Research Article

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Characterization of Kuttiyadi ecotype of coconut (*Cocos nucifera* L.) using morphological and microsatellite markers

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Abstract

West Coast Tall (WCT) is the most popular coconut cultivar grown by the farmers in Kerala, which occupies over 95 per cent of the area under coconut. The long history of coconut cultivation throughout Kerala state has resulted in the development of many ecotypes of WCT. The present work compares the similarity/diversity of the morphological and molecular characteristics of the Kuttiyadi ecotype growing in the hilly, midland region of Kozhikode District, Kerala with those of the WCT cultivar of the coastal region of Kasaragod District, Kerala, using vegetative, reproductive and fruit component characters and microsatellite markers. Geographically, these two locations show a wide range of variation for soil and climactic factors. The vegetative, reproductive and fruit component characteristics and microsatellite markers showed wide variations between selected WCT palms from Kasaragod and Kuttiyadi. The similarity index based on Dice's coefficient, obtained after pair-wise comparison of Kuttiyadi and WCT samples with 15 SSR markers, revealed that the percentage similarity varied from the coefficient range 0.20 to 0.97 between the WCT and Kuttiyadi palms. UPGMA clustering clearly distinguished the two populations with WCT and Kuttiyadi forming separate clusters. STRUCTURE analysis was also carried out, which also showed that the two populations studied were distinct.

Keywords: Genetic diversity, coconut, ecotype, morphological characters, microsatellite markers

Introduction

The coconut palm (*Cocos nucifera L.)* is one of the most useful crops supporting the livelihood security of millions of small holder and marginal farmers (and associated processors) in over 93 countries of the world. Coconut is mainly an oil crop containing lauric acid but has various other uses in addition to commercial oil production (Harries, 1995). It is a monocotyledonous, woody perennial and a solo species of the genus *Cocos*, within the tribe of Cocoeae, family Arecaceae order Arecales (Palmae). Within this genus, two main groups are recognized, the tall palm, *C. nucifera* var. *typica*, and the dwarf palm, *C. nucifera* var. *nana*. The chromosome number of coconut is 2n=32. The centre of diversity for coconut is in the Asia/West Pacific region (Gunn *et al*., 2011) with ample morphological variations. The seed nuts might have dispersed by floating on ocean currents (Persley, 1992), but were taken by boat to regions where they could not float and carried to inland and upland locations where they needed human assistance to survive.

Studies on genotype-environment interactions are very important, since they provide information about adaptation and stability of varieties in specific locations. In India, the coconut growing area extends from the coast to interior regions varying in altitudes. The geographical area extending from Maharashtra to Kerala has different ecological zones with differential selection pressures on the coconut varieties adapted to these areas. Weather factors like sunshine hours, light intensity, temperature, humidity, rainfall and soil factors play a significant role in fluctuations of coconut yield (Coomans, 1975; Murray, 1977).

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Kerala is the major coconut growing state in the country and West Coast Tall (WCT) is the most popular cultivar grown by the farmers here, which occupies over 95 per cent of the area under coconut (Remany, 2003). WCT is a tall sturdy palm giving satisfactory yield up to an age of 70 years. It is widely accepted that farmers' perception of coconut varieties provides the basis for conserving biodiversity. The long history of cultivation throughout Kerala has resulted in the development of many ecotypes in WCT. These ecotypes are known by the locations where they are grown. Such local ecotypes with the adaptations to the environment are rich source of valuable genes for coconut breeding (Remany, 2003).

Reliable knowledge of the genetic diversity of breeding material is important for a plant breeder, in order to select parents for new breeding cycle. Genetic diversity is desirable for long term improvement in yield and resistance to pests and diseases. In order to develop effective conservation strategies in coconut, it is important to obtain knowledge on the amount and extent of genetic diversity. The use of morphological traits in coconut for assessment of genetic diversity has now been augmented by molecular markers. Recently, molecular marker tools such as ISTR (Rohde *et al.,* 1995), RFLP (Lebrun *et al*., 1998), RAPD (Upadhyay *et al*., 2004), AFLP (Perera *et al*., 1998) and SSR (Teulat *et al*., 2000; Perera *et al*., 2003; Meerow *et al*., 2003) have been used to study the genetic diversity and population pattern in coconut. The advantages of molecular markers are that they are not influenced by the environmental factors.

Microsatellites or simple sequence repeats (SSR) have been recognized as powerful and informative genetic marker in coconut (Rajesh *et al.,* 2008a; 2008b). They consist of tandemly repeated units of short nucleotide motifs that are 1-6 bp long. Di-, tri- and tetra- nucleotide repeats *i.e.,* $(CA)_n$, $(AAT)_n$ and $(GATA)_n$ respectively, are the most common and are widely distributed throughout the genome of plants. These markers are abundant, co-dominant, highly polymorphic even within populations, spread throughout the genome, easily amplified by PCR and the great majorities are selectively neutral.

The present work compares the similarity/ diversity of Kuttiyadi ecotype of coconut growing in the hilly, midland region of Kozhikode district, Kerala with those of the West Coast Tall (WCT) cultivar of the coastal region of Kasaragod District, Kerala, using vegetative, reproductive and fruit component characters and microsatellite markers.

Materials and methods

Location of study area and plant materials

For this study, palms were selected from two locations in Kerala state *viz*. WCT palms growing in coastal region (CPCRI, Kasaragod) and Kuttiyadi ecotype growing in a hilly region of Kozhikode district. The WCT populations maintained at CPCRI, Kasaragod is geographically located at 12.31°N latitude and 74.51°E longitude and at an altitude range from 15-17 m above mean sea level. Average temperature is 31.5 °C during summer and 21.3 °C in winter. Mean annual rainfall is 3400 mm, spread over 132 days. The South-West monsoon is predominant at this location. High relative humidity prevails at this location with an annual average of 88 per cent. Soil is sandy loam with low clay content, with a pH of 4.4. The soil contains low organic carbon (0.58%) and low nitrogen content (0.034%). Kuttiyadi is located at 11.41°N latitude and 75°E longitude and at an altitude of 80-350 m above MSL. Average temperature is 32.8 °C during summer and 21.3 °C in winter. Mean annual rainfall is 3266 mm, spread over 130 days. South-West monsoon is predominant at this location. High relative humidity prevails at this location with an annual average of 91 per cent. Soil is laterite, with a pH of 5.05 containing high carbon (1.93%) and nitrogen (0.23%) contents (Chandy, 1995).

Morphological analysis

The age of the palms selected for the study was between 40-50 years. A total of 200 palms in each location were identified for observations (200x2 = 400 palms). Base data, such as morphological and reproductive characters were observed in the field and recorded. Fully matured nuts were harvested from the selected palms for fruit analysis. Morphological characters were observed on the basis of coconut descriptors (Ratnambal *et al*., 1995).

All experimental data were analyzed statistically using SAS software (Local, W32, and VSPRO). A general linear model univariate ANOVA

was run for every data set to find significant differences between the two populations. Mean values and critical differences (CD) were calculated for WCT and Kuttiyadi populations.

Molecular analysis

Spear leaves were collected from 36 selected palms (20 palms for Kuttiyadi and 16 palms for WCT) for DNA extraction and analysis. A set of 15 hyper-polymorphic coconut SSR markers (Table 1), distributed in different coconut chromosomes, were used for microsatellite 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 in an Eppendorf

Table 1. List of coconut specific SSR markers, their sequence ad the annealing temperature (T_m)

Sl. No.	Primer name	Sequence (5'-3')	T_m (ºC)
1	CnCir 87F	ATAACATCCTCCAACCTG	55
	CnCir 87R	GACTGAATCCAACCCTT	
2	CnCir 74F	GAGATCCTCACCTCCAC	52
	CnCir 74R	CGGCAACAAAGAGAAC	
3	CnCir G11F	AATATCTCCAAAAATCATCGAAAG	52
	CnCir G11R	TCATCCCCACACCCTCCTCT	
4	CnCir E4F	GCATGGTATTCGGATTTG	54
	CnCir E4R	ATGGTTCAGATTTGGACAGT	
5	CnCir E10F	TTGGGTTCCATTTCTTCTCTCATC	59
	CnCir E 0R	GCTCTTTAGGGTTCGCTTTCTTAG	
6	CnCir C3F	AATATCTCCAAAATCATCGAAAG	59
	CnCir C3R	GTGGGGCATGAAAAGTAAC	
7	CnCir 2F	AGTCCTAAAAGTGTTGGC	56
	CnCir 2R	GTAATCCTATGGCTGCTT	
8	CnCir B12F	GCTCTTCAGTCTTTCTCAA	57
	CnCir B12R	CTGTATGCCAATTTTTCTA	
9	CnCir E2F	TCGCTGATGAATGCTTGG	55
	CnCir E2R	GGGGCTGAGGGATAAACC	
10	CnCir B6F	GAGTGTGTGAGCCAGCAT	59
	CnCir B6R	ATTGTTCACAGTCCTTCCA	
11	CnCir 56F	AACCAGAACTTAAATGTCG	51
	CnCir 56R	TTTGAACTCTTCTATTGG	
12	CNZ 10F	CCTATTGCACCTAAGCAATTA	54
	CNZ 10R	AATGATTTTCGAAGAGAGGTC	
13	CNZ 05F	CTTATCCAAATCGTCACAGAG	50
	CNZ 05R	AGGAGAAGCCAGGAAAGATTT	
14	CNZ 04F	TATATGGGATGCTTTAGTGGA	52
	CNZ 04 R	CAAATCGACAGACATCCTAAA	
15	CNZ 17F	ATGTAAAGAAAGTAGGGAGGC	60
	CNZ 17 R	CATAGGTTATCATGCAGAGCT	

gradient thermal cycler with a PCR profile of 94 ºC for 5 min followed by 30 cycles of 1 min at 94 ºC, 2 min at the different annealing temperatures standardized for the individual SSR locus, and 2 min at 72 ºC with a final extension for 5 min at 72 ºC. After amplification, a volume of 3 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. The amplified products were run on 3 per cent agarose gel, stained with ethidium bromide and were visualized in a gel documentation system.

Data analysis

The alleles were scored individually based on comparison with the molecular ladder. The size of the amplicons was compared using a 100 bp ladder (M/s Bangalore Genei, India). Each band generated by SSR primers was considered as an independent locus. Clearly resolved, unambiguous bands were scored visually for their presence or absence with each primer. The scores were obtained in the form of a matrix with '1' and '0', which indicate the presence and absence of bands respectively in each sample. Based on the number of polymorphic bands, percentage polymorphism was calculated for each primer. The binary data scored was used to construct a dendrogram. The genetic associations between populations were evaluated by calculating the Dice similarity coefficient for pair wise comparisons based on the proportions of shared bands produced by the primers (Dice, 1945). Similarity matrix was generated using the NTSYS-PC software, version 2.0 (Rolf, 1998). The similarity coefficients were used for cluster analysis and dendrogram was constructed by the unweighted pair-group method (UPGMA). Principal component analysis (PCA) was done to obtain both 2-D and 3-D images, in order to visualize the difference between the individuals. They were generated using the NTSYS-PC software 2.0 (Rolf 1998).

For the microsatellite data, the number of alleles, number of effective alleles, Shannon's information index, the observed and expected heterozygosity (Levene, 1949), Wright's fixation indices, gene flow were estimated using the software POPGENE version 1.32 (Yeh *et al*., 1999) GENEPOP V4.0 software was used to test data sets for deviations in Hardy-Weinberg Equilibrium (HWE) and for linkage disequilibrium (Rousset, 2008). Deviations from HWE were estimated using both the exact test and the FIS statistic estimations, using Markov chain Monte Carlo (MCMC) runs for 1000 batches, each of 2000 iterations (Rice, 1989).

STRUCTURE 2.3.3 software (Prichard *et al*., 2000), was used to assume the sample of individuals comprising K unknown populations to which individual genotypes or fractional genotypes were assigned. The admixture model of STRUCTURE and the option of correlated allele frequencies between populations were used. The correct number of clusters (K) was determined by testing K values from 1 to 10 and performing 15 repeats for each K. The burn-in period consisted of $1x10^5$ iterations followed by $1x10^5$ MCMC repeats. Finally, estimated log probabilities of data Pr (X | K) for each value of K were evaluated by calculating ΔK, the rate of change in the log probability of data between successive K values (Evanno *et al.,* 2005). The STRUCTURE output files were first processed using STRUCTURE HARVESTER v0.3. This produces an output consisting of a series of files, including graphical files representing, per K and per repeated run, the estimated Ln probability of each run, and three other Ln based estimates that allow the selection of the most optimal values (Evanno *et al.,* 2005). Samples were analyzed without any prior population information, but are sorted by their sampling population once STRUCTURE is completed.

Results and discussion

Morphological characterization

Palm vegetative characters:

A total number of nine vegetative characters, eight reproductive characters and 21 fruit component characters were studied from 400 palms, 200 each of WCT and Kuttiyadi. The results obtained from the above studies were statistically analyzed and are presented in Tables 2 and 3.

Plant height, girth at the base of the stem and numbers of fully opened leaves on the crown were significantly higher in Kuttiyadi compared to WCT (Table 2). Leaf characters like length of petiole, length of leaflet bearing portion, number of leaflets

Table 2. Vegetative and reproductive characters of WCT and Kuttiyadi ecotype

Sl. No.	Characters observed	WCT	Kuttiyadi	CD
1	Plant height** (cm)	1229.64	1554.22	79.91
2	Girth at base** (cm)	102.06	110.52	7.75
3	Number of leaves on the crown**	29.94	34.81	1.63
4	Length of petiole (cm)	108.17	107.97	—
5	Length of leaflet bearing portion (cm)	343.89	342.66	—
6	Number of leaflets	112.68	111.21	—
7	Breadth of leaflet (cm)	5.63	5.47	—
8	Length of leaflet** (cm)	115.99	110.17	5.06
9	Leaf scars at one meter**	14.06	11.59	0.94
10	Length of inflorescence** (cm)	91.62	96.10	4.49
11	Length of spikelet portion (cm)	34.92	34.39	—
12	Length of stalk (cm)	44.96	40.29	—
13	Length of spikelet*(cm)	38.94	37.10	1.60
14	Number of spikelet	34.19	33.62	—
15	Number of female flowers**	23.79	20.16	3.39
16	Number of bunches**	11.60	15.07	0.83
17	Number of nuts per year**	92.58	82.88	9.90

(** Significant at 0.01 level * Significant at 0.05 level)

and breadth of leaflets were similar in WCT and Kuttiyadi. Length of leaflet was high in WCT compared to Kuttiyadi (Table 2). Several other earlier investigations have also reported the variations of these characters among the coconut varieties. Height of coconut palms varies with genotype, climate and soil condition and the attention bestowed to cultivation and manuring (Menon and Pandalai, 1958). According to Patel (1938), cultivation, manuring and better soil conditions favor the production of taller stems. Close planting was also reported in palms with taller stems (Menon and Pandalai, 1958). Greater stem girth is a typical character of tall varieties as reported by Rathnambal *et al*. (1995) and Arunachalam *et al*. (2001). Ramanathan (1984) reported that the stem height and girth of the stem were positively correlated with yield. The differences in these characters of WCT and Kuttiyadi may be due to difference in the soil, climatic or environmental condition.

Variations were also noticed in number of leaf scars on the stem of WCT and Kuttiyadi. From the analyzed data, it was observed that number of leaf scars in one meter of the stem was significantly higher in WCT compared to Kuttiyadi. The reports of earlier studies on coconut revealed that number of leaf scars denotes the number of internodes and widely spaced leaf scars are always associated with a long drooping habit of the leaves and closely spaced leaf scars are associated with short, strong and well oriented leaves (Pieris, 1934).

Palm reproductive characters:

The reproductive characters showed a wide range of variation among WCT and Kuttiyadi ecotypes (Table 2). The length of inflorescence was high in Kuttiyadi compared to WCT, but length of spikelet bearing portion and length of stalk were similar in WCT and Kuttiyadi. Variations were noticed in length of spikelets and number of spikelets between WCT and Kuttiyadi, which were significantly higher in WCT compared to Kuttiyadi. Number of female flowers and number of nuts per year were also found to be high in WCT, but the number of bunches on the palms were high in Kuttiyadi compared to WCT. Sathyabalan *et al.* (1993) found that the yield potential of the palm could be judged from their initial yields and also from the height of the palm and number of functional leaves in the crown. Flowering percentage is correlated with characters such as total number of leaves and the number of functional

leaves in the crown (Rajagopal *et al*., 1990). Stem height and number of leaves in the crown are positively correlated with nut yield in coconut (Sathyabalan *et al.,* 1993).

Based on palm morphology (combining vegetative and reproductive characters), a dendrogram was constructed, which shows segregation of WCT and Kuttiyadi populations for most of the palms used in the study. A few Kuttiyadi and WCT palms were inter-mixed and placed between the segregated Kuttiyadi and WCT populations (Fig. 1). According to the dendrogram, the segregated WCT and Kuttiyadi populations show maximum distance. From the intermixed palms, it is seen that some of the WCT and Kuttiyadi palms are very close. This suggests that even though Kuttiyadi population has segregated from WCT, a few palms of WCT and Kuttiyadi populations still show genetic affinity.

Fruit component character:

The fruit is a fibrous drupe developed from a tricarpellate ovary and takes about one year from spathe open to fruit maturity. It has a tough pericarp and a thick fibrous mesocarp that together constitute the husk. The nut inside the husk has a hard endocarp (shell) lined with firm white endosperm

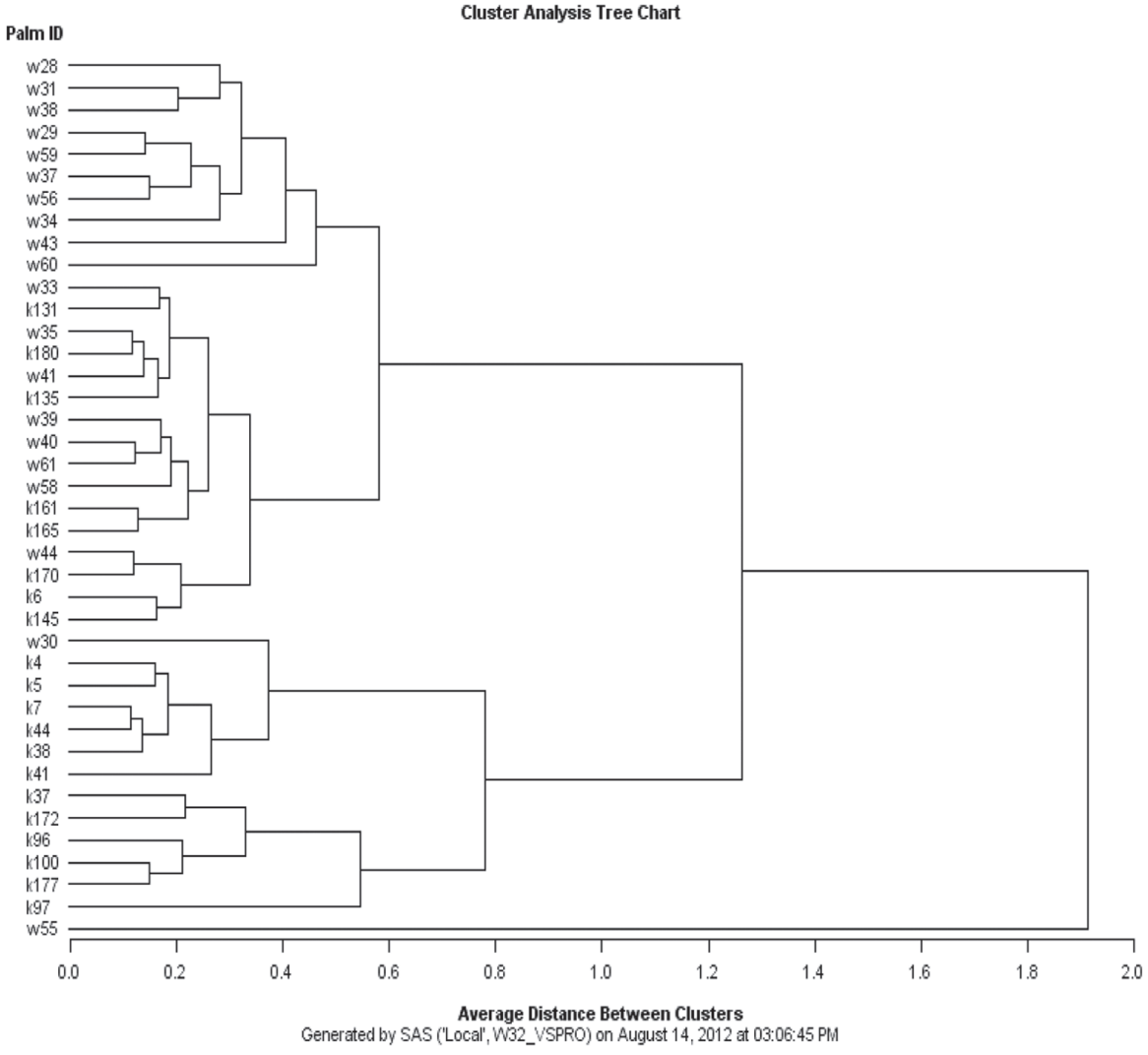

Fig. 1. Clustering of WCT (w) and Kuttiyadi (k) palms based on palm morphology

Table 3. Fruit component characters studied

Sl. No.	Characters studied	WCT	Kuttiyadi	CD value
1	Fruit weight** (g)	994.56	819.24	46.21
2	Fruit length** (cm)	20.71	17.39	0.47
3	Fruit breadth** (cm)	15.45	12.34	0.47
4	Fruit length at polar view** (cm)	55.90	52.06	1.20
5	Fruit length at equatorial view** (cm)	49.04	44.21	1.30
6	Husk thickness** (cm)	3.16	2.75	0.12
7	Husk weight**	418.68	375.40	26.40
8	Husk percentage**	42.84	45.37	1.78
9	Nut weight.**(g)	575.88	443.20	29.92
10	Nut length (cm)	10.64	10.76	—
11	Nut breadth** (cm)	9.68	9.02	0.27
12	Nut polar length** (cm)	32.61	31.98	0.61
13	Nut equatorial length** (cm)	31.59	29.86	0.71
14	Shell weight**	143.21	118.22	6.24
15	Shell thickness (cm)	0.47	0.45	—
16	Endosperm thickness** (cm)	1.38	1.33	0.03
17	Cavity diameter polar region* (cm)	7.80	6.14	0.30
18	Cavity diameter equatorial region** (cm)	6.71	9.27	0.36
19	Cavity volume** (mL)	174.91	115.54	11.00
20	Copra weight.** (g)	181.93	149.96	7.96
21	Oil percentage*	65.72	68.35	1.90

(** significant at 0.01 level * significant at 0.05 level)

(kernel) surrounding a central cavity containing residual liquid endosperm (water). Fruit component analysis was carried out at a specified stage of ripeness, after which the endosperm can be dried to produce copra for storage and oil extraction. Fruit component observations of 21 characters recorded in this study were analyzed statistically (Table 3). The result revealed that fruit weight, fruit length, fruit breadth, fruit circumference (polar and equatorial), husk thickness, husk weight, nut weight, nut breadth, nut circumference (polar and equatorial), shell weight endosperm thickness, cavity diameter on the polar region, cavity volume and copra weight were significantly higher in WCT compared to Kuttiyadi (Table 3). The characters husk percentage, cavity diameter of nut in the equatorial region and oil percentage in copra were significantly higher in Kuttiyadi compared to WCT. Characters such as length of the nut and thickness of the shell were similar in WCT and Kuttiyadi (Table 3).

The dendrogram constructed based on fruit component characters (Fig. 2), though showing segregation of WCT and Kuttiyadi populations, the resolution is not as clear as the case of clustering based on palm morphological characters. There is a grouping of WCT and Kuttiyadi populations and the groups are inter-mixed in the dendrogram. The diversity existing within the two populations extended as Kuttiyadi moved towards the hilly region.

The dendrogram developed by combining palm morphological and fruit component characters reveal the grouping and separation of Kuttiyadi and WCT palms (Fig. 3). The dendrogram shows one group of Kuttiyadi palms sandwiched between two groups of WCT palms suggesting segregation within WCT lead to the development of Kuttiyadi.

Molecular characterization

Allele richness of SSR loci

Fifteen polymorphic SSR markers were used to amplify DNA of Kuttiyadi and WCT palms. SSR

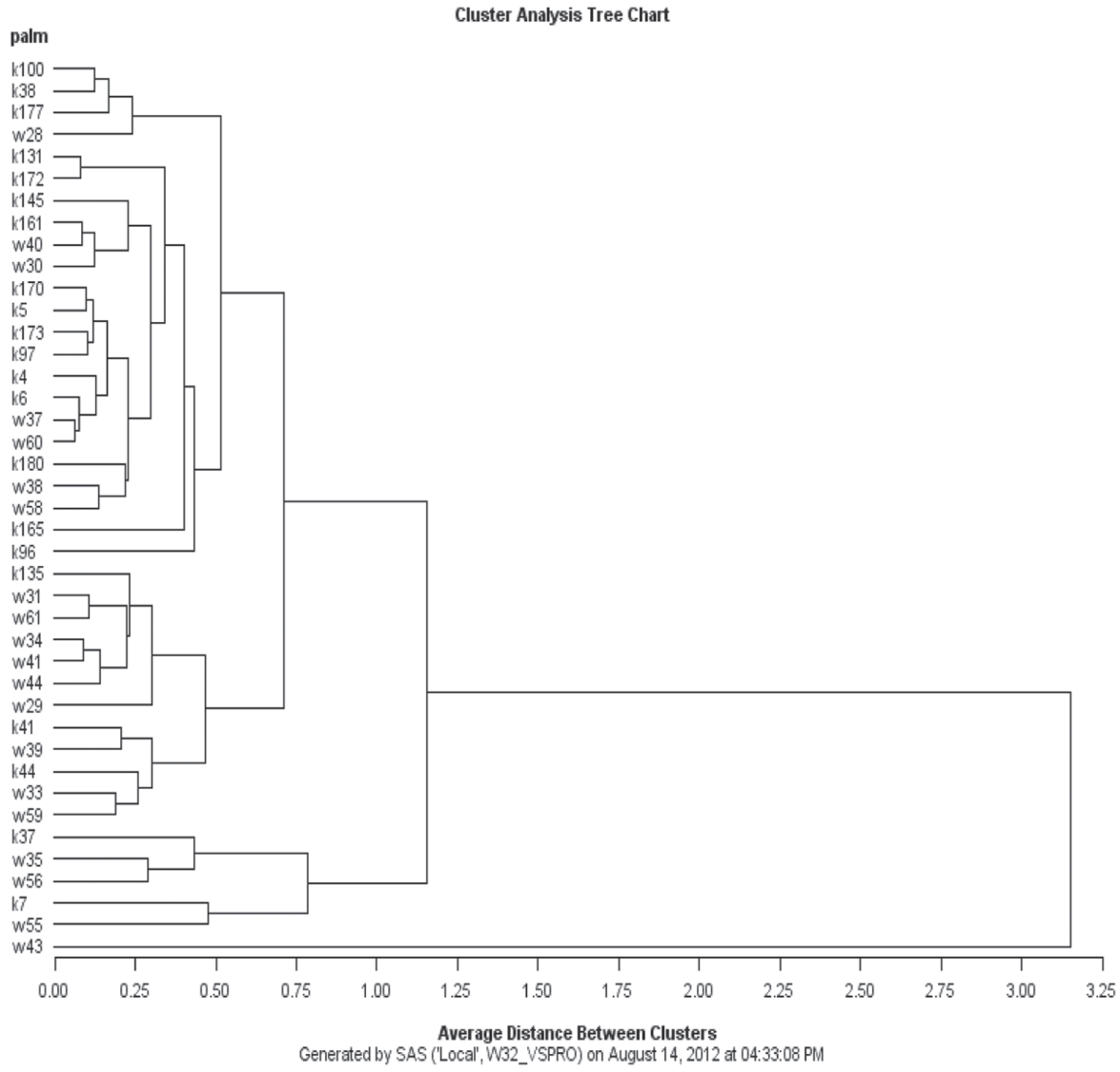

Fig. 2. Clustering of WCT (w) and Kuttiyadi (k) palms based on fruit components

profile of the palms with the primer CNZ04 is given in Figures 4a and 4b. Alleles were detected with all the markers revealing two alleles or more with a mean of 2.6 alleles per locus. The effective number of alleles per locus (N_e) ranged from 1.0 (CNZ 17) to 3.53 (CnCir87) with a mean of 2.029. Shannon's information index ranged from 0.00 (CNZ05) to 1.314 (CnCir87) with a mean of 0.709 (Table 4).

F_{IS} for six of the loci was below zero, with a mean of 0.1038. Mean F_{ST} (0.5296) indicated that the populations were highly differentiated. The mean gene flow (Nm), based on mean F_{ST}, was very low (0.749) indicating the absence of extensive gene flow among the two populations (Table 5).

The genetic structure of plant populations reflects the interactions of various factors, including the long-term evolutionary history of the species (shifts in distribution, habitat fragmentation and population isolation), genetic drift, mating system, gene flow and selection (Schaal *et al*., 1998). The founding number, probability of common origin, kin structure, and inbreeding within populations all have significant effects on genetic differentiation among populations (Whitlock and McCauley, 1990).

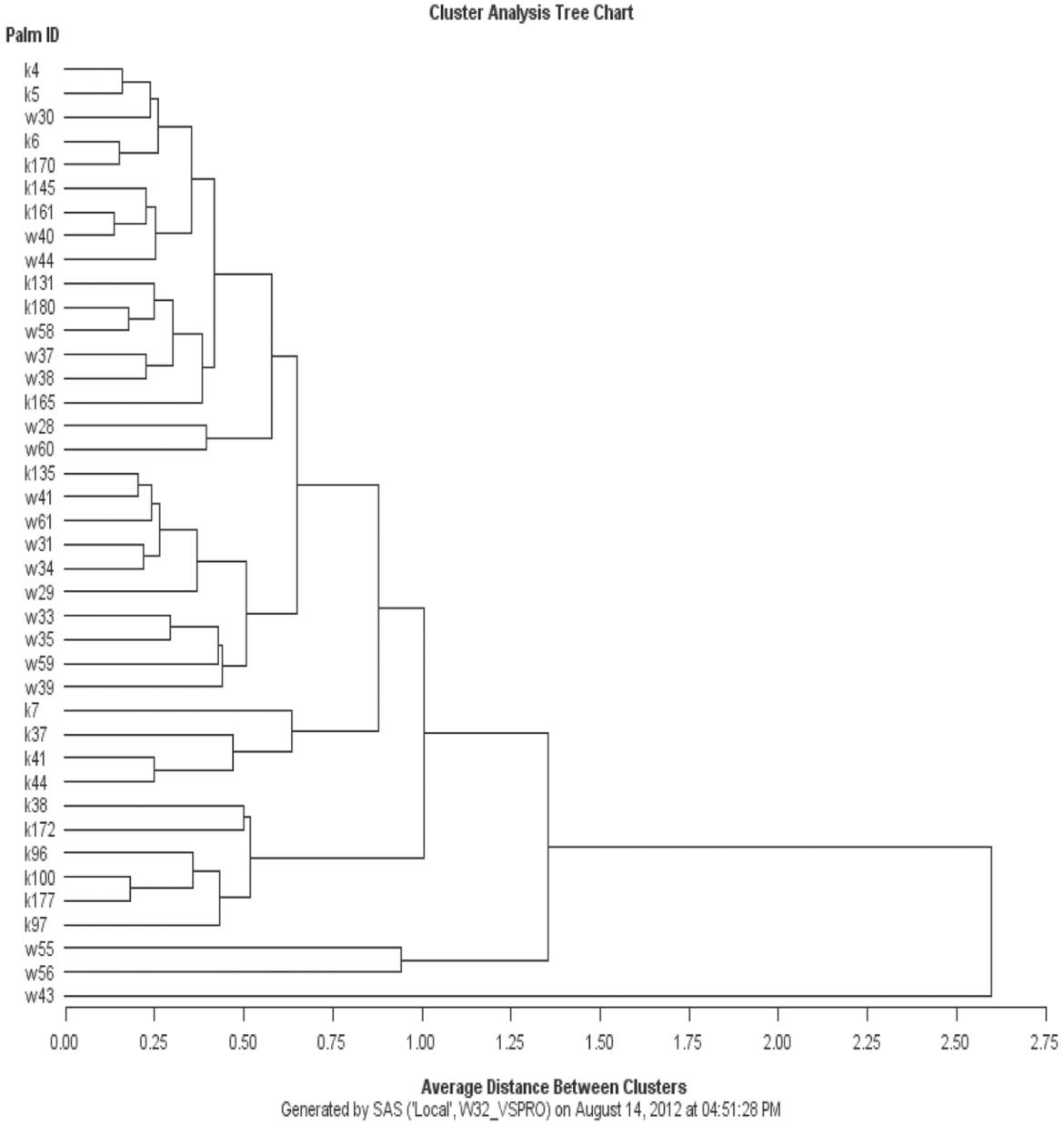

Fig. 3. Clustering of WCT (w) and Kuttiyadi (k) palms based on palm morphology and fruit components

Differentiation or speciation has mainly occurred during periods when habitats were fragmented (Bridle *et al.,* 2004). A high F_{ST} value (0.25) indicated pronounced genetic differentiation among the two studied populations.

In this study, the relatively high genetic differentiation and low levels of gene flow detected (Nm=0.529) strongly indicated that genetic drift had greatly affected the genetic composition of individual populations. In coconut, gene flow between populations is mostly *via* pollen movement. Between-population gene flow was limited by pollen and seed dispersal. Being primarilly an insect-pollinated plant, pollen dispersal is limited by the short flight ranges of the insects. Moreover, the limited seed dispersal contributes to the restricted gene flow and increases the probability that individuals in close physical

Table 4. Observed number of alleles, effective number of alleles and Shannon's information index for the 15 microsatellite loci

Sl. No	Locus	na*	ne*	I*
1	CnCir87	4.00	3.535	1.314
2	CnCirG11	2.00	1.993	0.691
3	CNZ04	3.00	2.373	0.963
4	CnCirE4	3.00	2.384	0.942
5	CnCir74	3.00	2.120	0.831
6	CnCirB6	2.00	1.152	0.257
7	CnCirE10	3.00	2.438	0.991
8	CNZ26	3.00	2.793	1.062
9	CNZ10	3.00	1.986	0.792
10	CnCirE2	3.00	2.354	0.963
11	CNZ17	1.00	1.000	0.000
12	CnCirB12	4.00	2.177	0.867
13	CNZ05	1.00	1.000	0.000
14	CN1H2	2.00	1.945	0.679
15	CnCir2	2.00	1.185	0.292
16	Mean	2.60	2.029	0.709
17	St. Deviation	0.91	0.707	0.395

*na= Observed number of alleles; *ne= Effective number of alleles *I= Shannon's information index

Table 5. Summary of F-Statistics and gene flow for the 15 microsatellite loci

Sl. No	Locus	FIS	FIT	FST	Nm*
1	CnCir87	-0.104	0.169	0.248	0.755
2	CnCirG11	0.345	0.483	0.210	0.940
3	CNZ04	0.076	0.131	0.059	3.970
4	CnCirE4	0.181	0.763	0.710	0.101
5	CnCir74	0.565	0.844	0.641	0.139
6	CnCirB6	-0.185	-0.084	-0.084	2.700
7	CnCirE10	0.477	0.480	1.004	61.360
8	CNZ26	-0.008	0.181	0.188	1.076
9	CNZ10	0.227	0.701	0.673	0.157
10	CnCirE2	0.961	0.135	0.043	0.450
11	CNZ17	–	–	0.000	–
12	CnCirB12	-0.305	0.000	0.234	0.816
13	CNZ05	–	–	0.000	–
14	CN1H2	-0.066	0.874	0.882	0.033
15	CnCir2	-0.124	-0.100	0.020	11.740
16	Mean	0.103	0.391	0.320	0.529

*Nm=gene flow estimated from Fst = 0.25(1-Fst)/Fst

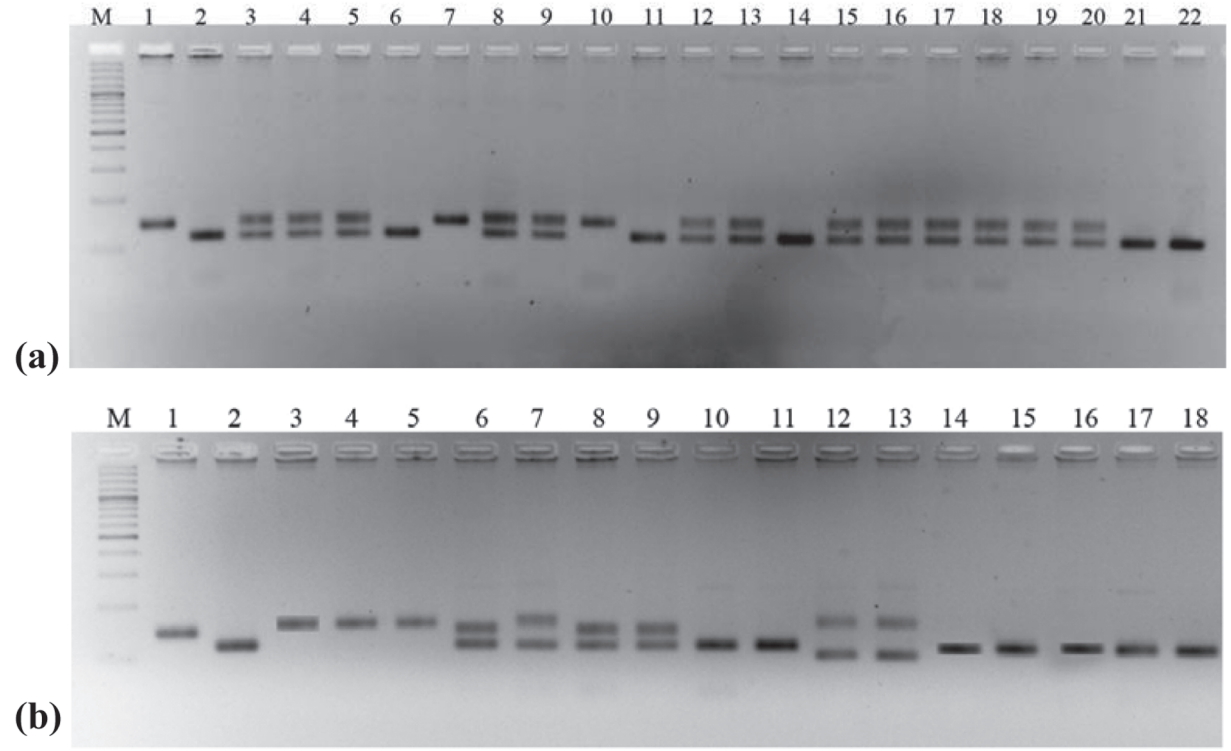

Fig. 4. SSR marker profile of Kuttiyadi and WCT palms using primer CNZ 04. (a) Kuttiadi palms: Lanes 1, 2 WCT palms; 3- 22: Kuttiyadi palms M: Ladder (100bp); (b) WCT palms: M: Ladder (100bp); Lanes 1, 2 Kuttiyadi palms; 3-18: WCT palms

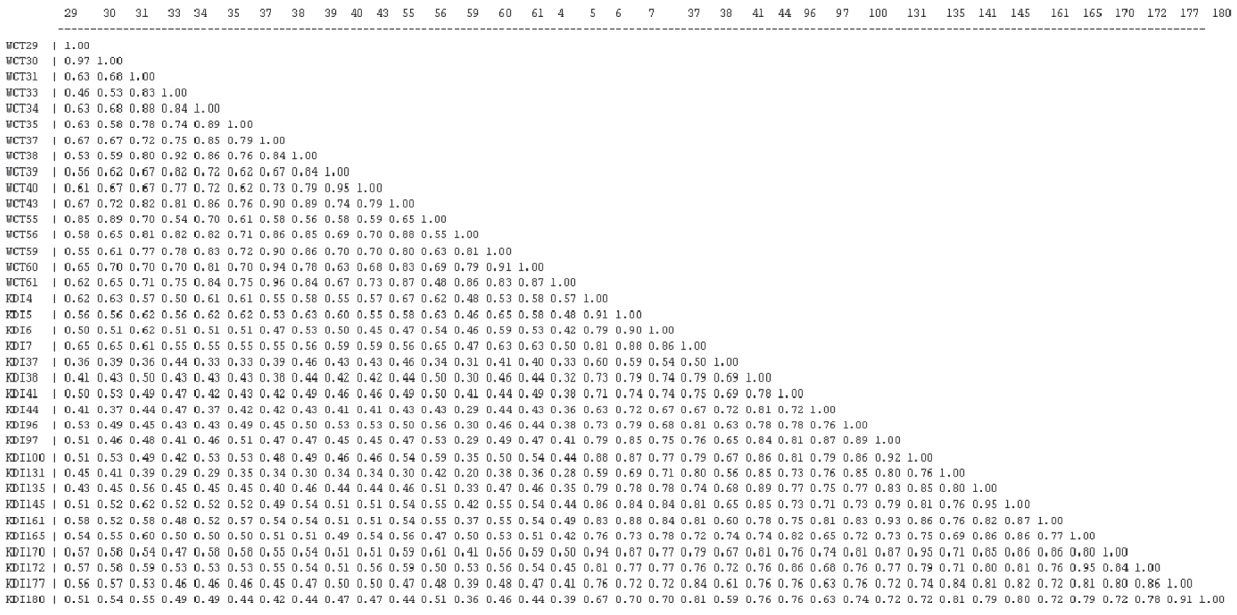

Fig. 5. Similarity index of Kuttiyadi and WCT palms based on Dice's coefficient

proximity mated with one another. Both effects would promote inter-population differentiation.

Knowledge of genetic diversity within and among the populations is crucial for conservation purposes, when interpreted within a broader ecological and organismic context. Considering the high level of genetic differentiation among populations, preservation of any one population would not protect all the variation in the species. Therefore several populations throughout the entire range should be considered for conservation.

Results of the Fisher's exact test for Hardy-Weinberg (HW) equilibrium across loci, considering heterozygote excess as the alternative hypothesis, showed that only four of the loci had significant ($p < 0.001$) departures from HW proportions.

Similarity index – Kuttiyadi and WCT palms

The similarity index based on Dice's coefficient, obtained after pair wise comparison of Kuttiyadi and CPCRI WCT samples are presented in Figure 5. The percentage similarity coefficient varied from 0.20 to 0.97 between the WCT and Kuttiyadi palms. Maximum similarity was observed between WCT29 and WCT30 (0.97) and minimum (0.20) was between WCT56 and Kuttiyadi 131.

Clusters based on dendrogram – Kuttiyadi and WCT palms

Cluster analysis was performed using a software NTSYS in order to get a dendrogram (Fig. 6). The clustering was based on UPGMA. On clustering it was found that the WCT and Kuttiyadi formed two major clusters at 0.40 similarity coefficient. Cluster 1 contains WCT samples and cluster 2 contains the Kuttiyadi samples. 2-D principal co-ordinate analysis was performed for both WCT and Kuttiyadi samples (Fig. 7). From the 2-D plot it was clear that all the WCT and Kuttiyadi differed with each other in genetic makeup.

Genetic diversity within populations

The fixation index ranged (f) from 0.033 (Kuttiyadi) to 0.21 (WCT) with a mean of 0.132

Table 6. Expected and observed heterozygosity and fixation index in the two populations

Population	He	Ho	f
West Coast Tall	0.343	0.273	0.210
Kuttiyadi	0.271	0.262	0.033
Mean	0.307	0.267	0.132

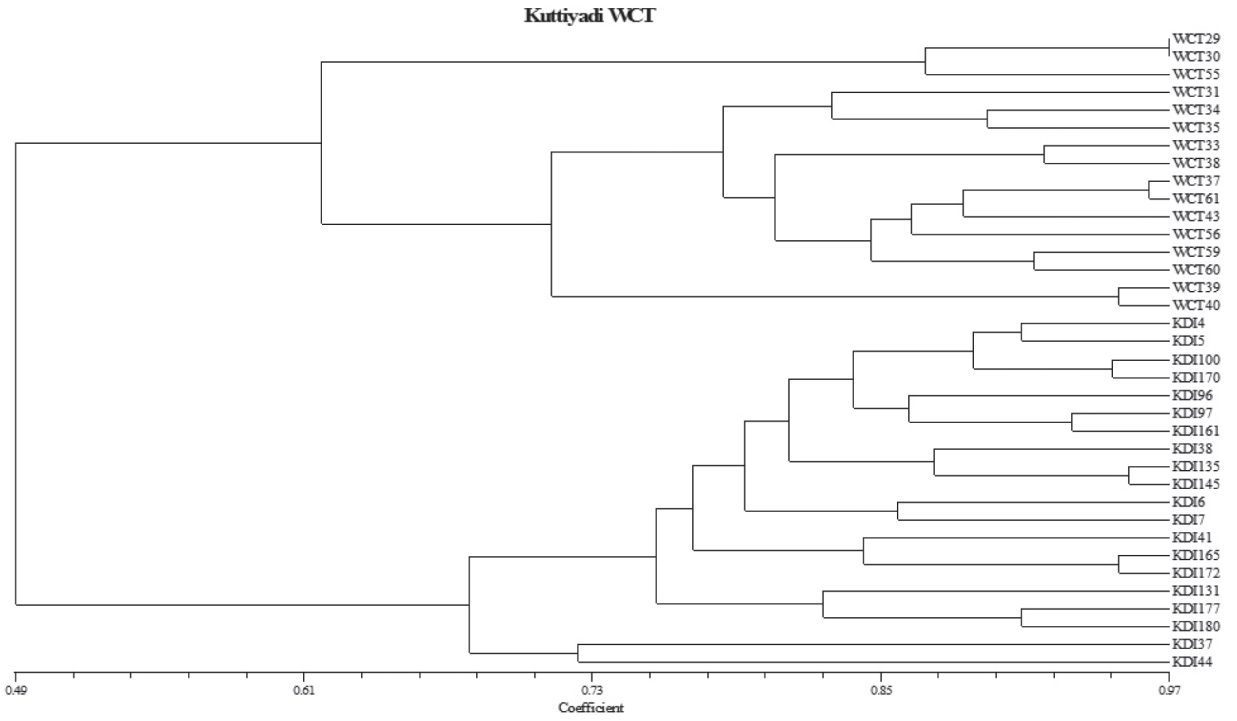

Fig. 6. UPGMA dendrogram of Kuttiyadi and WCT palms based on Dice's coefficient

(Table 6). The mean observed heterozygosity was same as expected and fixation index (f) was almost equal to zero indicating that the population is randomly mating. Specifically, observed and expected heterozygosity will be the same if the level of external gene flow is the same across all populations.

Population structure

Using STRUCTURE program, the population structure of the coconut populations was investigated by estimating the number of genetically distinct populations. An *ad hoc* statistical analysis, which was based on the second-order rate of change of the likelihood function with respect to K (ΔK) (Evanno *et al.,* 2005), was used to calculate the most appropriate K value using Structure Harvester v0.6.92. The log probability data of data (L (K)) for the admixture and correlated frequencies model under exhaustive sampling (averaged over 15 replicates) obtained in STRUCTURE package is shown in Table 7. The highest L (K) averaged over replicates running for each value of K (K from 1 to 20) was observed for K=2 (Table 7) indicating that both the populations were highly distinct.

Microsatellite marker technique has been used successfully to characterize the genetic diversity of the coconut population (Rivera *et al*., 1999; Teulat *et al*., 2000; Perera *et al*., 2000; Perera *et al*. 2003; Meerow *et al*., 2003; Rajesh *et al*., 2008a, 2008b). These markers are reproducible, enabling their parallel analysis in different laboratories, and exchange of the resulting data. Microsatellite forms an ideal marker system creating complex banding patterns by simultaneously detecting multiple DNA loci. PCR-based SSR markers are becoming the marker of choice for fingerprinting and genetic diversity studies for a wide range of plants (Gupta *et al.,* 1996).

West Coast Tall (WCT) developed on the West coast of India and came to be known by the region where it is cultivated. Though the origin of WCT is not traceable to any particular area, it is obvious that sea journey was involved in its spread. The cultivar over the years was taken from coastal region to the interior areas, which also resulted in further adaptation and diversity in the cultivar. Such adapted WCT populations in certain localities are designated with local names by farmers to differentiate it from other WCT populations.

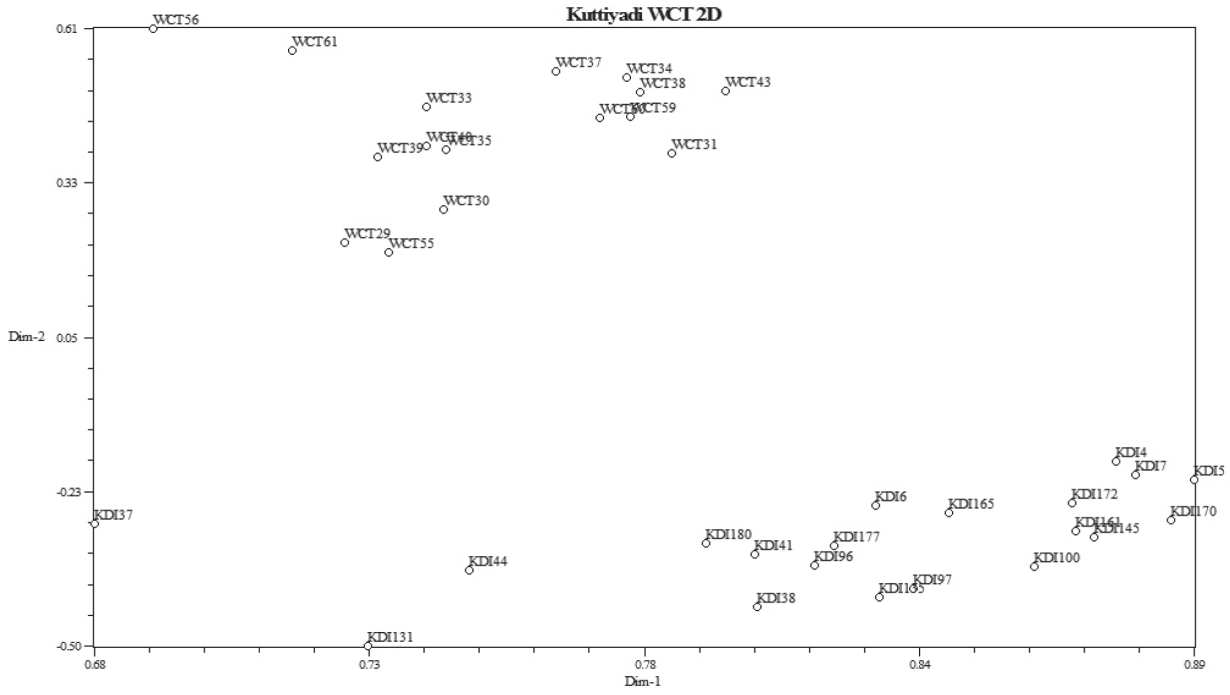

Fig. 7. 2-D Principal co ordinate analysis plot of Kuttiyadi and WCT palms

This work presents the use of microsatellite DNA markers to investigate the level of genetic diversity, distribution of genetic variation, and genetic relatedness in coconut genotypes in WCT and Kuttiyadi palms. A key observation is the large number of alleles detected with coconut microsatellites providing a multi-allelic, co-dominant marker system. According to Harries (1995), the difference between coconut populations and the variability within each population can be accounted for by the natural evolution of the wild type, its widespread dissemination by floatation, the selection for high liquid endosperm content of the domestic type, its distribution by man, the introgression of these two types, the predominance of the intermediate recombinant forms, the resegregation of the extreme types and the selection of minor variants. This is distinctly seen in habitats which are drastically disturbed by man and serves to prevent the formation of stable coconut varieties, because each type is able to cross, backcross, sib and self pollinate, so that there is a repeated

Table 7. Summary of STRUCTURE results

K	Reps	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	|Ln"(K)|	Delta K
1	10	-766.34	0.6346	NA	NA	NA
2	10	-548.08	0.7540	218.26	199.32	264.366770
3	10	-529.14	6.6657	18.94	28.34	4.251617
4	10	-538.54	34.9256	-9.40	13.34	0.381955
5	10	-534.60	49.6931	3.94	17.23	0.346728
6	10	-547.89	34.1967	-13.29	16.48	0.468226
7	10	-544.70	30.2653	3.19	18.67	0.616877
8	10	-560.18	37.6139	-15.48	23.04	0.612539
9	10	-552.62	46.6285	7.56	46.36	0.994241
10	10	-591.42	81.1931	-38.80	NA	NA

re-assortment of genes at each generation. In the same way, the present study has given a result where both the WCT samples are different according to the region by their alleles. The WCT and Kuttiyadi palms may be differed due to the climatic change, pH of the soil, annual rainfall or any other environmental factors or even human involvement. It can be also due to the cross or self pollination of the progenies resulting in the formation of new type of breeds which can or cannot give a better yielding variety of coconut. The total genetic variation of a species is likely to be distributed among populations as the impact and direction of natural selection varies from one to another, due to environmental variation and genetic drift (Lawrence and Rajanaidu, 1985). Therefore with germplasm conservation programmes, it is imperative to accurately measure the amount of genetic diversity and its distribution within and between populations. To these ends, molecular markers provide an efficient and unbiased estimate of these statistics, free of environment effects. The microsatellites used in this study appeared to possess a significant potential in this respect.

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