Assessment of the genetic diversity of Indian coconut accessions and their relationship to other cultivars, using microsatellite markers

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Summary

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Coconut cultivation in the Indian subcontinent over many centuries has given rise to diverse cultivar types, identified based on discernible morphological traits, geographical locations and farmers' choice. The Indian coconut germplasm base has also been enriched through introductions from other major coconut growing regions of the world. This study assessed the genetic diversity in a range of coconut accessions, and their relationship to other major coconut cultivars, using microsatellite markers. Microsatellite assay was used for 23 Indian accessions (15 Talls and 6 Dwarfs) and for 21 exotic accessions (18 Talls and 6 Dwarfs). A total of 48 alleles were detected in the Indian accessions by using 8 microsatellite primers (average 6 alleles per locus). More alleles were detected in the Indian accessions than in the exotics, indicating the presence of a wide allelic spectrum in the Indian accessions. Mean gene diversity ranged from 0.00 for 'Chowghat Green Dwarf' to 0.59 for 'Lakshadweep Ordinary Tall', with an overall mean of 0.32. The within-population variation was slightly higher (53%) than the between-population variation (47%), suggesting recent local adaptation and subsequent divergence among Indian cultivars. An UPGMA dendrogram separated the Indian accessions into two groups, one clustering with South-east Asian cultivars, which are introgressed types, and another clustering with African and Sri Lankan cultivars. The results are discussed in terms of diversity of Indian coconut accessions and relationships to other major coconut cultivars.

Key words: Coconut, *Cocos nucifera*, Indian coconut germplasm, microsatellite

Résumé

Évaluation de la diversité génétique d'accessions de cocotier indien et de leur relation avec d'autres cultivars, en utilisant des marqueurs microsatellites

La culture du cocotier dans le sous-continent indien depuis plusieurs siècles a entraîné l'apparition de divers types de cultivars, identifiés sur la base de caractères morphologiques discernables, de localisations géographiques et de la préférence des agriculteurs. Le matériel génétique des cocotiers indiens a également été enrichi par des introductions provenant d'autres grandes régions de culture du cocotier dans le monde. La présente étude évalue la diversité génétique dans une gamme d'accessions de cocotier, et leur relation avec d'autres cultivars importants de cocotier, en utilisant des marqueurs microsatellites. Un test basé sur des microsatellites a été appliqué à 23 accessions indiennes (15 de grande taille et 6 naines) et 21 accessions exotiques (18 de grande taille et 6 naines). Un total de 48 allèles a été détecté dans les accessions indiennes en utilisant 8 amorces de microsatellites (moyenne de 6 allèles par locus). Davantage d'allèles ont été détectés dans les accessions indiennes que dans les accessions exotiques, indiquant la présence d'un large spectre allélique dans les accessions indiennes. La diversité génique moyenne va de 0,00 pour le « Chowghat Green Dwarf » à 0,59 pour le « Lakshadweep Ordinary Tall », avec une moyenne globale de 0,32. La variation au sein de la population est légèrement plus élevée (53 %) que la variation entre populations (47 %), suggérant une adaptation locale récente et une divergence ultérieure parmi les cultivars indiens. Un dendrogramme UPGMA permet de séparer les accessions indiennes en deux groupes, l'un rassemblant les cultivars du sud-est asiatique, qui sont de types introgressés, et l'autre regroupant les cultivars africains et sri-lankais. Les résultats sont discutés en termes de diversité des accessions de cocotier indien et de leurs relations avec d'autres cultivars importants de cocotier.

Resumen

Evaluación de la diversidad genética de accesiones de coco de la India y su relación con otros cultivares, empleando marcadores microsatélites

El cultivo del coco en el subcontinente indio ha dado origen a diversos tipos de cultivares a lo largo de muchos siglos, identificados sobre la base de rasgos morfológicos discernibles, ubicaciones geográficas y preferencia de los agricultores. El germoplasma base del coco de la India ha sido también enriquecido introduciendo germoplasma proveniente de otras principales regiones productoras de coco en el mundo. Este estudio establece la diversidad genética de una serie de accesiones de coco y sus relaciones con otros cultivares principales de coco empleando marcadores microsatélites. Los ensayos de microsatélites se utilizaron en 23 accesiones de la India (15 altas y 6 enanas) y en 21 accesiones exóticas (18 altas y 6 enanas). Se detectó un total de 48 alelos en las accesiones de la India empleando 8 microsatélites cebadores (un promedio de 6 alelos por locus). Se detectaron más alelos en las accesiones de la India que en las exóticas, lo que indica la presencia de un amplio espectro de alelos en las accesiones de la India. La diversidad genética media iba de 0,00 para la "Chowghat Green Dwarf" a 0,59 para la "Lakshadweep Ordinary Tall", con una media total de 0,32. La variación era ligeramente mayor dentro de la población (53%) que entre las poblaciones (47%) lo que sugiere una adaptación local reciente y la subsiguiente divergencia entre los cultivares de la India. Un dendrograma UPGMA separó las accesiones de la India en dos grupos, uno que se agrupa con los cultivares de Asia sudoriental, que son tipos introgredidos, y el otro con los cultivares de Africa y Sri Lanka. Se debate el resultado en términos de diversidad de las accesiones de coco de la India y sus relaciones con otros cultivares principales de coco.

Introduction

Coconut cultivation in India dates back to at least 1200 BC, the post-Vedic era in an Indian context (Menon and Pandalai 1958). Coconut currently occupies about 1.87 million hectares in India (Ministry of Agriculture 2003) and is mainly cultivated by small-scale and marginal farmers. Its multiple uses for food, oil, cosmetics and fuel, coupled with its tolerance of primitive husbandry and its role as provider of year-round employment for rural households, makes it one of the most useful plants among tropical cultivated crops.

Coconut cultivars can be classified into Talls, Dwarfs and intermediate types. In India, Talls are the predominant cultivated form, although Dwarfs occupy a significant area.

The various coconut growing countries of the world have varying levels of germplasm collection and conservation activities. The International Coconut Genetic Resources Network (COGENT), established in 1992 under the aegis of the International Plant Genetic Resources Institute (IPGRI), acts as a facilitator in collecting and exchanging germplasm among the member countries, along with many other activities. The long gestation period associated with acclimatization and evaluation of exotic germplasm, coupled with coconut's low seed-multiplication rate, makes coconut hybridization with exotic accessions time consuming and currently not very practical. Moreover, the risk of introducing new pests, such as phytoplasmas and eryophid mites, is of major concern when considering the introduction of exotic materials. Until recently, embryos were considered to be a safe mode of coconut germplasm exchange, but the recent identification of phytoplasma in coconut embryos (Cardova et al. 2002) makes it risky to import coconut embryos. The best strategy is now to conserve and exploit the diversity available in local populations, either to develop hybrids or to improve traditional cultivars. The conservation of indigenous diversity has therefore become more important, not least in the context of the genetic erosion that has been occurring in recent years following changes in land use and the replacing of senile palms with high yielding varieties and hybrids.

Narayana and John (1949) attempted the first systematic classification of Indian germplasm. Menon and Pandalai (1958) identified 12 forms of Tall and 5 of Dwarf coconut in India. Since then, germplasm prospectors have documented various coconut types in different agro-ecological regions in India, such as Orissa (Panda 1982), Andaman and Nicobar Islands (Balakrishnan and Pillai 1979) and Lakshadweep islands (Jacob and Krishnamoorthy 1981). There are also reports on distinct cultivars identified in a particular location, such as 'Spicata' (Jacob 1941) and 'Ayiramkachi' (Ramachandran et al. 1977). All the different forms of coconut collected from different agro-ecological zones of India have been categorized by location of collection or cultivation, or by specific traits, such as small-fruited nuts (e.g. 'Laccadive Micro Tall'), large quantity of nut water ('Kappadam Tall'), spikeless character with a large number of female flowers ('Spicata') and Dwarfs for their distinct nut colour (e.g. green, yellow or orange-red). Thus, much of the earlier grouping of Indian coconut germplasm has been based on morphological characteristics.

It is essential to understand the genetic diversity within Indian coconut cultivars in order to utilize effectively them for breeding purposes. Knowledge of relatedness among different coconut cultivars will also help in identifying cultivars and thus avoiding duplication in the continuing screening programme for endemic root (wilt) disease (Jacob et al. 1998). Knowing relationships among Indian cultivars and other major cultivars will also help when formulating future breeding strategies involving exotic cultivars.

Upadhyay et al. (2004) reported a narrow range of genetic diversity present in the Indian coconut accessions, using random amplified polymorphic DNA (RAPD) techniques. Microsatellite markers or simple sequence repeats (SSR) have become the preferred molecular markers for studying genetic diversity in many crops because of its co-dominant (genotyping of homozygosity or heterozygosity for a particular loci is possible) multi-allelic nature, ease of use and repeatability. In coconut, its efficiency and application in diversity analysis and for genotyping various cultivars had been demonstrated by previous workers (Rivera et al. 1999; Perera et al. 2000; Teulat et al. 2000; Baudouin and Lebrun 2002; Perera et al. 2003; Cardena et al. 2003).

The main aim of this study was to use microsatellite markers to identify the genetic diversity present within Indian cultivars and their relationship to other important coconut cultivars in germplasm collections.

Materials and methods

Plant material

Leaf samples were collected from the coconut germplasm collection maintained at the Central Plantation Crops Research Institute (CPCRI), Kasaragod. The accessions (listed together with their acronyms in Table 1) were collected as open-pollinated nuts from their respective geographical locations, except for few cultivars through germplasm exchange, such as 'Kulasekharam Orange Dwarf' and 'Cameroon Red/Orange Dwarf', which are secondary introductions. The leaf samples of 'Assam Green Tall' were collected from the collection maintained at the Assam Agricultural University campus at Kahikuchi, Assam. A total of 45 accessions were included in this study, of which 21 (15 Talls, 5 Dwarfs and 1 intermediate type) were indigenous cultivars and 24 were exotic cultivars (18 Talls and 6 Dwarfs). Sample size varied from 3 to 6 palms per accession. The exotic cultivars were mainly from major coconut areas (South-east Asia (11), South Pacific (4), Sri Lanka (2), Africa (5) and South America (2); see Table 1).

DNA extraction

DNA was extracted from the spear leaf following the procedure of Upadhyay et al. (1999), whereby 5 g of spear leaf tissue was ground in liquid nitrogen and transferred to extraction buffer containing 10% sodium dodecyl sulphate (SDS). The contents were boiled at 65°C, cooled and extracted with an equal volume of 24:1 chloroform:isoamyl alcohol mixture. The supernatant was transferred to a new tube and DNA was precipitated with 70% ethanol.

Table 1. Indian and exotic coconut accessions used in the study

Accession (Region or country of origin)	Code	No of samples	Genetic diversity	
Indian accessions				
Talls				
Andaman Ordinary Tall	ADOT	4	0.33	0.19
Assam Green Tall (Assam)	AGT	6	0.36	0.30
Ayiramkachi Tall (Tamil Nadu)	AYRT	4	0.33	0.31
Benalium Tall (Goa)	BENT	4	0.33	0.15
Calangute Tall (Goa)	CALT	4	0.36	0.35
East Coast Tall (Tamil Nadu)	ECT	4	0.49	0.50
Gangapani Tall (Andhra Pradesh)	GPNT	3	0.34	0.42
Kappadam Tall (Kerala)	KPDT	6	0.42	0.51
Laccadive Ordinary Tall	LCT	4	0.59	0.62
Laccadive Micro Tall	LMT	4	0.30	0.23
Orissa Giant Tall (Orissa)	OGT	4	0.30	0.20
Orissa Tall (Orissa)	SKGT01	4	0.40	0.50
Tiptur Tall (Karnataka)	TPT	4	0.40	0.24
West Coast Tall (Kerala)	WCT	4	0.35	0.43
Spicata (Kerala)	WCT01	4	0.39	0.47
Dwarfs				
Andaman Yellow Dwarf	AYD	4	0.06	0.13
Chowghat Green Dwarf (Kerala)	CGD	4	0.00	0.00
Chowghat Orange Dwarf (Kerala)	COD	4	0.07	0.03
Gangabondam Green Dwarf (Andhra Pradesh)	GGBD	4	0.17	0.06
Kulasekharam Orange Dwarf (Tamil Nadu)	MRD02	4	0.58	0.78
Kenthali Orange Dwarf (Karnataka)	KTOD	4	0.06	0.13
Exotic accessions				
Talls				
Borneo Tall (South-east Asia)	BOR	4	0.23	0.25
Cochin China Tall (South-east Asia)	COC	4	0.28	0.35
Ceylon Tall (Sri Lanka)	CT	4	0.30	0.44
Fiji Tall (South Pacific)	FJT	4	0.32	0.50
Guam Tall I (South Pacific)	GUBTI	4	0.55	0.57
Guam Tall II (South Pacific)	GUBTII	4	0.39	0.29
Java Tall (Southeast Asia)	JVT	4	0.37	0.28
Kenya Tall (Africa)	KNT	4	0.33	0.16
Philippines Lono (South-east Asia)	P.Lono	4	0.36	0.38
Philippines Ordinary Tall (South-east Asia)	PHOT	4	0.20	0.50
Sanramon Tall (South-east Asia)	SNRT	4	0.20	0.30
Straits Settlements Green (South-east Asia)	SSG	4	0.43	0.54
Surinam Tall (South America)	SUT	4	0.46	0.24
Federated Malayan States (South-east Asia)	MLT01	4	0.36	0.22
West African Tall (Africa)	WAT	4	0.10	0.06
Zanzibar Tall (Africa)	ZNT	4	0.46	0.14
Gonthembili Tall (Sri Lanka)	GT	4	0.33	0.15
New Guinea Tall (South Pacific)	NG	4	0.32	0.22
Dwarfs				
Malayan Green Dwarf (South-east Asia)	MGD	4	0.19	0.16
Malayan Orange Dwarf (South-east Asia)	MOD	4	0.11	0.09
Malayan Yellow Dwarf (South-east Asia)	MYD	4	0.18	0.09
Nigerian Green Dwarf (Africa)	NIGD	4	0.23	0.38
Cameroon Red/Orange Dwarf (Africa)	CRD	4	0.49	0.60
Surinam Brown dwarf (South America)	SUBD	4	0.37	0.54
Mean			0.315	

PCR assay and gel analysis

For microsatellite analysis, each well received 12.5 ng of DNA, 200 µM deoxynucleotide triphosphate (dNTPS), 1 unit of *Taq* polymerase (Bangalore Genie, India) and 1 µM of each primer. The polymerase chain reaction (PCR) conditions were identical to those of Perera et al. (2000). The amplified products were resolved in a 5% denaturing polyacrylamide gel and the bands were visualized by silver staining (Bassam and Caetano-Anollés 1993). The microsatellite bands were scored manually and the alleles were sized with reference to a 30–330 bp ladder (Gibco Brl).

Statistical analysis

The calculation of genetic diversity values and construction of the unweighted pair group method with arithmetic mean (UPGMA) dendrogram using Nei's genetic distance (Nei et al. 1983) was carried out using the POWERSSR v 1.2 software (Liu 2001). The PCA plot was constructed for all the accessions using Nei's genetic distance (1972) with gene frequency data using NTSYS pc v.2.02i software (Applied Biostatistics Inc, USA). The analysis of molecular variance was done using GENALEX software (Peakall and Smouse 2001) with a significance setting permutation value of 999.

Results and discussion

Microsatellite polymorphism and unique alleles

The eight coconut microsatellite loci distinguished a total of 48 alleles, with an average of 6 alleles per locus (Table 2). The CAC8 locus had a maximum of 10 alleles, while CAC3, CAC11 and CAC13 had 4 alleles each. Only one unique allele of 214 bp with CAC8 primer was found in accession 'Spicata', with a frequency of 0.125. Another allele of 166 bp with CAC13 primer was found only in one sample each from 'Kulasekharam Orange Dwarf' (MRD02) and 'Laccadive Ordinary Tall', with a frequency of 0.125 each.

Genetic diversity

The genetic diversity for each population across loci ranged from 0.00 for 'Chowghat Green Dwarf' to 0.59 for 'Laccadive Ordinary Tall', with an average of 0.31. The average mean diversity was 0.23 among Dwarfs and 0.35 among Talls. Among Dwarfs, 'Kulasekharam Orange Dwarf' showed a high mean diversity of 0.58, which is significantly higher than many Talls (Table 1).

A study by Bavappa and Sukumaran (1983) of floral biology and breeding systems in some of the Indian cultivars, using intra- and inter-spadix overlapping of male and female phases, revealed that 'Chowghat Green Dwarf' is 100% overlapping, while 'Laccadive Ordinary Tall' had a range of 0 to 35%. The extent of selfing or crossing in coconut is dependent on the overlapping of the inter- and intra-spadices of male and female flowers. Thus the genetic diversity estimate is essentially a reflection of the breeding nature of the accession. The genetic diversity values obtained through microsatellite studies are in agreement with the above findings, except for 'Kulasekharam Orange Dwarf', which was the reverse of previous findings.

'Kulasekharam Orange Dwarf' was imported into India from Sri Lanka in the early 1900s by some private estate owners (Nair 1960). 'Kulasekharam Orange Dwarf' was included in the germplasm collection during the 1950s, and the palm samples analysed for this study were derived from the core collection. With a Nei diversity index of 0.58 and a heterozygosity rate of 0.78, it is possible that the 'Kulasekharam Orange Dwarf' individuals are in reality 'naturally-crossed Dwarfs' (i.e. hybrids obtained by natural pollination). This hypothesis seems to be confirmed by 'Kulasekharam Orange Dwarf' sorting into the same cluster as most Indian Talls (see Figure 1). It is quite unusual for a Dwarf to have such a high heterozygosity value, but it is possible because of admixtures or through pollen parents of local origin.

Genetic diversity in Indian germplasm accessions

The overall mean genetic diversity of all the accessions was a moderate 0.315. The genetic diversities were comparable between Indian and exotic accessions.

Heterozygosity levels among populations

'Kulasekharam Orange Dwarf' showed the highest heterozygosity (0.78), while 'Chowghat Green Dwarf' was

Table 2. Details of microsatellites, alleles detected and gene diversity in Indian and exotic coconut accessions

Micro-satellite	No. of alleles detected	Motif of the microsatellite†	Alleles (bp)	Gene diversity
CAC2	7	$(CA)_{12}(AG)_{14}$	210, 228, 232, 234, 240, 246, 248	0.362
CAC3	4	$(CA)_{13}$	187, 197, 199, 201	0.346
CAC4	8	$(CA)_{19}(AG)_{17}$	182, 186, 188, 200, 204, 208, 212, 216	0.301
CAC6	7	$(AG)_{14}(CA)_{9}$	150, 152, 154, 156, 158, 160, 162	0.389
CAC8	10	$(AG)_{10}(CA)_{9}$	188, 196, 198, 200, 202, 204, 206, 208, 210, 214	0.377
CAC10	5	$(TA)_{6}(CATA)(CA)_{11}(TA)_{8}$	195, 197, 201, 203, 205	0.355
CAC11	4	Complex $(CA)_{n}(TA)_{n}$	156, 158, 162, 170	0.246
CAC13	3	$(CA)_{9}(TA)_{5}A(TA)_{4}(CA)_{6}$	158, 162, 166	0.146
Mean	–	–	–	0.315

† For details of primer sequences and repeat types, see Perera et al. (2000).

0.00 (Table 1). Lebrun et al. (1998) reported that the Dwarfs were generally homozygous. Rivera et al. (1999) reported that there was some degree of heterozygosity in the Dwarfs, and our study supports this finding. All the other Dwarfs, with the sole exception of 'Chowghat Green Dwarf', showed some degree of heterozygosity. The highly homozygous nature of 'Chowghat Green Dwarf' was also supported by additional data on a large number of 'Chowghat Green Dwarf' samples (40 samples) using the same primers (data not shown). The other Dwarf cultivars exhibited some variation. The high homozygosity of 'Chowghat Green Dwarf' means that several such Dwarf palms can be used as the female parent in a cross involving a common pollen donor to create a backcross mapping population (Lebrun et al. 2001). This is particularly significant, as 'Chowghat Green Dwarf' is the widely grown Dwarf palm in the endemic root (wilt) region of Kerala. 'Kulasekharam Orange Dwarf' showed the highest heterozygosity among Dwarfs, followed by 'Cameroon Red/Orange Dwarf'. 'Cameroon Red/Orange Dwarf' entered the CPCRI germplasm collection in 1970s as a secondary collection from Côte d'Ivoire. Both 'Kulasekharam Orange Dwarf' and 'Cameroon Red/Orange Dwarf' are secondary introductions in the germplasm and possible admixtures in their respective areas of collection might explain the discrepancy in the results. Normally, Dwarfs are considered highly self-pollinating and therefore breed true to type. Sometimes, insufficient care is taken to manually self-pollinate when rejuvenating, and this may be a possible cause for the above findings.

Analysis of molecular variance

An analysis of molecular variance (AMOVA) analysis was carried out to partition the genetic diversity between populations through Rst analysis (Slatkin 1995). Significant differences ($p<0.001$) were detected (Table 3). The within-population variation was found to be higher (58%) than the among-population variation (42%). Among Indian populations, the within-population diversity was found to be 53% and the between-population variation was found to be 47%. In both cases, the within-population components of variance dominated the AMOVA, accounting for slightly greater than 50% of the variation. Earlier studies, using RAPD markers in South Pacific coconut populations, reported that the between-population variation was higher than within-population variation. (Ashburner et al. 1997). Based on amplified fragment length polymorphism (AFLP) analysis in Sri Lanka coconut populations, Perera et al. (2000) reported that there is equal partitioning of within- and between-population variations. This difference could be attributed to the different marker systems and the different populations used in the analysis. Microsatellites have been reported to be superior in such genetic population assignment tests and for discerning the fine-scale genetic differentiation between subpopulations (Loughheed et al. 1999). In coconut, SSRs have been reported to be more informative in revealing allelic differences within and between populations (Teulat et al. 2000). The slightly higher within-population variation observed in our study is possibly a founder effect acting on different populations, and their subsequent divergence.

UPGMA cluster analysis

Unweighted Pair Group Method with Arithmetic Mean (UPGMA) cluster analysis (Figure 1), constructed using Nei's genetic distance (1972), resulted in a phenotype dendrogram (phenogram) that separated the accessions into two major groups, i.e. a Dwarf group and a Tall group, with a few anomalies, such as 'Assam Green Tall' and 'Guam Tall II' in the Dwarf group, and 'Kulasekharam Orange Dwarf' and 'Malayan Green Dwarf' in the Tall group. The outliers are 'Andaman Ordinary Tall', 'Nigerian Green Dwarf' and 'Surinam Brown Dwarf'.

Among the Talls, two sub-clusters could be identified, namely South-east Asian Talls and Indian Ocean Talls. Exceptions among these groups were 'Kappadam Tall', 'Spicata' and 'Zanzibar Tall', which are Indian Ocean Talls that clustered with South-east Asian Talls. A South-east Asian Tall cultivar, 'Java Tall', clustered with the Indian Ocean Group.

Based on fruit component analysis, Harries (1978) hypothesized that 'Kappadam Tall', a large-fruited nut, is probably of South-east Asian origin, which became introgressed with Indian cultivars upon introduction into India, and it was classified in the Niu Vai group, characterized by bigger nuts with less husk. 'Kappadam Tall' is the only cultivar from India that was included in the Niu Vai group. In our study, 'Kappadam Tall' appeared to be closer to South-east Asian Tall

Table 3. Analysis of molecular variance (AMOVA) for the different Indian and exotic coconut populations used in the study

Source of variation	Df	SS	MS	Est. Var.	t-Value	Prob.
All accessions						
Among populations	44	4 360	99.1	10.7		
Within populations	309	4 554	14.74	14.7	0.42	0.001
Indian Accessions						
Among populations	20	2 214	110.7	11.9		
Within populations	151	2 009	13.30	13.3	0.47	0.001

Df = degrees of freedom; SS = sum of squares; MS = mean sum of squares; Est. Var. = estimated variation; Prob. = probability.

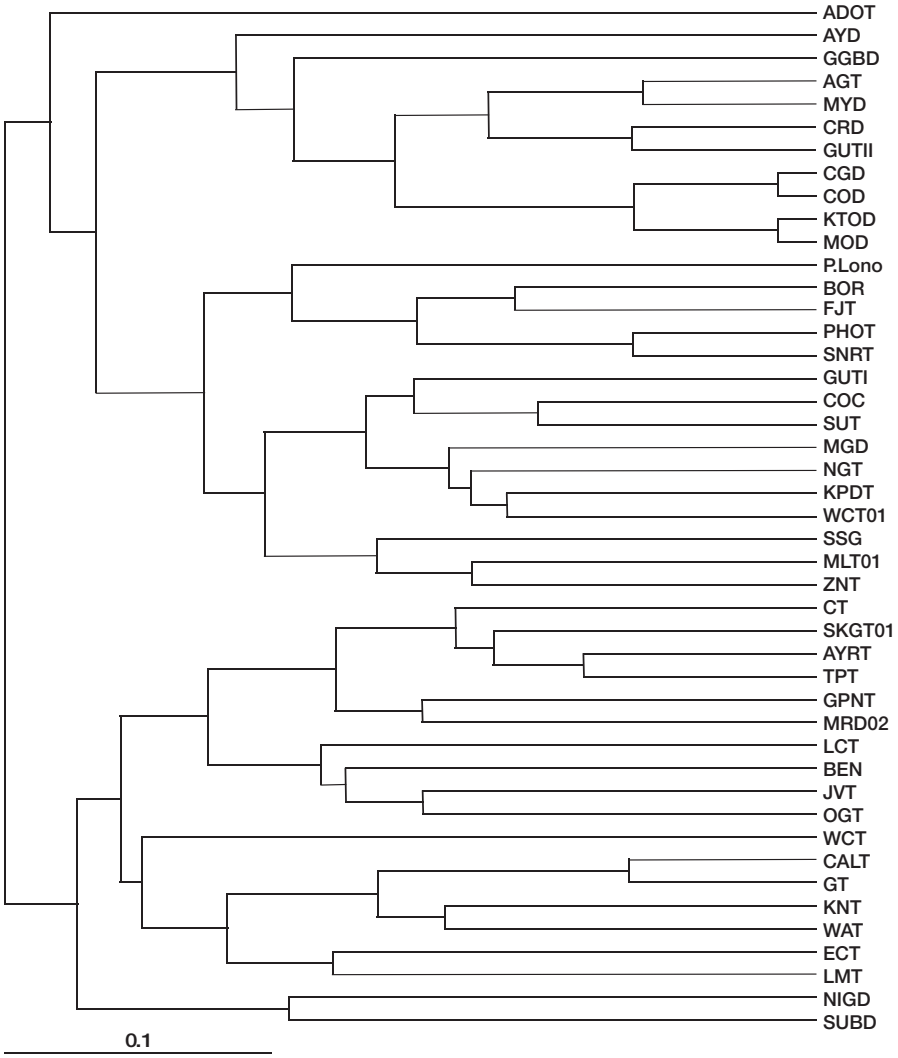

Figure 1. Unweighted pair group method with arithmetic mean (UPGMA) dendrogram showing the relationship between Indian and exotic coconut accessions. For key to cultivar names, see Table 1.

cultivars. In contrast, Teulat et al. (2000) placed 'Kappadam Tall' in the Indian Ocean group of coconut populations. One possible explanation is that, in our study, fewer microsatellites were used. The number of microsatellites and the discriminating capacity of microsatellites can play a role in identifying such introgressed forms of coconut accessions.

'Spicata', considered to be a variant of 'WCT' according to the COGENT Coconut Genetic Resources Database, also showed a clear proximity to South-east Asian cultivars. Perera et al. (2000) and Meerow et al. (2003) had included 'Spicata' in their study and found it to cluster with South-east Asian cultivars. Both of them had used 'Spicata' from South-east Asia and the Americas (which were introductions from South-east Asia). 'Spicata' is characterized by a large number of female flowers and very few male flowers. The study of their reproductive biology shows that it is strictly cross-pollinated (Ratnambal et al. 1995). It is quite possible that the 'Spicata' cultivars of coconut may have a common origin, as for Dwarfs (Lebrun et al. 1998).

In the present study, the only South-east Asian accession that grouped with the Indian Ocean group was 'Java Tall'. Java, in Indonesia, had historical connections with the Indian subcontinent during the 6th and 7th centuries, with the establishment of the Sailendra Empire by the rulers of Orissa State, India. A great deal of cultural exchange took place during this period and coconut, being a cultural and religious symbol of the rulers, might have spread to the islands. This hypothesis is supported by the subgrouping of 'Java Tall' with 'Orissa Giant Tall' in the phenogram. A similar result was obtained using RAPD analysis of Indian coconut germplasm by Upadhyay et al. (2004), where 'Java Tall' clustered in the same group as 'Orissa Giant Tall'. Harries (1978), based on the available morphological data, indicated that the coconut forms of Bali, Indonesia—also influenced by nearby Java and Sulawesi—were notable for their large nuts and attributed this to the religious isolation of this region.

'Malayan Green Dwarf' has been reported to be cross-pollinating (Lebrun et al. 1999), and in our study this cultivar clustered with the Talls from the South-east Asian and South Pacific regions.

'Andaman Ordinary Tall' is from a geographical region where the Indian Ocean group and the South-east Asian group meet. Because of the proximity of this region to the South-east Asian region, this represents an introgressed type.

'Assam Green Tall' and 'Guam Tall I' are the only Talls that clustered with the Dwarf group. 'Assam Green Tall' is a typical Tall cultivar cultivated in the north-eastern region of India. The Assam region is not traditional coconut growing area and the coconuts there are believed to have come from the South-east Asian region. Morphologically, 'Assam Green Tall' resembles South-east Asian cultivars.

PCA analysis of accessions

The first axis of the principal components analysis (PCA; Figure 2) separated all the Tall and Dwarf accessions. The second and third axes distinguished the South-east Asian Talls from other Indian Ocean (Indian and African) Talls. The first, second and third axes showed 33.9%, 20% and 12.7% variation, respectively. Three Dwarfs—'Kulasekharam Orange Dwarf', 'Malayan Green Dwarf' and 'Cameroon Red/Orange

Dwarf'—lie centrally between the Dwarf and Tall groups, which is indicative of their hybrid nature. Overall, the pattern of grouping in PCA is similar to the phenetic cluster groups obtained through UPGMA. 'Java Tall' separated further from Indian accessions and is located at the farther end of the PCA graph, which can be attributed to genetic isolation and local adaptation.

Similarity between cultivars

'Chowghat Green Dwarf' and 'Chowghat Orange Dwarf' are homozygous at all the loci except one (CAC2 locus, 234 and 246 bp alleles for 'Chowghat Orange Dwarf' and 246 bp homozygous allele for 'Chowghat Green Dwarf') where the 'Chowghat Orange Dwarf' is heterozygous. Likewise, 'Malayan Orange Dwarf' and 'Kenthali Orange Dwarf' are similar for all the loci except two (CAC5 and CAC3). The Chowghat Dwarfs are indigenous coconut accessions, mainly found in the Kerala region of India. The high level of similarity between these two cultivars means that either one is a derivative of the other. It is likely that the 'Chowghat Orange Dwarf' is a mutant form.

Coconut dissemination

The UPGMA phenetic tree and the PCA analysis are consistent with the morphological classification of coconuts and their dissemination (Harries 1978). In general, the two coconut groups could be distinguished. The clustering of 'Surinam Tall' with the South-east Asian group reinforces the hypothesis of an eastward spread of coconut.

Conclusions

In summary, the results of our work validate previous work on coconut microsatellite markers and are consistent with the morphological classification of coconut. Based on the eight microsatellites, it was found that the genetic diversity of the Indian Talls and Dwarf coconut accessions were comparable with genetic diversity estimated for exotic Talls and Dwarfs. In general, the dwarfs showed less gene diversity, except two—MRD02 and 'Cameroon Red/Orange Dwarf'—that are secondary introductions. The UPGMA cluster analysis distinguished, although not tightly, the Dwarf and Tall coconut populations, and separated Talls into two groups, namely Indian Ocean Talls and South-east Asian Talls. The introgressed forms from the regions where the two groups meet, such as 'Andaman Ordinary Tall', 'Surinam Brown Dwarf' and 'Nigerian Green Dwarf', could be distinguished in the UPGMA cluster diagram.

The Niu Vai type South-east Asian Tall accessions were poorly represented in the germplasm collections. Only five to

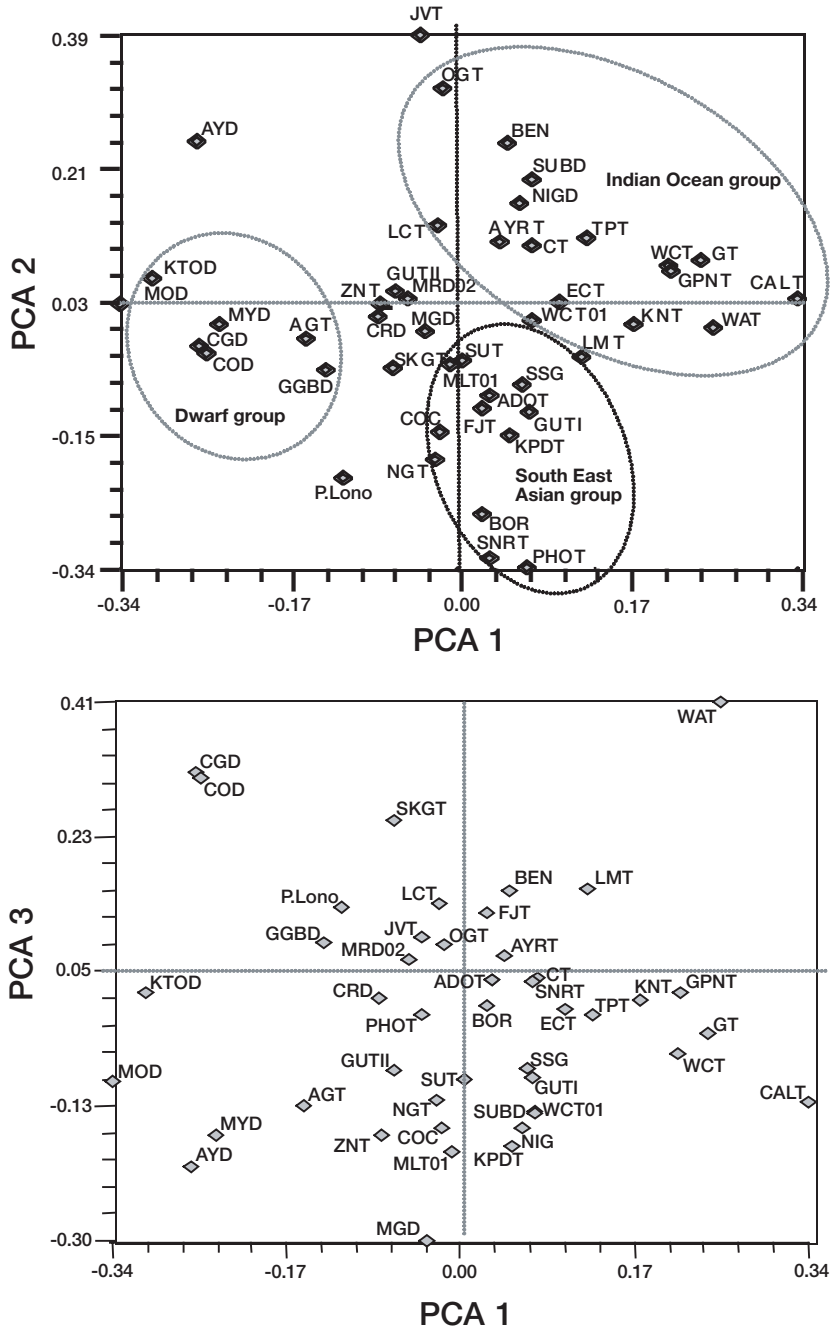

Figure 2. Two-dimensional plot of PCA 1 vs. PCA 2, showing three groups of coconut accessions. For key to cultivar names, see Table 1 (top). Two-dimensional plot of PCA 1 vs. PCA 3 for Indian and exotic coconut accessions. For key to cultivar names, see Table 1 (bottom).

ten individuals of South-east Asian coconut germplasm are present in the germplasm collections, making it imperative to import more South-east Asian Tall accessions to provide a wider germplasm repertoire and potentially use them in the Indian coconut breeding programme.

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