Diversity in Mohachao Narel, *a Sweet Endosperm Coconut (*Cocos nucifera *L.) Population from Maharashtra, India*

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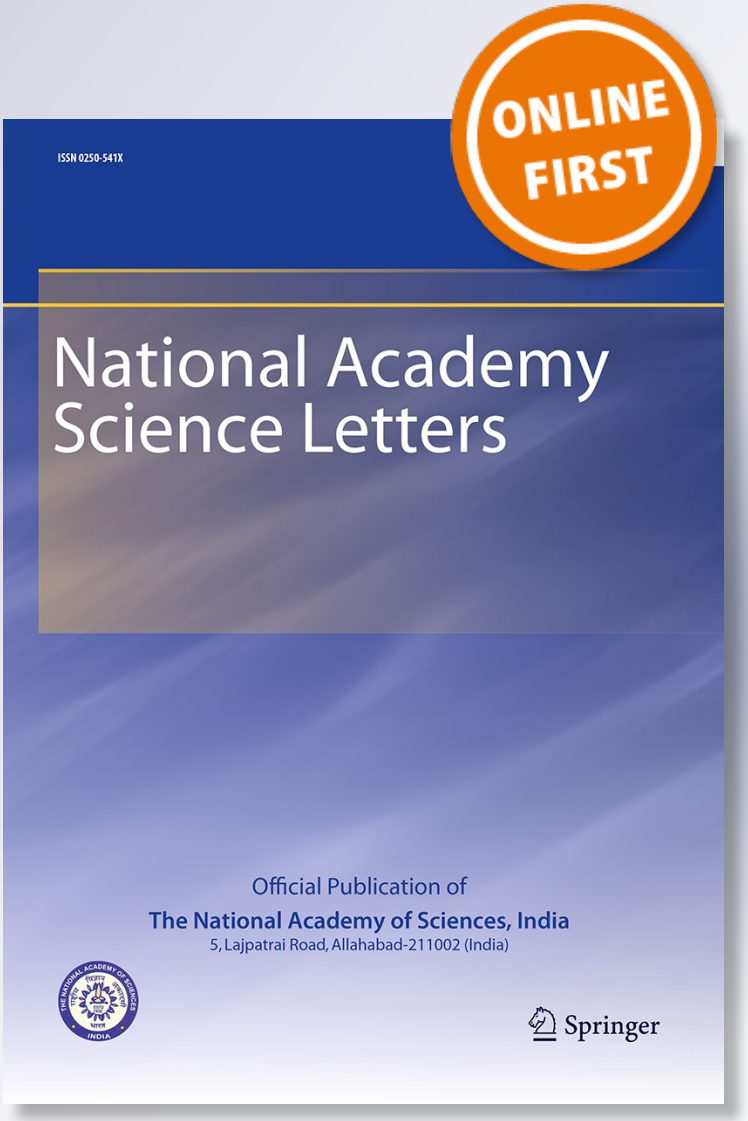

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RESEARCH ARTICLE

Diversity in *Mohachao Narel*, a Sweet Endosperm Coconut (*Cocos nucifera* L.) Population from Maharashtra, India

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Abstract '*Mohachao Narel*', a coconut variant characterized by sweet and soft kernel with less fibre content, has been reported from Guhaghar taluk of Ratnagiri district of Maharashtra State in India. Farmers of the area get a premium price for sweet endosperm nuts and the sweet kernel is mainly consumed directly. A total of 28 mother palms possessing nuts with sweet kernel have been identified in these areas. The number of nuts with sweet endosperm per bunch varies from 10 to 77 percent in these 28 palms. In this study, 12 palm characters including vegetative as well as reproductive characters, 14 fruit characters and 14 SSR loci, distributed uniformly throughout the coconut genome, were used to assess the genetic diversity of these palms. Palms exhibited 18 and 33 % coefficient of variation in vegetative and reproductive characters respectively. In the case of vegetative characters, highest variation was detected in plant height followed by number of leaf scars per meter. Among the reproductive characters, number of female flowers per bunch possessed highest amount of variation followed by number of nuts per bunch. Among the 14 fruit characters studied, high variation was observed in the weight of embryo, husk weight, copra weight and oil content. The 14 SSR loci detected a total of 35 alleles with an average of 2.5 alleles per primer. All the primers were 100 % polymorphic. The analysis microsatellite revealed that palms of sweet kernel type had only 45 % similarity among them indicating the existence of a high level of genetic diversity between the palms. The results can pave the way for devising strategies for conservation and management of the sweet kernel coconut population and their use in future breeding programmes.

Keywords Sweet kernel coconut · Palm character · Fruit character · SSR

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Introduction

Due to its multifarious uses, coconut (*Cocos nucifera* L.) symbolizes an important plant for the rural communities in developing tropical countries. It provides the basis for food production and by-product utilization in addition to its uses in industrial processing [1]. Every part of this 'tree of life' is beneficial to mankind in one manner or other, the most extensively used part being the endosperm and its derivatives. Coconut endosperm, which is hard and white in colour, is rich in proteins, amino acids, sugars, vitamins, minerals and growth factors. The endosperm is mostly used for extraction of coconut oil and culinary purposes.

Certain coconut palms produce nuts containing soft, jelly-like endosperm, called *Makapuno* in Philippines [2], which has been commercially exploited for product diversification especially in confectionary industries. Mutants similar to *Makapuno*-type have also been reported from other coconut-growing regions. Some of them are Coco Gra (Seychelles), Kopyor (Indonesia), *Thairu* or *Nei Thengai* (India), Dikiri Pol (Sri Lanka), Mapharao Khati (Thailand), Sap (Vietnam), Niu Garuk (Papua New Guinea), and Pia (Polynesia) [3–5].

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The *Makapuno* trait results in abortion of embryo and is known to occur because of the effect of lethal recessive gene [6].

Recently, another variant with sweet and soft endosperm, named '*Mohachao Narel*' has been reported from Guhaghar taluk of Ratnagiri district of Maharashtra State in India [7, 8]. Studies on fruit component traits of sweet and normal nuts of this population revealed that nuts possessing sweet endosperm types had slightly less fruit and husked fruit weight compared to normal nuts [8]. Shell thickness was similar in both, but the shell weight was more in normal types. Likewise, endosperm thickness was similar in both, but the endosperm weight was more in normal types [8]. Copra weight and copra recovery was more in normal nuts compared to sweet endosperm types at similar age of maturity (Fig. 1) [8]. Embryo weight of sweet endosperm nuts was significantly lower than normal endosperm. Total soluble sugars (Brix values) were same in both type of nuts, but organoleptic test showed that water in sweet endosperm nuts was poor in taste. Sweet endosperm nuts get a premium price and are mainly used for raw consumption [8].

Twenty-eight palms of '*Mohachao Narel*' were located in Guhaghar taluk of Ratnagiri district (N17° 28′55″ to N17° 29′50″ and E73° 11′03″ to E73° 19′50″) and one palm was near the Ratnagiri city (N16° 58′15.3″ and E73° 19′50.4″) in the state of Maharashtra. These 29 tall palms, with the stem height ranging from 7 m to 27 m, are aged between 40 and 80 years and found randomly distributed in the population. The number of nuts with sweet endosperm per bunch varied from 10 to 77 percent in different palms [8].

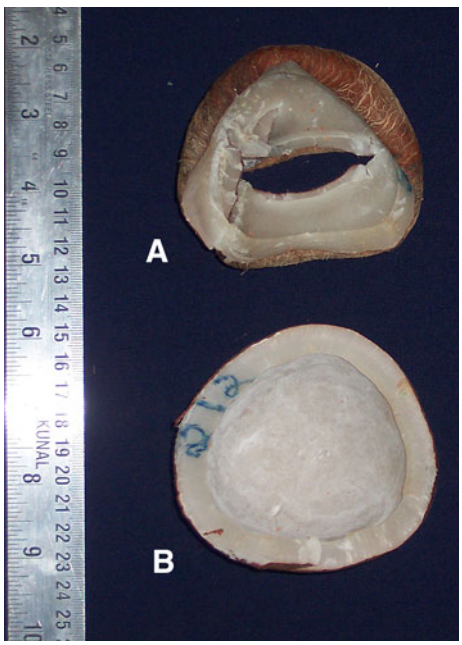

Fig. 1 **A** Sweet kernel copra, **B** normal kernel copra

Assessment of genetic diversity of these rare palms will aid in effective collecting and gene-banking strategies. Diversity analysis in coconut palm has been carried out earlier using morphological traits, biochemical and molecular markers. Molecular markers have proven to be more sensitive, rapid, cost-effective and reliable techniques for assessment of genetic diversity in coconut [9]. Molecular characterization of coconut accessions have been performed with a repertoire of techniques viz. RAPD, RFLP, AFLP, ISSRs and Microsatellites/SSRs. Among these techniques, SSRs were found to be the most efficient in the diversity analysis of coconut varieties. The present work was undertaken to estimate the genetic diversity among a population of coconut variants, possessing sweet kernel endosperm from Maharashtra, based on vegetative, reproductive and fruit component traits; as well as using SSR technique.

Materials and Methods

Study Area and Plant Materials

A field survey was conducted in the initial stage of the study, in Ratnagiri region of Maharashtra. A total of 29 mother palms possessing nuts with sweet kernel have been identified in this area. The identified palms were marked for further studies. Mean age of the palms was 50 years and population age ranged between 40 and 80 years. In this study, 12 palm characters including vegetative as well as reproductive characters, 14 fruit characters and 14 SSR loci distributed uniformly throughout the coconut genome were used to assess the genetic diversity in 28 sweet kernel palms.

Study of Morphometric Traits

The morphological characters of the palms were studied from the location itself. The vegetative as well as reproductive traits viz., height of plant (cm), girth of plant (cm), number of leaves, length of petiole (cm), length of leaf (cm), number of leaf scars per meter, length of internode (cm), length of inflorescence (cm), length of inflorescence stalk (cm), number of spikelet per inflorescence, number of female flowers per inflorescence, number of nut per bunches were recorded on the mother palms as described in coconut descriptors [10].

As many mature nuts as possible from each of the 28 palms were harvested, for studying the fruit component traits. The collected nuts were transported to CPCRI, Kasaragod. After 2 weeks of natural drying, the nuts were used for the fruit analysis. Fourteen fruit component measurements like fruit shape index, fruit weight (g),

average husk thickness (cm), percentage of husk to whole fruit weight (husk %), nut weight (g), nut shape index, shell weight (g), shell thickness (cm), endosperm thickness (cm), endosperm weight (g), copra weight (g), copra recovery (g), weight of embryo (g), and oil percentage were recorded to study the variability in the population.

Shape of the fruits and nuts was quantified as ratio between equatorial circumference and polar circumference. The perfect round ones will have the value of one while the elongated ones will have value smaller than one and the broader ones will have value more than one. Weight of the husk was estimated by deducting nut weight (with water) from fruit weight. Husk percentage was estimated on dry weight basis. The mean, standard deviation and coefficient of variation were computed and the data was subjected to analysis of variance as per standard procedure. Clustering analysis was carried out using SAS software.

Microsatellite Analysis

Spindle leaf samples of 26 coconut genotypes possessing sweet kernel collected from Ratnagiri region of Maharashtra, were used for molecular study. Leaf samples were also collected from three genotypes possessing normal nuts from the same location to serve as control. DNA was extracted from spindle leaves of palms following the SDS-protocol [11].

A total of 14 highly polymorphic SSR primer pairs specific to coconut were used in the present study the details of which are given in Table 1. Microsatellite analysis was conducted as per the standardized procedure [12, 13]. In brief, PCR was 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), 1× buffer [10 mM Tris–Hcl

Table 1 Details of SSR primers, their banding patterns, % polymorphism and PIC value

S. No.	Primer name	Sequence (5′–3′)	Annealing temperature (°C)	Total bands	Polymorphic bands (nos.)	% Polymorphism	PIC value
1.	CAC2F	AGCTTTTTCATTGCTGGAAT	51	2	2	100	0.27
	CAC2R	CCCCTCCAATACATTTTTCC					
2.	CAC3F	GGCTCTCCAGCAGAGGCTTAC	56	3	3	100	0.48
	CAC3R	GGGACACCAGAAAAAGCC					
3.	CAC4F	CCCCTATGCATCAAAACAAG	55	3	3	100	0.52
	CAC4R	CTCAGTGTCCGTCTTTGTCC					
4.	CAC6F	TGTACATGTTTTTTGCCCAA	56	3	3	100	0.47
	CAC6R	CGATGTAGCTACCTTCCCC					
5.	CAC8F	ATCACCCCAATACAAGGACA	60	3	3	100	0.64
	CAC8R	AATTCTATGGTCCACCCACA					
6.	CAC10F	GGAACCTCTTTTGGGTCATT	56	2	2	100	0.29
	CAC10R	GATGGAAGGTGGTAAGGCTCC					
7.	CAC13F	GGGTTTTTAGATCTTCGGC	56	2	2	100	0.33
	CAC13R	CTCAACAATCTGAAGCATCGG					
8.	CnCirH9F	CACAATCCTTACATCAAA	56	3	3	100	0.54
	CnCirH9R	TCTCAAGTTCTTACAGCAGT					
9.	CnCir56F	AACCAGAACTTAAATGTCG	56	2	2	100	0.39
	CnCir56R	TTTGAACTCTTCTATTGGG					
10.	CNZ02F	CTCTTCCCATCATATACCAGC	58	2	2	100	0.37
	CNZ02R	ACTGGGGGGATCTTATCTCTG					
11.	CNZ03F	CATCTTTCATCATTTAGCTCT	54	2	2	100	0.49
	CNZ03R	AAACCAAAAGCAAGGAGAAGT					
12.	CNZ04F	TATATGGGATGCTTTAGTGGA	60	3	3	100	0.60
	CNZ04R	CAAATCGACAGACATCCTAAA					
13.	CNZ06F	ATACTCATCATCATACGACGC	57	2	2	100	0.47
	CNZ06R	CTCCCACAAAATCATGTTATT					
14.	CNZ10F	CCTATTGCACCTAAGCAATTA	54	3	3	100	0.36
	CNZ10R	AATGATTTTCGAAGAGAGGTC					
Total				35	35	100	

(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., India). 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 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 3 µl of 6× loading buffer (10 mM Tris–HCl pH 7.6, 0.03 % per cent each of xylene cyanol FF and bromophenol blue, 60 % glycerol, 60 mM EDTA) was added to each of the amplified product. The amplified product were run on 3.0 per cent agarose gel, stained with ethidium bromide and was visualized in a gel documentation system.

Table 2 Palm vegetative characters

S. No.	Palm no.	Plant height (cm)	Girth (cm)	No. of leaves	Petiole length (cm)	Leaf length (cm)	Leaf scars	Inter node length (cm)
1.	NSD 1	692	104	29	157	423	12	9
2.	NSD 2	1,700	98	33	114	374	12	9
3.	NSD 3	1,654	115	33	110	390	9	9
4.	NSD 4	1,434	94	29	90	292	13	8
5.	NSD 5	1,500	90	28	105	360	14	7.2
6.	NSD 6	1,965	100	28	88	294	14	6.7
7.	NSD 7	2,130	105	30	86	310	13	8.3
8.	NSD 8	1,870	120	30	85	292	11	8.2
9.	NSD 9	1,750	78	32	87	310	9	11
10.	NSD 10	2,180	88	30	87	330	19	5
11.	NSD 11	2,000	101	35	105	432	13	8
12.	NSD 12	2,190	117	32	115	390	14	7.8
13.	NSD 13	1,350	70	27	122	350	12	6.9
14.	NSD 14	1,050	76	35	112	318	10	10
15.	NSD 15	2,250	114	35	170	350	14	7.5
16.	NSD 16	2,227	95	30	98	360	18	5
17.	NSD 17	868	81	28	106	352	18	5.5
18.	NSD 18	1,555	89	26	105	350	20	4.8
19.	NSD 19	1,370	82	28	90	390	21	6.5
20.	NSD 20	1,080	105	29	119	386	20	6
21.	NSD 21	2,035	88	32	116	395	14	6.4
22.	NSD 22	1,589	90	30	110	360	12	8.2
23.	NSD 23	2,211	98	28	98	310	14	7.6
24.	NSD24	1,660	85	28	87	388	14	7.5
25.	NSD 25	1,812	91	27	95	415	18	5.7
26.	NSD 26	2,675	107	28	90	410	11	7.4
27.	NSD 27	1,991	109	30	85	335	18	6.6
28.	NSD 28	1,760	87	35	93	363	14	6.5

Table 3 Diversity in palm vegetative characters

S. No.	Character	Average	SD	CV	Min	Max
1.	No. of leaves	30.18	2.68	8.88	26.00	35.00
2.	Leaf length (cm)	358.18	40.79	11.39	292.00	432.00
3.	Girth (cm)	95.61	13.00	13.60	70.00	120.00
4.	Petiole length (cm)	104.46	20.41	19.54	85.00	170.00
5.	Inter-node length (cm)	7.33	1.50	20.51	4.80	11.00
6.	Leaf scars	14.32	3.39	23.66	9.00	21.00
7.	Plant height (cm)	1,733.86	460.05	26.53	692.00	2,675.00

Table 4 Diversity in palm reproductive characters

S. No.	Character	Average	SD	CV	Min	Max
1.	Inflorescence length(cm)	89.81	14.53	16.18	66.00	123.00
2.	Stalk length (cm)	41.41	6.42	15.50	30.00	56.00
3.	Spikelet/inflorescence	33.81	6.25	18.47	16.00	43.00
4.	Female flower/inflorescence	16.81	13.38	79.55	7.00	81.00
5.	Nuts/bunch	10.11	2.97	29.42	4.00	17.00
6.	Percentage of sweet kernel	46.00	17.53	38.11	10.00	77.00

Table 5 Palm reproductive characters

S. No.	Palm no.	Inflorescence length (cm)	Stalk length (cm)	Spikelet/ inflorescence	Female flower/ inflorescence	Nuts/ bunch	Percentage of sweet kernel
1.	NSD 1	108	56	33	81	17	52
2.	NSD 2	101	50	40	20	12	50
3.	NSD 3	89	45	43	20	15	44
4.	NSD 4	66	31	16	20	12	70
5.	NSD 5	75	45	34	13	7	50
6.	NSD 6	87	38	29	15	12	50
7.	NSD 7	109	40	39	12	12	50
8.	NSD 8	77	41	32	10	13	54
9.	NSD 9	90	41	34	17	10	55
10.	NSD 10	94	42	36	12	14	14
11.	NSD 11	123	50	30	15	9	25
12.	NSD 12	97	40	40	18	10	62
13.	NSD 13	106	46	35	16	12	33
14.	NSD 14	85	35	36	19	12	60
15.	NSD 15	96	50	40	16	11	29
16.	NSD 16	67	30	26	12	8	44
17.	NSD 17	89	44	25	9	6	36
18.	NSD 18	77	35	31	7	4	25
19.	NSD 19	78	35	26	13	9	30
20.	NSD 20	89	50	30	8	5	10
21.	NSD 21	96	40	42	20	12	45
22.	NSD 22	89	41	41	12	8	57
23.	NSD 23	112	45	38	15	10	67
24.	NSD 24	86	41	32	11	7	10
25.	NSD 25	102	40	35	15	9	64
26.	NSD 26	68	32	39	13	8	54
27.	NSD 27	78	37	29	19	10	19
28.	NSD 28	81	39	36	13	9	77

Each band generated by SSR primers were 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 palm. Based on the number of polymorphic bands, percentage polymorphism was calculated for each primer.

Polymorphism information content (PIC) for each SSR was calculated by the following equation:

$$\text{PIC} = 1 - \sum X_i^2$$

where X_i is the relative frequency of the *i*th allele of the SSR loci and is summed over *n* alleles.

The binary data scored was used to construct a dendrogram. The genetic associations among palms were evaluated

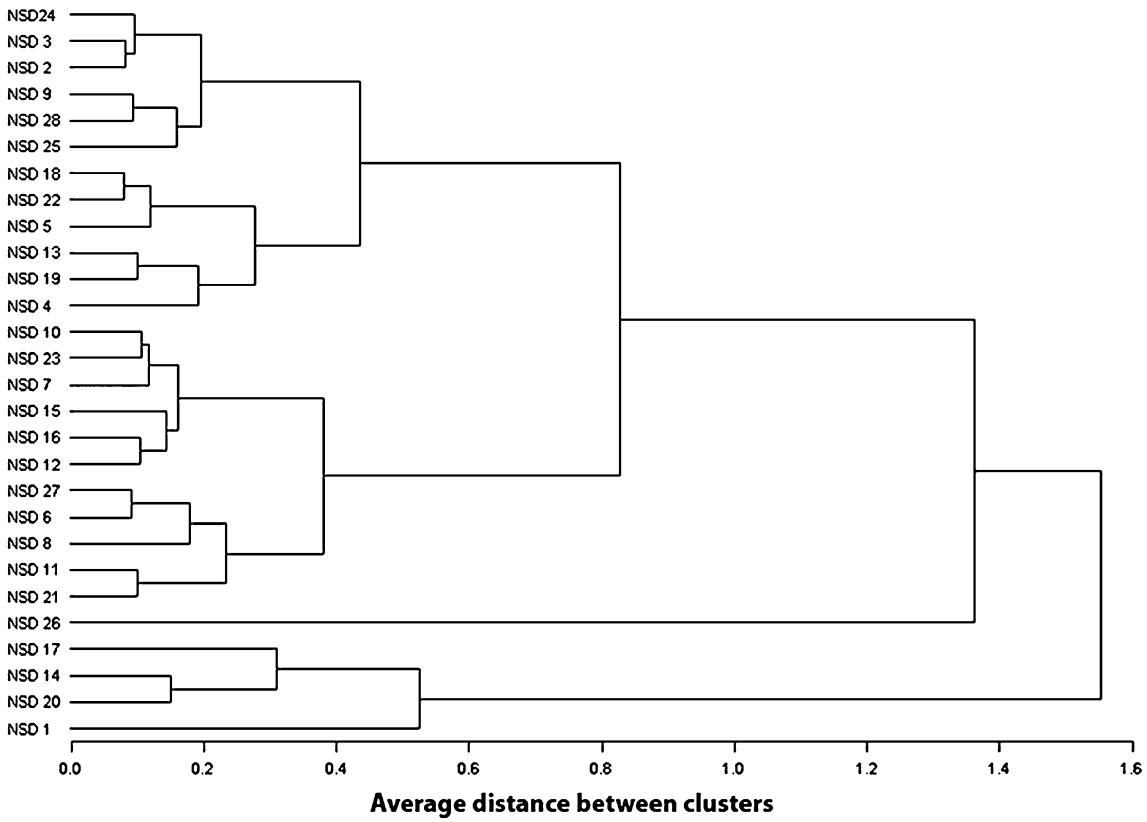

Fig. 2 Dendrogram representing relationships among the sweet kernel coconut genotypes based on average linkage obtained using palm morphological characters

Table 6 Clusters based on palm characters

Cluster1	Cluster2	Cluster3	Cluster4
NSD 3	NSD 8	NSD 1	NSD 26
NSD24	NSD 6	NSD 17	
NSD 2	NSD 27	NSD 14	
NSD 9	NSD 11	NSD 20	
NSD 28	NSD 21		
NSD 25	NSD 7		
NSD 13	NSD 10		
NSD 19	NSD 12		
NSD 4	NSD 23		
NSD 5	NSD 16		
NSD 18	NSD 15		
NSD 22			

by calculating the Dice's similarity coefficient for pair wise comparisons based on the proportions of shared bands produced by the primers. Similarity matrix was generated using the NTSYS-PC software, version 2.02 [14]. The similarity coefficients were used for cluster analysis and dendrogram was constructed by the unweighted pair-group method (UPGMA) [15].

Results and Discussion

The present study was undertaken to evaluate the extent and range of genetic diversity in 28 palms of a coconut population possessing sweet endosperm, based on vegetative, reproductive and fruit component traits; as well as using highly polymorphic SSR primers specific to coconut. Wide variability was observed for most of the vegetative and reproductive traits studied as reflected in the coefficient of variation. In the case of vegetative characters, highest variation was detected in plant height followed by number of leaf scars per meter and inter-node length. The number of leaves showed least variability among palms. Plant height ranged between 692 cm (NSD1) and 2,675 cm (NSD26). Maximum number of leaf scars per meter (21) was observed in NSD19 and minimum (9) in NSD3 and NSD9. The highest value (11 cm) for inter-node length was observed in NSD9 and the lowest (4.8 cm) in NSD18 (Tables 2, 3). Among the reproductive characters, number of female flowers per inflorescence had the highest amount of variation followed by percentage of sweet kernel fruits and then by number of nuts per bunch. Inflorescence stalk length showed low variance among the palms (Table 4). Maximum number of female flower per inflorescence (81)

Table 7 Correlation coefficient of palm characters with sweet kernel

Palm characters	Correlation	Palm characters	Correlation	Palm characters	Correlation
Plant height	0.078	Leaf scars	−0.500*	Spikelet/inflorescence	0.162
Girth	0.030	Internode length	0.389	Female flower/inflorescence	0.182
Leaves	0.168	Inflorescence length	−0.055	Bunches	0.276
Petiole length	−0.084	Stalk length	−0.227	Nuts/bunch	0.260
Leaf length	−0.209				

*Significant correlation at 1 % level

Table 8 Diversity in fruit and nut characters

S. No.	Characters	Mean	SD	CV	Max	Min
1.	Nut shape index	0.94	0.03	3.52	1.01	0.89
2.	Endosperm thickness	1.32	0.07	5.34	1.43	1.17
3.	Fruit shape index	0.85	0.05	5.70	0.92	0.76
4.	Copra recovery	49.64	5.86	11.80	63.86	35.78
5.	Husk %	45.79	5.98	13.06	55.34	35.44
6.	Shell thickness	0.37	0.05	13.44	0.53	0.29
7.	Husk thickness	3.05	0.47	15.59	3.95	2.38
8.	Shell weight	137.60	24.81	18.03	181.25	74.37
9.	Endosperm weight	306.12	56.96	18.61	422.25	175.36
10.	Oil %	46.59	9.28	19.92	71.80	18.20
11.	Embryo weight	0.09	0.02	20.21	0.15	0.07
12.	Nut weight	547.50	116.03	21.19	759.63	292.45
13.	Fruit weight	1,008.23	224.26	22.24	1,452.19	584.70
14.	Copra weight	151.94	34.10	22.44	226.25	86.10

was recorded in NSD1 and minimum (7) in NSD18. Percentage of fruits in a bunch with sweet kernel varied among palms from 77 in NSD28 to 10 in NSD24 and NSD20. Number of nuts per bunch also showed significant variation, ranging between 4 in NSD18 and 17 in NSD1 (Tables 5, 4). Based on palm morphological characters, an attempt was made to group the palms using average linkage between genotypes. Four groups could be visualized from the dendrogram generated by SAS software (Fig. 2; Table 6). The average coefficient of variation before clustering was 23 for the palm characters. In the first group, there were 12 genotypes with an average coefficient variation of 19. The second group consisted of 11 genotypes and had coefficient variation of 17. NSD 26, the tallest among all, was the only member in the third group. Fourth group had four members of diverse types with an average coefficient variation of 30.8. Female flower production was reported as one of the factors contributing to genetic divergence in coconut [16]. Inflorescence traits were found as major factors contributing to divergence of coconut genotypes [17]. Similarly, the length of inflorescence was reported to contribute towards variation among the coconut populations of Indian Ocean Island [3]. These findings are in themselves indication of the variability existing in different coconut population for different traits. The palm morphological characters exhibited lot of variations among the individual palms of '*Mohachao Narel*' making it difficult to correlate any of the character with percentage of sweet kernel fruits. A significant negative correlation of 0.50 magnitude was found between number of leaf scars and percentage of sweet kernel fruits, indicating that the lesser the number of leaf scars (a character of tall palm stature) the higher the percentage of sweet kernel fruits (Table 7).

Among the 14 fruit characters studied in 26 genotypes, high variation was observed in copra weight, fruit weight, nut weight, embryo weight, oil content, endosperm weight and shell weight (Table 8). Copra weight was ranging from 86 g in NSD1 to 226 g in NSD11. Fruit weight ranged from 584.7 g in NSD4 to 1,452.2 g in NSD3. Husked fruit (Nut) weight ranged from 292.5 g in NSD4 to 759.6 g in NSD11. Embryo weight ranged between 0.07 g in NSD12 and 0.15 g in NSD26. Percentage of oil also showed variation with maximum yield of 71.8 % in NSD27 and minimum of 18.2 % in NSD16. Endosperm weight ranged from 175.4 g in NSD1 to 422.3 g in NSD11. Shell weight

Table 9 Fruit characters of individual palms

S. No.	Palm	Fruit weight	Fruit shape index	Husk thickness	Husk %	Embryo weight
1.	NSD 1	704.60	0.89	2.38	51.94	0.09
2.	NSD 2	1,284.75	0.86	3.91	55.34	0.08
3.	NSD 3	1,452.19	0.90	3.90	50.97	0.11
4.	NSD 4	584.70	0.85	2.95	49.98	0.07
5.	NSD 5	812.38	0.90	2.53	35.44	0.08
6.	NSD 6	1,124.45	0.92	3.05	46.58	0.10
7.	NSD 7	1,202.33	0.79	2.99	55.31	0.08
8.	NSD 8	1,056.00	0.84	3.37	46.37	0.10
9.	NSD 9	910.58	0.79	2.55	41.05	0.08
10.	NSD 10	1,212.57	0.83	3.14	43.16	0.12
11.	NSD 11	1,189.38	0.83	2.79	36.13	0.13
12.	NSD 12	927.08	0.79	2.95	47.30	0.07
13.	NSD 13	831.67	0.91	2.86	36.95	0.10
14.	NSD 14	681.00	0.83	2.55	38.62	0.08
15.	NSD 15	829.07	0.83	2.97	43.00	0.09
16.	NSD 16	663.33	0.76	2.68	41.66	0.08
17.	NSD 17	1,158.86	0.82	2.82	38.94	0.11
18.	NSD 18	1,107.00	0.80	2.50	39.41	0.11
19.	NSD 21	1,119.55	0.91	3.00	50.21	0.10
20.	NSD 22	1,114.14	0.91	3.83	50.62	0.09
21.	NSD 23	1,302.67	0.82	2.58	50.44	0.07
22.	NSD 24	930.00	0.76	3.69	42.84	0.10
23.	NSD 25	1,192.64	0.85	3.95	52.81	0.10
24.	NSD 26	775.92	0.83	2.91	49.24	0.15
25.	NSD 27	984.41	0.87	2.94	50.33	0.10
26.	NSD 28	1,062.62	0.92	3.42	45.78	0.08

ranged from 74.4 g in NSD4 to 181.3 g in NSD18 (Tables 8, 9, 10).

An attempt was made to correlate the fruit characters with sweet kernel occurrence. Characters like copra weight, oil %, copra recovery, embryo weight and shell weight had significant correlation with sweet kernel (Table 11). Average linkage between genotypes based on fruit characters was used to cluster the palms (Fig. 3; Table 12). The average coefficient of variation before clustering was 15 for the fruit characters. Variability in fruit characters were less compared to palm morphological characters. The higher the variability, the lesser is the likelihood that it contributes to the sweet fruit character. Three groups could be visualized from the dendrogram generated by SAS software. In the first group, there were 11 genotypes with an average coefficient variation of 14. The second group consisted of 14 genotypes and had coefficient variation of 12. NSD 3, with heavy fruit and nut, was the only member in the third group. Earlier studies on fruit component traits of sweet and normal nuts of this population revealed that nuts possessing sweet endosperm types had slightly less fruit and husked fruit weight compared to normal nuts. Shell thickness was similar in both, but the shell weight was more in normal types. Likewise, endosperm thickness was similar in both, but the endosperm weight was more in normal types. Copra weight and copra recovery was more in normal nuts compared to sweet endosperm types. Embryo weight of sweet endosperm nuts was significantly lower than normal endosperm [8]. Palms with lighter fruits have more chance of having higher proportion of sweet kernel fruits. From the clustering, it was clear that the average value for fruit weight, nut weight, shell weight, endosperm weight, copra weight and embryo weight was less in cluster 1. Hence palms in cluster 1 have more chance of producing sweet kernel fruits.

Variation in allele frequencies at many unlinked loci is the preferred way to assess genetic diversity and differentiation and to estimate the strengths of the various forces shaping those [18]. Among the various DNA marker methods currently available [19] that can be used to examine the genetic diversity at the molecular level, the most informative polymorphic marker system to date is

Table 10 Husked fruit (nut) characters of individual palms

S. No.	Palm	Nut weight	Nut shape index	Shell weight	Shell thickness	Endosperm thickness	Endosperm weight	Copra weight	Oil %	Copra recovery %
1.	NSD 1	338.64	0.94	123.20	0.33	1.20	175.36	86.10	46.50	49.10
2.	NSD 2	573.75	0.95	162.38	0.53	1.43	305.75	143.50	45.78	46.93
3.	NSD 3	712.06	0.99	157.69	0.40	1.40	379.88	191.56	51.45	50.43
4.	NSD 4	292.45	0.93	74.37	0.43	1.18	192.37	93.21	37.05	48.45
5.	NSD 5	524.50	1.01	142.29	0.35	1.34	311.71	141.57	54.30	45.42
6.	NSD 6	600.64	0.99	152.90	0.37	1.27	310.19	150.40	45.25	48.49
7.	NSD 7	586.18	0.94	151.09	0.38	1.34	302.73	126.36	46.60	41.74
8.	NSD 8	566.31	0.90	136.77	0.33	1.35	316.31	147.69	48.10	46.69
9.	NSD 9	585.55	0.94	130.55	0.34	1.33	335.27	153.18	38.31	45.69
10.	NSD 10	689.21	0.95	167.29	0.39	1.37	367.57	205.21	49.97	55.83
11.	NSD 11	759.63	0.91	180.25	0.40	1.35	422.25	226.25	47.70	53.58
12.	NSD 12	488.58	0.91	125.73	0.39	1.36	290.73	152.00	45.93	52.28
13.	NSD 13	524.33	0.97	157.33	0.40	1.20	273.00	174.33	48.20	63.86
14.	NSD 14	418.00	0.94	102.80	0.32	1.37	261.40	142.40	47.83	54.48
15.	NSD 15	472.57	0.92	125.68	0.39	1.35	267.18	141.04	49.13	52.79
16.	NSD 16	387.00	0.89	108.89	0.38	1.34	246.56	115.78	18.20	46.96
17.	NSD 17	707.57	0.90	157.21	0.32	1.34	391.93	181.64	48.95	46.35
18.	NSD 18	670.75	0.93	181.25	0.40	1.38	351.75	208.25	57.20	59.20
19.	NSD 21	557.45	0.99	124.09	0.32	1.28	346.82	161.73	40.90	46.63
20.	NSD 22	550.14	0.97	151.67	0.41	1.24	299.76	137.17	46.03	45.76
21.	NSD 23	645.67	0.94	116.00	0.30	1.17	341.00	122.00	46.22	35.78
22.	NSD 24	531.60	0.90	131.30	0.35	1.35	302.20	158.50	51.90	52.45
23.	NSD 25	562.82	0.91	133.64	0.33	1.35	313.82	140.00	47.20	44.61
24.	NSD 26	393.85	0.93	109.08	0.35	1.26	226.25	109.25	50.05	48.29
25.	NSD 27	519.50	0.94	125.56	0.29	1.38	316.81	186.50	71.80	58.87
26.	NSD 28	576.15	0.90	148.69	0.40	1.36	310.54	154.85	30.80	49.86

Table 11 Correlation coefficient of fruit characters with sweet kernel

Fruit character	Correlation	Fruit character	Correlation	Fruit character	Correlation
Fruit weight	−0.119	Nut shape index	0.009	Copra weight	−0.632*
Fruit shape index	0.256	Shell weight	−0.442*	Oil %	−0.489*
Husk thickness	0.042	Shell thickness	0.0154	Copra recovery	−0.586*
Husk %	0.380	Endosperm thickness	−0.325	Embryo weight	−0.515*
Nut weight	−0.345	Endosperm weight	−0.363		

*Significant correlation at 1 % level

microsatellites or SSRs. In this study, Fourteen SSR primers were used for screening all the 26 palms possessing nuts with sweet kernel ('NSD') and the three control palms ('CON'). The 14 primers detected a total of 35 alleles with an average of 2.5 alleles per primer (Table 1). All the primers were 100 % polymorphic. The profile generated by the microsatellite loci CNZ4 is given in Fig. 4.

The similarity index, based on Jaccard's coefficient, was obtained after pair wise comparison among the 26 sweet kernel and three normal coconut palms. The highest similarity index of 0.96 was observed between the coconut palm NSD13 and NSD24 and lowest similarity index 0.21 was observed between CON1 and NSD3.

Cluster analysis was performed based on similarity index calculated using Jaccard's coefficient (Fig. 5). The

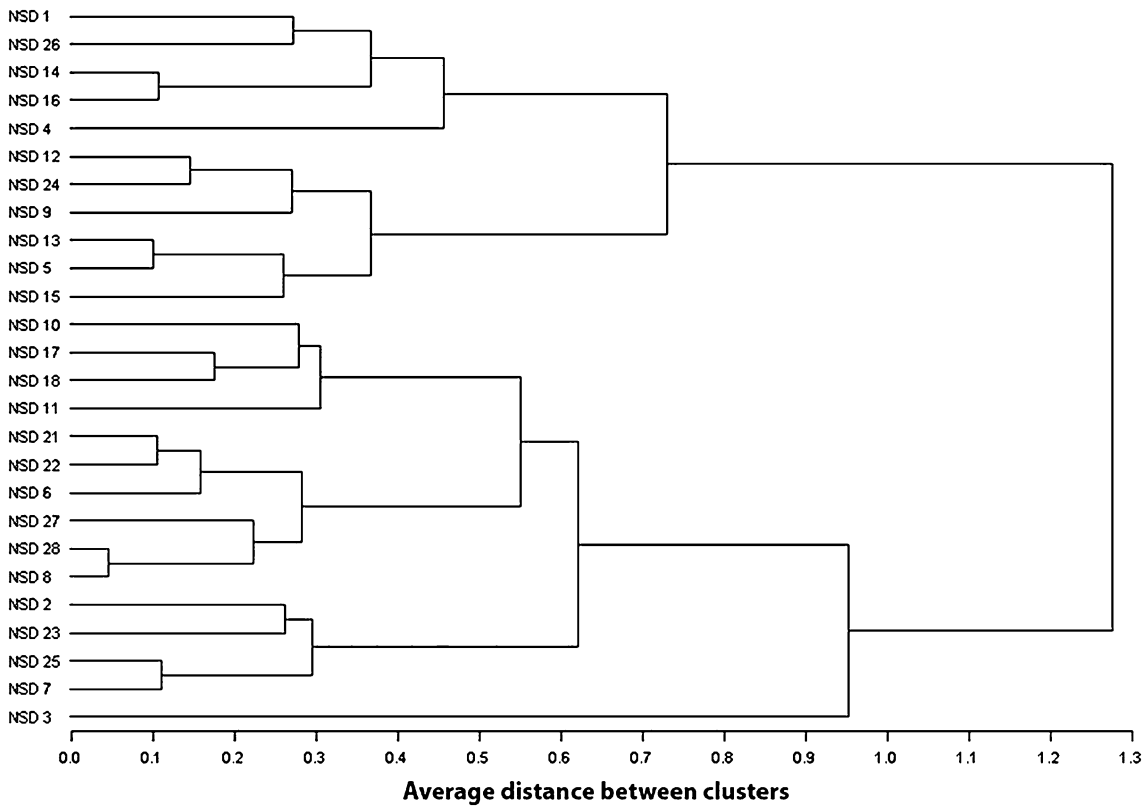

Fig. 3 Dendrogram representing relationships among the sweet kernel coconut genotypes based on average linkage obtained using fruit characters

analysis revealed that palms of sweet kernel type had only 45 % similarity among them. Earlier studies using SSR markers also detected higher heterozygosity values for indigenous tall coconut populations [11, 12]. The dendrogram showed five major clusters. The first cluster had three palms viz. NSD2, NSD3 and NSD4 and they were closest to the second group, but most diverged from fifth group. The second cluster consisted of seven palms viz. NSD6, NSD8, NSD7, NSD5, NSD9, NSD10 and NSD12 and was close to the third group followed by first group. Third cluster comprised of six palms NSD23, NSD21, NSD22, NSD20, NSD18, and NSD19 with two palms (NSD23 & NSD21) showing 95 % similarity. The fourth cluster included eight palms NSD17, NSD15, NSD14, NSD13, NSD24, and NSD26. In addition, fourth cluster also included three normal (non-sweet type) palms (CON1, CON2 and CON3). Two of the sweet type (NSD13 & NSD24) in this group had 100 % similarity. Fifth group had three palms viz. NSD27, NSD28 and NSD29 in it. Two of the palms in this group were 93 % similar while third palm had 66 % similarity to the first two.

However, the frequency of occurrence of sweet kernel fruits in palms had no correlation with clustering obtained based on fourteen SSR primers studied. For example,

Table 12 Clusters based on fruit and nut characters

	Cluster1	Cluster2	Cluster3
Palm numbers	NSD 1	NSD 23	NSD 3
	NSD 4	NSD 7	
	NSD 26	NSD 22	
	NSD 16	NSD 25	
	NSD 15	NSD 2	
	NSD 5	NSD 8	
	NSD 14	NSD 6	
	NSD 12	NSD 28	
	NSD 24	NSD 21	
	NSD 9	NSD 17	
	NSD 13	NSD 27	
		NSD 10	
		NSD 18	
		NSD 11	
Fruit weight	786.4	1,150.8	
Nut weight	450.6	759.6	
Shell weight	121	149	
Endosperm weight	262	335.5	
Copra weight	133.4	163.7	
Embryo weight	0.09	0.10	

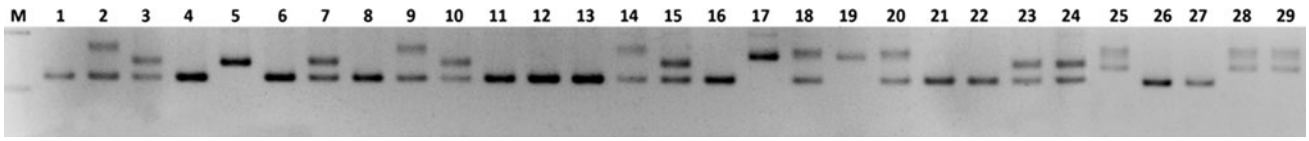

Fig. 4 PCR amplification pattern of microsatellite loci CNZ4 with sweet kernel coconut genotypes *M* molecular weight ladder, *lanes 1–26* sweet kernel coconut population, *lanes 27–29* control palms

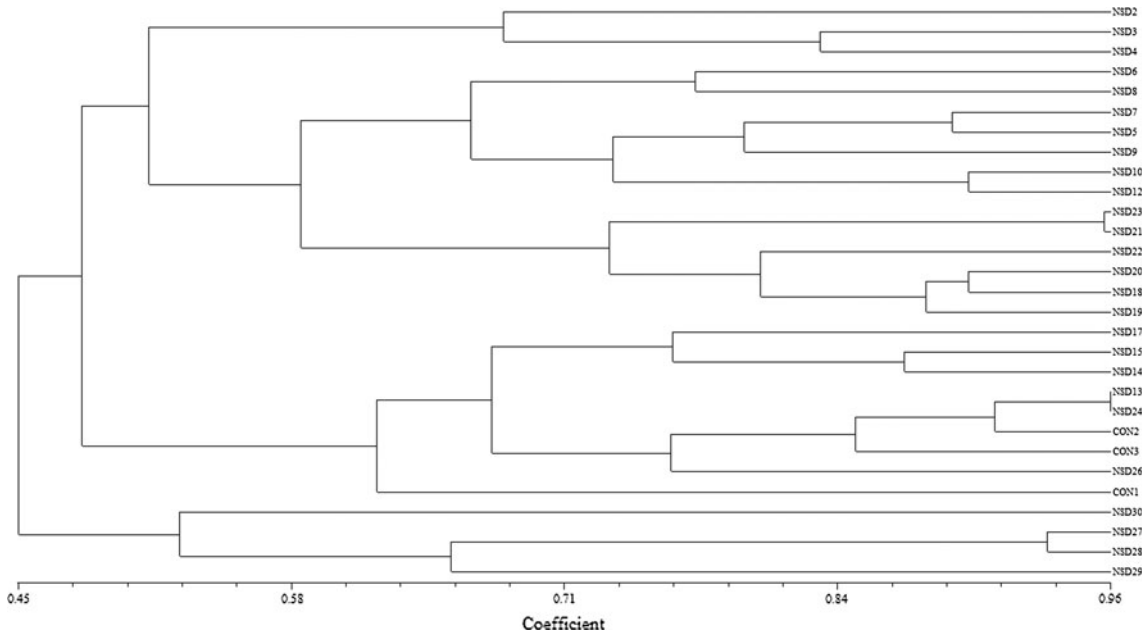

Fig. 5 Dendrogram (UPGMA) representing genetic relationships among the sweet kernel coconut genotypes based on Dice's coefficient obtained using SSR technique

NSD27 had only 19 % sweet kernel fruits while NSD28 had 77 % but were clustered together. Similarly, in the first cluster, percentage of sweet kernel fruits varied among palms from 14 to 70. These facts points to the sporadic development of such palms, possibly from the normal type. This argument is supported by earlier report that embryos of these coconuts were very small and they do not germinate under the natural conditions [7]. The *Makapuno* trait of coconut, reported from the Philippines, is due to the effect of a lethal recessive gene [6]. The sweet kernel trait reported in this study may also be due to a recessive gene, but this requires further detailed studies using progenies of the palms identified.

Coconut plays a very important role in the agrarian economy of India. Besides serving as the source of food and nutritious drink, coconut provides raw material for a variety of industrial activities. India is endowed with a wealth of coconut collections. Although these collections are a substantial asset for breeding, their very size makes it necessary to design a reliable strategy for their utilization. As a first step, it is necessary to identify a sub-set of populations' representative of the diversity of the palm since conserving and describing all the cultivars is both labour and land-intensive. This is the first report of diversity in a sweet endosperm coconut population from India. Farmers of the area get a premium price for sweet endosperm nuts and the sweet kernel is mainly used for raw consumption. Exploitation of sweet kernel trait will help in product diversification in coconut which will lead to profitability of coconut industry.

Though there was no clear correlation of sweet fruit character with clusters developed using palm, fruit or molecular data, a few fruit characters, viz. copra weight, oil percentage, copra recovery, embryo weight and shell weight, had significant correlation with sweet kernel. Further research is required to study the frequency of sweet endosperm nuts in consequent bunches and the seasonal variation in production of nuts with sweet kernel is to be further studied. Seed nuts from identified palms were collected and a population was conserved at CPCRI, Kasaragod for further evaluation and possible utilization in the coconut improvement programme. Presently, we are

focusing on estimation of total soluble sugars, reducing sugars, fatty acid composition and fibre content in both normal and sweet kernel fruits. The presence of entophytic bacteria in sweet kernel coconut is also being investigated. In addition to the above, future research will be targeted towards studies on differences in gene expression patterns between normal and sweet kernel types. Results from these studies may throw a light on the possible development of sweet kernel endosperm.

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