal Oval Tall
B6	210	0.25	Rounded Point Tall
B6	214	0.50	Trinket Tall
G11	186	0.16	Nicobar Auck Chung Tall

population that clustered with two dwarfs, viz. Andaman Yellow Dwarf and Nicobar Orange Dwarf.

The A&N Islands have large coconut stands, which are deposited by oceanic currents and are mostly wild coconut populations. Wild-type coconut populations have been earlier reported from the Philippines[29], Australia[30], Tuvalu[31], Kiribati[32] and Cocos Islands[33]. Presence of the endangered robber crab (*Birgus latro* L.) has been reported from the Nicobar Islands, where wild coconuts are found[34]. Close biological association between coconut diversity and the robber crabs (which are specific on coconuts) has been reported[35]. This region originally belonged to the ancient Gondwanaland from where fossil coconuts were recovered[36,37]. The present-day distribution of the Cococeae tribe suggests an origin in western Gondwanaland[38]. The Nicobar Islands are part of a great island arc created by the collision of the Indo-Australian Plate with Eurasia[39]. The collision lifted the Himalayas and most of the Indonesian Islands, and created a long arc of highlands and islands, which include the Arakan Yoma range of Myanmar, the A&N Islands, and islands off the west coast of Sumatra, including the Banyak Islands and Mentawai Islands. The southernmost point of Nicobar Islands is just 189 km from the Indonesian Island of Sumatra to the southeast, one of the regions considered as the putative centre of origin of coconut. All these may account for the rich diversity of coconut palms in these islands.

Several of the A&N Islands were inundated after the tsunami following the 2004 Indian Ocean earthquake, resulting in the loss of valuable plant species. The present

study using molecular markers confirms that the A&N region represents a large gene pool for coconut, which warrants urgent conservation efforts and formulation of strategies for their evaluation and utilization in breeding programmes for tolerance to biotic/abiotic stress.

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