

Evaluation of Morphological Diversity of Conserved Tall Coconut (*Cocos nucifera* L.) Germplasm in Sri Lanka

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ABSTRACT: Coconut is the most widespread plantation crop in Sri Lanka. Characterisation of conserved coconut germplasm has been undertaken globally for identification of important features of different accessions for them to be effectively used in coconut breeding. The current study aimed at characterizing conserved coconut germplasm in Sri Lanka for quantitative traits with morphological descriptors pertaining to stem, leaf and inflorescence characters. Twelve coconut accessions belonging to variety *Typica* (Tall) conserved *ex-situ* gene bank in Pallama, Coconut Research Institute of Sri Lanka were used for recording morphological data. A total of 19 descriptors listed by Bioversity International for stem, leaf and Inflorescence morphology were recorded. Results analysis was performed in SAS v8 and Minitab v14. Analysis of variance revealed significant differences for 13 traits and Principal Component Analysis revealed the traits which contributed in higher magnitudes for the observed variation. Results revealed morphologically diverse accessions among the studied germplasm and provided evidence for the morphological variation among the different accessions having different origins although all the accessions were primarily of the same coconut variety. This indicates the effectiveness of sampling in the conservation process. Furthermore, the information derived in the present study will be useful in coconut breeding for desirable traits and also in formulating further conservation strategies.

Key words: Coconut, Germplasm diversity, Morphological Characterisation, Multivariate Discrimination

INTRODUCTION

Coconut (*Cocos nucifera* L.) is a perennial palm offering a multitude of uses for the mankind and therefore is important for many in the Asia and Pacific region. This crop provides food and beverage and numerous other raw materials to coconut based industries. The coconut palm has about 60 years of economic life span and has been recognized as a crop with tremendous potential for alleviating poverty in the third world (Everard *et al.*, 2000). Therefore, conservation of coconut is of primary importance in the world.

Coconut is the most widespread plantation crop in Sri Lanka. According to Batugal *et al.* (2010), coconut occupies 20% of the cultivated land area and the total extent under coconut cultivation is approximately 394,836 ha (Anon, 2002). The first systematic classification of coconut germplasm in Sri Lanka was reported in 1958 (Liyanage, 1958). According to the traditional classification, coconut varieties are basically divided as Tall (*typica*), Dwarf

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(*Nana*) and Intermediate (*Aurantiaca*) based on tree habit. ‘Dwarfs’ represent about 5% of coconut palms and are cultivated worldwide (Gunn *et al.*, 2011). Some of the distinct characters of Dwarf coconuts are; shorter stature, smaller circumference, lack of a root bole, shorter leaves and self-pollination breeding behaviour. They are typically found near human habitation and show traits closely associated with human selection. In contrast, the tall coconuts are more common, out-crossing and grow faster than ‘Dwarfs’, resulting in greater heights and root boles at the base of the trunk. Many ‘Talls’ are grown for copra (approximately 200 g/nut) for oil extraction and coir for fibre; while actively cultivated, these varieties lack the obvious domestication traits of the self-pollinating Dwarfs (Gunn *et al.*, 2011).

Coconut germplasm conservation has become a vastly important area in recent years in a rapidly changing world. The Coconut Research Institute initiated a systematic collection and conservation of coconut germplasm in 1984. Initial country wide surveys were undertaken in 1986/87 to locate populations having different characters; priority was given to drought tolerant material as changing weather patterns have caused severe losses in coconut production. This provided a good opportunity for identifying palms, which withstood the drought at least to some degree.

Being a perennial crop with a persistent capacity for sexual reproduction, coconut gene pools serve in two ways; as a collection for breeder’s work and as a base collection for conservation (Bandaranayake, 2002). Therefore, global interest also heavily focuses on the identification, collection and conservation of germplasm which is identified as a priority in order to safeguard the genetic diversity of the coconut palm grown in Sri Lanka. Field gene banks are the only viable option for *ex-situ* conservation of coconut largely due to recalcitrant nature of the coconut seed (Ekanayake, 2010).

Characterisation of conserved germplasm is another key area to be focused on for the genetic resources to be effectively used and for formulating further conservation strategies. Characterisation is beneficial for identifying specific traits of accessions for the conserved genetic resources to be effectively used in commercial cultivation. Furthermore, characterisation is essential in selecting parents for breeding programs, identification of the duplications of conserved accessions, decision making related to further collection, management of the field gene banks and the formulation of future conservation programmes (Perera *et al.*, 2009). Therefore the objective of this study was to quantitatively characterize conserved coconut germplasm in an *ex-situ* field gene bank in Sri Lanka with morphological descriptors pertaining to stem, leaf and inflorescence characters.

MATERIALS AND METHODS

Twelve coconut accessions belonging to the variety *Typica* (Tall) conserved *ex-situ*, in Pallama gene bank of the Coconut Research Institute of Sri Lanka were used for collection of morphological data. Each accession was represented by 50-70 palms in the field gene bank; out of which 20-30 palms were randomly selected for data collection (Table 1). The palms were 10-15 years old at the time of data collection and were managed with average standard management practices for coconut (Anon, 2005). A total of 19 descriptors (Table 2) for coconut outlined by Bioversity International (formerly IPGRI) for stem, leaf and Inflorescence morphology were recorded for all the above accessions.

Table 1. Details of the selected accessions for the study (WZ=wet zone, IZ=Intermediate zone and DZ=Dry zone)

Number	Accession	Abbreviations used in data analysis	International Accession number	District (Zone)
1	Theladiriya	TLD	CRI SLT59	Ratnapura (WZ)
2	Kalawewa	KW	CRI SLT55	Anuradhapura (DZ)
3	Galenbidunawewa	GBW	CRI SLT52	Anuradhapura (DZ)
4	Ambakelle Special	AS	CRI SLT50 I3	Puttalam (IZ)
5	Sidurupitiya	SP	CRI SLT58	Ratnapura (WZ)
6	Wanathawilluwa	WW	CRI SLT61	Puttalam (DZ)
7	Ihalakagama	IHKG	CRI SLT54	Hambanthota (DZ)
8	Vijithapura	VP	CRI SLT60	Anuradhapura (DZ)
9	Blackstone	BS	CRI SLT51	Matale (WZ)
10	Hangiliagama	HG	CRI SLT53	Anuradhapura (DZ)
11	Mahawalatenna	MT	CRI SLT57	Ratnapura (WZ)
12	Lanlib	LL	CRI SLT56	Puttalam (IZ)

Table 2. Descriptors listed by Bioversity

Number	Descriptors	Abbreviation
1	Girth at 20 cm (cm)	G20
2	Girth at 1.5 m (cm)	G150
3	Stem height (cm)	SH
4	Length of 10 internodes (cm)	L10INT
5	Petiole length (cm)	PL
6	Petiole thickness (mm)	PT
7	Width of petiole (mm)	PW
8	Rachis length (cm)	RL
9	No. of leaflets	NL
10	Width of leaflet (cm)	LW
11	Length of leaflet (cm)	LL
12	Peduncle length (cm)	PedL
13	Peduncle diameter (mm)	PD
14	Spikelets w/ female flowers	SWFF
15	Spikelets w/out female flowers	SWOFF
16	No of female flowers	NFF
17	Female flower distribution	FFD
18	Length of central axis (cm)	LCA
19	Length of spikelets (cm)	LOS

The data were analysed using analysis of variance and general linear models procedure followed by mean separation procedure; Duncan's multiple range test with a statistical software package SAS version 8. Minitab version 14 was used for principal component analysis. Distance and correlation matrices were derived by cluster analysis and dendrogram based on squared Euclidean distances was drawn.

RESULTS AND DISCUSSION

Descriptive statistics- analysis of variance

In the ANOVA, all the scored stem characters revealed statistically significant differences at 5% probability level (Table 3).

Table 3. Means of the statistically different stem and leaf parameters

Acc	G20	G150	SH	L10INT	PL	LW
TLD	116.8 ^{de}	77.0 ^b	411.8 ^c	65.5 ^{bc}	149.1 ^a	5.4 ^{cde}
KW	111.6^e	76.0^b	405.8 ^c	62.3 ^{bc}	151.9^a	5.2 ^{de}
GBW	134.4 ^{ab}	78.0 ^b	402.6^c	66.5 ^{bc}	150.6 ^a	5.1^e
AS	125.1 ^{abcde}	78.2 ^b	404.9 ^c	57.7 ^c	126.2^c	5.6 ^{bcd}
SP	118.5 ^{cde}	81.5 ^{ab}	453.2 ^{bc}	65.0 ^{bc}	128.3 ^c	5.6 ^{bcd}
WW	135.4 ^{ab}	80.7 ^{ab}	408.9 ^c	56.9^e	153.4^a	5.9 ^{abc}
IHKG	123.4 ^{bcd}	76.1 ^b	416.0 ^c	59.6 ^{bc}	147.0 ^{ab}	5.6 ^{bcd}
VP	138.5^a	76.0^b	406.5 ^c	57.2 ^c	149.6 ^a	5.6 ^{bcd}
BS	127.9 ^{abcd}	85.4^a	518.7^a	66.6 ^{bc}	129.6 ^c	6.0 ^{ab}
HG	116.3 ^{de}	77.5 ^b	458.1 ^{bc}	68.5 ^{ab}	134.1 ^c	5.6 ^{bcd}
MT	131.6 ^{abc}	80.6 ^{ab}	490.3 ^{ab}	76.7^a	126.8 ^c	5.7 ^{abc}
LL	117.5 ^{de}	77.6 ^b	477.2 ^{ab}	63.3 ^{bc}	135.6 ^{bc}	6.1^a

Means with the same letter for each character are not significantly different (p=0.05)

Highest and lowest values are bolded

The highest average G20 value recorded was 138.45 for Vijithapura (VP) followed by Wanathawilluwa (WW) which was 135.44. Blackstone (BS) showed the highest mean value (85.40) for G150 followed by Sidurupitiya (SP) which was 81.53. The highest average Stem Height (SH) recorded was 518.70 for Blackstone (BS) followed by Mahawalatenne (MT). However both were not statistically different from each other. The highest mean value for L10INT was shown by Mahawalatenne (MT) (i.e. 76.7).

Considering leaf morphology; PT, PW, RL, NL and LL (Table 2) were not significantly different at 5% probability level. But, PL and LW were significantly different among the accessions at the same 5% probability level (Table 3). Further, the highest mean value for PL was recorded in the accession Wanathawilluwa (WW) followed by Kalawewa (KW). The highest mean value 6.13 for LW was recorded in Lanlib (LL).

All inflorescence characters except PD exhibited significant differences among accessions (Table 4). The highest mean values for; NFF, FFD and LOS were recorded in the single accession Hangiliyagama (HG) exhibiting mean values of 32.15, 0.91 and 47.59 respectively. Further, the highest mean values for SWOFF (20.35) and LCA (42.47) were recorded in Blackstone.

Table 4. Means of the significantly different inflorescence parameters

Acc	PedL	SWFF	SWOFF	NFF	FFD	LCA	LOS
TLD	24.1 ^{ab}	20.8 ^{abc}	18.5 ^{ab}	29.2 ^{ab}	0.7 ^{ab}	38.1 ^{abcde}	45.3 ^{ab}
KW	23.6 ^b	17.7 ^{abcd}	17.7 ^{ab}	20.7 ^{abc}	0.6 ^b	34.7^e	45.7 ^{ab}
GBW	24.7 ^{ab}	17.4 ^{bcd}	17.4 ^{ab}	23.4 ^{abc}	0.6 ^{ab}	40.9 ^{abc}	45.4 ^{ab}
AS	25.9 ^{ab}	16.9 ^{bcd}	19.6 ^{ab}	18.3 ^{bc}	0.5^b	36.0 ^{cde}	40.5 ^{cd}
SP	27.0 ^{ab}	24.2^a	15.2 ^{abc}	27.0 ^{ab}	0.7 ^{ab}	41.3 ^{ab}	42.1 ^{bcd}
WW	22.7^b	13.3^d	18.6 ^{ab}	13.4^c	0.4 ^b	34.9 ^{de}	40.2 ^d
IHK	23.0 ^b	15.2 ^{cd}	17.3 ^{ab}	17.2 ^{bc}	0.6 ^b	36.8 ^{bcde}	39.3^d
G							
VP	26.9 ^{ab}	17.3 ^{bcd}	15.4 ^{abc}	21.2 ^{abc}	0.7 ^{ab}	39.1 ^{abcde}	45.7 ^{ab}
BS	27.0 ^{ab}	17.8 ^{abcd}	20.4^a	20.7 ^{abc}	0.5 ^b	42.5^a	44.6 ^{abc}
HG	26.9 ^{ab}	22.4 ^{ab}	11.2^c	32.2^a	0.9^a	41.6 ^{ab}	47.6^a
MT	28.4^a	20.3 ^{abc}	14.7 ^{bc}	30.5 ^{ab}	0.9 ^a	40.3 ^{abc}	46.4 ^{ab}
LL	26.4 ^{ab}	20.5 ^{abc}	14.8 ^{bc}	25.4 ^{abc}	0.7 ^{ab}	40.0 ^{abcd}	45.3 ^{ab}

Means with the same letter for each character are not significantly different ($p=0.05$)

Highest and lowest values are bolded

The analysis of variance for the scored morphologies revealed the quantitative variations among accessions. While several accessions were identified as the highest or the least scoring for one or a few traits as highlighted in Tables 3 and 4, the accession TLD scored average values for all the parameters scored in the present study.

Principal component analysis

Principal component analysis quantifies each parameter to calculate the Principal Components (PCs) which help in describing the variation in the dataset. The first 4 principal components (PC1, PC2, PC3 and PC4) accounted for 39.4%, 17.5%, 12.8% and 9.4% of the variation, respectively accumulating to a total of 79.1% of the total variability among the coconut accessions evaluated (Table 5).

All the stem characters, except G20 were negatively correlated with PC1 having the loadings of the highest magnitude of -0.284 for L10INT with PC1. But, all the stem characters, except L10INT were negatively correlated with PC2 having the loadings of the highest magnitude of -0.435 for G150 (Table 5).

Table 5. Matrix of Eigen values and vectors of Principal components

		Principal Components (PC)			
		PC1	PC2	PC3	PC4
	Eigen value	7.4845	3.334	2.4261	1.782
	Proportion	0.394	0.175	0.128	0.094
	Cumulative	0.394	0.569	0.697	0.791
	Variable				
stem	G20	0.110	-0.185	0.161	-0.539
	G150	-0.081	-0.435	0.277	0.057
	SH	-0.247	-0.324	0.165	0.002
	L10INT	-0.284	0.003	0.163	-0.173

Leaf	PL	0.245	0.272	-0.004	-0.206
	PT	-0.104	-0.151	-0.511	0.193
	PW	0.180	0.005	-0.171	-0.578
	RL	0.038	-0.177	-0.116	-0.052
	NL	-0.053	-0.169	-0.439	-0.202
	LW	-0.001	-0.498	-0.036	-0.044
	LL	-0.249	-0.165	-0.353	-0.053
Inflorescence	PedL	-0.299	-0.173	0.077	-0.122
	PD	-0.245	0.301	0.179	0.003
	SWFF	-0.329	0.092	-0.053	0.206
	SWOFF	0.258	-0.162	0.242	0.143
	NFF	-0.332	0.154	-0.040	-0.067
	FFD	-0.316	0.168	-0.119	-0.102
	LCA	-0.282	-0.062	0.323	-0.103
	LOS	-0.238	0.170	0.040	-0.332

All the leaf characters except PL, PW and RL were negatively correlated with PC1 with the highest loading of -0.249 for LL with. PL and PW were positively correlated with PC2 while all the other leaf characters were negatively correlated with PC2 having the loadings of -0.498 for LW with PC2 (Table 5).

All the inflorescence characters except SWOFF showed high negative loadings for the PC1 and recorded the highest loading of -0.332 for NFF with PC1. PedL, SWOFF and LCA recorded higher negative correlation with PC2 while PD, SWFF, NFF, FFD and LOS recorded higher positive correlation with PC2 and showed the loadings of higher magnitude of 0.301 for PD with PC2 (Table 5).

Phenetic tree for stem characters

Phenetic tree is derived based on complete linkage and Euclidean distance is presented in Fig. 1 to 4 for stem, leaf, inflorescence and all characters, respectively. The dendrogram given in Fig. 1 displays the relative positions of accessions based on the stem morphology scored. It is shown that Blackstone grouped separately and the data deposited in International Coconut Genetic Resources Database, refers to Blackstone as collected from Matale District and having larger, spherical nuts. Further, dendrogram showed that Sidurupitiya, Hangiliyagama, Mahawalatenna and Lanlib closely related with Blackstone.

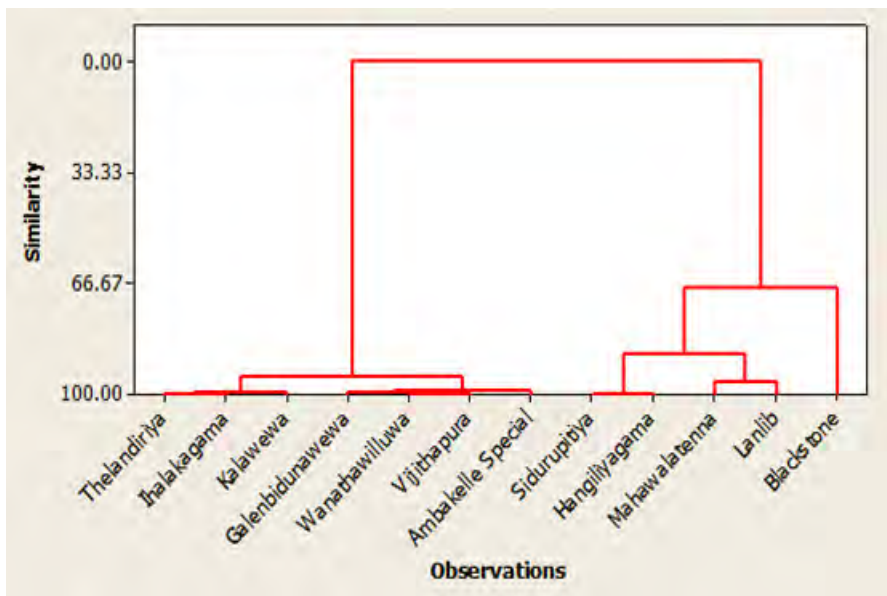


Fig. 1. Dendrogram based on Squared Euclidean Distance for Stem characters

The Dendrogram given in (Fig. 2) displays the clustering of accessions based on the leaf morphology. It is shown that Blackstone, Hangiliyagama, Mahawalatenna and Sidurupitiya grouped together as in (Fig. 1).

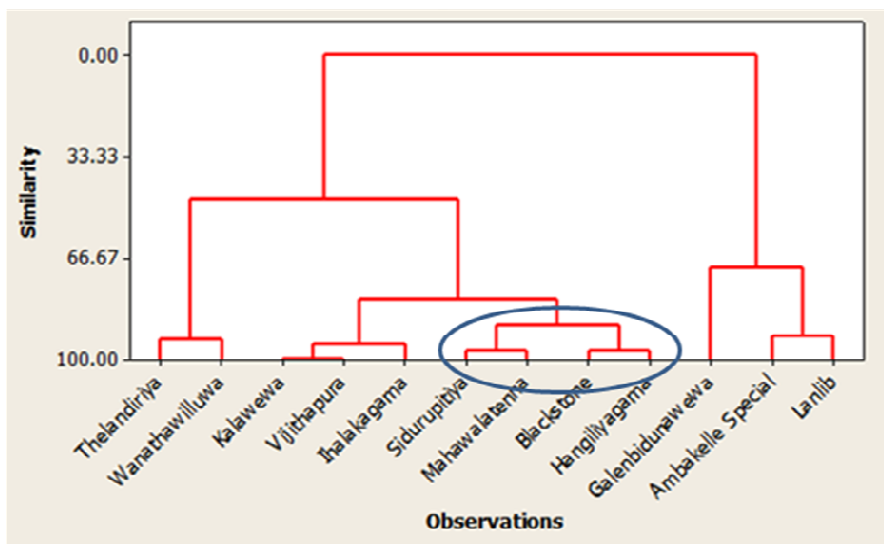


Fig. 2. Dendrogram based on Squared Euclidean Distance for Leaf characters

The dendrogram given in (Fig. 3) displays the relative positions of accessions based on the inflorescence morphological characters scored. Here again two major clusters were formed with one major cluster forming two separate groups.

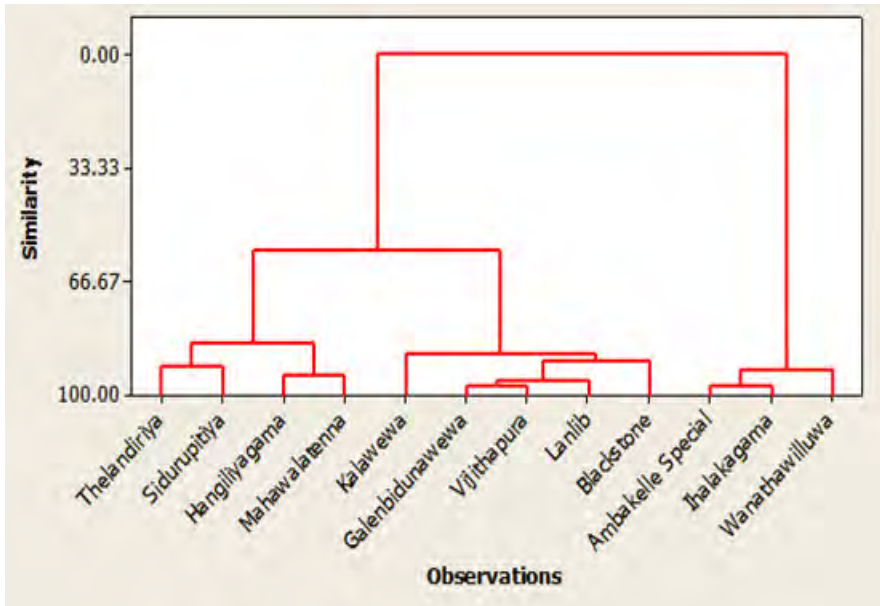


Fig. 3. Dendrogram based on Squared Euclidean Distance for Inflorescence characters

Fig. 4 displays the dendrogram based on Squared Distance for all morphological descriptors scored for all 12 tall accessions. Two major clusters were observed in this dendrogram with Blackstone, Sidurupitiya, Mahawalatenna, Hangiliyagama and Lanlib forming one major cluster and the rest forming the second major cluster.

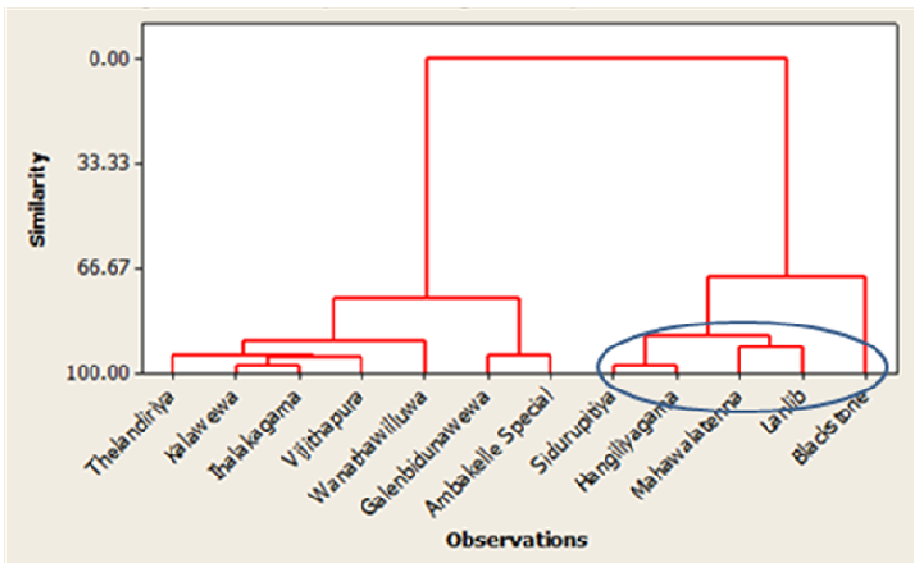


Fig. 4. Dendrogram based on Squared Euclidean Distance for all morphological variables

Correlation matrix and the distance matrix

Pearson correlation coefficients among the quantitative traits were derived (correlation matrix is not shown in the paper). Very strong positive correlations (0.922) were observed between FFD and NFF. There were no very strong negative correlations (>-0.9) between any of the scored variables. Strong positive correlations (0.7 to 0.8) were observed between G150 and SH (0.70), LL and PT (0.714), L10INT and NFF (0.788), NFF and PT (0.718), L10INT and FFD (0.767), SWFF and FFD (0.766), LCA and SH (0.701), LCA and PL (0.74) and LOS and NFF (0.735). Strong negative correlations (-0.7 to -0.8) were observed between SH and PL (-0.731) as well as in PL and PedL (-0.768).

Pair-wise distance matrix (not shown in the paper) revealed the highest distance between Blackstone (BS) and Galenbindunawewa (GBW) with the magnitude of 15946.3 and the lowest distance of 393.5 between the accessions Ihalakagama (IHKG) and Kalawewa (KW) among all the accessions studied.

Further, all these grouping patterns revealed that the variation is not explained by the geographical regions (Table 1). Thus the observed variation is attributed to the specificity of each population.

CONCLUSIONS

All the accessions evaluated in the present study were of the same variety, namely Sri Lanka Tall. Within Sri Lanka tall, the different accessions originated covering different geographic locations within the country. This study provided evidence for the morphological variation among the different accessions having different origins although all the accessions were primarily of the same coconut variety. This indicates the effectiveness of sampling in the conservation process. Furthermore, the information derived in the present study will be useful in coconut breeding for desirable traits and also in formulating further conservation strategies.

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