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# Genetic Variation of Coconut Tall (*Cocos nucifera* L., Arecaceae) in Bali, Indonesia Based on Microsatellite DNA.

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**Abstract**

The coconut has important roles for economics, traditional medicine and culture, especially for Hindu's ceremonial purpose in Bali Island (Indonesia). Each coconut cultivar has unique characteristics. The aimed of this research was to determine genetic variation of coconut tall (*Cocos nucifera* L., Arecaceae) in Bali, based on DNA microsatellite. Six pairs microsatellite markers were used to determine heterozygosity.
The results showed that total of 80 alleles were detected by microsatellite with an average of 13.33 alleles per locus, there were 12 alleles on microsatellite locus CnCirA3, 12 alleles on locus CnCirC3, 16 alleles on locus CNZ05, 14 alleles on CNZ09 , 17 alleles on CNZ21, and 9 alleles on microsatellite primer pairs CNZ51. The mean values of gene diversity (He) and observed heterozygosity (Ho) were 0.8835 and 0.5421, respectively. The highest heterozigosity was on *bulan tall* coconuts cultivar (0.816), the lowest heterozygosity was on *bluluk tall* coconuts (0.35).
**Keywords:** unique characteristic, microsatellite, allele, heterozygosity, coconut

## 1.Introduction

Coconut (*Cocos nucifera* L.) is one of the primary sources of income for most Indonesian farmers. Coconut fruit is processed into various products like vegetable oil, raw material for food, industry, medicine, cosmetic and oleo chemicals. Total land area plantation of coconut is about 3.7 million hectares, with 97% of overall production from farmers (Novarianto, et al., 2005). In Bali, beside of its economic value, parts of coconut tree (leaves, fruit, young flower) also used for ceremonial and medicinal purposes, which called "*nyuh madan*" . Morphologically, some coconut plants differ in some characters, which is very unique and specific to its individual plant. Based on the unique characters, there were more than twenty unique coconuts identified that differ individually. Some of them are *ancak* which has branches on its trunk; the *be julit* has plicated lamina leaves, the *bingin* 's root grow from nodes of stem, the *Bojog* 's fruit husk is colored like the hair of long tailed monkey. Differences in fruit color such as white, green, yellow and red were used to distinguish between the *bulan*, *gadang*, *gading* and *surya* coconuts respectively. Inflorenscentia spicata was characteristic of *bluluk*, while the *udang* and *mulung* were characterized by red mesocarpium. The *Rangda* coconut has petiole and the apex of the stem were twisted. The *sudamala* was characterized by many kinds of unique characters, which some of them are doubled spatha, and flat apex of male spikelet (Kriswiyanti, 2012).
To study its genetic diversity, simple sequence repeats (SSR) microsatellite markers were used. This technique had been used successfully by many scientists to characterize the genetic diversity of the coconut population (Perera et al. 2001; Manimekalai and Nagarajan 2006; Kumar et al., 2011). The microsatellite analysis of the 9 coconut populations with 8 primers revealed a total of 37 alleles (Devakumar et.al., 2010). From the used 26 SSR markers, detected a total of 188 alleles with an average of 7.23 per locus (Liu, et.al., 2011). In this research, genetic variation of *tall coconut* (*Cocos nucifera* L., Arecaceae) was determined based on DNA microsatellite, using alleles size on each individual of tall coconut. The alleles data from each accession can be applied to predict natural breeding for unique coconut conservation purpose.

## Materials and Methods

*Plant material used*: Leaf samples were collected from Klungkung, Bangli, Karangasem, Gianyar, Tabanan, Badung, Jembrana residence and Denpasar, Bali province, Indonesia. Of the 58 samples from twenty cultivars

 

were 'tall coconut' category (*nyuh ancak, biasa, bulan, bingin, gadang, gading, kebo, srogsogan, mulung, rangda, bluluk, sudamala, surya, udang, kapas, be julit, bojog, macan, naga, pudak* ). DNA extraction and detection of microsatellite polymorphisms were analysed at Forensics and Primata Laboratory Udayana University, Bali Indonesia.

**DNA extraction:** DNA was extracted from fresh coconut young leaves using a CTAB based protocol modified from Doyle and Doyle (1987). The primer sequences and associated information are given in Table 1 (Pandin, et.al., 2008).

**SSR analysis**

Polymerase Chain Reactions (PCR) assay and gel analysis : DNA was amplified in 13 µL reactions containing 2 µL sample, 3.5 µL $H_2O$, 6.5 µL Mastermix (Qiagen), and 1 µL primer. The PCR programmed for 30 cycles of 45 seconds each at 94°C, 1 min at the different annealing temperatures standardized for the individual SSR locus, and extension for 1 min 30 seconds at 72°C. The first cycle was preceded by a 5 min denaturation at 94°C and the last cycle ended with 5 min extension at 72° C. Reaction products were separated on 6% polyacrylamide (denatured) and visualized with silver nitrate staining (Tegelstorm, 1984).The alleles were scored based on the size of each PCR amplified fragment by electrophoresis all samples in a single gel. Allele size were determined by semilog plotting of distance migration of amplicon on PAGE (Hutchinson, 2001). Diversity values based on allele frequencies were calculated for each microsatelite locus and coconut cultivar using Nei's methods (1987).

Table .1 Details of microsatelites primer used in the analysis

| **Primer** | Urutan Basa | |
|---|---|---|
| | **Forward (5'-3')** | **Riverse (5' – 3')** |
| CNZ 05 | CTTATCCAAATCGTCACAGAG | AGGAGAAGCCAGGAAAGATTT |
| CNZ 09 | ATCTACCAGTGTGGTCCTCTC | ACCAGGAAAAAGAGCGGAGAA |
| CNZ 21 | ATGTTTTAGCTTCACCATGAA | TCAAGTTCAAGAAGACCTTTG |
| CNZ51 | CTTTAGGGAAAAAGGACTGAG | ATCCATGAGCTGAGCTTGAAC |
| CnCir A3 | AATCTAAATCTACGAAAGCA | AATAATGTGAAAAAGCAAAG |
| CnCir C3 | AGAAAGCTGAGAGGGAGGATT | GTGGGGCATGAAAAGTAAC |

**Results**

From 58 extracted DNA samples, some sample were successfully amplified on each primer used. For examples locus CNZ09 on figure 1, C and D were not successfully amplified.

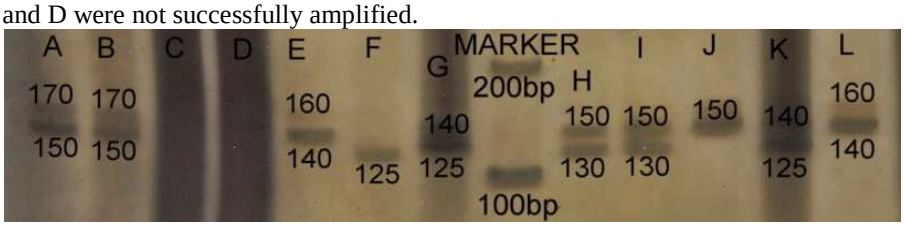


Figure 1. An example of allelic polymorphisme at microsatellite locus CNZ09 in 12 individuals coconut tall from some village in Bali island . A.*Be Julit tall* Babung, B *Be Julit tall* Buruan, C *Be Julit tall* Tuwed D. *Be Julit tall* Tamblang, E.*Biasa tall* Pejeng, F.*Bingin tall* Jelekungkang, G.*Bingin tall* Babung, H. *Biasa tall* Babung, I.*Ancak tall* Pikat, J.*Surya tall* Jelekungkang, K.*Gadang tall* Babung, L.*Rangda tall* Babung

**Genetic Variation (Heterozigosity)**

Total number of investigated alleles, number alleles, heterozygosity, and variation alleles length of twenty tall coconut cultivars for each SSR locus were showed in Table1. The combination of six SSR loci generated a total of 80 alleles, with a mean of 13.33 allele per locus and ranging from nine (CNZ51) to seventeen alleles (CNZ21).

Gene diversity range from 0.8561 for the loci CNZ51 to 0.9204 for the loci CNZ21, with an overall mean 0.8835. The loci CnCirC3 and CNZ05 presented the lowest (0.45) and the highest value (0.7619) respectively, for observed heterozygosity, with a mean value of 0.5421.

 

Table 1. Number of alleles per locus, heterozigosity and variation in allele length of Bali tall coconut, using 6 SSR loci

| Primer | Number of amplified samples | Number of allele | Gene diversity/ Expected Heterozygosity (He±SE) | Observed Heterozygosity (Ho) |
|---|---|---|---|---|
| CnCirA3 | 32 | 12 | 0.8922±0.0001 | 0.4687 |
| CnCirC3 | 40 | 12 | 0.862±0.0005 | 0.45 |
| CNZ05 | 42 | 16 | 0.9093±0.0012 | 0.7619 |
| CNZ09 | 47 | 14 | 0.8612±0.0003 | 0.4893 |
| CNZ21 | 42 | 17 | 0.9204±0.0002 | 0.5714 |
| CNZ51 | 43 | 9 | 0.8561 ±0.0005 | 0.5116 |
| Mean±SD | | 13.333±2.9 | 0.8835±0.027 | 0.5421 |

Heterozygosity among the coconut cultivars was showed in Table 2. The expected heterozygosity range 0.35 ( Bluluk tall) to 0.8166 (Bulan tall), with a mean of 0.5941.

The highest heterozygosity was in Bulan tall (0.776) and the lowest value in Pudak tall and Kapas tall (0.33).

Table 2. Description and heterozigosity of tall coconuts from Bali, Indonesia

| Coconut Name | Description | Expected Heterozigosity | | Observed Heterozigosity | Number of sample |
|---|---|---|---|---|---|
| | | (He) ± SD | | (Ho) | |
| Ancak tall | branched trunk | 0.6458 | 0.2186 | 0.5766 | 3 |
| Bejulit tall | plicated lamina of young leaves | 0.5989 | 0.163 | 0.6833 | 3 |
| Biasa tall | Ordinary tall | 0.4835 | 0.351 | 0.264 | 3 |
| Bingin tall | the root growth from nodes trunk | 0.6331 | 0.259 | 0.6067 | 3 |
| Bojog tall | fruit husk colored like the hair of long tailed monkey | 0.6043 | 0.177 | 0.4716 | 3 |
| Bulan tall | White fruit | 0.8166 | 0.154 | 0.776 | 3 |
| Gadang tall | Green fruit with sweet water | 0.6777 | 0.333 | 0.6067 | 3 |
| Gading tall | Golden yellow fruit | 0.8042 | 0.2106 | 0.526 | 3 |
| Srogsogan tall | Damage endosperm = kopyor | 0.4525 | 0.225 | 0.36 | 3 |
| Mulung tall | Green fruit with red mesocarp | 0.5805 | 0.2061 | 0.608 | 3 |
| Rangda tall | petiole and the apex of the stem were twisted | 0.4199 | 0.363 | 0.358 | 3 |
| Bluluk tall | Inflorescentia spicata | 0.35 | 0.418 | 0.415 | 4 |
| Sudamala tall | Double spatha, reduction of some male spikelet , tip of primordial leaves like hook | 0.6833 | 0.178 | 0.553 | 3 |
| Surya tall | Red fruit and tip lamina (old) | 0.7137 | 0.208 | 0.555 | 3 |
| Udang tall | Brown fruit with red mesocarp | 0.6102 | 0.131 | 0.34 | 3 |
| Kapas tall | Edible white husk (immature) | 0.4166 | 0.52 | 0.333 | 2 |
| Kebo tall | Small nut with thick husk | 0.6498 | 0.392 | 0.5 | 3 |
| Macan tall | Black spot on fruit skin | 0.6748 | 0.186 | 0.416 | 3 |
| Naga tall | Green fruit colour, angled of stem | 0.6833 | 0.2185 | 0.4666 | 3 |
| Pudak tall | Brown fruit colour, spatha>2 | 0.3832 | 0.525 | 0.33 | 2 |
| Mean | | 0.5941 | | 0.4872 | |

 

**Discussion**

The alleles size of several locus from tall coconut that explored during this research differed to that found in other studies of coconut tree population using same SSR markers. Alleles size on locus CnCirA3 range 210-280 bp, with highest frequency on 255bp allele (0,203). Rajesh *et.al*.,(2008) and Ribeiro et al (2010) found 228-240bp alleles sizes. The alleles sizes on the CNZ21 locus that range 180-300bp with highest frequency on 240 bp (0,207), on locus CNZ51 range 110-230 bp with the highest frequency 0,221 on 140 and 160 bp. Pandin et al (2009) found 270bp on loci CNZ21 and 110bp on loci CNZ5 .

Total alleles were found in this research were 80 elleles , with range 9-17 alleles and mean values was 13,3. This result had more variation than others. Perera et al. (2001) found 56 alleles sizes range 3-10 on 33 samples coconut in Srilangka used 8 loci. Thirty seven alleles were found on tall and dwarf from nine population from Agatti and Kavaratti Island, Lakshadweep, India (Devakumar et.al., 2010). Other research that used 8 microatellites loci from 14 accession from Kidu India coconut population, found 28 alleles with mean two alleles per locus (Kumar et al., 2011). Overall, genetic diversity in tall coconuts in Bali was very high (Table 1). Mean values of gene diversity (He) and observed heterozygosity (Ho) were 0.8835 and 0.5421, respectively. The highest expected heterozygosity was on loci CNZ21 (0.9204), and the lowest gene diversity on CNZ51 (0.8561). Genetic variation found in this result higher than others research, e.g research coconut population in Brazil, Phillipina, India, Sri Langka (Perera et al, 2003; Devakumar et al., 2010; Liu, at al.,2011; Xiao, et al., 2013).

Genetic variation on the tall coconut cultivars range value 0.35-0.8166 (Table 2), high expected heterozygosity on bulan tall (0.816), and lowest on bluluk tall (0.35). The estimate of heterozygosity was high (0.590) for Laccadive Micro Tall and Laccadive Small Tall was lowest (0,240) (Devakumar, et.al 2010). Genetic diversity of 10 coconut accessions from six location in Hainan province, China; expected heterozygosity of Haikou Green Tall accession was significantly higher (0.4753) than other tall type (Liu et al., 2011)

Higher heterozygosity was determined by number of alleles variation and frequency. Higher diversity in tall coconuts in Bali, Indonesia was caused by germplasm variation or natural mutation.

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