An Analysis of Genetic Diversity in King Coconut, *Cocos nucifera, Var. Aurantiaca* Using SSR Markers

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ABSTRACT

The coconut variety *Aurantiaca*, generally known as *Thembili* or King Coconut, comprises of a number of morphologically distinguished coconut forms that lack a reliable scientific classification. Several already established *Tall* coconut forms are also termed as *Thembili* by the locals. Thirty-five individuals representing eight of these phenotypically divergent *Thembili* forms including two *Tall* forms with the extension *Thembili* collected from different locations were analyzed with eight microsatellite (SSR) primer pairs to study the genetic diversity and genetic relationships among them. All primers generated 1 to 3 alleles per locus and a total of 19 alleles for all coconut forms. Overall genetic diversity ranged between 0.136 and 0.508. *Bothal Thembili*, King Coconut and *Rathran Thembili*, indicated the lowest genetic diversity ranging from 0.136 to 0.139 and grouped with *Dwarfs*. This low level of genetic diversity could be attributed to their predominant inbreeding nature. Known *Tall* forms, *Ran Thembili* and *Gon Thembili* grouped with predominantly outbreeding *Tall* coconuts along with *Rath Gon Thembili* and *Labu Thembili*. With a genetic diversity of 0.192, inbreeding *Nawasi Thembili* which had previously been classified under coconut variety *Aurantiaca*, grouped with *Talls* as well. The study revealed that the *Thembili* forms, King Coconut, *Rathran Thembili* and *Bothal Thembili* are more related to inbreeding *Dwarfs* than *Talls*, though they are considered to form a separate coconut variety, *Aurantiaca*. The recently identified coconut form *Bothal Thembili* from the southern province, despite its outstanding morphological characters, grouped with other two known *Aurantiaca* or *Thembili* forms confirming its *Thembili* identity. *Ran Thembili* and *Gon Thembili* further confirmed their relatedness to *Tall* coconuts. Out of all SSR primers used, CNZ06 and CNZ29 gave informative genetic profiles for all individuals while the least suited was CNZ44.

INTRODUCTION

King Coconut

King Coconut or *Thembili*, *Cocos nucifera*, variety *Aurantiaca*, a coconut variety whose liquid endosperm is highly accepted as a fresh natural drink, is indigenous to Sri Lanka and for centuries it has been playing a major role in various cultural aspects of the local communities. Ayurvedic medicine along with traditional psychotherapeutic practices and wizardry has found King Coconut to be an inevitably compulsory item, which may be the most possible reason behind for locals to consider the palm a "living pharmacy" and to have at least a single palm planted in their home gardens. The palm also seems to have won a remarkable place in landscaping as an ornamental item.

In the preparation of various concoctions, laxatives and many other medicinal preparations, local Ayurvedic physicians exploit the unique composition of the liquid endosperm which accounts for 6-7% sugars, minerals, vitamins and many other nutrients (Table 2.)when harvested at the correct maturity stage. Nut water comes naturally sterile and during World War II, both the Americans and the Japanese military doctors found out that in an emergency they could inject nut water instead of sterile glucose solutions directly into person's veins. Even today, modern doctors consider King Coconut water a good antidote to some of the powerful drugs that are being administered to some patients. Recent studies suggest that King Coconut water is far more superior to injectable potassium salts. Although comparatively low in the amount extracted (Table 1.), King Coconut oil is considered a well-known hair growth promoter and used increasingly in the cosmetics industry.

The chemical composition of nut water varies depending on the stage of maturity (Nathanael 1970, Mohandas 1982). A large portion of total sugars comprises of reducing sugars and a very small percentage consists of non-reducing sugars (Mohandas 1982).

Table 1. Oil percentage of the three coconut varieties grown in Sri Lanka on dry basis

Coconut Variety	Oil percentage (Dry basis)
Typica (Talls)	68.95
Nana (Dwarfs)	69.5
Aurantiaca (Thembili)	65.62

Source: Liyanage 1958 and Nathanael 1959

Changes in the sugars are more marked than any other constituent in nut water (Jayalakshmy *et al.*, 1988). As ripening proceeds, the total sugars steadily fall and sucrose increasingly makes its appearance, and finally in the ripest bunch, the total sugars may go down to about 2% (Nathanael 1952). The item most responsible for the sweetness in nut water is sucrose. Even though the percentage of sucrose increases with the storage time, palatability decreases owing to changes in other components in nut water. At 7-8 months after pollination nut water has the maximum concentration of sugars, optimum levels of minerals and vitamins and has a neutral pH (Table 2.) (Anon.1952; Subrahmanyan and Swaminathan 1959; Gunawardena 1973; CRI 1983; Jayalakshmi *et al.*1988). Therefore, the best stage of maturity to harvest King Coconut for beverage purposes would be 7-8 months after pollination.

Palms of the variety *Aurantiaca* are characterized with less trunk girth, short leaflets, lean leaf stalks and sparsely arranged leaves in the crown. But the rate of photosynthesis of this variety is high despite the lesser chlorophyll content. Wimalasekara *et al.*, (1997) showed that the nitrate reductase activity of King Coconut, which was 1.47 nmolg^{-1}h^{-1}, is distinctive from other coconut varieties at the seedling stage. Nitrate reductase is an enzyme which stimulates the process of nitrogen assimilation. The inducible nitrate reductase activity has been shown to have a highly significant and positive correlation with the annual yield for mature coconut in India (Shivashankar and Ramadasan 1988; Ramadasan *et al.* 1993). This can be confirmed through the high nut production in King Coconut which is about 18 bunches

Table 2. Some important nutritional components in King Coconut water at 7-8 months after pollination

Mineral	Amount (mg/100ml)	Amino acid	Amount (% of dryProtein)	Vitamin	Amount-units
Sodium	105	Glutamic	9.76-14.5	C	2.2-3.7mg/100ml
Potassium	312	Arginine	10.75	B	
Calcium	29	Leucine	1.95-4.18	Nicotinic acid	0.64ug/ml
Magnesium	30	Lysine	1.95-4.57	Pantothenic acid	0.52ug/ml
Iron	0.14	Proline	1.21-4.12	Biotin	0.02ug/ml
Copper	0.04	Aspartic	3.60	Riboflavin	<0.01ug/ml
Phosphorus	37	Alanine	2.41	Folic acid	0.003ug/ml
Sulphur	24	Histidine	1.95-2.05	Thiamin	0.003 traces
Chloride	183	Phenylalanine	1.23	Pyridoxin	0.003 traces
		Serine	0.59-0.91		
		Cystine	0.97-1.17		
		Tyrosine	2.83-3.0		

per palm per year. Hence, the reason for the characteristic less vigorous appearance of King Coconut palms may be the lower proportion of dry matter allocation for vegetative growth and a higher portion for the formation of nuts. Generally, *Thembili* is believed to be seasonal in fruiting (Liyanage 1958) although the observations of regular bearing palms are not rare(Manthrirathne 1965). The observations at Coconut Research Institute, Sri Lanka, also support the conclusion that a majority of King Coconut palms under well-managed conditions generally do not exhibit seasonality in fruiting (Bandaranayake, C.K and Fernando, W.M.U. 1999). Under poorly managed lands, a considerable number of palms may appear to be seasonal. King Coconut is generally susceptible to drought (Liyanage *et al.* 1988) and it may be out of production during dry weather. But the recovery is faster with the onset of rains.

The worldwide demand for drinks is shifting from the artificial to the natural due to serious health hazards caused by artificial drinks. In this picture, with its sucrose-rich (Liyanage1960) liquid endosperm, King Coconut remains to be a potential income generator for the future.

Phenotypic diversity

A remarkable morphological diversity exists among the coconut forms known as *Thembili* (Figure 1.and 2.). Generally, *Thembili* palms exhibit two statures; one similar to that of *Tall* coconuts and the other having in-between characters of both *Tall* and *Dwarf* coconuts. Due to these intermediate characters, *Thembili* forms are also termed as *Intermediate* coconuts. However, with the stature alone, it would be difficult to determine the identity of a particular form since the diversity is best pronounced by the morphology of nuts.

Nut shape, along with many other qualitative and quantitative parameters such as thickness of the husk and the kernel, weight and size of nuts and the composition of liquid endosperm, specially the sugar percentage, varies among populations, among the nuts of the same palm and even among the nuts of the same bunch (C.K. Bandaranayake, W.M.U. Fernando, 1998). These nut-related features are highly induced by the environment. Differences in caratinoid distribution result in *Thembili* nuts of many combinations of orange, green, yellow, red and brown colors. King Coconut, *Rathran Thembili* and *Bothal Thembili* nuts are characterized by a button-like projection at their distal ends.

A prominent pink coloration (Figure 2.) can be observed in the husk including the perianth region of *Ran Thembili* and *Rathran Thembili* when cut-open. The husk of *Nawasi Thembili* nuts gets softer as the ripening proceeds and the perianth region of their tender nuts are sweet and edible. All these evidences of morphological diversity suggest high level of diversity that might be existing at the DNA level in all *Thembili* forms. Liyanage *et al.*, (1958) included two *Thembili* forms, namely King Coconut and *Nawasi Thembili* under variety *Aurantiaca* or *Intermediate* coconuts and another two, *Gon Thembili* and *Ran Thembili*, under *Tall (Typica)* coconuts considering their morphological traits. However, the classification has got complicated and confusing with the identification of new *Thembili* forms. Recent studies using DNA markers have revealed that *Ran Thembili* and *Gon Thembili* are closely related to *Tall* coconuts while King Coconut and *Rathran Thembili* are more related to *Dwarf* coconuts (Perera *et al.*, 2004). However, no proper diversity analysis has been done including all available *Thembili* types and therefore, systematic and reliable classification on *Thembili* has become a need of the hour.

Though *Thembili* is having a significant economic potential for the future as a fresh and natural beverage, not much effort has been made to improve the desirable characters of this variety. Conventional varietal improvement and desirable trait identification programs are so laborious, time-consuming and unpredictable since they entirely depend upon the morphological markers which are simply the identifiable traits or characteristics observed in individuals. The selection of individuals depending on these may not always be accurate as such traits are highly environment-dependent. So,the probability for a trait of interest to be a genetically determined, i.e., heritable one is very much unpredictable. The time required for the expression of a trait of interest changes with the growth rate of a particular species. This reason exists as an extremely limiting factor for perennial crops such as coconut. Bred generations of individuals may have to be maintained in large populations under well-managed conditions for a long time in large blocks of land or specially created artificial conditions such as green houses, until they express the particular trait. This is simply due to the fact that these traits mostly are expressed at a particular phase of plant development only, for instance,fruiting. Therefore, measuring the amount and distribution of genetic diversity prior to deciding on where and how many samples to collect, is of major importance.

Figure 1. The remarkable diversity exhibited by different *Thembili* forms

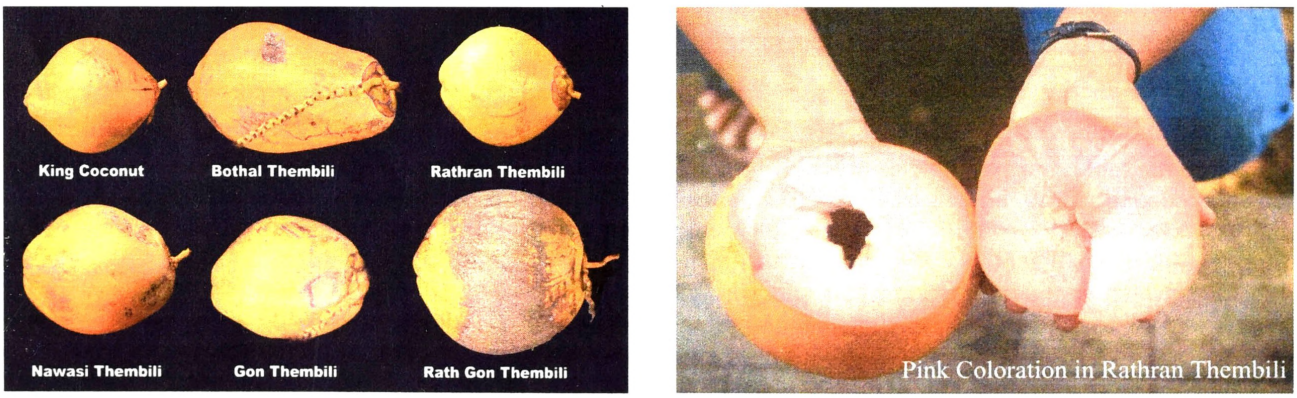

Figure 2. Nut morphology in different *Thembili* forms and pink coloration in *Rathran Thembili*

Molecular Markers

Molecular markers are based on naturally occurring polymorphisms in DNA sequences (i.e., base pair deletions, substitutions, additions or patterns). Various methods exist to detect and amplify these polymorphisms. Molecular markers are superior to all other forms of markers (morphological and biochemical markers) as they are relatively simple to detect, abundant throughout the genome even in highly bred cultivars, completely independent of environmental conditions and can be detected virtually at any stage of plant development and therefore, it is possible to identify the genetic profiles of individuals with maximum precision. Comparing the sequence of bases in the chromosome of two individuals of the same species, most of the base pairs would be identical. However, no two individuals have the same exact sequence of bases in each of the millions of base pairs that make up the genetic material. At certain sites along the chromosome, the sequence would vary among individuals. These sites where differences in DNA sequences occur, are known as molecular markers. When these differences occur within genes, they have the potential to affect the function of the gene and hence,

the phenotype of the individual. However, most of the molecular markers are not associated with a visible phenotype. They can be used for a number of different applications including germplasm characterization, genetic diagnostics, characterization of transformants, study of genome organization and phylogenic analysis.

At present, different types of DNA markers have been developed to evaluate characters of interest more precisely and effectively. More than 20 different DNA marker techniques are being employed in various studies at DNA level world over. The phenomenon called polymorphism is the basis of all of these techniques. Polymorphism is simply the variation at the DNA base sequences or the restriction sites (sites where restriction enzyme cleavage takes place) due to natural occurrence of mutations. Several combinations of PCR-based (Polymerase Chain Reaction) molecular techniques reveal the existence of this polymorphism or variation with a high degree of resolution and this variation forms the basis of the screening to ascertain the identity of individual plants and their relationship to the population. Among DNA markers available today, one of the most promising and reliable is SSRs (highly polymorphic) (Powell *et al.*, 1996a and b). SSRs are the most informative marker system available today. They are multi-allelic, co-dominant, highly polymorphic and abundant throughout the genome although they are developmentally expensive as prior sequence knowledge of the crop under study is essential.

Up to date, more informative and systematic study on all coconut types within the group *Thembili* has not been made. Studies based upon more precise and predictable innovative techniques should, therefore, be employed in order to accelerate population analyses that will ultimately help plan breeding programs yielding successive and promising individuals with valuable economic traits. As King Coconut seems to possess a number of potential uses other than having a liquid endosperm of a demanding beverage value, it is worthwhile scrutinizing the diversity within *Thembili* in search of forms or individuals having desirable features for the current artificial-to-natural shift of food items world over is in progress. The best method available for screening the greater diversity within the *Thembili* today would be DNA markers as they produce results with maximum accuracy, in contrast to traditional morphological and biochemical markers. This study targets the proper classification of divergent *Thembili* forms and authors believe that this work will motivate the initiation of further studies such as mapping of the genome and identification of more informative DNA markers so that more sensitive phylogenic analyses will be possible. Application of modern biotechnological techniques and concepts in the perennial crop improvement programs may also be stressed. Together, all these may eventually help meet the accelerated demand for *Thembili* the future possibly would give rise to.

MATERIALS AND METHODS

Plant material was obtained from eight different *Thembili* forms selected upon their morphological traits from the southern province and *ex-situ* gene bank of the Coconut Research Institute of Sri Lanka including several adjacent areas.

Each form consisted of 2-6 individuals making the total number 35 (Table 3.). Healthy leaf samples were collected from each individual and stored at 0°C until use.

DNA Extraction

About 0.5g of fresh green leaf samples was taken from each individual and crushed into a fine powder using liquid nitrogen. The ground material was transferred into 1.5ml tubes containing 1ml of pre-warmed CTAB with 2% Monothioglycerol (MTG)(500µl of MTG per 25ml of CTAB). Each sample was incubated at 65°C for 30 minutes in the water bath. Samples were allowed to cool down to room temperature and 300µl of chloroform/isoamylalcohol, 24:1(V/V) was added and mixed gently followed by centrifugation at 13,000rpm for 2min. The same step was repeated for the remaining leaf material in each tube for re-extraction. 600µl of cold(-20°C) isopropanol was added to the supernatant. Each sample was gently mixed and left at room temperature for 1hr. DNA was pelletted by centrifugation for 5min. The supernatant was removed and 1ml of wash buffer was added followed by vigoruos agitation to release pellets and the samples were left still for 30mins. Finally, samples were centrifugated for 2min at 13,000rpm. Resulting pellets were washed with 70% ethanol. Samples were air-dried. Each sample was diluted adding 40µl of sterilized distilled water and stored at -20°C prior to use.

Table 3. Different *Thembili* forms, codes and sample numbers

Name	Code	Sample number
01.Rathran Thembili	RRT	1-6
02.Rath Gon Thembili	RGT	7,8and35
03.Gon Thembili	GT	9-13
04.Bothal Thembili	BT	14-19
05.King Coconut	KC	20-24
06.Nawasi Thembil i	NT	25-27
07.Ran Thembili	RT	28-32

DNA quantification

The quality and quantity of extracted DNA was determined by 1% Agarose gel electrophoresis with Lambda DNA as standards.All samples except for few contained over 100ng/µl DNA.

PCR assay for SSR primers and Gel Electrophoresis

PCR amplification was performed for eight microsatellite loci (Table 4). PCR regime consisted of an initial denaturation (94°C for 4mins), 35 cycles each consisting of 30s denaturation at 94°C, 30s annealing (52-58°C according to the primer used)(Table 4.) and 1min extension (72°C). At the end of the final cycle an extension period of 10min at 72°C was included. Final soak temperature was 4°C. Using a BIORAD Sequi-Gen® GT apparatus polyacrylamide gel electrophoresis was performed as follows; the removable and fixed glass plates including spacers were cleaned thoroughly with 70% ethanol. A few ml of 2% Binding Silane was applied thoroughly and uniformly all over the inner surface of the outer plate. RepelCoat (BDH) was applied onto the outer surface of fixed plate. Placing two spacers at both sides of the fixed plate, plates were fixed tightly turning the clamps on with polished surfaces facing each other. The base portion that connects syringe and the inter-plate space was fixed and the sequencing gel mix (60ml of

acrylamide /bis-acrylamide, 600µl of 10% ammonium persulfate (APS) and 60 µl of TEMED) was injected with light tapings at the gel front to avoid air bubble formation until the gel took the whole inter-plate space. The comb was inserted at the top of the gel with teeth facing outwards. The gel was allowed to set for an hour and the syringe and the base portion were removed. The gel was placed in gel tank with 1X TBE buffer. The comb was removed and re-inserted with teeth facing inwards forming wells. Gel was pre-warmed for 30 min at 80W. Samples were denatured at 94°C for 6 min after adding 2µl of gel loading buffer into each PCR tube. At the end of electrophoresis for 2 hrs at 80W, the gel was removed. Buffer also removed and only the outer plate containing the gel was taken out. For the visualization of bands the gel was first fixed dipping in a solution containing 10 ml of glacial acetic acid and 1990ml of distilled water for 20 min. The gel was washed with distilled water for several times. It was then dipped in the staining solution (3g of silver nitrate ($AgNO_3$) dissolved in 2l of distilled water). Staining was done for 20min shaking the solution in a tray. The gel was washed once with distilled water and developed dipping in a solution containing 12g of NaOH, 8 ml of formaldehyde and 2000ml of distilled water in a separate tray.

DATA ANALYSIS

Microsatellite loci were scored individually and the different alleles were recorded for each sample screened. With scored data, a distance matrix was constructed using the software GENECLASS2, Version 2.0.g(31-Jan-2005)(http: //www.montpellier .inra.fr/CBGP/softwares) according to the Nei's standard distance criterion (1972), based on the proportion of shared alleles at each locus and meaned across all loci. This distance matrix was uploaded into the software Neighbor and again into the software Drawgram of the PHYLIP Phylogeny Inference Package (Version 3.2)(Joseph Felsentein, University of Washington, USA.), in order to construct the dendrogram (phenogram). Dendrogram construction was based on the Unweighted Pair Group Method with Arithmetic Averages (UPGMA) embedded in the software Neighbor.

Table 4. The Eight SSR markers used in the study

Locus	Repeat motif	Annealing temperature (°C)	Fragment size (bp)
CAC10	$(TA)_8CATA(CA)_{11}(TA)_8$	58	198
CAC20	$(CA)_{19}$	52	128
CAC65	$(CA)_{15}$	53	151
CNZ04	$(CT)_{39}TT(CA)_{10}$	53	162
CNZ06	$(CT)_{13}$	53	85
CNZ10	$(CT)_{18}(GT)_{17}$	53	148
CNZ29	$(GT)_{22}(GA)_2CA(GA)_{11}$	51	135

RESULTS AND DISCUSSION

Population Analysis Study

Eight microsatellite primer sets (Table 4.) were used to assess thirty-five individuals from eight coconut forms known as *Thembili*. Some forms were made up of individuals from different locations. The collection included several known *Tall* coconut forms since they are generally considered *Thembili* by the Locals.Two examples of SSR polymorphism are shown in Figure 3. A pair for each, *Dwarf* and *Tall* DNA samples were used as controls. Genetic diversity for each form and the number of alleles detected along with their means are shown in Table 5. The genetic diversity for all samples studied ranged between 0.136 of *Rathran Thembili* and 0.508 of *Rath Gon Thembili*. 0.136 of *Rathran Thembili* and 0.139 of both King Coconut and *Bothal Thembili* (Identified recently by the Genetics and Plant Breeding Division-GPBD, CRISL) were among the least genetic diversities of all forms. *Nawasi Thembili, Gon Thembili, Ran Thembili, Labu Thembili and Rath Gon Thembili* (Identified recently by the GPBD, CRISL) exhibited comparatively higher genetic diversities; 0.192, 0.228, 0.383,0.250 and 0.508 respectively. *Nawasi Thembili* and *Gon Thembili* indicated genetic diversities lower than that of *Dwarfs*(0.250) while *Labu Thembili* had the same diversity value of *Dwarf* coconuts. The eight SSR primers revealed a total of 19 alleles in all populations. The best suited microsatellite markers out of the eight used the study revealed to be CNZ06 and CNZ29 while the least suited was CNZ44 which yielded no genetic diversities for any of the *Thembili* forms (Table 5.).Other markers appeared to be moderately informative.

The dendrogram (phenogram) (Figure 4.) divided all *Thembili* types into two distinct groups. *Rathran Thembili, Bothal Thembili* and King Coconut grouped with *Dwarf* control and all the other types grouped with *Tall* control.

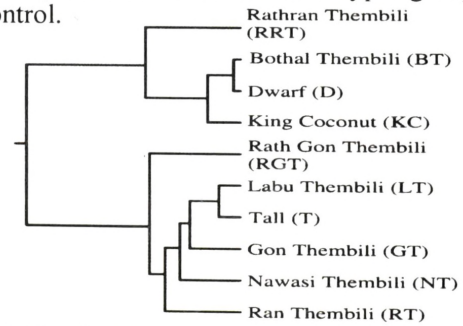

Figure 4. The Phenetic tree constructed based on Nei's genetic distance (1972)

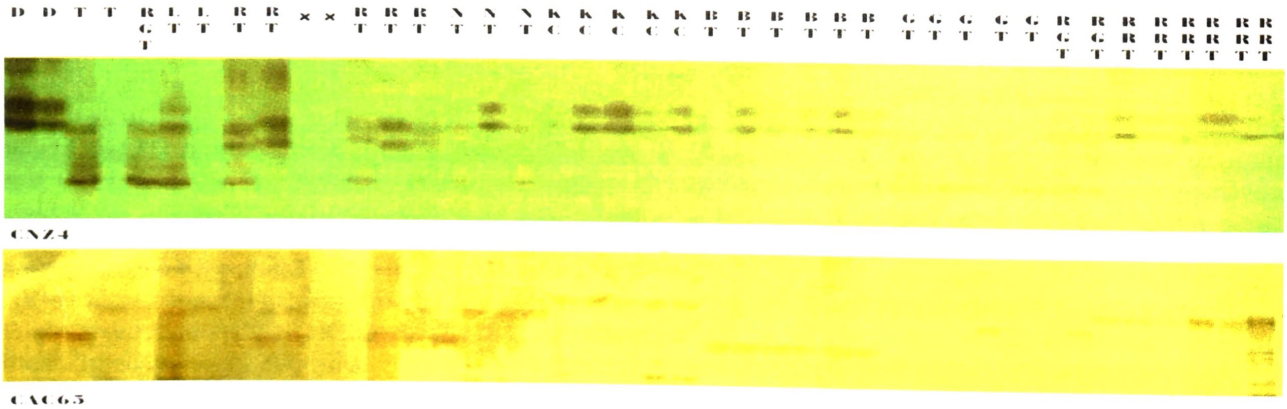

Figure 3. Polymorphism detected by primer pair CNZ 04 and CAC65

Table 5. Genetic diversity and number of alleles detected by eight SSR primer pairs in all populations.

Loci	RRT		RGT		LT		GT		BT		KC		NT		RT		Means		Total
	Allele	#D	Allele#	D	Allele#	D	Allele#	D	Allele#	D	Allele#	D	Allele#	D	Allele#	D	Allele#	D	
CNZ44	1	0.0	1	0.0	1	0.0	1	0.0	1	0.0	1	0.0	1	0.0	1	0.0	1.0	0.0	1
CNZ06	2	0.545	2	0.667	2	1.0	2	0.55	2	0.556	1	0.556	3	0.7	3	0.689	2.125	0.662	3
CNZ04	1	0.0	2	0.6	1	0.0	1	0.0	1	0.0	2	0.0	1	0.0	2	0.533	1.375	0.142	2
CNZ29	2	0.545	2	0.667	2	1.0	2	0.556	2	0.556	1	0.556	3	0.8	3	0.689	2.125	0.671	3
CAC65	1	0.0	3	0.773	1	0.0	1	0.0	1	0.0	2	0.0	1	0.0	2	0.467	1.5	0.155	3
CNZ10	1	0.0	2	0.533	1	0.0	3	0.711	1	0.0	1	0.0	1	0.0	2	0.356	1.5	0.2	3
CAC20	1	0.0	2	0.533	1	0.0	1	0.0	1	0.0	1	0.0	1	0.0	1	0.0	1.125	0.066	2
CAC10	1	0.0	2	0.333	1	0.0	1	0.0	1	0.0	1	0.0	1	0.0	2	0.337	1.25	0.083	2
All loci	1.25	0.136	2.0	0.508	1.250	0.250	1.500	0.228	1.25	0.139	1.250	0.139	1.5	0.192	2.000	0.383	-	-	
Total	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	19

D = Genetic diversity

Dwarf and *Aurantiaca* (*Thembili*) coconuts are known to be predominantly inbreeding (Liyanage *et al.*, 1958). Based on the phenotypical diversity, Liyanage *et al.*, (1958) included *Nawasi Thembili* and King Coconut under the group *Intermediate* or *Aurantiaca*. Recent studies based upon more reliable DNA marker techniques have shown that *Rathran Thembili* and King Coconut are closely related to *Dwarf* coconuts (Perera *et al.*, 2004).

This study further confirms this fact by grouping *Rathran Thembili* and King Coconut together with recently identified *Bothal Thembili* from the southern province in the same way with *Dwarfs*. The self-fertilization causes comparatively smaller genetic variations in populations and this could be attributed to the lesser genetic diversities observed among *Thembili* forms coming under *Dwarf* coconuts. Irrespective of the fact that both *Bothal Thembili* and *Rathran Thembili* were made up of individuals from different locations, they exhibit smaller diversities implying the existence of almost true-to-type individuals in the locations they were collected from. Although King Coconut and *Bothal Thembili* express a greater phenotypic diversity in nut morphology (Figure 2), the study reveals that they share a similar genetic diversity. All individuals of the King Coconut population were selected from inbred palms of the *ex-situ* gene bank of CRISL.

Being classified under the *Dwarf* group, with a genetic diversity of 0.139, King Coconut confirms the fact that all forms under *Dwarf* group are predominantly inbreeding. Dendrogram expresses the fact that *Bothal Thembili* is the most *Dwarf*-related *Thembili* form out of all under study while *Rathran Thembili* remains to be a distant member. *Nawasi Thembili*, known to be inbreeding (J. Somarathne, Personal communication) has grouped with the *Tall* coconuts revealing its relatedness to *Talls*. This contrasts with the classification made by Liyanage *et al.*, (1958) based upon morphological markers. *Nawasi Thembili* nuts and the stature of the palm are morphologically similar to that of *Talls*. These samples were selected from inbred individuals of the same *ex-situ* gene bank of CRISL and it may be the most possible reason for them to exhibit a genetic diversity of 0.192 which is smaller than that of *Dwarfs*. *Ran Thembili* and *Gon Thembili* samples were isolated from the same location and they exhibited medium genetic diversities. For *Rath Gon Thembili* and *Labu Thembili*, individuals were selected from several distant locations. Both these categories consisted of individuals from the southern and the north western provinces of the island. Although individuals within each group appear to be similar phenotypically to each other, it is possible for them to have different genetic makeups as they have probably evolved under different environmental conditions over a long period of time and as a result, they may be indicating comparatively high levels of genetic diversities. *Tall* coconuts exhibit greater levels of genetic diversities as they are predominantly outbreeding. However, *Ran Thembili* and *Rath Gon Thembili* indicate genetic diversities closer to that of *Talls*, 0.54. High levels of within population variation are a common observation in many other outbreeding crops also, for instance, in *Theobroma cacao* (Allen, J.B. 1998), more than 90% of the variation is observed within population.

The study reveals that out of all available *Thembili* types, King Coconut, *Rathran Thembili* and *Bothal Thembili*, being closely related to inbreeding *Dwarf* coconuts; make up the *Intermediate* or *Aurantiaca* coconut group. These three *Thembili* forms can be considered true *Thembili* types known up to date in Sri Lanka. *Nawasi Thembili* has previously remained as a misidentified *Thembili* form. *Ran Thembili* and *Gon Thembili* further confirmed their relatedness to *Talls*. They only seem to be sharing a phenotypic similarity with true *Thembli* forms and it may be their appearance which may have led locals to draw wrong categorical conclusions. This may be the case regarding *Nawasi Thembili* also. It is possible for true *Thembili* forms to have crossed with *Tall* types giving rise to individuals with varying characteristics. Liyanage *et al.*, (1958) has stated about a cross between King Coconut and a Green Tall coconut which yielded a coconut type with reddish brown nuts, just like that of *Rath Gon Thembili*, and the same way, natural crosses along with various adaptational mutations may have developed new coconut forms we observe today over thousands of years. The study was based on relatively small number of samples for each of the coconut forms considered. More accurate results could be generated with large sample sizes and due to time constrain, construction of large sample sizes was impossible during the study. More complete and accurate study must be conducted followed by a survey on all available *Thembili* forms distributed all over the island. All SSR markers except for one successfully analyzed the genetic diversity within the common coconut form *Thembili* in Sri Lanka. The study stresses the effective and potential use of microsatellites in the genetic diversity analysis of *Thembili*.

An effort was made to direct an Amplified Fragment Length Polymorphism (AFLP®) analysis for all samples using AFLP® Core Reagent Kit and AFLP® Starter Primer Kit (GIBCO BRL) from LIFE TECHNOLOGIES. Successful results generated for just a few samples and the procedure terminated due to the time lapse. The procedure is to be completed by Genetics and Plant Breeding Division, Coconut Research Institute of Sri Lanka after further optimization.

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