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RESEARCH ARTICLE

Analysis of coconut (*Cocos nucifera* L.) diversity using microsatellite markers with emphasis on management and utilisation of genetic resources

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Abstract: Sixteen SSR markers were used to identify genetic relationships of 43 coconut accessions conserved *ex-situ* in field gene banks of the Coconut Research Institute of Sri Lanka (CRISL). The 16 SSR markers clearly unveiled the genetic relationships of Sri Lankan coconut populations. Gene diversity and polymorphism information content (PIC) were relatively higher in the common 'tall' coconut and Pacific tall coconut than in autogamous dwarf form of coconut. The SSR assessment unveiled the genetic lineages based on evolutionary mechanisms signifying the narrow genetic base of coconut germplasm, with most of the diversity confining to 'tall' coconut. The main genetically different coconut groups identified were 'tall', 'San Ramon and alike' and 'dwarf'. These have already been utilised in coconut improvement programmes and the study emphasizes the need for enrichment of the gene pool by exotic introductions. The overall results also supports the hypothesis that coconut disseminated from it's center/s of origin in far east to Indo Atlantic regions via America.

Keywords: Coconut, genetic diversity, germplasm, microsatellite, Sri Lanka

INTRODUCTION

Coconut palm (*Cocos nucifera* L.) plays a significant role in the daily life of people over 80 countries in the tropics. The Consultative Group of International Agricultural Research (CGIAR) identified coconut as one among the most valued 22 crops in the world and emphasized the need for conservation of its germplasm and effective utilisation[1]. Potential of the coconut palm for alleviating poverty in rural coconut growing communities was aptly demonstrated in the Asian Development Bank (ADB)/ International Plant Genetic Resources Institute (IPGRI)/ Coconut Genetic Resources Network (COGENT) assisted project recently concluded in eight developing countries including Sri Lanka[2].

Coconut plays a more prominent role in the the social, economic and cultural life of the people in Sri Lanka than in any other country. It provides livelihood directly and indirectly for many thousands of people in the three sectors: production, processing and marketing. Coconut occupies 25% of the cultivable land, second only to rice, the staple food in Sri Lanka. It is the major source of edible oils and fats providing 22% of the daily caloric requirement of an average adult[3].

The origin and dispersal of coconut was long debated until recent DNA analyses provided substantial evidence for its origin as the atolls in the far-east or Pacific[4-8]. Coconuts have gone through a severe selection process assisted by man for over many generations for traits such as nut yield, fruit constituents, quantity and quality of oil.

There are two main types of coconut: 'tall', the naturally cross pollinating group with more economic value and 'dwarf', the naturally self pollinating group with reduced size and growth habit. It is believed that the 'dwarf' originated from earliest 'tall' coconuts in atolls of the far east and maintained most of its original genome because of its autogamous behaviour[6-8]. Thus, dwarf coconuts are of similar stature and fruit features irrespective of the geographical location. However, 'tall' genome has undergone many changes because of bottle-neck effects of selection, though it had retained

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the tall stature and fruit characteristics irrespective of its dispersion from far east to Indo-Atlantic regions across Africa[4]. The predominately cultivated Sri Lanka Tall (SLT) coconut resembles Indo-Atlantic coconut.

Due to high international demand for coconut, IPGRI mandated COGENT to develop and implement an international mechanism to coordinate research activities of national, regional and global significance with regard to exploration, collecting, conservation and enhancement of coconut germplasm. Sri Lanka benefited by the efforts of the COGENT and succeeded in establishing coconut field gene banks with accessions exceeding over a hundred. A thorough knowledge on genetical relationships of these accessions is needed for adopting effective gene-banking strategies and germplasm utilisation. Characterization and evaluation of coconut germplasm by morphological descriptors alone have failed to elucidate an accurate picture of true genetic variation because of time, land and cost constraints for carrying out controlled evaluation experiments. Earlier attempts made however, have shown a few inflorescence descriptors and fruit components as useful descriptors in assessing coconut germplasm[6]. Efforts made to reveal coconut genetic diversity by isozyme analysis too had met with meagre success, mainly due to technical limitations[9,10].

Studies on DNA polymorphisms have shown considerable success in analyzing coconut genetic diversity in the recent past because of the rapid advances in DNA marker-technologies. Rhode *et al*. sequenced repetitive DNA regions and successfully designed primers for amplifying inter-repetitive regions (Inverse Sequence-tagged Repeat or ISTR)[11,12]. This method was successfully used as a multiplex system for assessment of coconut genetic diversity and lately, in constructing a preliminary linkage map of coconut[13,14]. There were a number of successful studies using randomly amplified polymorphic DNA (RAPDs)[6,15,16], restriction fragment length polymorphisms (RFLPs)[7], amplified fragment length polymorphisms (AFLPs)[7] and microsatellite polymorphisms (SSRs)[8,18,19-21] that led to the understanding of the genetic diversity of coconut world over and its dissemination. Genetical relationship of coconut germplasm in Sri Lanka has so far not been assessed on a wider scale despite a few studies made on small samples of morphologically distinctive coconut groups[15,17,19]. The present study is the first attempt made to elucidate the genetic relationships of *ex-situ* coconut germplasm in Sri Lanka.

METHODS AND MATERIALS

Plant material used: Leaf samples were obtained from 43 coconut accessions established in *ex-situ* gene banks of the Coconut Research Institute of Sri Lanka (CRISL). The names of accessions, abbreviated names and characteristic features are described in Table 1. Among the 43 accessions, 32 were of the 'tall' category and 8 were of the 'dwarf' category in addition to 03 members of the intermediate 'king coconut' category. The genotypes, San Ramon (Clovis) and San Ramon likes are strictly 'tall' coconuts by stature and reproductive behavior but they distinctly differ from common Sri Lanka Tall (SLT) in the extremely tall stature and fruit size and shape.

DNA extraction and detection of SSR polymorphisms: DNA was extracted from fresh coconut leaves using a CTAB based protocol modified from Doyle and Doyle[22] by Dasanayake *et al*.[23]. Polymerase chain reactions were carried out with 16 SSR primers, pre-selected from a pool of 35 primers[18,20]. Sequence information of primers is given in Table 2. DNA was amplified in 10 µL reactions containing 1µM forward primer, 1 µM reverse primer, 1 unit of *Taq* DNA polymerase (Promega), 0.2 mM each dNTP (Pharmacia), 1x PCR buffer supplied with enzyme (Promega), 1.5 mM $MgCl_2$ supplied with enzyme (Promega), 30 ng template DNA in a thermal cycler (PTC 100 - MJ Research Inc) programmed for 30 cycles of 60 seconds each at 94^0C, 51-58^0C (depending on primer) and 72^0C. The first cycle was preceded by a 3 min denaturation at 95^0C and the last cycle ended with 5 min extension at 72^0C. Reaction products were separated on 6% polyacrylamide (denatured) and visualized by staining with 11.2 mM $AgNO_3$. The alleles were scored based on the size of each PCR amplified fragment by electrophoresing all samples in a single gel.

Data analysis: The alleles amplified by SSR primers were scored for each primer across all genotypes. The number of alleles per locus, gene diversity and Polymorphism Information Content (PIC) were calculated using PowerMarker version 3.25 (Liu and Muse 2006)[24]. Genetic distances and cluster analysis were also estimated using the same software. Shared allele distances were calculated and cluster analysis was performed with Neighbor joining method and the TreeView software (TreeView 1.6 version for WXP)[25] was used to construct the tree diagram. Bootstrap values were also computed for the NJ tree constructed in the PowerMarker software. The genetic relationships were also determined by Principal Coordinate Analysis in the NTSYS-pc[26].

Table 1: Description of 43 coconut accessions from *ex-situ* gene banks of the Coconut Research Institute of Sri Lanka

Name of accession	Variety	Characteristic feature
Ran thembili – RAT	Typica*	Green nuts, pink mesocarp (in immature nut), rarely occur
Bodiri – BOD	Typica	Small nuts, profusely bearing (> 50/bunch), partly autogamous, rarely occur.
Kamandala – KMD	Typica	Large nuts, sparsely bearing (< 5/bunch), rarely occur
Gon thembili – GNT	Typica	Golden yellow nuts, frequently occur in small numbers
Porapol – POP	Typica	Thick shell, rarely occur
Nawasi – NAW	Typica	Soft edible mesocarp in tender nuts, rarely occur
SLT Mahawalatanna – MWT	Typica	Sri Lanka Tall ecotype in Ratnapura District
SLT Ihalakagama – IHK	Typica	Sri Lanka Tall ecotype in Anuradhapura District
SLT Lanlib – LLB	Typica	Sri Lanka Tall ecotype in Puttalam District
SLT Wanathawillu – WAW	Typica	Sri Lanka Tall ecotype in Puttalam District
SLT Hangliyagama – HNG	Typica	Sri Lanka Tall ecotype in Anuradhapura District
Mirishena semi tall – MHT	Typica	Semi Tall coconut in Kalutara Distrcit
Wilhelmina – WHM	Typica	Large nuts with big husk found in Puttalam District
SLT Yodakandiya – YOK	Typica	Sri Lanka Tall ecotype in Hambantota District
SLT Dadalla – DAD	Typica	Sri Lanka Tall ecotype in Galle District
SLT Iranawila – IRW	Typica	Sri Lanka Tall ecotype in Puttalam District
Nipuni – NIP	Typica	San Ramon like**** palm from Colombo District
Indian – IND	Typica	San Ramon like palm from Gampaha District
SLT Bandrippuwa – TBE	Typica	Sri Lanka Tall ecotype in Puttalam District
SLT Akuressa – AKU	Typica	Sri Lanka Tall ecotype in Matara District
SLT Kasagala – KAS	Typica	Sri Lanka Tall ecotype in Hambantota District
SLT Deberayaya – DEB	Typica	Sri Lanka Tall ecotype in Hambantota District
SLT Maliboda – MLB	Typica	Sri Lanka Tall ecotype in Ratnapura District
SLT Goyambokka – GYM	Typica	Sri Lanka Tall ecotype in Hambantota District
SLT Damana – DMN	Typica	Sri Lanka Tall ecotype in Ampara District
SLT Uhana – UHN	Typica	Sri Lanka Tall ecotype in Ampara District
SLT Deegawapi – DWP	Typica	Sri Lanka Tall ecotype in Ampara District
Dickwella – DIK	Typica	San Ramon like collection from Matara District
Margaret – MGT	Typica	San Ramon like collection from Puttalam District
Blackstone – BLS	Typica	San Ramon like collection from Matale District
San Ramon Ran thembili - SRT	Typica	San Ramon like with pink mesocarp
Clovis – CLV	Typica	San Ramon collection introduced from Philippines
Dwarf green – DWG	Nana	Green small nuts with less meat, high bearing
Dwarf brown – DWB	Nana**	Brown small nuts with less meat, high bearing
Dwarf red – DWR	Nana	Red small nuts with less meat, high bearing
Dwarf yellow – DWY	Nana	Yellow small nuts with less meat, high bearing
Green dwarf Kundasale - GDK	Nana	Green dwarf ecotype in Kandy Distrct
Mirishena dwarf – MID	Nana	Green dwarf with a semi tall stature in Kalutara District
Cameroon red dwarf – CRD	Nana	Red dwarf believed to be introduced from Cameron
Brazilian green dwarf – BGD	Nana	Green dwarf believed to be introduced from Brazil
King coconut – KCT	Aurantiaca***	Orange nuts with sweet water, high bearing
Rathran thembili – RRT	Aurantiaca	King coconut like with pink mesocarp
Nawasi thembili – NWT	Aurantiaca	King coconut like with edible husk

* All typica accessions are characterized by tall stature and naturally out crossing behaviour, ** All nana accessions are characterized by dwarf stature and naturally inbreeding behaviour, *** All aurantiaca are characterized by intermediate stature and inbreeding behaviour, **** All San Ramon like are characterized by extremely tall stature and large round shaped nuts

Table 2: Primer information of sixteen SSR loci used for amplification of DNA isolated from 43 accessions of coconut germplasm.

Locus	Repeat	Sequence (5'-3')	Size of the product (bp)	Source of information sequence
CAC06	$(AG)_{14}(CA)_9$	FP TGTACATGTTTTTTGCCCAA RP CGATGTAGCTACCTTCCCC	158	
CAC08	$(AG)_{10}(CA)_9$	FP ATCACCCCAATACAAGGACA RP AATTCTATGGTCCACCCACA	190	
CAC23	$(CA)_8$	FP TGAAAACAAAAGATAGATGTCAG RP GAAGATGCTTTGATATGGAAC	192	
CAC38	$(CA)_{13}(CT)_{17}$	FP ACCCTACTTCTAACTGTTCACTC RP CAGCTTGATAAATATCATCCAT	155	Perera, 1999 and
CAC50	$(TA)_6(CA)_{21}$	FP CTTACTCACCCCATAACAAAG RP TTGTAGTTGCCCATATCTCTT	153	Perera *et al*. 1999
CAC65	$(AC)_{15}$	FP GAAAAGGATGTAATAAGCTGG RP TTTGTCCCCAAATATAGGTAG	151	
CAC68	$(CA)_{13}$	FP AATTATTTTCTTGTTACATGCATC RP AACAGCCTCTAGCAATCATAG	142	
CAC77	$(CA)_{15}(CT)_{11}$	FP CAGAGGTCACAACCATATTG RP CTTTAGCTATTTGTTCCAAGG	131	
CNZ04	$(CT)_{29}TT(CA)_{10}$	FP TATATGGGATGCTTTAGTGGA RP CAAATCGACAGACATCCTAAA	162	
CNZ06	$(CT)_{15}$	FP ATACTCATCATCATACGACGC RP CTCCCACAAAATCATGTTATT	85	
CNZ10	$(CT)_{18}(GT)_{17}$	FP CCTATTGCACCTAAGCAATTA RP AATGATTTTCGAAGAGAGGTC	148	
CNZ12	$(CT)_{15}$	FP TAGCTTCCTGAGATAAGATGC RPGATCATGGAACGAAAACATTA	214	Rivera *et al.* 1999
CNZ29	$(GT)_{22}(GA)_2CA(GA)_{11}$	FPTAAATGGGTAAGTGTTTGTGC RP CTGTCCTATTTCCCTTTCATT	135	
CNZ43	$(GA)_{21}$	FP TCTTCATTTGATGAGAATGCT RP ACCGTATTCACCATTCTAACA	197	
CNZ44	$(GA)_{15}$	FP CATCAGTTCCACTCTCATTTC RP CAACAAAAGACATAGGTGGTC	165	
CNZ46	$(CT)_{24}$	FP TTGGTTAGTATAGCCATGCAT RP AACCATTTGTAGTATACCCCC	116	

RESULTS

Sixteen primer pairs identified 79 alleles, averaging 4.9 alleles per locus ranging from 3 to 10 simple sequence repeat polymorphisms among the 43 coconut accessions assessed. All 16 loci were polymorphic and a total of 76 alleles were observed in tall category, ranging from 3 to 10 with an average of 4.7 alleles per locus. A total of 29 alleles were observed in dwarf category ranging from 1 to 3 with an average of 1.8 alleles per locus. The thirty alleles observed in the king coconut (*aurantiaca* group) ranged from 1 to 3 with an average of 1.8 alleles per locus (Table 3). Gene diversity, often referred to as expected heterozygosity is defined as the probability that two randomly chosen alleles from the population become different. Forty-three accessions showed a mean gene diversity of 0.64 with the 'tall' group showing the highest gene diversity, 0.55. The diversities of dwarf and king coconut groups were 0.21 and 0.32 respectively.

Polymorphism Information Content (PIC) is also a measure of genetic diversity estimated, and the overall PIC of the 16 SSR loci in the 43 accessions was 0.58. The respective PIC values of the three groups; tall, dwarf and king cocont were 0.5, 0.18 and 0.26 respectively (Table 3). Among the 16 SSR loci assessed, six and four were respectively uni-allelic among dwarf and king coconut accessions. Loci CAC50 and CNZ12 were uni-allelic in both dwarf and king coconut accessions. All the 16 SSR loci were multi allelic in tall accessions. The gene diversity and PIC were high in loci; CAC50, CAC68, CAC77, CNZ10, CNZ43 and CNZ44. However, the gene diversities were predominant among the tall accessions. High gene diversities were observed in only a few loci (i.e CAC65, CNZ29) among dwarf and king coconut accessions.

Allele frequencies of each SSR locus for the three groups 'tall', 'dwarf' and 'king coconut' (*Typica*, *Nana* and *Aurantiaca*) are presented in Table 4. There are 37 unique alleles in the 'tall' group while no unique alleles were observed in dwarf and king coconut groups. The frequency distributions of alleles were remarkably high in the tall group compared to dwarf and king coconut groups. The most frequent allele of the 'tall' group was CNZ46-5, which is also found in low frequencies in the other two groups. CNZ06-3 and CNZ29-4 were also very frequent among the tall group. Six alleles were observed with maximum frequency in the dwarf group (CAC06-2, CAC23-3, CAC50-6, CAC68-6, CNZ04-1 and CNZ12-2) of which CAC50-6 and CNZ12-2 were also observed to be maximum in king coconut group. In addition the frequency of two alleles, CAC38-2 and CNZ06-1 were also found to be maximum in the king coconut group.

Shared allele distances varied from 0.13 to 1.00 among the 43 accessions. The distances were low among pairs within the dwarf group (mean = 0.18, range = 0.13 to 0.41). The six pairs; Dwarf Green and Cameroon Red Dwarf, Dwarf Red and Cameroon Red Dwarf, Dwarf Red and Dwarf Green, Mirishena Dwarf and Cameroon Red Dwarf, Mirishena Dwarf and Dwarf Green and Porapol and Gon thembili had the lowest shared allele distance (0.13). Sri Lanka Tall and king coconut accessions averaged distances of 0.55 (0.13 – 0.96) and 0.24 (0.38-0.69) respectively. A total of 40 pairs, all between tall and dwarf recorded the maximum (GD = 1).

Table 3: Number of alleles, gene diversity and polymorphism information content in 16 SSR loci of 43 coconut germplasm accessions

SSR Locus		No of alleles				Gene Diversity				Polymorphism information content			
		All	Tall	Dwarf	King coconut	All	Tall	Dwarf	King coconut	All	Tall	Dwarf	King coconut
1	CAC06	5	5	1	2	0.65	0.58	0.00	0.44	0.58	0.53	0.00	0.35
2	CAC08	5	4	2	2	0.60	0.41	0.22	0.44	0.54	0.39	0.19	0.35
3	CAC23	3	3	1	2	0.66	0.58	0.00	0.44	0.58	0.50	0.00	0.35
4	CAC38	6	6	2	1	0.59	0.47	0.22	0.00	0.51	0.44	0.19	0.35
5	CAC50	10	10	1	1	0.80	0.83	0.00	0.00	0.78	0.81	0.00	0.00
6	CAC65	4	3	3	2	0.55	0.42	0.41	0.44	0.48	0.37	0.37	0.35
7	CAC68	6	6	1	2	0.74	0.70	0.00	0.28	0.70	0.66	0.00	0.24
8	CAC77	4	4	2	2	0.70	0.72	0.38	0.44	0.64	0.66	0.30	0.35
9	CNZ04	4	4	1	2	0.57	0.51	0.00	0.44	0.48	0.45	0.00	0.35
10	CNZ06	3	3	2	1	0.52	0.35	0.22	0.00	0.43	0.31	0.19	0.00
11	CNZ10	5	5	3	2	0.73	0.70	0.45	0.28	0.68	0.65	0.41	0.2
12	CNZ12	3	3	1	1	0.57	0.44	0.00	0.00	0.48	0.40	0.00	0.00
13	CNZ29	4	4	3	3	0.57	0.39	0.53	0.44	0.51	0.36	0.47	0.35
14	CNZ43	7	6	2	2	0.76	0.69	0.38	0.44	0.73	0.65	0.30	0.35
15	CNZ44	4	4	1	2	0.73	0.70	0.22	0.44	0.68	0.65	0.19	0.35
16	CNZ46	6	6	3	3	0.50	0.25	0.32	0.67	0.45	0.25	0.29	0.59
	Total	79	76	29	30								
	Average	4.93	4.75	1.81	1.87	0.64	0.55	0.21	0.32	0.58	0.50	0.18	0.26

Table 4: Number of alleles observed and frequency of each allele in 16 SSR loci scored among 43 accessions of coconut germplasm

SSR Locus and allele		Count				Frequency				Standard deviation			
Locus	Allele	All	Tall	Dwarf	King coconut	All	Tall	Dwarf	King coconut	All	Tall	Dwarf	King coconut
CAC06	1	1	1				0.01	0.02			0.01	0.02	
	2	27	11	16		0.31	0.17	1.00		0.07	0.06	0.00	
	3	14	10		4	0.16	0.16		0.67	0.05	0.06		0.27
	4	41	39		2	0.48	0.61		0.33	0.07	0.08		0.27
	5	3	3			0.03	0.05			0.02	0.03		
CAC08	1	48	48			0.56	0.75			0.07	0.07		
	2	6	6			0.07	0.09			0.04	0.05		
	3	25	7	14	4	0.29	0.11	0.88	0.67	0.07	0.05	0.12	0.27
	4	4		2	2	0.05		0.13	0.33	0.03		0.12	0.27
	5	3	3			0.03	0.05			0.03	0.03		
CAC23	1	35	33		2	0.41	0.52		0.33	0.07	0.09		0.27
	2	24	24			0.28	0.38			0.07	0.09		
	3	27	7	16	4	0.31	0.11	1.00	0.67	0.07	0.05	0	0.27
CAC38	1	4	4			0.05	0.07			0.04	0.05		
	2	27	7	14	6	0.36	0.13	0.88	1.00	0.07	0.06	0.12	0.00
	3	40	38	2		0.53	0.70	0.13		0.08	0.09	0.12	
	4	5	5			0.07	0.09			0.03	0.05		
CAC50	1	1	1			0.01	0.02			0.01	0.02		
	2	3	3			0.04	0.05			0.02	0.03		
	3	4	4			0.05	0.07			0.03	0.04		
	4	1	1			0.01	0.02			0.01	0.02		
	5	1	1			0.01	0.02			0.01	0.02		
	6	26	4	16	6	0.33	0.07	1.00	1.00	0.07	0.04	0.00	0.00
	7	10	10			0.13	0.18			0.05	0.06		
	8	5	5			0.06	0.09			0.04	0.05		
	9	13	13			0.17	0.23			0.06	0.07		
	10	14	14			0.18	0.25			0.06	0.08		
CAC65	1	10	4	2	4	0.12	0.06	0.13	0.67	0.05	0.04	0.12	0.27
	2	51	47	2	2	0.59	0.73	0.13	0.33	0.07	0.07	0.12	0.27
	3	25	13	12		0.29	0.20	0.75		0.06	0.06	0.15	
CAC68	1	3	3			0.03	0.05			0.03	0.03		
	2	19	18		1	0.22	0.28		0.17	0.05	0.06		0.14
	3	28	28			0.33	0.44			0.06	0.07		
	4	8	8			0.09	0.13			0.04	0.05		
	5	2	2			0.02	0.03			0.02	0.03		
	6	26	5	16	5	0.30	0.08	1.00	0.83	0.06	0.03	0.00	0.14
CAC77	1	8	8			0.09	0.13			0.04	0.05		
	2	36	22	12	2	0.42	0.34	0.75	0.33	0.07	0.07	0.15	0.27
	3	21	13	4	4	0.24	0.20	0.25	0.67	0.06	0.06	0.15	0.27
	4	21	21			0.24	0.33			0.06	0.07		
CNZ04	1	35	15	16	4	0.41	0.23	1.00	0.67	0.07	0.07	0.00	0.27
	2	6	6			0.07	0.09			0.03	0.04		
	3	1	1			0.01	0.02			0.01	0.02		
	4	44	42		2	0.51	0.66		0.33	0.07	0.07		0.27
CNZ06	1	32	12	14	6	0.37	0.19	0.88	1.00	0.07	0.06	0.12	0.00
	2	4	2	2		0.05	0.03	0.13		0.03	0.03	0.12	
	3	50	50			0.58	0.78			0.07	0.06		

Table 4: Continued.

SSR Locus and allele		Count				Frequency				Standard deviation			
Locus	Allele	All	Tall	Dwarf	King coconut	All	Tall	Dwarf	King coconut	All	Tall	Dwarf	King coconut
CNZ10	1	2	1		1	0.02	0.02		0.17	0.02	0.02		0.14
	2	15	15			0.18	0.23			0.06	0.07		
	3	31	29	2		0.37	0.45	0.14		0.07	0.07	0.13	
	4	12	10	2		0.14	0.16	0.14		0.05	0.05	0.13	
	5	24	9	10	5	0.29	0.14	0.71	0.83	0.07	0.06	0.17	0.14
CNZ12	1	8	8			0.09	0.13			0.04	0.05		
	2	32	10	16	6	0.37	0.16	1.00	1.00	0.07	0.06	0.00	0.00
	3	46	46			0.53	0.72			0.07	0.07		
CNZ29	1	4	2	2		0.05	0.03	0.13		0.03	0.02	0.12	
	2	23	9	10	4	0.27	0.14	0.63	0.67	0.06	0.05	0.17	0.27
	3	8	4	4		0.09	0.06	0.25		0.04	0.03	0.15	
	4	51	49		2	0.59	0.77		0.33	0.07	0.07		0.27
CNZ43	1	4	4			0.05	0.06			0.02	0.03		
	2	6	6			0.07	0.09			0.03	0.04		
	3	23	15	4	4	0.27	0.23	0.25	0.67	0.06	0.06	0.15	0.27
	4	14		12	2	0.16		0.75	0.33	0.06		0.15	0.27
	5	5	5			0.06	0.08			0.03	0.04		
	6	3	3			0.03	0.05			0.02	0.03		
	7	31	31			0.36	0.48			0.06	0.07		
CNZ44	1	25	7	14	4	0.30	0.11	0.88	0.67	0.07	0.05	0.12	0.27
	2	21	19	2		0.25	0.31	0.13		0.06	0.07	0.12	
	3	27	25		2	0.32	0.40		0.33	0.07	0.08		0.27
	4	11	11			0.13	0.18			0.05	0.07		
CNZ46	1	1	1			0.01	0.02			0.01	0.02		
	2	3	2	1		0.04	0.03	0.06		0.02	0.02	0.06	
	3	3	1		2	0.04	0.02		0.33	0.03	0.02		0.27
	4	2	2			0.03	0.03			0.02	0.03		
	5	54	50	2	2	0.68	0.86	0.13	0.33	0.07	0.06	0.12	0.27
	6	17	2	13	2	0.21	0.03	0.81	0.33	0.06	0.03	0.12	0.27

The tree diagram (Figure 1) clearly depicts the relationship of the 43 accessions though strictly not defining an evolutionary pathway. The tree comprised three main branches of which the first two branches grouped 16 accessions of the Sri Lanka Tall group. The third branch was further subdivided into two main groups, the first comprising six accessions of Sri Lanka Tall and the second consisting of accessions belonging to dwarf, king coconut, San Ramon and San Ramon like types. The only exception is grouping of Ran thembili and bodiri, conventionally named as forms of Sri Lanka Tall (SLT) with above accessions.

The PCoA plot clearly showed two groups, the first comprising 22 accessions of Sri Lanka Tall and the second having nine accessions of dwarf and king coconut (Figure 2). Rest of the accessions were scattered either as individuals or pairs or smaller groups very much similar to the tree diagramme (Figure 1).

DISCUSSION

Phenotypic assessment has been the main criterion for characterizing coconut until recent advances in molecular marker technology. Earlier, Liyanage[27] identified three main groups (varieties) of coconut in Sri Lanka based on stature and reproductive behaviour and named three varieties; *typica* or tall, *nana* or dwarf and *aurantiaca* or king coconut. In addition, an exotic collection, San Ramon (Clovis) from the Philippines was noted and incorporated to the breeding programme.

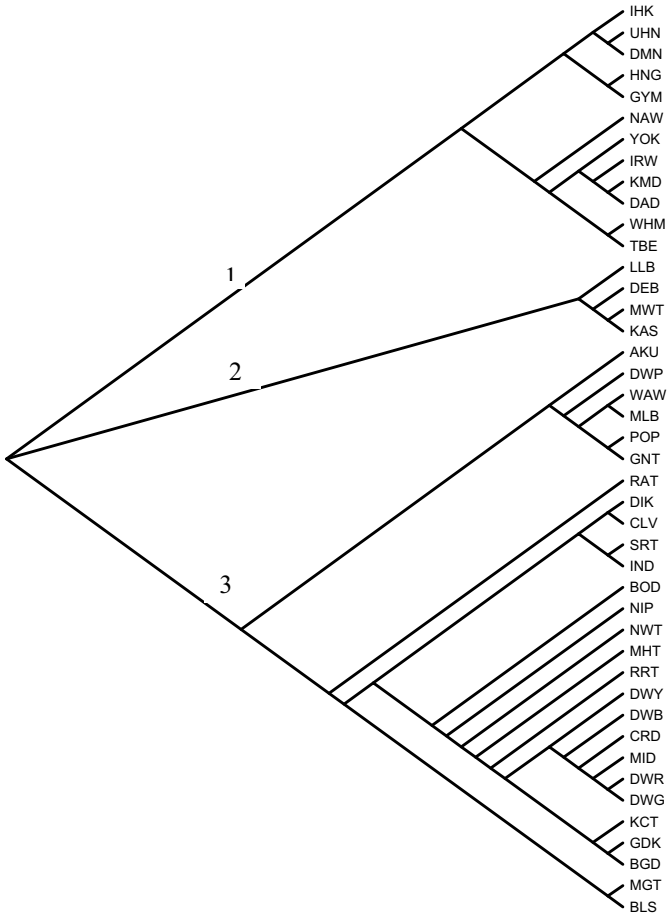

Figure 1: Dendrogram of 43 *ex-situ* conserved coconut germplasm generated by the Neighbor-Joining method.

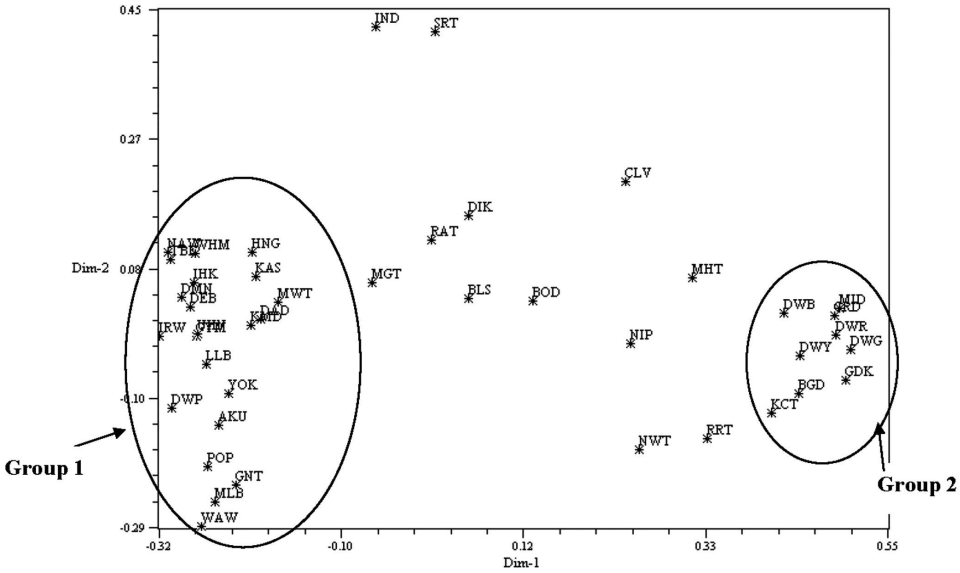

Figure 2: Two–dimensional principal coordinate analysis plot of 43 *ex-situ* conserved coconut germplasm accessions.

Systematic collection of coconut germplasm in Sri Lanka began in 1986 and *ex-situ* field gene banks have been established since then with a wider representation of commercial 'tall' (SLT ecotypes) and distinctive coconut phenotypes[28]. Characterization of these germplasm by morphological descriptors alone has failed to elucidate genetic relationships of these *ex-situ* conserved accessions of coconut.

The present study unveils a clear picture of the coconut germplasm in Sri Lanka with information that is similar to information of the coconut genetic resources world over. Identification of 79 alleles (4.9 per locus) by 16 coconut specific SSR primers aptly demonstrated the ability of micro-satellite markers to identify polymorphisms in coconut. Merrow *et al*.,[29] observed almost the same, 4.5 alleles per locus with 15 SSR primers within Florida's coconut germplasm. Comparative averages on coconut worldwide, 6.4 by Perera *et al*. [8] and 9.14 by Teulat *et al*.[21] were much higher as expected because of the relative slenderness of the coconut genetic base in Sri Lanka.

The low gene diversity and PIC of dwarf (0.21 and 0.18 respectively) and king coconut (0.33 and 0.26) accessions is attributed to the autogamous nature of these forms, which was also described by morphological means[30]. This observation agrees well with SSR polymorphisms in 'dwarf' coconut in other countries[18,20,21,29]. Conversely a moderate level of gene diversity and PIC, 0.55 and 0.5 respectively were observed in the predominantly allogamous 'tall' coconut group including San Ramon and alikes.

The grouping of 43 accessions based on shared allele distances depicts the genetic relationships of coconut germplasm accessions more in accordance with geographic representation morphological descriptor states. Grouping of accessions into three main groups with one and two having all 'tall' coconut and the third with a sub group having all dwarf, king coconut, semi tall, Bodiri, Nipuni and Clovis forms exemplify the above fact. The clustering of all 'Sri Lanka Tall' accessions in a single group and separation of San Ramon and alikes was also revealed in the PCoA analysis. All the dwarf accessions and king coconut closely placed in the PCoA plot.

Low gene diversity and high allele sharing in dwarf coconuts world over has already been revealed [7,8,18,20,21]. This was attributed to the autogamous breeding habit of the dwarf group under natural conditions. Perera[31] hypothesized the possibility that dwarf coconut has evolved from a small number of tall palms because almost all the alleles present in dwarf coconut are shared by 'tall' coconut[31]. Close clustering of dwarf and San Ramon and San Ramon like 'tall' coconut suggests a common putative origin of dwarf coconut and the coconut from Philippines or perhaps those of the center/s of origin in the South East Asia. Presence of bodiri, conventionally classified as a Sri Lankan Tall form in the dwarf predominant cluster suggests this as a separate introduction or more likely a semi tall, domesticated as an ornamental due to its prolific bearing capacity and good taste of water. This suggestion is further validated partly by the autogamous nature of bodiri. Positioning of king coconut forms (King Coconut, Ratharan Thembili and Nawasi Thembili) in the conventional classification as an intermediate between 'tall' and 'dwarf' appears to be weaker because evolutionary mechanisms play a more significant role in determining the genetic structure of the coconut in Sri Lanka. King coconut is certain to be an introduction similar to all other dwarf forms with less lineage with 'tall' prevalent in commercial plantations in Sri Lanka.

Close clustering of Sri Lanka Tall ecotypes and other SLT forms clearly indicate SLT coconut as a separate introduction with a close genetic lineage to Indo Atlantic Tall coconut[7]. The dwarf group in the African region not being grouped with African Talls, Whitehead's[5] suggestions on the route of coconut dissemination as Pacific coast to America from the presumed center/s of origin in the Southeast Asia also favours close relationship of SLT with African 'tall' coconut.

Presence of San Ramon like accessions, Margaret, Blackstone, San Ramon-RT, Indian, Ran Thembili and Dickwella in a sub group with Clovis (the San Ramon coconut brought from Phillipines) suggests that these are pure or mixed populations of Paciffic coconut. These coconut too are characterized by relatively large more round shaped coconut similar to Clovis and SLT x San Ramon progeny[32]. Here again the earlier classification of Ran Thembili as a SLT form becomes less acceptable in terms of genetic relationships. The separation of San Ramon and San Ramon like tall coconut from the Sri Lanka Tall group by the PCoA also indicates the genetic relationships of coconut in Sri Lanka and is well determined by evolutionary relationships than phenotypic differences even as acute as tall/short stature or autogamous / allogamous reproductive behavior.

In addition to the clear elucidation of genetic relationships of coconut in Sri Lanka the results of this study also lead to several implications on effective conservation and breeding of coconut in Sri Lanka. Close clustering of SLT coconut from the rest irrespective

of tall or dwarf morphotypes clearly signifies SLT as a heterozygous group sharing a narrow genetic base. Therefore, the current effort made to collect SLT from different geographical locations for conservation in large blocks of land with 60 or more palms/accession appears somewhat futile. A single countrywide collection of SLT with small samples from different locations appears a better option for forming a core of SLT coconut diversity with minimum duplication. A molecular marker assessment across populations in different geographical locations prior to collecting germplasm for conservation therefore, is a more efficient strategy for identifying unique populations with specific relevance for breeding.

As revealed by this study the entire genetic diversity of coconut in Sri Lanka is confined within the widely grown commercial 'tall', more ornamental type, 'dwarf' and the solitary collection from the Clovis estate, San Ramon. SLT has very little opportunities for further genetic improvement by selecting within itself. First released coconut cultivar, CRIC60, which is a selection of SLT has failed to demonstrate a significant yield increase over unselected SLT. Early studies of CRISL has revealed heterosis by combining SLT, dwarf and San Ramon in varying combinations for economic traits such as early flowering/bearing, nut yield and kernel weight[32]. This investigation therefore, emphasizes the dearth of genetic diversity in Sri Lanka for extensive utilisation as germplasm. The option now is for genetic enrichment by introduction of exotic germplasm. Wide genetic base of Pacific coconut revealed by previous SSR studies[8] and loosing of genes during domestication in the African region due to bottle necks of selection further strengthens the need for introducing germplasm particularly, from far east, the presumed centres of origin.

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