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RESEARCH ARTICLE

Characterisation of Sri Lanka Yellow Dwarf Coconut (*Cocos nucifera* L.) by DNA fingerprinting with SSR markers

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Abstract: Coconut (*Cocos nucifera* L.) is classified into three main varieties in Sri Lanka as tall, dwarf and intermediate with each variety including several forms of coconut. Sri Lanka Yellow Dwarf (SLYD) is a coconut form included under the variety dwarf and it is a parent of the improved coconut hybrid CRIC65. Morphological differentiation of SLYD from Sri Lanka Yellow Semi Tall (SLYST) and a mixed morphological group (ML) of SLYD and SLYST is impossible at the seedling stage. The objective of the present study was to derive DNA fingerprints for SLYD and SLYST for distinct identification at any stage during its life cycle.

A total of 30 palms were genotyped with sixteen polymorphic SSR primer pairs and data were analysed with the PowerMarker V3.25 software. The results revealed SLYD specific DNA fingerprints at SSR loci CAC65, CAC4, CNZ10, CnCirD8 and CNZ6. These markers scored specific homozygous alleles for SLYD with very high allelic frequency, and scored very low frequencies in SLYST and ML phenotypes. SLYD formed a clear separate cluster in the UPGMA dendrogram while SLYST and ML formed separate mixed clusters. Thus the present study was successful in generating specific DNA fingerprints for SLYD and this information will be of practical use in differentiating SLYD at the seedling stage, especially in the establishment of isolated coconut seed gardens for mass production of improved coconut cultivars having SLYD as a parent.

Keywords: DNA finger printing, genetic diversity, microsatellite markers, yellow dwarf coconuts.

INTRODUCTION

Coconut is the sole species of the genus *Cocos* belonging to the subfamily Cocoideae and the lower group of flowering plants referred to as monocotyledons. It is distributed throughout the tropics and is native to the coastal areas of South East Asia, the Pacific and South Asia and Africa in the Indian Ocean. Coconut is grown in over 80 countries throughout the world (Smith *et al.*, 2009) and every part of the coconut palm is of use in different ways. Consequently, coconut is termed as the 'tree of life'.

Based on the breeding behaviour and the stature, two groups of coconuts, referred to as Tall (*Typica*) and Dwarf (*Nana*) have been known the world over. Tall palms are tall in stature and cross-pollinate naturally while dwarf palms are short and self pollinating. In comparison with the tall palms, dwarf palms display a narrow genetic variability due to their naturally self pollinating breeding behaviour (Perera *et al*., 2003). In Sri Lanka, coconut has been classified into three varieties as Tall (*Typic*a), Dwarf (*Nana*) and Intermediate (*Aurantiaca*), and under these three varieties 13 different forms of coconut were recognised by Liyanage (1958). Later, more forms were added into the varietal classification of coconut in Sri Lanka (Liyanage *et al*., 1988; Perera *et al.*, 1997;

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Ekanayake *et al.*, 2010). The Sri Lankan dwarf variety consists of four fruit colour based phenotypes as, green, yellow, red and brown. The intermediate variety possesses mixed characters of tall and dwarf coconuts and it is not common in the world. Originally three different phenotypes of king coconut (*Thembili*) were included in the intermediate variety.

A recent study reported the morphological non-uniformity of the Sri Lanka Yellow Dwarf (SLYD) coconut population (Kamaral *et al.*, 2014; Perera *et al.*, 2015). Later, in addition to the pure SLYD, a new semi tall coconut phenotype was identified within this population bearing yellow coloured nuts. This new phenotype was named Sri Lanka Yellow Semi Tall (SLYST) and was included as a new coconut form in the variety *Aurantiaca* (Kamaral *et al*., 2016). The same studies revealed the presence of coconut phenotypes displaying mixed characters of SLYD and newly identified SLYST. In addition, molecular studies revealed the genetic variation of the coconut population bearing yellow colour coconuts in comparison to Sri Lanka tall, gon thembili tall and green dwarf as reference varieties (Kamaral *et al.*, 2014; Perera *et al.*, 2015).

Hybrid coconuts derived by crossing dwarf with tall coconuts are favoured by the majority of coconut growers in Sri Lanka due to their desirable attributes such as precocity, higher number of nuts/palm and copra productivity per unit land area. SLYD is one of the female parents of the recommended coconut hybrid CRIC65 (yellow), which is included in the national coconut hybridisation programme in Sri Lanka. This hybrid is mass produced by directed natural pollination at the Isolated Coconut Seed Garden (ISG) at Ambakelle, Sri Lanka.

Use of true-to-type parent material in seed gardens is essential to ensure the authenticity of the resulting hybrid planting material. In this respect it is vital to distinguish the different coconut forms especially at the seedling stage. Colour of the petioles is a reliable morphological marker in differentiating dwarf coconut forms; green, red, brown and yellow at the seedling stage. However, the seedlings of SLYD and SLYST are similar in morphology making it difficult to distinguish the two forms morphologically. Therefore, with the discovery of the novel coconut form SLYST, a need for separate identification of the seedlings of this variety form SLYD became a practical necessity. DNA fingerprinting using molecular markers is the most reliable method for definitive differentiation of life forms, and the previous studies on this population have not resulted in sufficient information for distinguishing the two phenotypes. Simple sequence repeat (SSR) markers are often used in current molecular biological studies mainly due to the ease of use, high genomic abundance and the co-dominance inheritance. Accordingly, the objective of the current study was to compare the DNA profiles of coconut phenotypes SLYD and SLYST at a higher number of SSR marker loci to distinguish the pure SLYD from SLYST and the rest of the dwarf and intermediate phenotypes bearing yellow coloured nuts.

METHODOLOGY

A random sample of 30 palms from a mixed population of SLYD, SLYST and mixed phenotypes planted in the *ex-situ* field gene bank of coconut, established at the Coconut Research Institute of Sri Lanka was selected for the study. The palms of the sample were at the age of 32 years. They were visually observed and categorised into three morphological groups; SLYD, SLYST and mixed group (ML) based on the stature of palm, presence/absence of a root bole (swollen part of the base of stem), and appearance of the crown (shorter fronds, drooping rachis ends *vs* longer fronds, non-drooping rachis ends).

Genomic DNA was extracted from the tender leaf tissues of the 30 palms using a modified CTAB DNA extraction method developed by Weising and Karl (1997), and Doyle and Doyle (1990). A total of 16 microsatellite primer pairs consisting of CAC (Perera *et al*., 2003), CNZ (Rivera *et al.*, 1999), and five CnCir markers (Baudouin *et al*., 2006) were used for genotyping (Table 1).

The polymerase chain reaction mixture contained 4 µL of (20 ng/µL) template DNA, 1x *Taq* PCR green buffer containing 2 mM $MgCl_2$, 1.25 U of *Taq* DNA polymerase (Dream *Taq*- Fermentas), deoxynucleoside triphosphates (0.35 mM each; Geneshun Biotech) and 0.6 µM primer pair (1st BASE) in a final volume of 25 µL. The PCR programme consisted of 4 min initial denaturation at 94 °C followed by 35 cycles of 30 s at 94 °C, 30 s at annealing temperature (depending on primer) and 1 min at 72 °C, and final extension at 72 °C for 5 min. The PCR was performed using thermal cycler (Applied Biosystems).

The PCR product was subjected to 6 % denaturing polyacrylamide gel electrophoresis followed by silver staining (Anolles & Petter, 1994). The genotypes were obtained by allele size (number of base pairs) differences in comparison to a standard marker (50 bp).

Table 1: Details of microsatellite primers used for genotyping Sri Lanka Yellow Dwarf coconut palms

Oligo name		Sequence	Size	Annealing temperature
CAC04	F	5′-CCC CTA TAG ATC AAA ACA AG-3′	182 – 216 bp	58 °C
	R	5′-CTC AGT GTC CGT CTT TGT CC-3′		
CAC06	F	5′-TGT ACA TGT TTT TTG CCC AA-3′	150 – 168 bp	52 °C
	R	5′-CGA TGT AGC TAC CTT CCC C-3′		
CAC08	F	5′-ATC ACC CCA ATA CAA GGA CA-3′	188 – 210 bp	56 °C
	R	5′-AAT TCT ATG GTC CAC CCA CA-3′		
CAC65	F	5′-GAA AAG GAT GTA ATA AGC TGG-3′	150 – 173 bp	54 °C
	R	5′-TTT GTC CCC AAA TAT AGG TAG-3′		
CNZ04	F	5′-TAT ATG GGA TGC TTT AGT GGA-3′	130 – 166 bp	53 °C
	R	5′-CAA ATC GAC AGA CAT CCT AAA-3′		
CNZ06	F	5′-ATA CTC ATC ATC ATA CGA CGC-3′	69 – 97 bp	52 °C
	R	5′-CTC CCA CAA AAT CAT GTT ATT-3′		
CNZ10	F	5′-CCT ATT GCA CCT AAG CAA TTA-3′	108 – 152 bp	56 °C
	R	5′-AAT GAT TTT CGA AGA GAG GTC-3′		
CNZ12	F	5′-TAG CTT CCT GAG ATA AGA TGC-3′	218 – 229 bp	54 °C
	R	5′-GAT CAT GGA ACG AAA ACA TTA-3′		
CNZ40	F	5′-CTT GAT TGC TAT CTC AAA TGG-3′	143 – 155 bp	56 °C
	R	5′-CTG AGA CCA AAT ACC ATG TGT-3′		
CNZ44	F	5′-CAT CAG TTC CAC TCT CAT TTC-3′	151 – 170 bp	52 °C
	R	5′-CAA CAA AAG ACA TAG GTG GTC-3′		
CNZ46	F	5′-TTG GTT AGT ATA GCC ATG CAT-3′	101 – 120 bp	56 °C
	R	5′-AAC CAT TTG TAG TAT ACC CCC-3′		
CnCir01	F	5′-TTG GTC TAT TGC ATG TTC-3′	150 bp	44 °C
	R	5′-TGG CAT TGA GAG GGT-3′		
CnCirC5	F	5′-ACC ACC AAA GCC AGA GC-3′	133 bp	50 °C
	R	5′-GCA GCC ACT ACC TAA AAA G-3′		
CnCirD8	F	5′GCT CTT GAT GTG GCT GCT-3′	250 bp	54 °C
	R	5′-AGG CGT GTT GAG ATT GTG A-3′		
CnCirHll	F	5′-TCA TTC AGA GGA CAA AAG TT-3′	150 – 200 bp	46 °C
	R	5′-TAA AAA TTC ATA AAG GTA AAA-3′		
CnCir51	F	5′-TCT CGT GGA TCT CGT C-3′	200 bp	48 °C
	R	5′-GCT CTT CCA GTT ACG TTT-3′		

Genotypic data were analysed with PowerMarker V3.25 software (Liu & Muse, 2005) to derive genetic relationships among individuals.

RESULTS AND DISCUSSION

The sample of 30 palms consisted of 10, 12 and 08 palms grouping into SLYD, SLYST and ML, respectively.

SSR marker analysis

All of the 16 SSR primer pairs scored polymorphic loci within the tested sample. A total of 58 alleles were scored resulting in four alleles per locus (Table 2). Among these SSR markers the highest number of alleles (5) was observed with CAC65, CAC04, CnCir01 and CnCirHll markers. The highest gene diversity value of 0.6173 was observed in CAC65 marker and the smallest value was observed in CAC06. A total of 74 genotypes were scored with a mean value of five. Heterozygosity ranged from zero to 0.1852 with a mean value of 0.09. These results are in par with the previous studies conducted on this population.

Summary statistics were calculated for the three phenotypes of SLYD, SLYST and ML separately and comparison was made between the coconut forms (Table 3).

Table 2: Summary statistics of genotypic data of the coconut palms assayed by SSR markers

Marker	Major allele frequency	Genotype no.	Allele no.	Gene diversity	Heterozygosity	PIC
CAC65	0.4821	7	5	0.6173	0.1429	0.5448
CNZ44	0.6667	4	3	0.4664	0.0741	0.3869
CAC04	0.5000	7	5	0.6269	0.1852	0.5626
CNZ04	0.8833	5	4	0.2150	0.1000	0.2076
CAC06	0.9000	4	4	0.1861	0.1333	0.1798
CAC08	0.8833	5	4	0.2150	0.0667	0.2076
CNZ10	0.5667	2	2	0.4911	0.0000	0.3705
CNZ12	0.8621	4	3	0.2449	0.1379	0.2272
CNZ40	0.8833	3	2	0.2061	0.1000	0.1849
CNZ46	0.8500	3	2	0.2550	0.0333	0.2225
CnCir01	0.8333	6	5	0.2983	0.0333	0.2883
CnCirC5	0.5500	5	4	0.5361	0.1000	0.4380
CnCir51	0.8500	5	4	0.2633	0.1000	0.2428
CnCirHll	0.8500	6	5	0.2689	0.1667	0.2564
CnCirD8	0.5000	2	2	0.5000	0.0000	0.3750
CNZ06	0.5000	6	4	0.5728	0.0667	0.4829
Total	-	74	58	-	-	-
Mean	0.7226	5	4	0.3727	0.0900	0.3236

PIC = polymorphic information content

Table 3: Summary statistics of the different phenotypes of Sri Lanka Yellow Dwarf coconuts

Form	Mean no. of alleles scored	Mean major allele frequency	Mean heterozygosity	Mean gene diversity	Mean PIC
SLYD	1.125	0.993	0.014	0.013	0.013
SLYST	3.313	0.729	0.125	0.410	0.364
ML	2.5	0.131	0.131	0.296	0.261

PIC = polymorphic information content

As given in Table 3 SLYST and ML were rich in allelic diversity compared to SLYD, recording higher values for mean number of alleles, heterozygosity, gene diversity and PIC in comparison to SLYD. In contrast SLYD recorded the highest mean major allele frequency compared to that of SLYST and ML. The results re-iterates the legitimacy of the purified SLYD by giving evidence for the homozygous nature and a reduction of alleles and gene diversity in SLYD in comparison to both SLYST and ML. Both SLYST and ML display certain morphological features of tall coconuts, which include more heterozygous individuals and heterogenous populations compared to dwarf coconuts. The current study thus provides molecular evidence for this phenotypic variation in SLYST and ML forms of coconut.

Cluster analysis

The UPGMA dendrogm drawn based on shared allele genetic distance method and showing the genetic relationships among the tested sample population based on 16 SSR markers, is presented in Figure 1.

The palms of the sample formed three major clusters (A, B and C) in the dendrogram. In all of these clusters, there were certain individuals, which were outgrouping from the majority of individuals in that cluster. All the SLYD individuals separated themselves forming a sub-cluster (cluster C) while SLYST and ML individuals made mixed combination in clusters A and B.

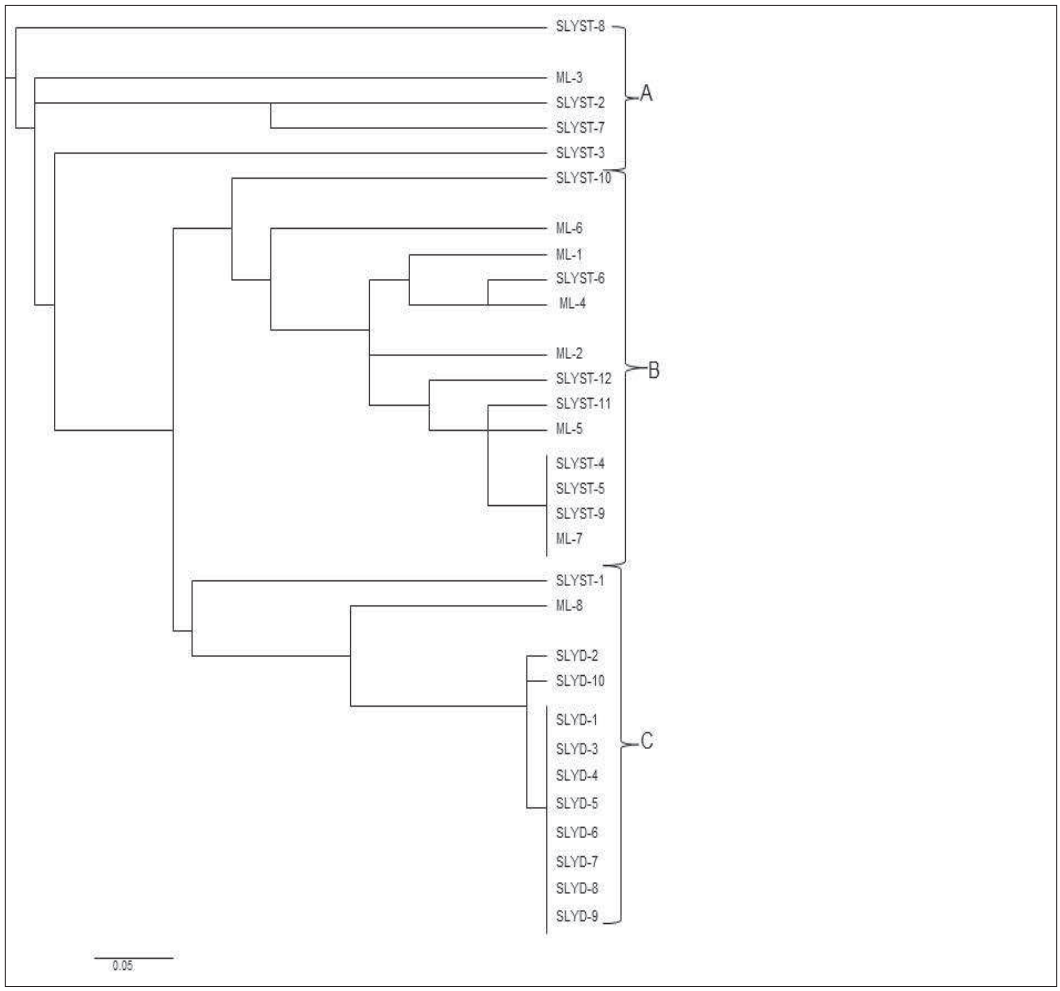

Figure 1: UPGMA dendrogram showing clustering pattern between different morphological groups within sample population of Sri Lanka Yellow Dwarf coconuts

Genotypic distinguishing of SLYD

The genotypes of each individual were observed for distinguishing different genotypes and the SSR loci CAC65, CAC04, CNZ10, CnCirD8 and CNZ06 resulted in specific identical homozygous genotypes for all the individuals in SLYD category (Figure 2). Alleles termed in this study as A1 at CAC65, C2 at CAC04, M2 at CNZ10, Z1 at CnCirD8 and D3 at CNZ06 SSR marker loci were found to be specific to SLYD in combination, and thus the combination of these SSR markers can be used to distinguish SLYD from the other two yellow coloured coconut phenotypes. However, no such specific allelic combinations were identified to distinguish the coconut form SLYST and ML groups at the 16 SSR loci that were tested in the present study indicating closer genetic relationship between SLYST and ML compared to SLYD.

The frequencies of the SLYD specific alleles with respect to each of the three different coconut phenotypes and the entire sample population are presented in Table 4.

Both SLYST and ML recorded lower frequencies of alleles (A1, C2, M2, Z1 and D3), which were identified to be specific to SLYD (Table 4). In contrast, SLYD recorded the highest frequency of 1.0 for all the specific alleles except for allele C2 that recorded a value of 0.938, which is close to one. Therefore, the SSR loci identified are highly specific and reliable to differentiate SLYD from SLYST and ML groups of coconut, which are actually genetically closer forms included in the two varieties *Nana* and *Aurantiaca*.

In a previous study conducted in Sri Lanka, the exotic tall coconut variety San Ramon, which is also used as a parent in improved coconut cultivars was distinguished

Phenotype	CAC65		CNZ44		CAC04		CNZ04		CAC06		CAC08		CNZ10		CNZ12		CNZ40		CNZ46		CnCir01		CnCirC5		CnCirF51		CnCirH11		CnCirD8		CNZ06	
SLYST-1	A1	A1	B2	B2	C2	C2	F1	F1	G2	G2	H2	H2	M1	M1	N2	N3	O2	O2	Q1	Q1	S2	S2	U5	U5	X4	X4	Y3	Y5	Z2	Z2	D4	D4
SLYD-1	A1	A1	B2	B2	C2	C3	F1	F1	G1	G1	H1	H1	M2	M2	N2	N2	O2	O2	Q1	Q1	S2	S2	U5	U5	X4	X4	Y3	Y3	Z1	Z1	D2	D3
SLYST-2	A4	A4	B2	B2	C3	C2	F1	F2	G1	G1	H1	H1	M1	M1	N3	N3	O1	O2	Q3	Q3	S2	S2	U4	U4	X3	X4	Y3	Y4	Z1	Z1	D2	D2
SLYD-2	A1	A1	B2	B2	C2	C2	F1	F2	G1	G1	H1	H1	M2	M2	N2	N2	O2	O2	Q1	Q1	S2	S2	U5	U5	X4	X4	Y3	Y3	Z1	Z1	D3	D3
SLYD-3	?	?	?	?	?	?	F1	F1	G1	G1	H1	H1	M2	M2	N2	N2	O2	O2	Q1	Q1	S2	S2	U5	U5	X4	X4	Y3	Y3	Z1	Z1	D3	D3
SLYST-3	A3	A4	B3	B3	C3	C3	F1	F1	G1	G5	H1	H1	M1	M1	N1	N2	O2	O2	Q3	Q3	S3	S3	U2	U2	X3	X3	Y3	Y3	Z2	Z2	D2	D2
ML-1	A3	A3	B2	B2	C1	C1	F1	F1	G1	G1	H1	H1	M1	M1	N2	N2	O1	O1	Q1	Q1	S4	S4	U5	U5	X4	X4	Y3	Y3	Z1	Z1	D2	D2
ML-2	A3	A3	B2	B3	C1	C1	F1	F3	G1	G1	H1	H1	M2	M2	N2	N2	O1	O2	Q1	Q1	S2	S2	U4	U5	X4	X4	Y3	Y3	Z1	Z2	D2	D2
ML-3	?	?	?	?	?	?	F4	F4	G1	G1	H1	H4	M1	M1	N1	N2	O2	O2	Q1	Q3	S1	S1	U1	U5	X1	X4	Y1	Y3	Z1	Z1	D1	D1
SLYST-4	A3	A3	B3	B3	C1	C1	F1	F1	G1	G1	H1	H1	M1	M1	N2	N2	O2	O2	Q1	Q1	S2	S2	U4	U4	X4	X4	Y3	Y3	Z2	Z2	D2	D2
SLYD-4	A1	A1	B2	B2	C2	C2	F1	F1	G1	G1	H1	H1	M2	M2	N2	N2	O2	O2	Q1	Q1	S2	S2	U5	U5	X4	X4	Y3	Y3	Z1	Z1	D3	D3
SLYD-5	A1	A1	?	?	?	?	F1	F1	G1	G1	H1	H1	M2	M2	N2	N2	O2	O2	Q1	Q1	S2	S2	U5	U5	X4	X4	Y3	Y3	Z1	Z1	D3	D3
SLYST-5	A3	A3	B3	B3	C1	C1	F1	F1	G1	G1	H1	H1	M1	M1	N2	N2	O2	O2	Q1	Q1	S2	S2	U4	U4	X4	X4	Y3	Y3	Z2	Z2	D2	D2
SLYD-6	A1	A1	B2	B2	C2	C2	F1	F1	G1	G1	H1	H1	M2	M2	N2	N2	O2	O2	Q1	Q1	S2	S2	U5	U5	X4	X4	Y3	Y3	Z1	Z1	D3	D3
SLYST-6	A3	A3	B2	B2	C1	C1	F1	F1	G1	G1	H1	H1	M1	M1	N2	N2	O2	O2	Q1	Q1	S2	S2	U5	U5	X4	X4	Y2	Y2	Z2	Z2	D2	D2
ML-4	A3	A3	B2	B2	C1	C1	F1	F1	G1	G1	H1	H1	M1	M1	N2	N2	O2	O2	Q1	Q1	S2	S2	U5	U5	X4	X4	Y3	Y3	Z2	Z2	D2	D2
SLYD-7	A1	A1	B2	B2	C2	C2	F1	F1	G1	G1	H1	H1	M2	M2	N2	N2	O2	O2	Q1	Q1	S2	S2	U5	U5	X4	X4	Y3	Y3	Z1	Z1	D3	D3
SLYD-8	A1	A1	B2	B2	C2	C2	F1	F1	G1	G1	H1	H1	M2	M2	N2	N2	O2	O2	Q1	Q1	S2	S2	U5	U5	X4	X4	Y3	Y3	Z1	Z1	D3	D3
SLYST-7	A4	A4	B2	B2	C3	C3	F1	F1	G1	G1	H1	H1	M1	M1	N3	N3	O1	O1	Q1	Q1	S5	S5	U4	U4	X3	X4	Y4	Y4	Z1	Z1	D3	D3
ML-5	A3	A3	B2	B2	C1	C1	F1	F1	G1	G1	H1	H1	M1	M1	N2	N2	O2	O2	Q1	Q1	S2	S2	U4	U4	X4	X4	Y3	Y3	Z2	Z2	D2	D2
SLYST-8	A3	A5	B2	B2	C1	C4	F3	F3	G1	G3	H2	H4	M2	M2	N2	O1	O2	Q3	Q3	Q3	S1	S4	U5	U5	X4	X4	Y3	Y4	Z2	Z2	D2	D2
SLYD-9	A1	A1	B2	B2	C2	C2	F1	F1	G1	G1	H1	H1	M2	M2	N2	N2	O2	O2	Q1	Q1	S2	S2	U5	U5	X4	X4	Y3	Y3	Z1	Z1	D3	D3
ML-6	A3	A3	B2	B3	C1	C1	F1	F1	G1	G1	H3	H3	M1	M1	?	?	O2	O2	Q3	Q3	S2	S2	U4	U4	X3	X3	Y3	Y3	Z2	Z2	D2	D2
SLYD-10	A1	A1	B2	B2	C2	C4	F1	F1	G1	G1	H1	H1	M2	M2	N2	N2	O2	O2	Q1	Q1	S2	S2	U5	U5	X4	X4	Y3	Y3	Z1	Z1	D3	D3
SLYST-9	A3	A3	B3	B3	C1	C1	F1	F1	G1	G1	H1	H1	M1	M1	N2	N2	O2	O2	Q1	Q1	S2	S2	U4	U4	X4	X4	Y3	Y3	Z2	Z2	D2	D2
ML-7	A3	A3	B3	B3	C1	C1	F1	F1	G1	G1	H1	H1	M1	M1	N2	N2	O2	O2	Q1	Q1	S2	S2	U4	U4	X4	X4	Y3	Y3	Z2	Z2	D2	D2
SLYST-10	A2	A3	B4	B4	C1	C5	F1	F1	G1	G3	H1	H1	M1	M1	N2	N3	O2	O2	Q1	Q1	S2	S2	U4	U4	X2	X4	Y3	Y4	Z2	Z2	D1	D2
ML-8	A1	A5	B2	B2	C2	C4	F1	F1	G1	G3	H1	H1	M1	M1	N2	N2	O2	O2	Q1	Q1	S2	S2	U5	U5	X4	X4	Y3	Y3	Z1	Z2	D2	D3
SLYST-11	A3	A3	B3	B3	C1	C1	F1	F1	G1	G1	H1	H1	M2	M2	N2	N2	O2	O2	Q1	Q1	S2	S2	U4	U4	X4	X4	Y3	Y3	Z2	Z2	D2	D2
SLYST-12	A3	A3	B3	B3	C1	C1	F1	F1	G1	G1	H1	H1	M1	M1	N2	N2	O2	O2	Q1	Q1	S2	S2	U4	U4	X4	X4	Y3	Y3	Z1	Z1	D3	D3

Figure 2: Representation of allelic combination of a sample of Sri Lanka Yellow Dwarf coconuts at 16 SSR marker loci (? Indicates missing scores)

Table 4: The frequencies of the SLYD specific alleles scored in different phenotypes

Marker	Allele	Frequency in SLYD	Frequency in SLYST	Frequency in ML	Frequency in entire sample population
CAC65	A1	1.0	0.083	0.071	0.375
CAC04	C2	0.938	0.083	0.071	0.333
CNZ10	M2	1.0	0.167	0.125	0.433
CnCirD8	Z1	1.0	0.25	0.25	0.5
CNZ06	D3	1.0	0.167	0.063	0.417

using SSR markers (Bandaranayake *et al*., 2005). Furthering the research findings in DNA fingerprinting of coconut, the current study reports the first effort in Sri Lanka to distinguish genetically close forms of the dwarf and intermediate coconuts.

CONCLUSION

The current study generated molecular information to distinguish the pure SLYD coconut form from the rest of the yellow-colour-nuts-bearing forms of coconut within varieties *Aurantiaca* and *Nana*. There are no reliable morphological markers for the differentiation of seedlings of SLYD from SLYST and ML phenotypes, and mitigating this problem, the current study was successful in deriving DNA fingerprints specific for SLYD facilitating the accurate identification of SLYD in seed garden establishment as well as for any other application.

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