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Full Length Research Paper

Characterization and diversity assessment in coconut collections of Pacific Ocean Islands and Nicobar Islands

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Characterization and genetic diversity assessment among 30 conserved coconut accessions collected from Pacific Ocean Islands and Nicobar Islands for morphological traits, physico-chemical traits of tender nut water, leaf biochemical parameters and molecular characterization by using random amplified polymorphic DNA (RAPD) markers were undertaken at World Coconut Germplasm Centre, Andaman. Significant variations were observed for most morphological and nut component traits. Out of the 30 accessions characterized, four accessions, namely, *Niu Leka, Hari Papua, Niu Oma* and *Nikkore* were dwarfs among which Niu Leka was identified as a unique dwarf with higher copra content. A dendrogram of genetic relationship obtained through RAPD markers showed distinct variation with 66% in all accessions. The average polymorphic information content (PIC) value of 13 selected primers was 0.29 and maximum and minimum PIC values was 0.46 and 0.17 for primers OPF-19 and OPH-25, respectively. Cluster analysis by UPGMA method grouped the accessions into two major clusters. The results will be useful in the selection among these conserved accessions for specific traits for future utilization in crop improvement initiatives based on their performance for different desirable traits and the level of diversity.

Key words: Coconut, genetic diversity, characterization, RAPD, cluster analysis.

INTRODUCTION

Cocos nucifera L. is one of the important plantation crops of tropical world grown in more than 93 countries and supports the livelihoods of millions of people. In India, it is predominantly cultivated in coastal states and islands. In Andaman and Nicobar islands of India, coconut covers 50% of the cultivable area of islands. Characterization of genetic resources in coconut is a pre-requisite for crop improvement initiatives in coconut as the breeding efforts are cumbersome in coconut owing to its perennial nature, high heterozygosity, long juvenile phase and requirement of large area and longer time duration for any evaluation trials. At present, morphological characters are mostly used for assessment of genetic resources for classification of pooled germplasm and use in further selection. Morphological diversity and geographical distribution of coconut have lead to the identification of more than 1374 assumed diverse coconut varieties/ populations (Coconut Genetic Resources Database Ver. 6/COGENT/IPGRI). Morphological traits, mainly fruit component (Foale, 1987), allozymes (Geethalakshmi et al., 2004) and polyphenols (Jay et al. 1989; Chempakam and Ratnambal, 1993) have also been used to evaluate genetic diversity in coconut from various regions. However, morphological traits and biochemical markers have the disadvantage of being influenced by environ-

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ment and are considered limited in number. In coconut, copra yield of the palm is the most important trait which is decided not only by the nut yield but also the nut characteristics. A wide morphological and physiological variation of this species has been described at both world-wide and regional levels (Harries, 1978; Ashburner et al., 1997a; Ovasuru, 1994; Zizumbo-Villarreal and Piñero, 1998). Evaluation of phenotypic traits is direct, cost effective and easy to observe but it also may be influenced by environmental factors and other factors (Perera et al., 2003). It is important to evaluate these materials by using DNA based molecular markers to provide complementary information.

Uses of molecular markers have a number of advantages over the morphological measurements for the assessment of genetic diversity. Molecular markers are powerful tool to study the evolution of the genome, and for understanding of genome structure and determinants of genetic diversity (Maghuly et al., 2004; Martínez-Gómez et al., 2005). Advances in molecular markers such as restriction fragment length polymorphism (RFLP) (Lebrun et al., 1998), RAPD (Ashburner et al., 1997b; Upadhyay et al. 2004; Ritto et al., 2008), amplified fragment length polymorphism (AFLP) (Teulat et al., 2000) and single sequence repeat (SSR) (Perera et al., 2003; Meerow et al., 2003; Rajesh et al., 2008) were used to analyze genetic diversity in coconuts. Among these markers, RAPD technique is a simple and effective tool for identifying differences and estimating diversity in different biological systems as they are abundant in genome, independent to stage and environment and lack requirement of DNA sequence information. Though wide variability and diversity for many traits have been reported in coconut, characterization of individual accessions at different localities is important to utilize them for further improvement programmes as the independently maintained populations at various localities are to be tested before going in for germplasm multiplication and utilization. A stable estimation of the genetic relationships between varieties and populations of coconut and the perfect estimation of the genetic diversity present in coconut populations are requirements for maintaining future coconut breeding and genetic resources conservation. Therefore, the present study was undertaken with the aim to characterize morphological, biochemical and nut component traits and to assess the diversity among 24 exotic and six indigenous in coconut accessions conserved at World Coconut Germplasm Centre (WCGC) in Andaman and Nicobar Islands which were planted in 1986 in order to explore the possibility of utilizing these genotypes for further conservation, multiplication and in future breeding programmes.

MATERIALS AND METHODS

Thirty (30) coconut accessions (24 Pacific Ocean collections and six accessions from Nicobar group of Islands, India) were characterized and used for this study (Table 1). These accessions were planted during 1986 at World Coconut Germplasm Centre, Sipighat farm, South Andaman (under the erstwhile Centre of Central Plantation Crops Research Institute, Kasaragod) presently under Central Agricultural Research Institute, Port Blair, India. The palms are the products of direct introductions brought from the respective place of origin and the observations were made from selected ten typical palms of each of the accessions.

Morphological and biochemical characterization

Observations on morphological, fruit component and yield traits were recorded as per the coconut descriptors (IPGRI, 1995). The total polyphenol content was analysed by Folin-Ciocalteau reagent method (10%, v/v) with some modifications; gallic acid was used as reference standard and results were expressed as mg of gallic acid equivalent (mg/100g sample) in fresh weight (FW). Leaf protein was determined as per the standard procedures (Sadasivam and Manickam, 1996).

The tender nuts of seven to eight months old were collected from different accessions and cleaned thoroughly with water. The young nuts were trimmed off at the centre. The nut water was collected and filtered through muslin cloth and the quantity was measured, stored and used for subsequent analyses. The biochemical parameters of tender nut water such as reducing sugars (Miller, 1972), total sugars (Hedge and Hofreiter, 1962), amino acid (Sadasivam and Manickam, 1996), potassium, sodium, TSS and organoleptic score were estimated with standard procedures. Organoleptic tests were conducted using method given by IPGRI (1995). The non-reducing sugar was estimated using the formulae (Total Sugar (mg) = Reducing sugar (mg) – Non reducing sugar (mg)) given by Moneruzzaman et al. (2008). Sodium and potassium levels were measured with the help of flame photometer (ESICO).

DNA extraction

The genomic DNA isolation was done by using the protocol of Upadhyay et al. (1999), with slight modifications. 3 g of leaf material was frozen in liquid nitrogen and ground to powder with the help of pestle and mortar. 0.25 g of polyvinyl(poly)pyrolidone was added to the ground powder, mixed well and transferred to a centrifuge tube containing 15 mL of extraction buffer (1M Tris (pH 8.0), 0.5 M EDTA, 5 M NaCl, 10% SDS (sodium dodecyl sulphate) and 20 µl of 0.2 M β-mercaptoethanol. The mixture was incubated at 65°C for 1 h with intermittent mixing. To this, same volume of chloroform: isoamyl-alcohol (24:1) were added and homogenized by inverting the tubes gently. The mixture was centrifuged at 8000 rpm for 15 min. Later the supernatant was transferred into equal volume of ice-cold isopropanol. The DNA spool was collected in 2 mL micro tube and washed with 70% ethanol, air-dried and resuspended in 200 µl Tris DTA (TE) buffer. To remove contaminant RNA, the sample was treated with 3 µl RNase and then incubated in water bath for 1 h at 37°C and after that equal volume of phenol: chloroform: isoamyl alcohol (24:1) were centrifuged at 8,000 rpm for 5 min at 4°C. DNA was precipitated by chilled absolute alcohol and pelleted by centrifugation at 5,000 rpm for 8 min. The precipitated DNA was spooled, rinsed with 70% ethanol and dissolved in 0.5 ml of TE buffer for further analysis. DNA concentration was measured in a UV spectrophotometer and the intactness was checked in 0.8% agarose gel.

PCR amplification

Initial screening was done with 45 random primers and only the expressed primers were chosen for this study. Amplification

Table 1. Coconut accessions used for characterization.

Accession number	Name of accession	Country of origin
WCGC 01	Solomon Tall	Solomon Island
WCGC 02	Rennel Tall	Solomon Island
WCGC 03	Fiji Tall	Fiji Island
WCGC 04	Niu Drau	Fiji Island
WCGC 05	Niu Leka Green Dwarf	Fiji Island
WCGC 06	Niu Bulavu	Fiji Island
WCGC 07	Local Tall	American Samoa
WCGC 08	Niu Oma Yellow Dwarf	American Samoa
WCGC 09	Local Tall (Tutiala)	American Samoa
WCGC 10	Niu Ui	American Tonga
WCGC 11	Niu Taukave	American Tonga
WCGC 12	Niu Hake	American Tonga
WCGC 13	Tahiti Tall	French Polynesia
WCGC 14	Local Tall (Pao pao)	French Polynesia
WCGC 15	Local Tall (Hae piti)	French Polynesia
WCGC 16	Local Tall (Bora Bora)	French Polynesia
WCGC 17	Rangiroa Tall (Avatoru)	French Polynesia
WCGC 18	Hari Papua Orange Dwarf	French Polynesia
WCGC 19	Rangiroa Tall (Tiputa)	French Polynesia
WCGC 20	Tall Kiriwana	Papua New Guinea
WCGC 21	Tall Muwa	Papua New Guinea
WCGC 22	Nikkore Orange Dwarf	Papua New Guinea
WCGC 23	Tall Kaveing	Papua New Guinea
WCGC 24	Natava Tall	Papua New Guinea
WCGC 25	Auck chang	Car Nicobar Island
WCGC 26	Tamaloo	Car Nicobar Island
WCGC 27	Kimios	Car Nicobar Island
WCGC 28	Kinmai	Car Nicobar Island
WCGC 29	Katchal	Katchal Island, Nicobar
WCGC 30	Campbell Tall	Great Nicobar Island

reactions were carried out in 20 µl volume containing 3 µl of template DNA, 10 µl of master mix (Ampliqon), 3 µl of random primer (Operon technologies, USA) and 4 µl sterile MilliQ water. Amplifications were performed in a Thermo Cycler (G-storm) programmed for an initial denaturation at 94°C for 5 min, 40 cycles of 1 min denaturation at 94°C, 1 min annealing at 37°C and 1 min extension at 72°C , a final extension of 10 min at 72°C and a holding for 1 h at 10°C (for cooling). The amplification products in RAPD analysis were separated by electrophoresis in 1.5% agarose gel containing ethidium bromide using 1X TBE buffer (pH 8.0). 1X TBE buffer was made by diluting the 10X TBE buffer 10 times (10X TBE was prepared by dissolving 108 g Tris base, 55 g boric acid and 40 ml of 0.5 M EDTA in single distilled water and volume was made up to 1 L). Separation was carried out by applying constant voltage at 70V/cm for 2 h. The sizes of amplified fragments were determined using standard (1 kb and 100 bp DNA ladder mix). Gel was photographed using a Gel documentation system (UPV).

Data analysis

Morphological and biochemical data were analysed using SAS software. 30 genotypes were compared with each other by using their amplification profiles and bands of DNA fragments were scored as present (1) and absent (0). Genetic similarity matrix was generated on the basis of Nei and Li's (1979) coefficients. Cluster analysis of genotypes was carried out on similarity coefficients using the unweighted pair group method arithmetic average (UPGMA) using NTSYS-PC, version 1.80 (Rohlf, 1995). The polymorphic information content (PIC) for each RAPD marker was calculated as PICi = 2fi(1-fi) as proposed by Roldan-Ruiz et al. (2000), where PIC is the polymorphic information content of the marker i, fi is the frequency of the marker bands present, and (1-fi) is the frequency of absent marker bands.

RESULTS AND DISCUSSION

Morphological and fruit component traits

All the accessions studied exhibited a high variability for most of the morphological characteristics (Table 2). This may be due to the wide range of collection sites and possible preferential collection or human selection for wide traits such as geographic location, dwarfness, colour and nut yield. The inheritance of these traits can

Table 2. Morphological traits of Pacific Ocean and Nicobar Islands coconut collections.

Accession	Palm height (cm)	Number of leaves/Palm	Length of Petiole	Length of leaflet bearing portion	Number of leaflets on one side of petiole	Length of longest leaflet (cm)	Breadth of longest leaflet at broadest portion (cm)	Girth of trunk at 1 m	Number of leaf scars in 1 m
WCGC 01	1157.66	29.35	113.44	392.03	109.60	118.60	5.00	93.19	21.00
WCGC 02	840.50	29.50	117.50	375.50	111.50	114.50	4.50	85.00	26.50
WCGC 03	1127.09	28.34	115.00	369.17	114.34	114.34	5.00	81.25	21.09
WCGC 04	1308.15	28.81	122.73	385.33	112.70	116.86	5.00	83.45	20.40
WCGC 05	484.25	32.25	98.00	279.50	97.75	103.75	5.50	103.25	33.25
WCGC 06	768.93	29.41	113.33	314.27	112.59	110.00	4.81	82.89	22.19
WCGC 07	723.43	28.75	109.95	373.16	109.06	108.01	5.59	81.97	22.40
WCGC 08	554.75	21.75	87.50	301.50	85.75	112.50	4.50	67.00	33.75
WCGC 09	777.09	26.50	113.42	361.17	105.67	109.92	4.92	81.17	23.59
WCGC 10	842.92	33.59	107.75	340.59	108.17	120.34	5.91	78.59	26.84
WCGC 11	787.50	28.09	101.50	347.84	111.34	100.34	4.59	76.67	21.84
WCGC 12	931.42	30.09	114.17	367.09	110.17	115.42	5.25	93.25	24.50
WCGC 13	829.17	33.67	114.09	383.25	106.59	116.34	5.66	79.50	25.09
WCGC 14	789.17	31.25	113.75	373.42	108.42	122.17	4.67	87.00	23.25
WCGC 15	927.92	33.92	127.50	379.67	117.59	113.34	5.09	89.17	19.59
WCGC 16	920.00	28.42	116.67	380.84	109.75	113.00	5.34	89.75	21.34
WCGC 17	871.67	28.09	118.75	400.42	105.34	124.50	5.54	83.84	25.59
WCGC 18	721.50	20.75	93.75	326.75	82.00	116.25	4.50	68.75	32.00
WCGC 19	794.17	28.00	108.00	383.67	104.75	108.75	4.84	78.09	20.92
WCGC 20	1235.00	28.17	116.67	378.34	106.59	121.25	5.00	76.17	19.84
WCGC 21	1112.50	28.34	119.25	387.42	111.84	122.50	5.76	87.67	19.84
WCGC 22	673.75	23.00	112.25	294.00	96.00	107.50	3.50	66.25	35.25
WCGC 23	1243.34	29.92	126.67	405.84	114.67	125.00	5.17	88.67	16.25
WCGC 24	1059.17	27.84	130.84	383.00	115.09	125.00	4.59	85.34	17.00
WCGC 25	1006.67	29.59	120.00	380.84	108.67	117.50	5.09	76.25	21.59
WCGC 26	852.50	29.67	117.09	400.42	112.59	109.75	4.84	75.17	21.17
WCGC 27	1065.21	31.84	115.21	447.63	124.00	118.75	5.09	94.34	20.00
WCGC 28	587.50	29.84	108.67	369.84	115.67	107.50	5.59	73.17	24.09
WCGC 29	985.00	30.09	115.42	370.42	117.75	113.75	5.67	83.34	20.59
WCGC 30	775.59	29.04	114.46	406.71	119.42	110.42	5.75	84.04	21.38
S.Ed	64.35	1.46	3.84	14.30	3.05	3.54	0.27	2.50	1.35
C.D. 5%	186.12	4.23	11.10	41.35	8.81	10.25	0.77	7.23	3.90

be observed without using specialized biochemical or molecular techniques. Morphological traits that are controlled by a single locus can be used as genetic markers provided their expression is reproducible over a range of environments (Kumar, 1999). The present study show that plant height ranged from 484.25 (WCGC 05) to1308.14 cm (WCGC 04) among the 30 accessions. The girth of trunk at 1 m height ranged from 66.25 (WCGC 22) to 103.25 cm (WCGC 05); both are the palms being dwarfs. Higher number of leaves on the crown (over 30) was observed in seven accessions indicating their potential for vigorous growth and WCGC 05 was the only dwarf among them. All the other dwarf accessions retained lesser number of leaves on the crown. Higher number of leaf scars per meter of trunk was recorded in dwarfs *viz*., WCGC 22, WCGC 08, WCGC 18 and WCGC 05 indicating their potential for dwarfness. Among the talls, WCGC 02 and WCGC 10 produced more number of leaf scars indicating their comparative short stature. Fruit component traits also showed wide variability among the accessions (Table 3). The length of fruit ranged from 18.08 to 28.18 cm and the general mean was 22.78 cm. Among the dwarf accessions, WCGC 05 had higher values for fruit length and breadth indicating the potential of this dwarf for production of larger nuts. The accession had also recorded higher fruit weight, weight of kernel, higher copra among the four dwarf accessions.

The lowest fruit weight of 422.09 g was recorded in WCGC 08, a dwarf of American Samoa and it was high in WCGC 01, a tall accession from Solomon Island. However, the copra content, a very important indicator for economic yield was higher in WCGC 07 followed by WCGC 02. Among the conserved coconut accessions in mainland India, generally, the dwarf accessions reported to produce lesser copra and the Cameroon Red Dwarf was the dwarf accession with more copra content of 220 g (Ratnambal et al., 2000). In the present investigation, WCGC 05 was found to produce 244.27 g of copra which is the highest among the dwarf accessions evaluated in India. All the Nicobar accessions in the study recorded more copra content with the highest in WCGC 26 (249.5 g) indicating the adoption of these types to the local environment and the role of human selection for superior types. Considering the morphological and fruit component traits studied, the accessions, WCGC 02, and WCGC 05 appeared to be promising among the talls and dwarfs respectively. Further selection in these types should be based on the testing for uniformity of progenies from these accessions. The dwarf accessions, WCGC 08, WCGC 18 and WCGC 22 have potential to be used as parents in new hybrid production programmes as they are more homogenous making the possibility of the hybrid progenies to be more uniform.

Biochemical parameters

The leaf polyphenols and leaf protein are indicators of plant health as they contribute positively to the stress factors. The leaf polyphenol content ranged from 106.58 (WCGC 12) to 299.77 mg (WCGC 09) indicating the potential of this trait for use in selecting the genotypes. Similarly, wide range of variability was observed for leaf nitrogen and leaf protein which will be helpful in assessing the genotypes for their response to the nutrition and capacity of nutrient uptake and assimilation (Table 4). The classification of coconut cultivars based on leaf polyphenol has suggested that the dwarfism is a relatively recent phenomenon in species evolution scale. In this study, the phenol content was higher in dwarfs (WCGC05) as compared to tall. Similar results were reported by Jay et al. (1989).

The coconut water is rich in energy sources and can be consumed as health drink. The potassium (K) and sodium (Na) levels in tender nut water ranged from 992.54 ppm (WCGC 27) to 2651.30 ppm (WCGC 22) and 55.60 ppm (WCGC 25) to 71.35 ppm (WCGC 05), respectively. The variation in the levels of K and Na might be due to the genetic makeup of the individual accessions and individual palms. The results of organoleptic tests in the 30 accessions (Figure 1) showed that accessions with more TSS (°Brix) was given more scores by the participants and hence TSS could be a useful trait in identifying the individual palms if not the accessions for better tender nut water quality from the point of view by consumers. Among the 30 accessions tested, the accessions with the organoleptic scores of 19 and above were grouped as very Good or Good category and hence these accessions could have potential for use in breeding programme aiming for tender nut qualities.

Molecular analysis

Molecular characterization of the 30 coconut accessions was done through RAPD markers. Out of the 45 primers screened, Only 13 primers (OPF-04, OPF-05, OPF-06, OPF-07, OPF-12, OPF- 13, OPF-19, OPF-20, OPH-24, OPH-25, OPH-28, OPH-29 and OPP-15) showed expression and all of them were used for polymorphism survey in pooled DNA of the 30 accessions of coconut collections which were highly reproducible and developed more bands for analyzing the genetic relationship. The generated amplicon size of all the 13 studied primers ranged from 170 to 1395 bp. The polymorphism obtained in the 30 genotypes with selected polymorphic RAPD markers showed distinct variation with 67.33% in all accessions. The highest number of bands was obtained in 28 accessions with primers OPF-04, OPF-13, OPF-19 followed by OPF-13 (27 accessions) while minimum number of bands was generated with OPH-29 and OPF-5 (6 accessions).

The average polymorphic information content (PIC) value of 13 selected primers was 0.29 and maximum and minimum PIC values was 0.46 and 0.17 for primers

Table 3. Fruit component traits of Pacific Ocean and Nicobar Islands coconut collections.

Accession	Number of nuts/ palm	Length of fruit (cm)	Breadth of fruit (cm)	Weight of fruit (g)	Thickness of husk (cm)	Weight of dehusked fruit (g)	Weight of kernel (g)	Weight of husk (g)	Thickness of kernel (cm)	Thickness of shell (cm)	Copra content/ nut (g)
WCGC 01	58.06	25.12	16.825	2091.40	2.62	984.37	386.25	243.280	1.18	0.54	181.09
WCGC 02	57.75	20.75	18.200	1675.00	3.05	825.00	425.00	200.00	1.55	0.55	255.00
WCGC 03	49.32	20.95	15.415	936.29	2.67	512.93	281.82	158.54	1.19	0.48	152.68
WCGC 04	70.53	23.64	16.440	1366.25	2.55	604.50	307.000	184.50	1.33	0.44	168.25
WCGC 05	45.14	21.91	17.695	1610.68	2.80	931.16	466.09	260.91	1.38	0.49	244.27
WCGC 06	57.16	19.68	16.070	1066.36	2.60	589.97	323.20	179.68	1.32	0.45	176.91
WCGC 07	43.42	23.96	18.085	1801.36	2.78	921.04	460.91	250.91	1.36	0.50	256.70
WCGC 08	82.40	18.75	10.965	422.08	1.51	267.71	147.710	103.96	0.95	0.44	71.66
WCGC 09	50.19	22.97	16.100	1284.37	2.47	609.37	312.50	170.62	1.32	0.52	179.37
WCGC 10	42.26	22.36	16.570	1580.00	2.44	830.25	420.75	212.00	1.32	0.43	214.75
WCGC 11	56.67	18.30	12.575	542.50	1.90	352.50	216.87	91.87	1.20	0.47	122.50
WCGC 12	45.79	22.15	17.190	1506.25	2.60	750.25	380.00	212.75	1.37	0.52	198.00
WCGC 13	51.73	20.51	16.310	1669.44	2.00	972.50	409.44	228.05	1.30	0.60	224.16
WCGC 14	37.02	21.24	16.550	1360.25	2.72	855.00	447.75	212.75	1.40	0.47	243.75
WCGC 15	50.17	23.56	17.405	1642.85	2.70	861.87	413.89	230.86	1.31	0.48	247.45
WCGC 16	37.09	22.96	16.600	1503.12	2.81	791.25	383.750	207.19	1.40	0.58	209.38
WCGC 17	37.91	25.37	16.595	1811.44	2.70	928.87	436.00	258.31	1.53	0.60	227.50
WCGC 18	45.69	18.06	13.365	900.66	1.65	560.00	260.83	261.67	1.28	0.51	102.08
WCGC 19	37.46	23.56	17.405	1642.85	2.70	861.87	413.89	230.86	1.31	0.48	247.45
WCGC 20	45.69	22.89	18.855	1705.11	2.83	929.29	432.57	243.25	1.29	0.48	227.95
WCGC 21	37.46	21.48	16.955	996.87	2.30	622.50	308.75	161.56	1.29	0.50	167.50
WCGC 22	70.48	18.07	11.940	545.00	1.47	359.69	207.81	106.25	1.15	0.38	101.56
WCGC 23	45.01	22.98	17.070	1437.75	2.53	805.50	372.00	224.25	1.28	0.46	192.00
WCGC 24	46.18	21.60	17.825	1388.12	2.70	775.00	378.12	200.00	1.25	0.45	192.50
WCGC 25	31.18	24.76	15.320	1771.00	1.92	765.25	365.50	224.25	1.19	0.51	178.00
WCGC 26	44.32	26.25	16.100	1809.25	2.47	899.25	439.00	250.75	1.35	0.47	249.50
WCGC 27	44.96	28.18	16.580	2099.25	2.76	964.75	433.50	255.50	1.39	0.57	234.75
WCGC 28	40.73	24.72	15.390	1623.25	1.95	828.25	404.50	233.25	1.28	0.48	210.50
WCGC 29	41.28	24.93	16.240	1814.25	2.55	890.25	417.75	250.25	1.38	0.52	217.75
WCGC 30	54.30	24.83	15.150	1369.5	2.06	688.75	332.7	224.50	1.24	0.52	182.50
S.Ed	6.2951	0.756	0.3185	116.30	0.122	56.63	18.23	12.811	0.0421	0.023	12.916
CD at 5%	12.87	2.188	0.9213	336.38	0.353	163.80	52.73	37.056	0.1219	0.068	37.358

Table 4. Biochemical traits in Pacific Ocean and Nicobar Islands coconut collections.

Accession	Leaf			Tender nut water							
	Leaf Phenol (mg/100 g F.W)	Leaf Nitrogen (%)	Protein (%)	Nut water	TSS (°Brix)	Free amino acid (mg/ml)	Total sugar (mg/100 ml)	Reducing sugar (mg/100 ml)	K (ppm)	Na (ppm)	*Overall organoleptic score
WCGC 01	128.63	0.66	4.13	570.00	6.10	1.60	250.33	226.07	1777.08	62.50	14.00
WCGC 02	89.12	0.53	3.31	720.00	5.60	1.37	267.89	232.69	1593.88	68.80	15.75
WCGC 03	205.10	0.94	5.84	245.00	4.30	1.66	238.02	212.77	2442.76	58.98	9.75
WCGC 04	127.44	0.73	4.53	370.00	5.50	1.82	278.37	230.70	2513.13	59.10	19.75
WCGC 05	259.86	0.30	1.84	470.00	5.40	1.48	252.74	202.53	2216.80	71.35	10.00
WCGC 06	196.66	0.73	4.56	525.00	5.20	1.35	257.60	231.20	2052.65	58.15	13.25
WCGC 07	229.59	1.24	7.75	495.00	4.70	1.37	461.66	204.72	2378.07	57.90	16.75
WCGC 08	255.78	0.76	4.75	117.00	5.70	1.44	232.75	211.95	2133.73	62.10	16.00
WCGC 09	299.77	0.59	3.66	445.00	6.40	1.54	222.07	219.15	2266.05	66.55	20.50
WCGC 10	263.27	0.39	2.41	691.50	6.30	2.09	232.75	200.48	2261.32	68.65	20.25
WCGC 11	240.93	0.52	3.22	475.00	5.30	1.78	232.50	221.03	2285.43	68.95	16.75
WCGC 12	106.58	0.74	4.63	557.50	6.10	1.51	235.78	203.88	2507.09	56.70	18.75
WCGC 13	211.45	0.31	1.94	690.00	5.30	1.36	231.81	230.62	1466.97	58.25	15.50
WCGC 14	196.26	1.12	7.00	915.00	6.40	1.44	232.85	204.94	2308.19	59.80	22.75
WCGC 15	206.52	0.80	5.00	547.00	6.80	3.50	218.93	201.45	2343.68	57.65	22.25
WCGC 16	167.97	0.73	4.56	550.00	5.70	1.34	286.05	203.45	2505.08	70.15	20.25
WCGC 17	187.36	0.39	2.41	425.00	6.40	1.95	227.65	203.76	2579.71	59.05	19.50
WCGC 18	200.23	0.29	1.78	154.50	6.30	1.52	227.79	198.89	2279.25	69.40	12.00
WCGC 19	160.15	0.39	2.44	575.00	4.60	1.50	255.82	222.04	2564.55	67.05	11.00
WCGC 20	266.27	0.88	5.50	592.50	6.60	1.47	266.26	211.75	2510.00	59.90	24.00
WCGC 21	235.66	0.79	4.94	500.00	6.20	1.56	372.56	329.15	2432.95	59.85	20.00
WCGC 22	240.53	0.84	5.25	198.00	4.90	1.44	238.32	202.07	2651.53	70.55	16.25
WCGC 23	267.18	0.98	6.09	335.00	5.00	1.77	264.47	216.56	2566.89	61.25	13.00
WCGC 24	223.70	0.79	4.94	527.50	4.90	1.57	247.97	201.21	1375.80	60.13	10.75
WCGC 25	186.79	0.52	3.25	560.00	5.30	1.80	257.90	230.80	1098.17	55.60	12.50
WCGC 26	143.48	0.38	2.34	458.50	5.60	1.37	233.95	208.11	1309.57	67.20	15.75
WCGC 27	147.79	0.18	1.09	697.50	5.80	1.68	225.65	206.03	992.54	59.45	19.75
WCGC 28	182.77	0.58	3.63	565.00	5.70	1.57	315.81	212.25	1318.27	63.05	20.75
WCGC 29	263.78	1.12	7.00	706.00	5.20	1.76	301.84	208.53	1116.81	70.40	16.00
WCGC 30	227.04	0.88	5.50	365.00	6.40	2.07	353.53	247.77	1818.74	61.40	20.00
SE d	33.38	0.1461	0.9132	143.94	0.7334	0.0306	0.6655	0.9273	305.33	6.836	4.6161
CD at 5%	68.28	0.9132	1.8678	294.40	1.5001	0.0626	1.3612	1.8966	624.50	13.981	9.4412

Table 5. Polymorphic information content value of RAPD primers.

RAPD Primer	Sequence	Polymorphic (number of accession)	PIC Value	Product size
OPF-04	GGTGATCAGG	14	0.26	400-1100
OPF-05	CCGAATTCCC	6	0.39	720-1300
OPF-06	GGGAATTCGG	16	0.31	290-1210
OPF-07	CCGATATCCC	21	0.26	350-665
OPF-12	ACGGTACCAG	22	0.22	390-790
OPF-13	GGCTGCAGAA	27	0.26	180-1070
OPF-19	CCTCTAGACC	28	0.46	190-1395
OPF-20	GGTCTAGAGG	23	0.26	185-1235
OPH-28	GGACCCAACC	20	0.41	170-1200
OPH-29	CGCCCGTTCC	6	0.24	100-1300
OPH-25	AGCGTCACCC	23	0.17	500-1150
OPH-24	TCGCCGCCCA	17	0.28	250-1000
OPP-15	GGAAGCCAAC	22	0.21	250-1150

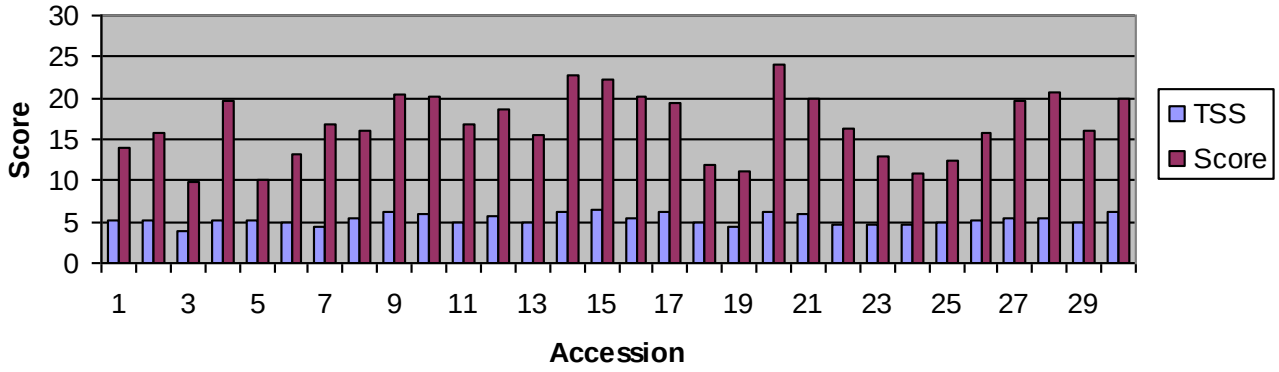

Figure 1. Organoleptic scoring of tender nut water in relation to the estimated TSS (°Brix).

OPF-19 and OPH-25, respectively, (Table 5). The RAPD analysis is in agreement with earlier results in coconut (Everard, 1999; Perera et al., 1998; Rivera et al., 1999; Upadhyay et al., 2002, 2004) for elucidating the variations, wherein dwarf accessions have grouped in concert and tall accessions showed more variation.

Cluster analysis was used to investigate further the inter-relationships of these coconut accessions. The unweighted pair-group arithmetic average method (UPGMA) and the complete-linkage method were carried out on the taxonomic distance matrix to establish relationship among the accessions (Figure 2). In cluster analysis, dendrogram showed that the accessions were grouped into two clusters.

The first cluster contained one dwarf accession WCGC 08 and second cluster contained the remaining 29 accessions. The clustering pattern did not show any geographical affinity. The dwarf accessions WCGC 05, WCGC 18 and WCGC 22 clustered along with the talls which indicate the need for purifying these materials through further selection before using these populations. The clustering pattern may be due to the fact that the studied palms are selected from open pollinated progenies of the respective collections. The diversity observed can be indicative only although they have been collected from different places using the typical palms selected based on morphological observations. Hence, further selection and *inter se* mating through artificial pollination among the selected palms will be ideally required for utilization of the accessions for specific traits. The mean performance for traits such as dwarfness, high copra content in selected accessions such as WCGC 05, appears to be promising although it was observed to cluster with the talls. Clustering of dwarf coconut accessions with talls were also reported earlier by several workers. Clustering of Niu Leka Dwarf (WCGC 05) in the present study was in line with earlier report (Manimekalai and Nagarajan, 2006). Dwarfs sampled from Sri Lanka was found to be clustered with South East Asian tall coconut accessions (Perera et al., 2000) and dwarfs separated from African talls and clustered with the talls of South Pacific coconuts (Lebrun et al., 1998).

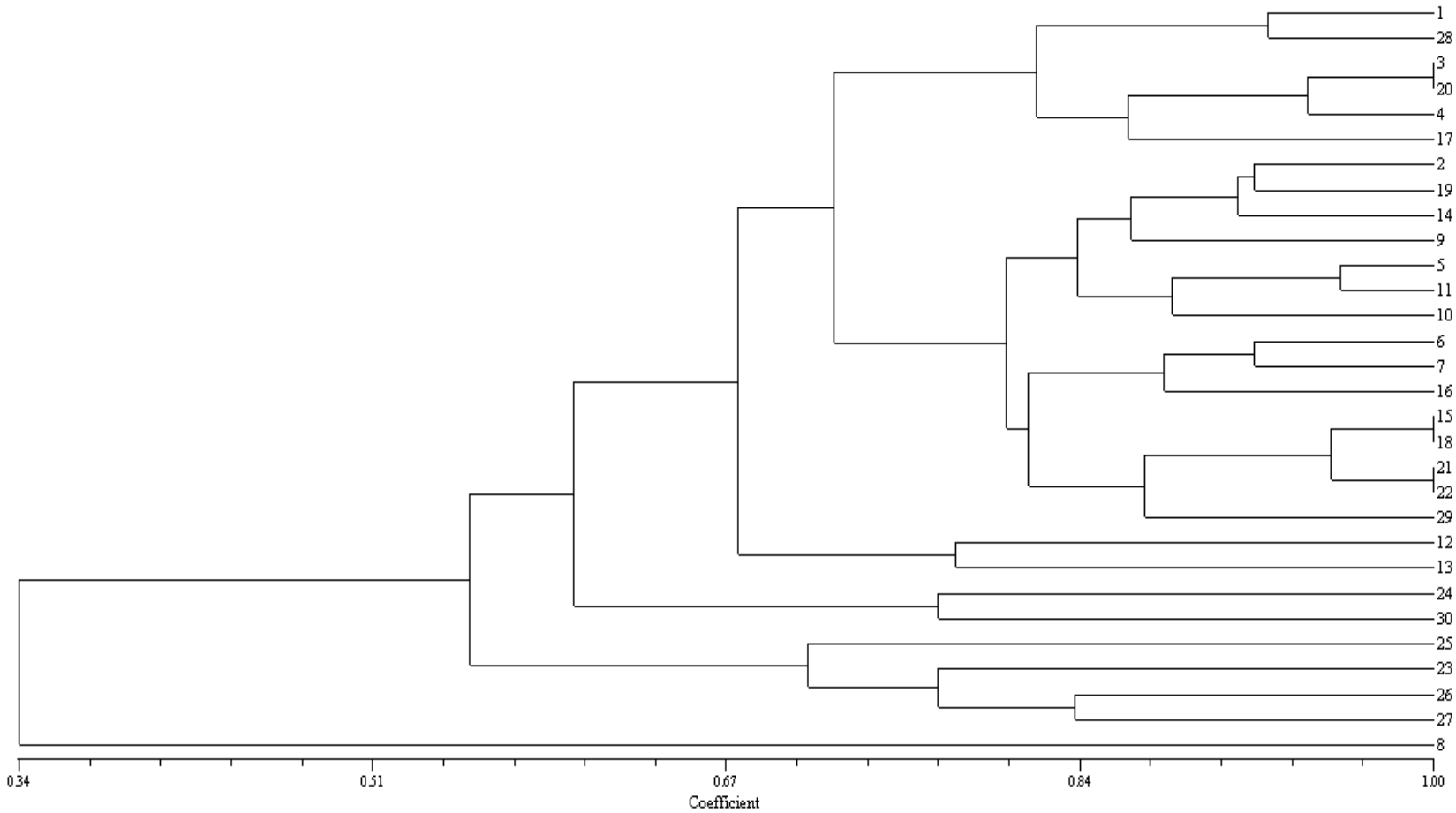

Figure 2. Dendrogram of the 30 accessions of coconut based on RAPD analysis.

Conclusion

The characterization exposed significant differences among the 30 coconut genotypes for morpho-agronomic traits and molecular markers which indicate the existence of genetic variation and robust characterization of the species diversity. The results provide information that is useful in utilizing the conserved coconut accessions at World Coconut Germplasm Centre, Port Blair for crop improvement to meet the diverse goals of producing cultivars with tender nut traits, dwarfness and increased yield. The grouping of genotypes had been found to be in congruence with few of the morphological traits which will further assist the breeders in planning future selections within these coconut populations. The characterization has given the range of variation for all the economic traits which will be useful in planning for further germplasm collection and conservation for new diversity.

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