Characterisation of the Newly-classified Coconut Forms in Sri Lanka for Fruit Morphology and Variation at SSR-marker Loci

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ABSTRACT

Four recently-classified farmers' varieties of coconut; Ran pol, Juwan, Bothal thembili and Murusi were evaluated in comparison to six coconut forms (Sri Lanka Tall, Bodiri, San Ramon, King coconut, Brown dwarf and Green dwarf) for their fruit morphology and genetic variation at SSR-marker loci to assess their potential as parents in the coconut breeding. Eight fruit morphologies measured by the shape of fruits and the weight of fruit components were scored in a sample of 60 nuts per phenotype. The data were analysed by ANOVA. DNA samples from two palms of each phenotype were subjected to Polymerase Chain Reaction amplification at 12 SSR loci. The data were analysed using Powermarker software. The phenotypes revealed a high diversity with respect to fruit morphology recording significant differences for all the traits scored. The data revealed new phenotype Ran pol to produce the largest and heaviest nuts. The new phenotype, Murusi was close to Green dwarf, and Bothal thembili was identified among the intermediate king coconut variety. Genetically, a rich allelic diversity was present in new phenotypes, and dwarfs and intermediates were distinguished at marker loci. The dwarfs, King coconuts and Pacific type tall Ran pol formed one main cluster and exotic San Ramon, Sri Lanka Tall and new phenotype Juwan formed the second main cluster in the dendrogram based on neighbour joining distances. The collated molecular and morphological data revealed the phenotypes Ran pol and Juwan to be closer to tall coconuts in the Pacific. It is recommended to consider the observed low levels of heterozygosity among talls, variations of fruit morphology and the evidences of origins of new phenotypes in designing future coconut breeding programmes.

Keywords: Coconut, Fruit components, Genetic diversity, SSR markers

INTRODUCTION

Coconut (*Cocos nucifera* L.) is a widely-cultivated tropical oil crop which offers a multitude of uses for the people in coconut-growing countries. Coconut provides food and shelter and material for many rural industries, and thus, it has been identified as a tree possessing a high potential for poverty alleviation in rural areas.

Coconut belongs to the family Arecaceae, and it is the only species of the genus *Cocos*. The wild relatives of the coconut palm are no longer in existence in the world. Two major groups of coconuts; talls and dwarfs have been identified world over, referring mainly to the height of the palm while certain other differences such as the breeding behaviour and coconut production have also been recorded between the two groups.

Consequently, the talls and the dwarfs form major groups in the classifications of coconut. The existing coconut classification in Sri Lanka categorised the Sri Lankan coconut germplasm into three varieties (Liyanage, 1958) as Typica (talls), Nana (dwarfs) and Aurantiaca (intermediate, king coconut forms) with each classified variety including several phenotypes named forms. This classification was based mainly on stature and pollination behaviour while certain other morphological differences among varieties and forms have also been considered.

A programme undertaken from the early 1980's for collection and conservation of coconut germplasm within Sri Lanka (Wickramaratne, 1984), resulted in over 100 coconut accessions belonging to above-mentioned varieties found within Sri Lanka. A predominant portion of these accessions comprised of Sri Lanka talls collected randomly covering different agro-ecological regions within the island, while a minority of accessions, were dwarf and intermediate varieties. The collected material has been conserved ex-situ in field gene banks (Everard, 2001) and morphological (Perera and Fernando 2000) and molecular characterisation (Perera *et al.*, 1998; Perera *et al.*, 2001; Dassanayaka *et al.*, 2003) of such material have also been undertaken over the years.

These studies revealed a narrow genetic base, especially within the Sri Lanka tall coconut germplasm despite the reasonably high number of accessions conserved (Perera *et al.*, 2001). The conserved germplasm constitute the breeders' collection of material, and the narrow genetic base of this collection resulted in inadequate diversity in parental material for the coconut breeding programme in Sri Lanka. This posed a serious limitation in the genetic improvement of the palm and created an imperative need for enrichment of the collection to move forward with the breeding of the coconut palm. In view of the continuous demand for new material for diverse uses of coconut, sustainability in coconut production especially in the scenario of climate change and the threat presented by the emergence of new pests (such as Aceria mite) and diseases (such as Weligama coconut Leaf Wilt Disease), the enrichment of the breeders' collection of coconut was urgently required.

The Coconut Research Institute of Sri Lanka adopted a two-way approach to enrich the breeders' coconut collection, namely, importation of exotic coconuts and search for farmers' varieties of coconut within the country. The latter approach resulted in identification of a number of new coconut forms and the newly-identified material were taxonomically classified and incorporated into the Sri Lankan coconut classification (Ekanayake *et al*, 2010). The novel genetic material need be characterised and evaluated for important traits, especially for fruit and nut characters for use in the breeding programmes. This paper discusses the characterisation of these novel phenotypes for their fruit morphology and their genetic variation at microsatellite (SSR)-marker loci.

MATERIALS AND METHODS

Ten coconut forms comprising of four farmers' varieties (Ekanayake *et al.*, 2010) and six comparative coconut forms were used in the current study (Table 1). A detailed fruit morphological study related to nut shape and nut size was carried out by scoring based on the Bioversity International descriptors (Anonymous, 1998), Fruit Polar Length (FPL), Fruit Polar Circumference (FPC), Fruit

Table 1 Details of the coconut forms used in the study

Coconut form	Type	Variety
Sri Lanka tall (SLT)	Local indigenous	*Typica* (Tall)
Sri Lanka Green Dwarf (GD)	Local indigenous	*Nana* (Dwarf)
Sri Lanka Brown Dwarf (BD)	Local indigenous	*Nana* (Dwarf)
Bodiri (BO)	Local indigenous	*Typica* (Tall)
San Ramon Tall (SR)	Exotic	*Typica* (Tall)
King coconut (KC)	Local indigenous	*Aurantiaca* (Intermediate)
Ran pol (RP)	Farmers' variety	*Typica* (Tall)
Murusi (M)	Farmers' variety	*Nana* (Dwarf)
Juwan coconut	Farmers' variety	*Typica* (Tall)
Bothal thembili (BT)	Farmers' variety	*Aurantiaca* (Intermediate)

Equatorial Length (FEL), Fruit Equatorial Circumference (FEC), Fruit Weight (FW), Split Fruit Weight (SFW), Split Husked Nut Weight (SHNW), Kernel Weight (KW) and Dry Weight (DW) in a sample of 60 nuts per phenotype. The analysis of variance and mean separation by Duncan's method were performed in statistical software SAS (V 8.0) to analyse the fruit morphological data.

For the molecular characterisation, genomic DNA was extracted from spear leaf tissues of 20 palms representing the ten coconut phenotypes as two samples per phenotype, using a modified C-TAB extraction method. Polymerase Chain Reaction (PCR) was carried out at twelve microsatellite marker loci namely, CNZ06, CNZ04, CNZ44, CNZ29, CNZ10, CNZ46, CNZ37, CNZ40, CAC23, CAC65, CAC08 and CAC20 (Table 2). All the sequences of CNZ markers and CAC markers were obtained from Rivera et al., (1999) and Perera *et al.*, (2001) respectively. The amplified PCR products were visualised by polyacrylamide gel electrophoresis followed by Silver staining. The genotypic data were analysed with PowerMarker software (Liu and Muse, 2005) to derive a dendrogram for the coconut phenotypes studied.

RESULTS AND DISCUSSION

Morphological characterisation of fruits and nuts

Among the new coconut phenotypes used in this study, Juwan coconuts are characterised by smaller round nuts and a higher number of female flowers. Plicata leaves (attached leaflets), larger fruits and the predominant reddish brown nuts are the specific features of *Ran pol*. *Murusi* coconuts are dwarf green-like coconuts having sweet nut water which is used as beverage coconuts. Bothal thembili is a type of a King coconut having long bottle-shaped nuts (Plates 1 to 4).

The analysis of variance revealed significant differences among the different coconut phenotypes for all the measured components of the fruit and nut indicating a higher diversity in size, shape and weight of the fruit and nut (Table 3).

Table 2 Forward (FP) and reverse (RP) primer sequences and annealing temperatures of the microsatellite markers used for genotyping

Primer	Sequence 5'- 3'	Annealing temperature (^{0}C)
CNZ04	FP - TATATGGGATGCTTTAGTGGA RP - CAAATCGACAGACATCCTAAA	53
CNZ06	FP - ATACTCATCATCATACGACGC RP - CTCCCACAAAATCATGTTATT	51
CNZ10	FP - CCTATTGCACCTAAGCAATTA RP - AATGATTTTCGAAGAGAGGTC	55
CNZ29	FP-TAAATGGGTAAGTAAGTGTTTGTC RP - CTGTCCTATTTCCCTTTCATT	55
CNZ37	FP - GTGGATAACTCATTTCAGGTG RP - TAAGAAAGCTGAGAGGGAGAT	58
CNZ40	FP - CTTGATTGCTATCTCAAATGG RP - CTGAGACCAAATACCATGTGT	53
CNZ44	FP - CATCAGTTCCACTCTCATTTC RP - CAACAAAAGACATAGGTGGTC	53
CNZ46	FP - TTGGTTAGTATAGCCATGCAT RP - AACCATTTGTAGTATACCCC	56
CAC08	FP - ATCACCCCAATACAAGGACA RP - AATTCTATGGTCCACCCACA	57
CAC20	FP - CTCATGAACCAAACGTTAGA RP - CATCATATACATACATGCAACA	55
CAC23	FP - TGAAAACAAAAGATAGATGTCAG RP - GAAGATGCTTTGATATGGAAC	56
CAC65	FP - GAAAAGGATGTAATAAGCTGG RP - TTTGTCCCCAAATATAGGTAG	56

Plate 1 The crown and nuts of Ran Pol

Plate 2 The crown ant nuts of Juwan Pol

Plate 3 The crown and nuts of Murusi

Plate 4 The crown and nuts of Bothal thembili

Table 3 Descriptive statistics (means, standard errors (SE) of the mean) of the scored fruit morphologies

	Ranpol	***Murusi***	**BT**	***Juwan* coconut**	**KC**	**SLT**	**GD**	***Bodiri***	**SR**	**BD**
FPL										
Mean	22.16c	19.37e	24.36a	14.64i	18.89f	20.41d	17.69g	16.71h	23.05b	19.42e
SE mean	0.071	0.102	0.097	0.097	0.093	0.144	0.1	0.166	0.145	0.12
FPC - Mean	66.80b	54.15e	60.33c	44.73i	51.34g	58.83d	48.11h	45.29i	68.66a	52.76f
SE mean	0.305	0.308	0.216	0.242	0.222	0.326	0.316	0.422	0.314	0.287
FEC- Mean	63.56a	44.93d	39.10g	41.8f	42.74e	50.20c	37.69h	36.02i	61.38b	42.24ef
SE mean	0.221	0.33	0.186	0.233	0.243	0.275	0.35	0.357	0.291	0.224
FEL - Mean	19.78a	14.01d	11.96g	12.95f	13.30e	15.57c	11.67h	11.09i	18.70b	12.95f
SE mean	0.086	0.114	0.063	0.075	0.077	0.093	0.121	0.12	0.088	0.076
FW- Mean	2318.3a	1050d	1080.4d	886.9e	1057d	1443.9c	555.5g	728.5f	2193.3b	1054.1d
SE mean	22.7	19.9	11.6	11.9	18.3	20.2	15.3	23.4	30.9	16.1
SFW-Mean	1847.2a	883.7c	909.46c	772.1d	796.2d	1248.8b	471.2f	523.5e	1831.3a	823.6d
SE mean	16.5	14.2	9.56	12.1	10.6	17.4	13.7	22.3	25.8	13.5
KW - Mean	494.92a	237.46e	230.26ef	204.02g	282.22d	338.47c	126.47i	174.21h	447.6b	219.92f
SE mean	5.62	4.67	2.97	2.62	3.89	4.84	3.85	3.78	6.2	3.27
DW - Mean	52.35c	52.09c	50.09d	55.45b	47.56e	56.75a	57.23a	56.51a	53.08c	47.81e
SE mean	0.282	0.4	0.358	0.456	0.459	0.252	0.42	0.443	0.315	0.512

Note: Means with the same letter (for each character) are not significantly different at 95% confidence level.

Fruit component parameters measured in this study represented two main aspects; nut/fruit shape and weights of the different components of the fruit. With respect to the shape of the fruit/nut, the new phenotype, BT had the most uncommon elongated fruit/nut shape recording the longest fruit polar length (FPL) of the fruit. Ran pol, followed by SR recorded the highest values for all the other fruit and nut length and circumference measurements except for FPC in which SR recorded the highest value followed by RP.

Fruit polar and equatorial lengths and circumferences given in Table 3 indicate the differences in nut shapes among the new coconut forms in comparison to the coconut forms classified earlier. Accordingly, Murusi nuts were bigger and more round in shape than its comparative varieties GD and BD. Similarly, Ran pol was also bigger and of more round shape than SLT which is one of its comparative varieties, bearing similarity to coconuts from the South-East Asian and Pacific region. SR which has a Pacific origin is the second comparative variety to Ran pol and there also the round shape of the nut was more prominent in Ran pol than SR. Juwan coconuts were smaller than SLT and more round in shape than the elongated SLT nuts. The nuts of Juwan coconuts, although of the same size as the comparative variety Bodiri, were more round in shape than that of the latter. KC was studied as the comparative variety for Bothal thembili. The comparison of these two phenotypes revealed the much elongated nut shape of BT. The new phenotypes RP and Juwan recorded typical Pacific tall-round nut shape similar to the exotic SR originated in the Pacific region while all the others recorded elongated nut shapes which is typical of the South Asian and African tall coconuts.

All the measured nut weight components followed a similar pattern among the different phenotypes. The weights of fruits revealed the new phenotype Ran pol to produce the heaviest nuts among all the phenotypes studied. The fruit and kernel weights of Ran pol recorded values much above the Sri Lankan tall coconut and even surpassed the heavy SR nuts (Figures 1 and 2). The weights of the fruits of Juwan coconuts were similar to Bodiri and lower than the rest of the talls scored, and fell in par with the dwarf phenotypes. The nut and kernel weights of KC and BT, the two intermediate types were statistically the same and were comparable to the two dwarfs Murusi and BD. The dwarf variety GD recorded the lowest values for weight components. In size, SLT recorded values following RP and SR for many of the traits. GD, Bodiri and the new phenotype, Juwan recorded the smallest fruit and nut weights. However, GD, SLT, Bodiri and the new phenotype Juwan recorded high dry weight of 100 grams of kernel, which reflects the per nut copra productivity.

Molecular characterisation

A total of 89 alleles were scored in the microsatellite genotyping ranging from 3-10 alleles per marker (Table 4).

Microsatellite genotyping revealed relatively low levels of heterozygosity for tall coconuts except for the marker locus CAC08.

The dendrogram drawn based on neighbour-joining distances is given in Figure 3. In this molecular analysis, two major groups were formed, the first including many of the talls and the second including all the dwarf, king coconut and two of the tall phenotypes. In previous studies, the exotic variety San Ramon has been reported to group with the dwarf and intermediate king coconuts (Perera *et al*, 2001;

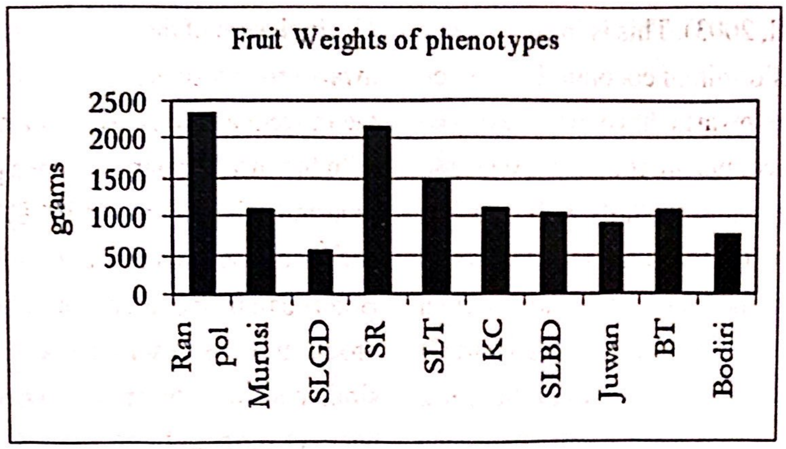

Figure 1 Weights of fruits of different coconut phenotypes

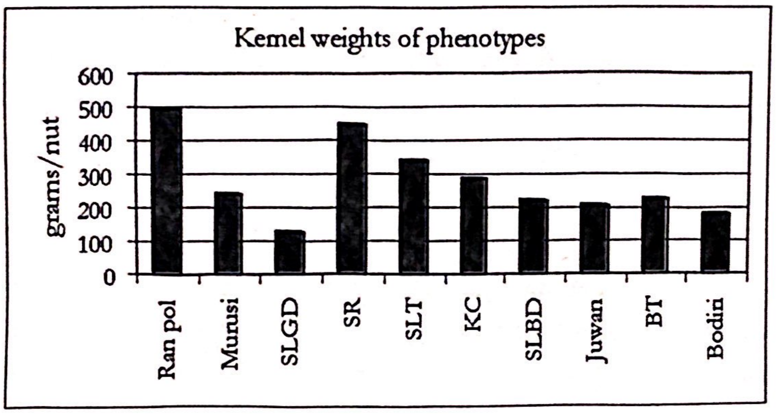

Figure 2 Kernel weights of the nuts of different phenotypes

Table 4 Details of alleles scored at each SSR locus

Microsatellite marker	Number of loci scored	% of heterozygosity in talls
CNZ-06	6	22.22
CNZ-04	4	14.29
CNZ-44	9	31.82
CNZ-29	7	18.18
CNZ-10	8	27.27
CNZ-12	4	14.29
CNZ-46	8	10.0
CNZ-37	8	8.33
CNZ-40	6	17.65
CAC-65	8	26.32
CAC-8	10	70.0
CAC-20	8	21.05
CAC-23	3	10.53

Dassanayake et al, 2003). This is in accordance with the theory of origin of coconut. It has been reported that the dwarfs have been evolved from the tall coconuts in the East Asia and Pacific region, and thus, the talls with a Pacific origin are genetically more similar to the dwarfs than the talls in the South Asia and Africa (Perera *et al*, 2001). San Ramon is a tall variety introduced to Sri Lanka from the Philippines, and thus, it is of Pacific origin. This was the reason for the grouping of the San Ramon tall in the dwarf group in previous studies. However, in the current study, San Ramon formed a separate sub-group within the tall group. This shows the occurrence of cross pollination of the exotic San Ramon with the Sri Lankan tall varieties over time resulting in mixed populations. Accordingly, this stresses the importance of being cautious in selecting proper true-to- type palms as parents in the breeding programme and also in the establishment of seed gardens.

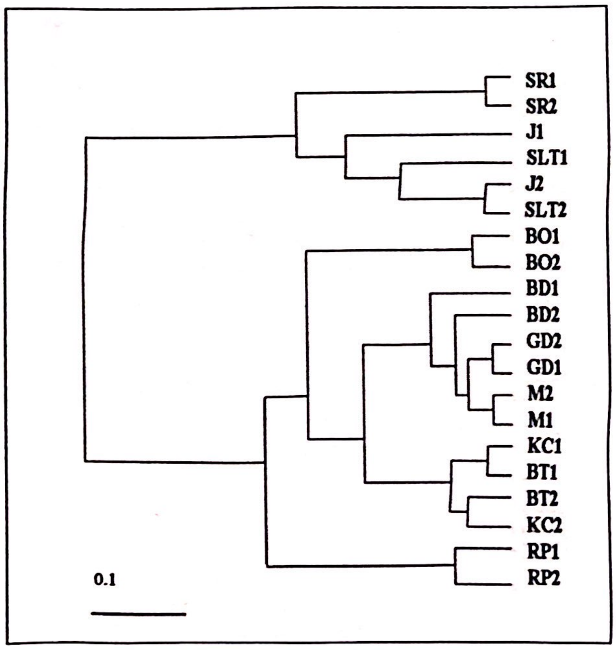

Figure 3 Dendrogram for genotypes based on neighbour joining distances

The inclusion of the tall phenotype Bodiri in the dwarf group also is a noteworthy observation in the current analysis. The similarities of Bodiri with the dwarf group also have previously been reported (Perera *et al*, 1998). Based on the results of the current study, it is proposed that Bodiri can be included genetically in the dwarf group, and it is observed that this form is much similar to the intermediate coconuts that have been described by Liyanage (1958) in the classification along with King coconut.

Yet another important observation in the molecular analysis is the grouping of the tall phenotype Ran pol in the dwarf group.

This genetic evidence as well as the visual morphological feature, nut shape indicates a Pacific origin for this new coconut phenotype. The differences in the origin will be highly useful in extracting hybrid vigour in the future coconut breeding programmes combining Sri Lankan material which are of South Asian origin.

Conclusion

The studied phenotypes revealed a high diversity with respect to nut size and shape. Genetically, a relatively rich allelic diversity was present within these coconut phenotypes and marker loci specific for dwarfs and intermediates were identified by the molecular characterisation. In addition, the observed low levels of heterozygosity and the evidences for the origins of new phenotypes should be considered in designing future coconut breeding programmes in Sri Lanka.

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