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Isolation and Purity DNA from Leaf Storage of *Lansium domesticum* Correspond for Barcoding Analysis

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Abstract

Lansium domesticum is one of tropical plant. Genetic studies of tropical plant species occurred very slowly due to some obstacles. Provided fresh sample for isolation step was one of impediment. Fresh sample was important conditions to avoid impure DNA template. A simple and rapid technique was needed to get a pure DNA from storage sample. The aims of this study is to see the purity of the DNA isolation results from Lansium domesti-cum mature leaf from South Sumatra that have been stored for 2 years and to test whether the DNA isolation results can be used for barcoding analysis by amplification using PCR method with rbcL primer. There are 11 sample of Lansium domesticum were taken from 8 districts in South Sumatra. The sample was the mature leaves of Lanisum domesticum. The leaves were stored in a freezer at -20oC for two years. There was one sam-ple stored within 2 weeks as a comparison. The results of the study showed the isolation of DNA from storage and mature leaves of Lansium domesticum used Plant Genomic DNA Kit DP305 had a good quantity for 7 sample. 3 sample had low purity and 1 sample contaminated by RNA. Despite electrophoresis showed the isolation result not intact and compact, nevertheless the PCR results showed 11 samples could be amplified using rbcL primer. This result indicated the DNA isolation method suit for barcoding analysis using PCR method.

Keywords: DNA Isolation, DNA Purity, DNA Barcoding, Lansium domesticum.

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1. Introduction

Lansium domesticum is a tropical plant that lives in lowland forests with temperature 22-35ºC and well distributed in the areas with rainfall around 2000-3000 mm. This plant originated from Southeast Asia, peninsula of Thailand, Malaysia, Indonesia, to Lazon's island in the Philippines. In Indonesia this plant also known as duku, pisitan, kokosan and langsat [1].

In Indonesia, the most famous local cultivar of *L. domesticum* is duku Palembang, South Sumatra province [2]. There are two variants of *L. domesticum* in South Sumatera, such as Duku and Langsat [3].

Genetic studies of tropical forest plant species occurred very slowly, this was due to some obstacles [4] Perennial crops from tropical forest, contain of high concentration of polysaccharide and polyphenol which will interfere enzymes activities in subsequent molecular analysis [5].

Isolation of DNA from plant usually can disrupt by contamination of secondary metabolites. Therefore, DNA isolation methods need to be adapted to each plant species and even to each plant tissue due to differences in the composition of these metabolites [6]. DNA extraction is a crucial step in genetic study of plant [7]. Plants are the primary source of human food and animal feed and also form the basis of numerous industrial and pharmaceutical products [8].

The purity of DNA Isolation was pure, intact and had high DNA quality were a basic rule that must be met in molecular analysis [4]. Several techniques and procedures had been published, but often they cannot be applied because the genus or even plant species is very specific [9].

The aims of this study to see the purity of the DNA isolation results from *L. domesticum* mature leave from South Sumatra that have been stored for 2 years and to test whether the DNA isolation results can be used for barcoding analysis by amplification using PCR method with rbcL primers.

2. Materials and Methods

Materials

The tools used in this research are gloves, aluminum foil, clear plastic, freezer -20^{o}C, hot plate, magnetic stirrer, erlenmeyer flask, freezer, water bath, micropipette, white microtip, yellow microtip, blue microtip, 0.2ml microtube, microtube 2ml, mortar, vortex, centrifuge, parafilm, electrophoresis, nanodrop thermo fisher, thermal cycler and UV-transimulator.

The equipment needed are *L. domesticum* leaves, Plant Genomic DNA Kit DP305, cloroform, 1% agarose, 1,8% Agaros, 1x TAE buffer, gel red, 6x loading dye (Tiagen), 2x My Taq HS Red Mix (Bioline Meridian Bioscience), Sizer 100 DNA marker, Lambda DNA/HindIII marker, ddH2O and primer.

Method

Sample Collection

The samples were taken from 8 districts in South Sumatra (Table 1). The mature leaves were taken using scissors. Leaves were stored in aluminum foil in dry condition and covered with plastic to make it airtight. Then the samples were stored in a freezer at -20^{o}C for two years. There was one sample stored within 2 weeks as a comparison, namely Getapan from Karang Agung, Lahat, South Sumatera (Table 1).

DNA Isolation

DNA Isolation was carried by using Plant Genomic DNA Kit DP305. Weighed 0.1 mg leaves, remove the bone leaves and mashed on ice using mortar. 700 µl of buffer GP1 at 65^{o}C was added to the tube containing the plant tissue and vortex for 10-20 seconds and incubated at 65^{o}C for 20 minutes. Mix by inverting the tube. 700 µl of chloroform was added and homogenized by inverting and centrifuged for 5 minutes with 12,000 rpm. The supernatant was taken into a new tube and added 700 µl of buffer GP2, homogenized by inverting. All solutions were transferred to the spin column CB3, then centrifuged for 30 seconds at 12,000 rpm.

The filtrate was discarded and added 500 µl of GD buffer, centrifuged at 12,000 rpm for 30 seconds. Then the filtrate was discarded and added 600 µl of PW buffer, centrifuged for 30 seconds at 12,000 rpm. Discarded the filtrate and the steps for adding the PW buffer was repeated. The spin column CB3 was transferred to a collection tube and centrifuged for 2 minutes at 12,000 rpm and the filtrate was discarded. Open the lid of spin column CB3 to dry and placed into a sterile tube. After that 150µl of TE buffer was added and incubated for 5 minutes at room temperature and last, centrifuged for 2 minutes at 12,000 rpm to elute the DNA.

Table 1. List of *L. domesticum* sample from South Sumatera

Code	Sample Name	Sample Origin
DB	Duku Baturaja	Baturaja, Ogan Komering Ulu (OKU)
DM	Duku Martapura	Martapura, Ogan Komering Ulu Timur (OKUT)
DP	Duku Pali	Penukal Abab Lematang Ilir (PALI)
DOKI	Duku OKI	Ogan Komering Ilir (OKI)
LEL	Langsat Empat Lawang	Tebing Tinggi, Empat Lawang
LL	Langsat Lahat	Pagar Gunung, Lahat
DL	Duku Lahat	Lahat
LM	Langsat Muaradua	Muaradua, Ogan Komering Ulu Selatan (OKUS)
DMD	Duku Muaradua	Muaradua, Ogan Komering Ulu Selatan (OKUS)
L	Duku Linggau	Lubuk Linggau, Musi Rawas
G	Getapan	Muara Payang, Lahat

Quantity and Quality of DNA

The quantity and quality of DNA were checked by using nanodrop thermo fisher and electrophoresis. Quantitative DNA measurement was calculated using nanodrop thermo fisher by adding 3 µl of DNA template on the sensor. The purity of the DNA solution

can be calculated using a nanodrop thermo fisher by comparing the wavelength of A260 nm with A280 nm. Pure DNA has a ratio of A260/A280 at 1.8 to 2 [10].

Measurement of DNA quality was carried out using gel electrophoresis using 1% agarose for isolation results and 1.8% agarose for PCR result [11].

DNA Amplification

The primers used in this process were rbcL f (ATGTCACCACAAACAGAGACTAAATG) and primer rbcL r (GTAAAATCAAGTCCACCRCG) [12]. The amplification process was carried out using thermal cycler with a total volume of 25 µl, consisting of: 2x My Taq HS Red Mix (Bioline Meridian Bioscience) 12.5 µl, ddH2O 5.5 µl, DNA template 5 µl and primer 2 µl. The PCR reaction used 35 cycles, with pre-denaturation 95^{o}C for 4 minutes, denaturation 94^{o}C 30 seconds, annealing 58^{o}C 45 seconds, extension 72^{o}C 1 minutes, final extension 72^{o}C 5 minutes and incubation 12^{o}C 13].

3. Results and Discussion

Based on the quantity and quality calculation data using a nanodrop thermo fisher, A260/A280 ratio of DNA isolation ranged from 1.73 to 2.28 (Table 2). Duku Linggau, Langsat Empat Lawang and Langsat Lahat have value less than 1.8. The low ratio of A260/A280 can be caused by contamination of polysaccharide, protein and some organic molecules. The DNA template with A260/A280 ratio below 1.4 indicated, there excess contamination from salt and also contamination from 70% ethanol during DNA washing in the isolation stage [14].

The nucleic acid has A260/A280 ratio around 1.8-2.2 [15]. Pure DNA has A260/A280 ratio around 1.8-2 [14]. Duku Batu Raja, Duku Martapura, Duku Pali, Duku OKI, Langsat Muaradua, Duku Muaradua and Getapan have good DNA purity.

The results of DNA isolation from Duku Lahat showed A260/A280 2.28. This indicated Duku Lahat sample had unpurified DNA. The RNA has A260/A280 ratio more than 2. Therefore, if A260/A280 ratio value is greater than 2, indicating contamination from RNA [10].

The concentration of the isolated DNA ranged from 6.6 ng/µl-65.7 ng/µl. The concentration of DNA isolation from Duku Lahat itself has a very low concentration value of 6.6. This low concentration indicated low DNA content in the isolation results [16]. The used of DP305 kit for extraction of DNA from raw soybean seed showed the lowest concentration than Plant Mini Kit, CTAB with modification and SDS with modification [17]

Table 2: Concentration and purity of DNA Isolation Result

Sample Name	DNA Concentration (ng/µl)	A260/A280
Duku Baturaja	39.2	1.87
Duku Martapura	58.7	1.91
Duku Pali	48.2	1.87
Duku OKI	65.7	1.9
Langsat Empat Lawang	48.2	1.78
Langsat Lahat	50.6	1.78
Duku Lahat	6.6	2.28
Langsat Muaradua	64.3	1.9
Duku Muaradua	59.9	1.83
Duku Linggau	32.1	1.73
Getapan	64.7	1.89

The time storage of leaves for two years or two weeks did not affect the quantity and quality of the DNA, as seen from the concentration, purity (Table 2) and electrophoresis (Figure 1) of Getapan which was only stored for two weeks not different from other samples that have been kept for two years. The used of different organ of plant would give different DNA isolations result [18]. DNA isolation from leaves section can produced purer DNA template than flower section [19]. Besides the quantity and quality of DNA isolation result also influenced by the age of the leaves, the use of young leaves has better isolation results compared to mature leaves since of the increased concentration of secondary metabolites in mature leaves such as tannins and phenols which become contaminated in DNA isolation [20].

Based on the results of visualization using electrophoresis (Figure 1), there is no DNA visualization from Duku Lahat, This due to the low concentration and unpurified of sample. In addition, other samples did not contain intact DNA bands, although they had a fairly high concentration and good purity. The used

1%-1.5% agar during electrophoresis, the isolated genome DNA will show a compact DNA band, high-molecular-weight band with no low-molecular-weight smears. Emersion of elongated smear with high proportion of material position towards the bottom of the gel indicated a degradation of DNA [21].

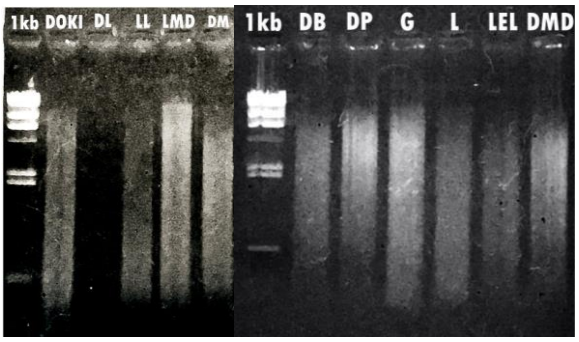

Figure 1: DNA band of DNA isolation result from *L. domesticum*

Despite the DNA isolation results showed incomplete electrophoresis, the PCR results showed 11 samples could be amplified using rbcL primers (Figure 2). According to the past research, the results of DNA isolation using the CTAB method with incomplete results can be applied to the PCR-RAPD method on *Manilkara zapota* (L) van Royen [22] and *Durio kutejensus* Becc [23]. The size of rbcL gene in *L. domesticum* was 600bp. The same size also found at *Dendrobium discolor* Lindl. [24] and several plant from Depterocarpaceae family [13].

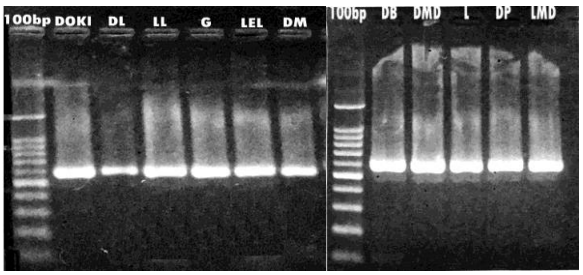

Figure 2: amplification result using rbcL primer

Duku Linggau which has a very low concentration 6.6 ng/µl with A260/A280 ratio 2.28 and no DNA band formed on electrophoresis showed a fairly bright DNA band in the amplification result. According to the past research, the small DNA concentration 1.8 ng/µl still be applied in DNA amplification in stored wood of *Falcataria moluccana* using the psbA-trnH intergenic spacer primer [25].

Modification conventional method CTAB produced DNA template that can be amplified used SSR and ISSR marker [26] [27] and also suitable for a range of high-throughput molecular assays such as SNP genotyping [28]. Moreover, the used of KIT isolation with modification was recommend due to the same quality of DNA isolation result compared with conventional technique [29]. Nevertheless, DNA isolation using the Wizard Genomic DNA Purification System Kit from Promega method has higher DNA quality than CTAB method in rice leave [30]. The used of Plant Genomic DNA Kit DP305 for DNA isolation from mature leaves of *L. domesticum* correspond for barcoding analysis using PCR method.

4. Conclusion

The isolation of DNA from storage and mature leaves of *L. domesticum* used Plant Genomic DNA Kit DP305 had a good quantity for 7 sample. 3 sample had low purity and 1 sample contaminated by RNA. Therefor this DNA isolation method suit for barcoding analysis using PCR method.

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