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Characterization of Conserved Coconut Germplasm in Sri Lanka with Morphological Descriptors

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Abstract

Characterization of conserved germplasm is of primary importance for genetic resources to be effectively used and for formulating further conservation strategies. The aim of the research reported in the current paper is to characterize 26 coconut germplasm accessions conserved *ex-situ* at Pottukulama field gene bank in Sri Lanka using morphological descriptors. A total of 17 stem, leaf and inflorescence traits listed for coconut by Bioversity International were scored in 30 randomly selected palms in each accession. Twenty six germplasm accessions evaluated included 5 dwarf accessions out of which 2 were of exotic origin and 21 Sri Lankan tall coconut accessions. Statistical analytical methods; principal component analysis, cluster analysis and correlation calculation were performed in Minitab version 11 while General linear models procedure and mean separation techniques were performed in SAS version 8. The first 3 principal components cumulatively explained 88.2% of the variation present among accessions. Stem, leaf characters and the length of central axis along with overlapping of male and female phases were identified as the main morphological traits distinguishing the evaluated germplasm. Talls and the dwarf groups separated clearly while the variation within the tall accessions was found to be very narrow. The exotic dwarf Brazilian green dwarf showed the highest distance with the tall accessions while the accession Brown dwarf was the furthest from the talls out of the local dwarfs indicating their potential for hybridizing with talls for maximum heterosis. Results further indicate that future germplasm collection should be based more on biased selection for distinct morphological features rather than random selections within tall populations in Sri Lanka.

Keywords: Coconut, germplasm, morphological characterization, genetic variation, multivariate discrimination.

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Introduction

Coconut (*Cocos nucifera* L.) is very important for many in the Asia and Pacific region so as for the coconut to be termed as, 'tree of life' or the 'palm from heaven'. Coconut has been a cultivated crop for over a long period of time and thus the coconut genetic resources in the world consist mainly of the cultivated material and the breeding stocks. Collection and conservation of coconut germplasm has been undertaken in many of the coconut growing countries extensively during the last two decades while in Sri Lanka, a systematic programme for collecting coconut germplasm has been in operation since 1984 (Wickramaratne, 1984). Populations to be conserved were mainly randomly selected to represent the geographic regions and secondly the biased selections were practiced for specific traits. Collected coconut germplasm has been conserved in *ex-situ* field gene banks, which is the only practical method for *ex-situ* conservation of the coconut germplasm in view of the recalcitrant nature of the coconut seednuts.

Characterization of the conserved germplasm is an essential requirement in several aspects. It is of primary importance in order to assess the genetic variability among the conserved material. Such knowledge will be of great value in identifying specific traits of accessions for the conserved genetic resources to be effectively used in commercial cultivation or in breeding programmes. In addition, the evaluation of the conserved materials reveal a gamut of important information which will help in 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.

There are several means by which the characterization of germplasm can be done, namely morphological, biochemical or the molecular methods. Biochemical or molecular methods involve costly laboratory procedures, they provide biochemical or the true genetic level information and these methods can be used at any age of the conserved material. On the other hand the morphological methods reveal the variation of the morphology of the palm. But for coconut, a crop with a long vegetative phase there will be a comparatively lengthy waiting period from the gene bank establishment for the populations to reach adult stage before it is possible for recording morphological traits such as inflorescence or fruit morphology.

Some of the coconut accessions conserved *ex-situ* in field gene banks in Sri Lanka has been subjected to extensive molecular characterization (Perera *et al.* 2001; Dassanayake *et al.* 2003) which has resulted in a gamut of information for establishing genetic relationships among the conserved material. As for the morphological characterization, Perera and Fernando (2000) reported the use of inflorescence descriptors for characterizing 12 conserved coconut accessions in Sri Lanka. Apart from this study, so far, there are no reports on the use of morphological descriptors for characterizing the coconut germplasm accessions conserved in Sri Lanka. The present study has been undertaken with the objective of characterizing 26 coconut germplasm accessions conserved *ex-situ* in Sri Lanka for stem, leaf and inflorescence morphology to measure the genetic diversity among the accessions.

Materials and methods

Twenty six coconut accessions belonging to the varieties *typica* (tall) and *Nana* (dwarf) conserved *ex-situ* in Pottukulama Research Station of the Coconut Research Institute of Sri Lanka were used for recording morphological diversity. Each accession was represented by 60-70 palms in the field gene bank. The names and the international codes (as given in the International Coconut Genetic Resources Database, CGRD) of the accessions, variety and the origin are given in table 1. The palms were in the age of 10-11 years at the time of

Table 1. Details of accessions used in the study

Accession name	International code	Variety	Origin
1. Moorock	SLT13	*Typica*	Sri Lanka
2. Pitiyakanda	SLT16	*Typica*	Sri Lanka
3. Palugaswewa	SLT15	*Typica*	Sri Lanka
4. Clovis	SNRT01	*Typica*	Sri Lanka
5. Namalwatta	SLT14	*Typica*	Sri Lanka
6. St. Annes	SLT19	*Typica*	Sri Lanka
7. Margaret	SLT12	*Typica*	Sri Lanka
8. Kasagala	SLT07	*Typica*	Sri Lanka
9. Debarayaya	SLT03	*Typica*	Sri Lanka
10. Green dwarf Kundasale	PGD02	*Nana*	Sri Lanka
11. Yellow dwarf Kundasale	CYD02	*Nana*	Sri Lanka
12. Red dwarf Kundasale	SLRD02	*Nana*	Sri Lanka
13. Mahena	SLT17	*Typica*	Sri Lanka
14. Ambakelle special	SLT02R2	*Typica*	Sri Lanka
15. Melsiripura	SLT10	*Typica*	Sri Lanka
16. Mangala Eliya	SLT11	*Typica*	Sri Lanka
17. Goyambokka	SLT05	*Typica*	Sri Lanka
18. Cameroon red dwarf	CRD	*Nana*	Cameroon
19. Goluwapokuna	SLT04	*Typica*	Sri Lanka
20. Brown dwarf	SLBD	*Nana*	Sri Lanka
21. Maliboda	SLT09	*Typica*	Sri Lanka
22. Horakelle	SLT06	*Typica*	Sri Lanka
23. Wellawa	SLT21	*Typica*	Sri Lanka
24. Brazilian green dwarf	BGD	*Nana*	Brazil
25. Walahapitiya	SLT20	*Typica*	Sri Lanka
26. Keenakelle	SLT08	*Typica*	Sri Lanka

recording data and had not yet attained the yield stability which prevented the scoring of fruit and yield components at the time.

A total of 17 descriptors listed by IPGRI (currently, Bioversity International) for stem, leaf and inflorescence morphology were recorded. Two stem characters, presence or absence of bole and the girth of the base at 20cm above ground; 7 leaf characters, namely, length of the petiole, width and the thickness of the petiole recorded at the point of leaflet origination, length of the rachis, average length of 4 leaflets and average width of 4 leaflets and the number of leaflets were scored as stem and leaf morphology. Inflorescences bearing receptive female flowers were used to score the 8 descriptors, peduncle length, peduncle diameter at the point where spikelets initiate, length of central axis, number of spikelets with female flowers, number of spikelets without female flowers, number of female flowers, average length of 4 spikelets and the presence or absence of the mature male flowers. Data were recorded in randomly selected 30 palms per accession following the sample sizes outlined in IPGRI descriptors for coconut germplasm characterization (Santos *et al.* 1997).

Multivariate data analytical methods, principal component analysis and cluster analysis were performed in statistical software Minitab version 11 to derive principal components, distance matrices (based on Euclidean distance) and to construct the

phenetic tree for the accessions used. Pearson's correlation coefficients were also calculated between pair-wise accessions using Minitab version11. General linear models procedure and mean separation procedures were performed in the statistical analytical software SAS to analyze the quantitative variables.

Results & discussion

Principal component analysis (PCA)

The first 3 principal components individually accounted for 63.3%, 16.3% and 8% respectively, of the variability among the measured traits, accumulating to 87.8% of the total variability among the 26 accessions evaluated (table 2). The traits bole, girth and all of the leaf characters excluding leaflet width had high negative loadings while the trait, presence of mature male flowers had a high positive loading for the first principal component. Inflorescence descriptors, number of female flowers and the number of spikelets with female flowers had high positive correlation while the number of spikelets had a high negative loading for the principal component 2. For the third principal component peduncle length had the highest magnitude which was positively correlated.

General linear models procedure revealed statistically significant differences among all of the quantitative traits scored. Means of the important traits which contribute in higher magnitudes for the morphological variation among accessions, as revealed by the PCA, are given in table 3. As expected, bole was present in all the tall accessions while dwarf accessions scored negative for the trait. On the other hand mature male flowers were still present on the inflorescences in dwarf accessions while the same had already fallen off in all the tall accessions. Inflorescences containing receptive female flowers were used for measurements and thus it confirms the cross pollinating behavior of all the tall accessions and the self pollinating behaviour of the dwarf accessions.

Pearson's Correlation coefficients

Calculated Pearson's correlations revealed strong positive/negative correlations between certain traits (table 4). Presence and the size of the bole (G20) showed strong positive correlations ($\geq \pm 0.8$) with quantitative traits, length of central axis, and spikelet length of the inflorescence and all the leaf related characters. The other qualitative trait presence of mature male flowers displayed strong negative correlation ($\geq \pm 0.8$) with the same characters. Combining these findings and correlating them with the means indicates the root bole to be the most prominent feature in the tall accessions while the presence of mature male flowers in the inflorescence bearing receptive female flowers (indicating self pollinating breeding behaviour) is identified to be a highly specific trait in the dwarfs.

Inflorescence characters peduncle length, peduncle diameter, number of spikelets with and without female flowers and the number of female flowers did not show any strong correlation ($\geq \pm 0.8$) with any of the other traits measured.

Distance matrix and the phenetic tree

Genetic diversity as revealed by stem, leaf and inflorescence descriptors was very low within the tall accessions conserved. Genetic distances greater than 5% were discovered only between the accessions belonging to the variety *typica* and *nana* (matrix of genetic distances is not given). Out of the 5 dwarf accessions evaluated, Yellow dwarf Kundasale showed the least distance with the tall accessions while Brazilian green dwarf which has an exotic origin had the greatest genetic distance with the talls with respect to the morphological data. The sequence of dwarf accessions with respect to the magnitude of distance compared to tall accessions in increasing order is Yellow dwarf Kundasale, Red dwarf Kundasale, Green dwarf Kundasale, Brown dwarf, Cameroon red dwarf and Brazilian green dwarf. These findings are further explained by the phenetic tree drawn using Euclidean distances (figure 1).

Table 2. Matrix of eigen values and vectors of principal components for morphological traits

	Principal components (PC)		
	PC1	PC2	PC3
Eigen values			
Variance	11.265	2.494	1.243
% individual contribution	66.3	14.7	7.0
% accumulated variation	66.3	80.9	88.2
Eigen vectors			
Bole	-0.294	0.025	-0.007
Girth (G20)	-0.264	0.123	-0.234
Peduncle length (PdL)	-0.163	0.102	0.637
Peduncle diameter (PD)	-0.191	0.253	0.095
Spikelets with female flowers (SWFF)	0.078	0.498	-0.396
Spikelets without female flowers (SWOFF)	-0.111	-0.486	-0.289
No of female flowers (NFF)	0.04	0.591	0.034
Length of central axis (LCA)	-0.251	0.019	-0.4
Spikelet length (SL)	-0.268	0.073	0.233
Presence of mature male flowers (PMMF)	0.294	-0.025	0.007
Petiole length (PL)	-0.282	0.085	-0.11
Petiole thickness (PT)	-0.278	-0.143	0.015
Petiole width (PW)	-0.279	-0.121	-0.007
No. of leaflets (NL)	-0.283	0.006	-0.111
Leaflet width (LW)	-0.253	-0.094	0.216
Leaflet length (LL)	-0.275	0.117	0.021
Rachis length (RL)	-0.29	0.067	-0.055

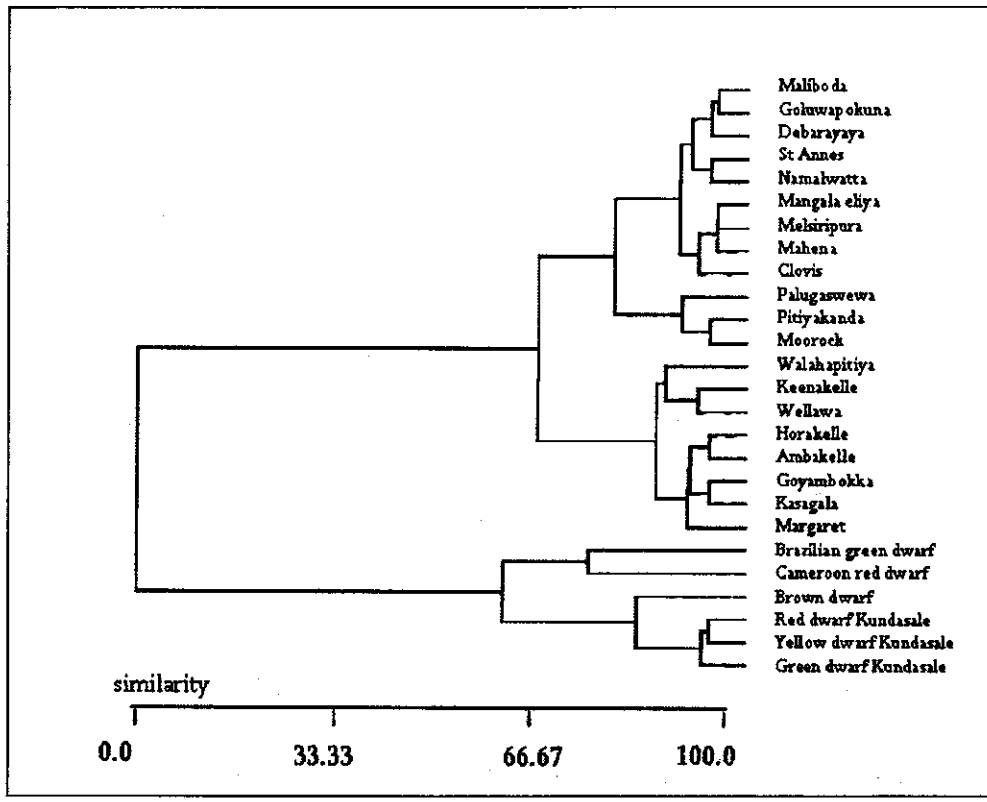
Table 3. Means of the traits accounting for major portion of the diversity among accessions (significant statistical differences among accessions were observed for all the traits)

Accession	PL	PT	PW	NL	LL	RL	NFF	PdL
1. Moorock	160.4	3.3	7.4	222.2	135.8	616.3	54.5	40.5
2. Pitiyakanda	156.8	3.5	7.4	217.5	128.6	561.7	56.7	35.9
3. Palugaswewa	157.0	3.4	7.6	227.5	134.9	589.5	66.7	31.0
4. Clovis	168.3	3.6	8.3	239.7	123.3	633.3	36.2	20.9
5. Namalwatta	163.8	3.6	7.2	243.2	127.9	605.4	30.5	36.1
6. St. Annes	159.5	3.6	8.0	231.7	134.1	594.0	33.4	32.7
7. Margaret	157.9	3.6	8.0	237.3	120.3	607.1	21.9	30.2
8. Kasagala	160.5	3.4	8.1	216.5	122.1	570.9	23.9	35.4
9. Debarayaya	157.9	3.5	8.4	236.7	123.7	572.8	33.6	25.7
10.Green dwarf Kundasale	115.1	2.4	5.3	171.8	86.8	376.6	37.6	21.9
11.Yellow dwarf Kundasale	129.1	2.8	6.1	178.7	89.9	428.7	30.1	22.0
12. Red dwarf Kundasale	125.7	2.8	6.0	168.0	100.0	415.0	36.1	16.7
13. Mahena	159.4	3.6	7.7	228.2	120.1	548.0	29.3	22.4
14. Ambakelle special	164.3	3.7	8.3	225.2	124.7	574.1	28.2	28.1
15. Melsiripura	169.7	3.5	8.1	225.5	122.5	606.2	27.4	24.1
16. Mangala Eliya	165.8	3.7	8.0	233.3	125.0	591.7	28.1	26.5
17. Goyambokka	166.9	3.6	7.9	219.8	120.6	562.8	20.4	24.6
18. Cameroon red dwarf	108.7	2.8	6.1	159.3	83.5	351.8	21.0	27.5
19. Goluwapokuna	162.5	3.7	8.1	220.7	123.2	583.0	34.8	30.1
20. Brown dwarf	124.7	2.3	5.5	180.5	94.5	411.3	64.4	23.8
21. Maliboda	167.7	3.4	8.0	223.5	125.3	575.8	33.1	28.0
22. Horakelle	168.8	3.8	9.2	232.0	127.7	603.4	26.7	37.5
23. Wellawa	165.2	3.5	8.2	220.7	131.1	590.3	28.9	26.6
24. Brazilian green dwarf	90.1	3.0	6.2	185.3	99.9	365.7	23.2	18.0
25. Walahapitiya	156.2	3.8	8.0	231.2	124.2	591.9	20.5	44.8
26. Keenakelle	166.8	3.6	9.0	230.5	122.7	598.7	28.1	25.5

Table 4. Correlation matrix of the traits scored

	bole	G20	PdL	PD	SWFF	SWOFF	NF	LCA	SL	PMMF	PL	PT	PW	NL	LW	LL
G20	0.88															
PdL	0.54	0.40														
PD	0.6	0.59	0.42													
SWFF	-0.2	0.05	-0.25	0.06												
SWOFF	0.35	0.30	-0.04	-0.10	-0.51											
NF	-0.08	0.03	0.09	0.19	**0.70**	**-0.746**										
LCA	**0.82**	**0.84**	0.11	0.56	-0.03	0.365	-0.128									
SL	**0.91**	**0.74**	0.65	0. 60	-0.28	0.136	-0.011	0.653								
PMMF	**-1**	**-0.88**	-0.54	-0.60	0.23	-0.351	0.075	**-0.821**	**-0.912**							
PL	**0.94**	**0.89**	0.45	0.69	-0.08	0.287	-0.038	**0.845**	**0.834**	**-0.936**						
PT	**0.91**	**0.79**	0.49	0.45	-0.40	0.493	-0.334	**0.756**	**0.813**	**-0.912**	**0.822**					
PW	**0.91**	**0.76**	0.42	0.54	-0.40	0.456	-0.287	**0.79**	**0.799**	**-0.905**	**0.867**	**0.93**				
NL	**0.95**	**0.87**	0.47	0.52	-0.20	0.395	-0.085	**0.861**	**0.804**	**-0.948**	**0.88**	**0.89**	**0.87**			
LW	**0.80**	0.61	0.53	0.58	-0.43	0.272	-0.263	0.665	**0.782**	**-0.801**	**0.767**	**0.83**	**0.90**	**0.73**		
LL	**0.94**	**0.83**	0.56	0.55	-0.11	0.237	0.11	**0.724**	**0.862**	**-0.94**	**0.861**	**0.84**	**0.82**	**0.90**	**0.71**	
RL	**0.97**	**0.91**	0.53	0.64	-0.17	0.314	-0.019	**0.84**	**0.851**	**-0.966**	**0.955**	**0.87**	**0.89**	**0.95**	**0.75**	**0.94**

Figure 1. Phenetic tree drawn using Euclidean distances



Two main groups of coconuts talls and the dwarfs formed the two major groups in the dendrogram. The talls once again divided into two groups while the dwarfs separated into two subgroups including local dwarfs in one cluster and exotic dwarfs in the second cluster. There again Brown dwarf was separated form the other local dwarfs.

Characterization of Sri Lankan coconut germplasm using molecular markers has revealed comparable results although not exactly with the same accessions (Perera *et al.* 2001; Dassanayake *et al.* 2003).

Implications

The results provide morphological evidence to the narrow genetic diversity within the Sri Lankan tall coconut populations. This finding should be carefully considered in decision making related to future germplasm exploration missions. Consequently random sampling covering different geographic regions will not be a fruitful exercise and on the contrary biased selection for specific traits should be the main criteria in selecting populations of coconut for conservation in future.

The findings will further be useful in selecting parents for hybridization programmes for selecting genetically diverse material. We have not scored for fruit and yield parameters in the current study. However, the material which are genetically apart for certain traits can be safely assumed to be genetically more distant for fruit and yield data also than the material which are genetically closer. So far Sri Lankan green and yellow dwarfs have been used for hybridization with talls in producing recommended coconut cultivars. The data shows greater genetic distances between brown dwarf (out of the local dwarfs) and the talls indicating a higher potential for extracting

heterosis by hybridizing brown dwarf with Sri Lanka talls. Furthermore, Brazilian green dwarf showed the highest genetic distance with the local talls. Therefore, satisfactory results can be expected from the ongoing research to produce hybrids between brown dwarf and the talls (Everard, 2003) and the Brazilian green dwarf and the talls (Perera, 2006).

Conclusions

Morphological characterization of conserved germplasm for stem, leaf and inflorescence morphology of Sri Lankan talls display narrow genetic diversity especially among the tall accessions. Hence, biased selection for specific traits would be a better option as against random sampling in selecting further material for conservation. Brown dwarf and Brazilian green dwarf will be promising choices to be used in hybridization programmes as they display greater genetic distances for the traits scored. The use of morphological descriptors for stem, leaf and inflorescence morphology listed by Bioversity International is successful in evaluating the diversity of coconut germplasm accessions. Breeding behaviour, bole measurement, most of the leaf parameters and the length of the central axis of inflorescences were identified as important traits in characterizing the listed Sri Lankan coconut germplasm and separating tall coconuts from the dwarfs.

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