Molecular Assessment of *Cocos nucifera* L. Var. Sri Lanka Yellow Dwarf for Genetic Purity and *Aceria* Mite Tolerance

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International Journal of Molecular Evolution and Biodiversity, 2014, Vol.4, No.1  doi: 10.5376/ijmeb.2014.04.0001

Received: 10 Dec., 2014
Accepted: 21 Dec., 2014
Published: 31 Dec., 2014

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Preferred citation for this article:
Perera et al., 2014, Molecular Assessment of *Cocos nucifera* L. Var. Sri Lanka Yellow Dwarf for Genetic Purity and *Aceria* Mite Tolerance, International Journal of Molecular Evolution and Biodiversity, Vol.4, No.1, 1-5 (doi: 10.5376/ijmeb.2014.04.0001)

Abstract World-over, coconut is classified broadly into tall and dwarf phenotypes. The dwarf coconut variety is further divided into forms based on the colour of the epicarp. The Sri Lanka Yellow Dwarf (SLYD) coconut variety was observed to have individuals of differing phenotypes and this variety also showed varying degrees of tolerance to *Aceria guerreronis* (Keifer), which is a pest infesting coconut causing economic losses. This study was aimed at the evaluation of the coconut form SLYD for varietal confirmation and the tolerance to *Aceria* mite at the microsatellite marker loci. Twenty five sample palms drawn randomly from four morphological groups based on stature and tolerance to *Aceria* mite were genotyped at seven SSR marker loci along with Sri Lanka Tall (SLT) and Sri Lanka Green Dwarf (SLGD) as reference varieties. Three SSR loci were highly informative for the SLYD population and the results revealed the presence of a population structure within the SLYD coconut population. A clear clustering was observed for tall like and the dwarf like groups with more variation within the tall like group. Clear allelic identifications were established at several SSR loci for the clustering based on stature and the markers were identified for distinguishing different groups. The study provided evidence for the need for reclassification and purification of SLYD coconut population. Certain associations were present for the character *Aceria* mite tolerance, indicating the potential for marker assisted selection.

Keywords Coconut; SSR markers; Varietal classification; Aceria mite; Marker assisted selection

Introduction Coconut palm is an important oil crop in the tropics. It is also important as a tree crop with diverse uses, such that every part of this palm can be economically used.

In its varietal classification, two main types of coconuts have been identified world-over, namely the tall (*Typica*) group and the dwarf (*Nana*) group. In addition to these two types several intermediate type coconut populations have also been identified in certain countries. Tall coconut varieties display tall stature, larger crowns and a prominent swelling at the base of the stem forming a root bole. In contrast, the dwarf coconut varieties are shorter in stature, produce a smaller crown and are lacking a root bole. The dwarf coconut varieties are further distinguished into four groups by the colour of the epicarp of the fruit as green (*pumila*), yellow (*eburnea*), red (*regia*) and brown (*braune*). The intermediate coconut varieties possess certain features from the tall group and the rest from the dwarf group (Liyanage, 1958). There are two sub groups within the tall coconuts; the South East Asian/Pacific group and the South Asian/African group. The dwarf coconuts are presumed to have evolved from the tall coconuts of the South East Asian/Pacific group (Perera et al., 2000). The dwarf varieties are mainly homozygous purelines while the tall coconuts are naturally cross-pollinating, heterogenous populations having varying degrees of heterozygosity. Consequently, the allelic diversity of tall coconuts is naturally higher while there is a reduction of allelic diversity in the dwarf coconuts.

In Sri Lanka all the four forms of dwarf coconuts, i.e. green, yellow, red and brown are found. These dwarf coconut varieties are not popular as commercial coconut cultivars but are extensively used as a parent in inter-varietal hybrid coconut production. The
observations in the parental palm pool of the Sri Lanka Yellow Dwarf coconut variety indicate morphological differences within the variety. In addition to the palms with typical dwarf features certain palms are observed to have taller stature, larger crowns and well-formed root boles which are specific features of the tall coconuts while the nuts in all of them are yellow coloured.

In addition to morphological differences, the palms in this population show a varying degree of tolerance/susceptibility to the coconut mite *Aceria guerreronis* (Keifer). *Aceria* mite is a pest that inhabits underneath the perianth of the fruit. It causes economic losses to the coconut by reducing the nut size due to its feeding on the soft tissues of the immature nut (Fernando et al., 2000).

The current study was conducted with the objectives of discriminating the different morphological groups within the Sri Lanka Yellow Dwarf coconut population by molecular means at microsatellite marker loci and to identify associations, if there are any between the same marker loci and the status of tolerance or susceptibility to *Aceria* mite infestation.

1 Methods and Materials

1.1 Sampling of palms

Sample palms were selected from a pool of Sri Lanka Yellow Dwarf parental palm population. They were categorised into four groups, and random sampling was performed within each group to select a total of 25 experimental palms (Table 1). In addition 01 Sri Lanka Tall (SLT) palm and 01 Sri Lanka Green Dwarf (SLGD) palm were included in the study for comparison purposes.

Table 1 Sampling of palms for molecular evaluation

| Group                        | Number of palms and the code |
|------------------------------|------------------------------|
| Tall-like *Aceria* mite tolerant | 07 (TLMT1 to TLMT7)          |
| Tall-like *Aceria* mite susceptible | 05 (TLMS1 to TLMS5)          |
| Dwarf- like *Aceria* mite tolerant | 06 (DLMT1 to DLMT6)          |
| Dwarf-like *Aceria* mite susceptible | 07 (DLMS1 to DLMS7)          |

Tall stature, larger crown with non-drooping frond tips and the presence of a root bole were the specific features used in identifying the tall-like category. Dwarf stature, smaller crown with drooping frond tips and the absence of a root bole were the criteria used for identifying the dwarf-like category.

Palms having more than two damaged nuts in the crown were classified as *Aceria* mite susceptible, while the palms with two or less damaged nuts in all bunches were classified as *Aceria* mite tolerant.

1.2 DNA extraction and PCR amplification

Genomic DNA of the 25 sample palms were extracted from the spear leaf tissues using a modified C-TAB DNA extraction method. PCR amplification was performed with 07 coconut specific microsatellite markers (Perera et al., 1999; Rivera et al., 1999) (Table 2). Amplified PCR products were visualized by Silver staining upon band separation by poly-acrylamide gel electrophoresis and the genotypes were scored.

Table 2 Forward (F) and Reverse (R) SSR primer sequences and their annealing temperatures

| Primer | Sequence                     | Annealing Temperature (°C) |
|--------|------------------------------|---------------------------|
| CAC 8  | F 5'-ATC ACC CCA ATA CAA GGA CA-3' | 57                        |
|        | R 5'-AAT TCT ATG GTC CAC CCA CA-3' | 54                        |
| CAC 20 | F 5'-CTC ATG AAC CAA ACG TTA GA-3' | 54                        |
|        | R 5'-CAT CAT ATA CAT ACA TCG AAC A-3' | 56                        |
| CAC 23 | F 5'-TGA AAA CAA AAG ATA GAT GTC AG-3' | 56                        |
|        | R 5'-GAA GAT GCT TTT ATA TGG ACC-3' | 56                        |
| CAC 65 | F 5'-GAA AAG GAT GTA ATA AGC TGG-3' | 56                        |
|        | R 5'-TTT GTC CCC AAA TAT AGG TAG-3' | 54                        |
| CNZ 12 | F 5'-TAG CTT CCT CCT GAG ATA AGA TGC-3' | 54                        |
|        | R 5'-GAT CAT GGA ACG AAA ACA TTA-3' | 53                        |
| CNZ 40 | F 5'-CTT GAT TGC TAT CTC AAA TGC-3' | 53                        |
|        | R 5'-CTG AGA CCA AAT ACC ATG TGT-3' | 53                        |
| CNZ 44 | F 5'-CAT CAG TTC CAC TCT CAT TTC-3' | 53                        |
|        | R 5'-CAA CAA AAG ACA TAG GTG GTC-3' | 53                        |
1.3 Data analysis

Powermarker (Liu and Muse, 2005) software was used for the analysis of genotypic data to derive allele and genotypic frequencies, genetic distances among the individuals and to generate the phenetic tree.

2 Results

A total of 21 alleles were scored in the population at the 07 microsatellite marker loci with a mean value of 03 alleles per locus (Table 3). Out of them 04 alleles representing 04 markers were specific to SLT control palm. The 21 alleles formed a total of 22 genotypes ranging from 2 to 5 genotypes per marker and a mean value of 3.1429 with 04 genotypes specific to SLT. Markers CAC8, CAC65 and CNZ12 formed the highest number of alleles, (4 alleles each) and the highest number of phenotypes also (5, 4 and 4 respectively).

Table 3 Summary statistics of genotypic data

| Marker  | No of alleles | No of genotypes | Gene diversity | Heterozygosity | PIC  |
|---------|---------------|-----------------|----------------|----------------|------|
| CAC8    | 4 (A1 - A4)   | 5               | 0.2688         | 0.1            | 0.2561|
| CAC20   | 2 (B1 - B2)   | 2               | 0.0713         | 0.0            | 0.0688|
| CAC23   | 2 (C1 - C2)   | 2               | 0.1975         | 0.0            | 0.1780|
| CAC65   | 4 (D1 - D4)   | 4               | 0.6693         | 0.125          | 0.6066|
| CNZ12   | 4 (E1 - E4)   | 4               | 0.4896         | 0.0833         | 0.4305|
| CNZ40   | 2 (F1 - F2)   | 2               | 0.0713         | 0.0            | 0.0688|
| CNZ44   | 3 (G1 - G3)   | 3               | 0.4170         | 0.0            | 0.3788|
| Total   | 21            | 22              |                |                |      |
| Mean    | 3             | 3.1429          | 0.3121         | 0.044          | 0.2839|

As reported in table 03 gene diversity values ranging from a minimum of 0.0713 at CAC20 and CNZ40 to a maximum of 0.6693 at the marker locus CAC65 were recorded with a mean value of 0.3121. CAC65 recorded the highest number of heterozygotes while four markers scored zero heterozygotes recording a mean value of only 0.044 among the observed individuals.

2.1 DNA fingerprints for the Sri Lanka Yellow Dwarf population

The single SLT palm scored alleles which were different to the SLYD population as expected at 04 of the marker loci. However, although naturally cross-pollinating and therefore expected to be highly heterozygous, SLT recorded homozygous alleles at all the marker loci examined in this study. This supports earlier findings for the comparatively low heterozygosity of Sri Lanka tall coconuts (Ekanayake, 2010). On the other hand although expected to be homozygous due to naturally self-pollinating nature, resulting in pure-lines, SLYD recorded a total of 05 heterozygous individuals at 07 different marker loci.

The second comparative coconut form Sri Lanka Green Dwarf (SLGD) scored identical alleles with the dwarf like SLYD (DL-SLYD) except at marker locus CAC65. Consequently, SLGD and DL-SLYD could be differentiated at the CAC65 marker locus where SLGD scored homozygous D1 alleles while DL-SLYD scored mainly D3 alleles. Microsatellite marker CAC65 could also be used to differentiate the DL-SLYD from the tall like group within the SLYD (TL-SLYD) (Table 4). TL-SLYD recorded a higher frequency of D1 followed by D2 alleles while DL-SLYD recorded a higher allelic frequency of D3. The results also display a higher allelic variation of TL-SLYD at the marker locus CAC65 compared to DL-SLYD.

Table 4 Allele frequencies at marker locus CAC65

| Allele | Frequency in SLYD | Frequency within DL-SLYD | Frequency within TL-SLYD |
|--------|-------------------|--------------------------|-------------------------|
| D1     | 34.091            | 10.0%                    | 54.167%                 |
| D2     | 13.636            | 0%                       | 25.0%                   |
| D3     | 45.454            | 80.0%                    | 16.667%                 |
| D4     | 6.818             | 10.0%                    | 4.167%                  |
2.2 Genetic distances and relatedness of coconut forms
Genetic distances among the individuals based on the Euclidean method revealed the highest distance of SLT with all the rest of the individuals. The second comparative coconut form SLGD, recorded closer genetic distances with DL-SLYD. Furthermore, the genetic distances within DL-SLYD were observed to be low while comparatively higher distances were observed between DL-SLYD and TL-SLYD. Also the genetic distances among the individuals within TL-SLYD were found to be comparatively high.

2.3 Phenetic tree
Figure 1 presents the dendrogram drawn by neighbour joining method using Euclidean distances.

Figure 1 Phenetic tree drawn based on Euclidean distance

As expected from the genetic distances SLT separated out as a single individual in the phenetic tree. Secondly, three individuals of TLMS group formed a clear separate cluster which was characterized by SSR marker CAC65 D2 alleles. From the rest of individuals six individuals of the seven within TLMT group formed a separate cluster which was characterized by homozygous D1 alleles of CAC65 and homozygous E3 alleles of CNZ12.

The rest of the individuals formed a larger group. Yet in that DLMS1, TLMS2 DLMT6 and TLMT2 did disperse from the main cluster and the results revealed that there were missing values, rare alleles or different allelic combinations in these four individuals. SLGD separated with DLMS4 and DLMS5, the two latter having several missing data points. Rest of the individuals within the main cluster, included all DL-SLYD and one individual from the TL-SLYD group. This group was characterized by CAC65 D3 allele and CNZ G3 allele and included both tolerant and susceptible individuals for Aceria mite infestation.

3 Discussion and Conclusions
The current study provides evidence for the presence of a genetic structure within the Sri Lanka Yellow Dwarf coconut population. The dwarf like group possesses specific dwarf like morphological characters described above and this group is genetically close having common alleles. This dwarf like group is genetically much similar to Sri Lanka Green Dwarf and share similar alleles in all but one of the loci observed in this study. As such this specific microsatellite locus, CAC65 can be used to distinguish SLGD from the DL-SLYD group. Moreover, their morphological features resemble the Malayan yellow dwarf coconuts, which is the most widely known yellow dwarf coconut variety in the world.

The tall like group on the other hand is morphologically similar to several of the phenotypic characters within the SLT coconut variety as described in materials and methods. However, they do not possess common alleles with the SLT coconuts indicating that they are more an intermediate type and mixed population despite being classified within SLYD. Therefore, the evidence strongly suggests the need for reclassification and purification of the coconut form SLYD. Furthermore, the allelic differences and the genetic distances are higher even
within the TL-SL YD indicating the possibility of genetic structure even within this group. Such observations have not previously been recorded in the world for dwarf coconut varieties.

The present study further evaluated the association of tolerance/susceptibility for the *Aceria* mite infestation with the molecular markers. In the phenetic tree two groups were identified with one group including the majority of individuals within the TLMT group and the other including the majority of individuals in the TLMS group with molecular markers and specific alleles defining such groups. No such grouping was evident for *Aceria* mite tolerance within the DL-SL YD group. Therefore, no conclusive associations were directly evident for *Aceria* mite tolerance/ susceptibility in the current study although certain grouping was clear within TL group. The results however, indicate the high possibility for identifying associations if tested with more molecular markers and the feasibility for subsequent marker assisted selection.

The coconut form SLYD has been used as a parent in developing hybrids in many of the coconut growing countries. It is a parent in the recommended coconut hybrid CRIC65 in Sri Lanka. Genetic fidelity of the parental population is a must for the resultant hybrid to be uniform and for the expected performance in the field. With the results elucidated in the current study purification of the variety is essential followed by the use of the DL-SLYD group as the parents in the hybrid production because the SLYD is the specified parent in the coconut hybrid CRIC65 in Sri Lanka.

Out of the SSR loci used in this study, CAC65, CNZ12 and CNZ44 were found to be highly informative and polymorphic to differentiate and distinguish the studied population and is recommended for other studies involving this population.

**Authors’ contributions**

SACNP conceived and designed the research, analysed data and prepared the manuscript. LCJK and WBSF conducted the field and lab work.

**Acknowledgements**

This research was funded by the Sri Lanka National Research Council under the grant no 11-042.

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