

# Description of correlation between quantitative and qualitative assays on candlenut DNA

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**Submission date:** 14-Mar-2021 06:14AM (UTC-0700)

**Submission ID:** 1532517838

**File name:** smiaty\_2020\_IOP\_Conf.\_Ser.\_3A\_Earth\_Environ.\_Sci.\_473\_012116.pdf (513.81K)

**Word count:** 3509

**Character count:** 17936

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To cite this article: Gusmiaty *et al* 2020 *IOP Conf. Ser.: Earth Environ. Sci.* **473** 012116

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## Description of correlation between quantitative and qualitative assays on candlenut DNA

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**Abstract.** DNA isolation is a crucial step in the molecular analysis, which influences the quantity and quality of the DNA. The objective of this study was to determine the correlation between DNA quantitative and qualitative assays on Candlenut (*Aleurites moluccana*) DNAs. This study was carried out in February up to April 2019. The research procedures consisted of sample collection, DNA isolation using CTAB method and Qiagen DNeasy Plant Mini Kit (QDPMK kit protocol), DNA quantitative assay using Qubit 3.0 fluorometer, and DNA qualitative assay using horizontal electrophoresis. No positive correlation was detected between DNA quantitative and DNA qualitative assays. The quantitative assay observed that the isolated DNA using QDPMK yielded more DNAs than CTAB ( $<0.2 \text{ ng}/\mu\text{l}$ ). Meanwhile, DNA qualitative assay reported QDPMK had better DNA quality than CTAB

### 1. Introduction

Candlenut, also known as *Aleurites moluccana* is Euphorbiaceae family that can grow at an altitude of 0-1200 m above sea level. This species does not require special requirements because it can grow in fields that are configured flat to bumpy and steep places, on fertile soils to infertile and in areas with dry climates to wet climates [1].

According to Sunanto's study [2], the benefits of candlenut are for cooking spices, industrial raw materials and the timbers are used to make household furniture, firewood, lighters as raw materials and pulp manufacture (paper making materials). This plant is suitable for reforestation, afforestation, and shelter for livestock in grazing areas. In addition, in the West Nusa Tenggara, it is utilized as an Industrial Plantation Forest (HTI). Plantation forests development that can produce good productivity by using quality seeds derived from seed sources from breeding programs such as seed gardens and pruning gardens. For supporting the mentioned plant above, accurate information about genetic diversity that will be chosen as parents is needed.

Genetic diversity knowledge is the basis for developing breeding strategies for a species. Genetic diversity is a very influential factor in developing tree breeding strategies. A molecular marker is one of the methods applied to obtain information about genetic diversity. Genetic investigation of molecular forestry begins with the process of isolation and amplification of DNA. Samples of plant DNA isolation come from young organs such as leaves, buds, flowers, buds, or cambium [3] which are good sources of DNA from plants.



Various analytical techniques in plant breeding and molecular biology-based on molecular hybridization or *Polymerase Chain Reaction* (PCR) require DNA with sufficient quantities and good quality. Because the secondary compounds in plant cells are different, each plant needs optimum isolation procedures to obtain genomic DNA that can be used as molecular analysis material [4].

Previous research about DNA quality assay has been carried out on Redwood (*Pterocarpus indicus* Willd), but the isolation results showed that DNA quality was low so the primer screening process gave suboptimal results and analysis of genetic Redwood diversity that cannot be continued [5][6]. Based on this, the candlenuts analysis of genetic diversity requires preliminary information about the quality and quantity of DNA isolation to minimize failure in the primer screening process and analysis of these plants genetic diversity.

The findings of this study are expected to obtain quality candlenut DNA so that it can contribute to the information development about the quality of DNA, particularly in candlenut plants, which can be applied in genetic diversity analysis. Moreover, it becomes a foundation for biotechnology development and tree breeding to support the sustainable management of plantations.

## 2. Materials and Methods

The tools used for sampling were cutter, pole, and scissors. The molecular analysis used scales, mortars, micropipette, vortex mixers, spatulas, tips, centrifuges, waterbath, freezer, gloves, and masks. Quality testing stages used horizontal electrophoresis, and DNA quantity used a Qubit 3.0 Fluorometric tool.

The material used was leaves from 50 Candlenut trees collected from Rompegading Village, Cenrana District, Maros Regency. Other materials used were 500  $\mu$ l CTAB buffer (100 mMTris-HCL + 1.4 M NaCl + 20 EDTA + 2% CTAB), 800  $\mu$ l Isopropanol, TE 500  $\mu$ l Buffer, 100  $\mu$ l chloroform, Isoamyl-alcohol + Chloroform (24%) : 1) 100  $\mu$ l, ddH<sub>2</sub>O 100  $\mu$ l, Sodium acetate 100  $\mu$ l, isopropanol 800  $\mu$ l, Aquades, Agarose, TAE buffer 1x, Gelred, loading day and Cup, AP1 buffer, RNase, P3 buffer, AW1 solution, AW2 solution, AE buffer, dsDNAqubit BR buffer, standard 1 and standard 2 dsDNAqubit, dsDNA reagent and working solution.

### 2.1. Sampling

Sampling was carried out in Rompegading Village and Limampocoe Village, Cenrana District, Maros Regency. Fifty trees were selected and each of which was taken 5-6 young leaves. Leaves taken samples were inserted into an envelope and coded based on the trees' code. The sample taken was put into a Coolbox with ice gel. Its function is that the leaf samples quality is maintained until DNA analysis in the laboratory.

### 2.2. DNA extraction

DNA extraction or isolation used two methods, namely CTAB (*CetylTrimethyl Ammonium Bromide*) and *Qiagen DNeasy Plant Mini Kit* with several modifications.

2.2.1. CTAB method. Implementation stages were as follows:

- 14 Samples of each candlenut leaf were weighed 0.3 g boneless leaves then crushed to be powder.
- After being powder, add 500  $\mu$ l of CTAB extraction buffer (100 mMTrisHcl pH 8.0; 20 Nm EDTA (*Ethylene Diamin Tetra Acetic Acid*), 2% CTAB; 1.4 M. NaCL) and vortexed for 15 seconds.
- The next process is the cell wall lysis process in the sample, which was done by incubating a tube containing leaf samples into a water bath at 65°C for 90 minutes.
- Each incubated sample was added to isoamyl alcohol: chloroform (24: 1) 100  $\mu$ l and mixed slowly then centrifuged at 10,000 rpm for 5 minutes.
- Supernatant or clear liquid which was above the centrifuged sample was moved into a new tube and added 800  $\mu$ l isopropanol

- f. The solution was then centrifuged at 10,000 rpm speed for 10 minutes, and the DNA precipitate was dried overnight
- g. The obtained DNA was purified by adding 500  $\mu$ l TE 1x buffer (10mM Tris-HCl pH 7.5 Mm EDTA), then centrifuged for 10 minutes at 10,000 rpm.
- h. The supernatant was transferred to a new 2 ml tube and 100  $\mu$ l chloroform was added.
- i. The supernatant solution was then centrifuged at 10,000 rpm for 10 minutes. The supernatant was taken and then added 100  $\mu$ l of sodium acetate 3 M and 800  $\mu$ l of isopropanol, then centrifuged for 10 minutes with 10,000 rpm.
- j. The precipitate was taken and dried overnight and then was added 100  $\mu$ l ddH<sub>2</sub>O and stored in a freezer with -20°C temperature.

2.2.2. *QDPMK method.* The implementation stages were as follows:

- a. Candlenut leaves were crushed then weighed for 0.1 grams.
- b. It was added 400  $\mu$ l AP1 buffer to each sample and then added 4  $\mu$ l of RNase.
- c. Then vortex and spindown until well mixed.
- d. It was incubated in a water bath at 65°C (every 10 minutes revortex-ed) for 60 minutes
- e. It then added P3 130  $\mu$ l and then vortex-ed for 60 seconds.
- f. It was incubated in the freezer for 45 minutes. After that, the solution was centrifuged for 5 minutes at 14,000 rpm (centrifuge was carried out twice).
- g. The supernatant was pipetted and transferred to a mini spin column (purple) in a 2 ml tube then centrifuged for 2 minutes at 14,000rpm. It was pipetted until 300 to 500  $\mu$ l of the solution.
- h. The column used was discarded and the upper fluid was taken.
- i. The solution was moved to a clear tube (2 ml tube) then an AW1 buffer was added with a volume of 1.5 x supernatant volume if the supernatant volume is 500  $\mu$ l, the buffer was added 750  $\mu$ l, then mixed-inverting.
- j. The solution was transferred to a mini spin column (filtered white tube), 650  $\mu$ l became the maximum number, if the samples less than 650  $\mu$ l, all solutions were taken, if more than 650  $\mu$ l, centrifuged twice. The centrifuge was carried out for 1 minute at 8,000 rpm speed.
- k. Colum was transferred to a new tube without a lid, then added 500 ml AW2. Then centrifuged for 1 minute at 8,000 rpm (rinse 1).
- l. After solution centrifuged was removed then added another 500 ml AW2 (rinse 2). It was centrifuged for 2 minutes at 14,000 rpm.
- m. The column membrane was transferred to a 2 ml tube and then 100 ml AE buffer was added (which has been heated in boiling water) then incubated at room temperature for 15 minutes and in the centrifuge for 1 minute with 8,000 rpm.
- n. Discard the membrane column and then the DNA template was stored in a freezer at -20°C.

2.2.3. *DNA quantity assay.* DNA Quantity assay used the Qubit 3.0 Fluorometer (Thermo Fisher Scientific) tool. This test uses DNA BR buffer, Qubit standard 1 and standard 2 dsDNAqubit, dsDNA reagent, and working solution.

- a. A working solution was made by mixing the dsDNA reagent and the dsDNA BR Buffer (1: 200).
- b. dsDNA standard 1 was made by mixing 10  $\mu$ l standard 1 and 190  $\mu$ l working solution.
- c. dsDNA standard 2 was made by mixing 10  $\mu$ l standard 2 and 190  $\mu$ l working solution.
- d. The sample solution preparation was done by mixing 1  $\mu$ l of DNA and 199  $\mu$ l of working solution.
- e. Each standard solution 1, standard 2, and the sample were put into a Qubit™ assay tube.
- f. The entire tube was vortex-d for 2-3 seconds
- g. Incubate the tube for 2 minutes at room temperature in the dark condition
- h. Put the tube in the fluorometer qubit and the analysis was done.

2.2.4. *DNA quality assay.* The next step is to conduct DNA quality assay which was the DNA isolation advanced stage. At this stage provides information about the DNA master that can show the DNA quality with 0.8% agarose concentration using horizontal electrophoresis, the steps were as follows:

- a. Agarose was weighed 1.4 grams and added 180 ml of 1xTAEbuffer.
- b. The solution was heated by using a Microwave for 6 minutes.
- c. After agarose dissolved, it was added 1.5  $\mu$ l geared and then let it warm.
- d. The solution was poured into a mold and given a comb then let it harden.
- e. The comb was then removed so that it was inserted into a 1x TAE Buffer solution filled the tank.
- f. DNA samples of 2  $\mu$ l + 1  $\mu$ l loading dye were put into the well. The end well contained a ladder.
- g. Electrophoresis was carried out for 90 minutes at 100 volts.
- h. The documentation was done in *Geldoc*.

### 3. Results and Discussion

#### 3.1. DNA quantitative assay

The DNA quantity is tested by looking at its concentration. This test was carried out using a Qubit 3.0 fluorometer. Quantity assay was done by comparing the concentration of DNA isolation results using the CTAB (*Cetyl Trimethyl Ammonium Bromide*) method and the QDPMK (*Qiagen DNeasy Plant Mini Kit*) method with several modifications. Based on the measurements, the concentrations obtained were very different between the two methods. Comparison of concentration values is depicted in Figure 1.



**Figure 1.** DNA concentration measurements based on (a) CTAB method, (b) QDPMK method

The test results above show differences in concentration using CTAB and QDPMK. Figure 1 shows that the CTAB method produced a low DNA concentration, it indicated that the concentration produced below 0.2 ng /  $\mu$ l, while the QDPMK method showed that the DNA concentration was very high at 366 ng /  $\mu$ l. Low concentrations (under 0.2 ng/ $\mu$ l) could not be diluted in the subsequent DNA amplification process [7].

Based on these results, no positive correlation between the quality assay and quantity assay. Figure 7 presents the quality assay of the QDPMK method of the sample that the K7 sample had the highest DNA concentration which was 404 ng/ $\mu$ l, while the K25 had the lowest concentration sample which was 6.68 ng/ $\mu$ l. Based on the quality of the bands producing from DNA amplification, K6 sample which had a concentration of 147.6 ng /  $\mu$ l had a thicker band quality compared to K7, even though it was smear but it appeared that the DNA that can be observed visually. This was contrary to Haris *et al* (2003) who stated that DNA concentration has an impact on the quality of bands producing from amplification [8]. Too low DNA concentrations produce bands that are very thin on the gel or even unseen visually, on the

contrary, too high DNA concentrations will produce thick bands so it is difficult to distinguish between one band and others.

The low concentration of DNA extraction results from the CTAB method can be influenced by two factors: the speed of extraction at the extraction process and the composition of the addition of buffer lysis. The extraction speed factor is the most influential factor because at the stage of cell lysis and precipitation supernatant must be done per sample so that some samples occur DNA deposition [9].

### 3.2. DNA qualitative assay

DNA extraction or isolation becomes the first step in the molecular analysis process. DNA isolation is very influential in providing good quality to plant DNA. It was carried out using two methods or protocols, namely the CTAB (*Cetyl Trimethyl Ammonium Bromide*) method and the QDPMK (*Qiagen DNeasy Plant Mini Kit*) method with several modifications. DNA isolation was done by grinding the young leaves of candlenut then weighing 0.1 grams. According to Wulansari (2014), young leaves contain much DNA because this part is actively carrying out the process of cell division and growth [10]. Young leaves also make it easier to grind because young leaves have a softer texture.

Optimization in DNA isolation is really needed in this regard. Total DNA isolated was tested qualitatively to see that the DNA did not experience contamination with other secondary metabolite compounds. Problems often arise in the DNA extraction process, namely the presence of contaminants in isolated samples such as polysaccharides, polyphenols, proteins, RNA, and secondary metabolites [11]. In the extraction process, DNA is often broken, DNA is degraded due to the presence of nuclease enzymes, contamination of polysaccharide compounds and the isolation of secondary metabolites are problems that often occur when extracting DNA [12]. Meanwhile, which extraction DNA can be inhibited by various processes ranging from DNA cutting, amplification to cloning, namely the contaminant compounds presence [13].

The DNA isolation results were visualized on 0.8% agarose gel electrophoresis. Agarose is colored with gelred.  $2 \mu\text{l} + 1 \mu\text{l}$  loading dye DNA samples were put into the well. The end well contains a ladder. Electrophoresis was run at 100 V for 90 minutes. The electrophoresis gel is inserted into the UV Transilluminator to see whether or not the DNA bands are presented [14].

The quality result of DNA from electrophoresis by two methods, namely the CTAB method and the QDPMK method with some modifications, is shown in Figure 2 and Figure 3. In Figure 2, the results showed unfavorable results. The absence of DNA bands that appear allegedly because the total amount of DNA extracted is quite low and degraded. Optimization of DNA isolation using the CTAB method is required at each stage of the process of DNA isolation, namely the stages of cell lysis, DNA extraction, and DNA purification. The optimal DNA isolation process will get DNA in sufficient quantity and quality, thus it can be used for a variety of molecular analyses[15].



**Figure 2.** Electrophoregram, DNA quality assay results by using the candlenut CTAB method  
Note: M = Markers and 1-25 = DNA



**Figure 3.** Electrophoregram, DNA quality assay results by using the QDPMK method

Note: M = Markers and 1-25 = DNA

Total DNA electrophoresis of candlenut plant samples showed very different results between the CTAB method and the QDPMK method. The figure showed that the CTAB method did not produce any amplified DNA that was caused by several factors. In a DNA isolation technique, there was still needed for steps to minimize the contaminant compounds that can interfere with PCR reactions such as polysaccharides and secondary metabolites [16]. This is due to the polysaccharides and secondary metabolites present in plants often making it difficult to isolate the origin of nucleic.

Unlike with the QDPMK method where the electrophoregram showed that DNA isolation had been successful, it produced DNA having bright and thick bands that could be observed on sample #1, #2, #3, #4, #5, #6, #7, #9, #10, #11, #12, #13, #14, #15, #16, #17, #18, #19, and #24. Whereas the thin and underexposed bands were found in sample #8, #20, #21, #22, #23, and #25.

#### 4. Conclusion

The quality and quantity assays showed no positive correlation between quality and quantity assays. Quantity assay detected that samples extracted using the QDPMK method have higher concentrations than the CTAB method which had concentrations under  $0.2 \text{ ng}/\mu\text{l}$ . DNA quality assay observed that the success of the QDPMK modification method had better DNA quality compared to the CTAB method which had not been modified.

#### Acknowledgement

The author would like to thank Universitas Hasanuddin who has funded this research through Hibah Internal Unhas Tahun 2019 Penelitian Dosen Penasehat Akademik (PDPA) Scheme.

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