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Comparative analysis of DNA extracted from mature leaves of rubber tree and application for seventeen tropical plant species for PCR amplification

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Abstract. Rubber tree is the main source of natural rubber and is the most important economic member of the Hevea genus. Polysaccharides and polyphenols in mature leaf can reduce the success of DNA extraction and downstream applications. This makes the isolation of high quality and molecular weight DNA from mature leaves of rubber tree challenging. The DNA yield and purity obtained using eight methods involving use of cetyltrimethylammonium bromide, SDS, and two commercial kits were compared. The modified procedure from Moreira & Oliveira (2011) consistently yields approximately 34 µg of high-quality amplifiable DNA with as little as 0.05 g fresh weight of mature leaf tissue of rubber tree. The key changes in the procedure were (1) a short grinding of leaf powder in mortar with extraction buffer after blending the leaf tissue in the presence of liquid nitrogen; (2) the optimization of the ratio of tissue (weight) to buffer (volume); (3) CTAB was added only once and (4) reduction in the incubation time of the macerated tissue in an extraction buffer including RNase A at 65 °C for 15 minutes. The procedure was also applicable to seventeen other tropical plant species. This helped to avoid the limitation of plant materials and could provide total DNA for further molecular studies.
Key Words: DNA extraction, mature leaf, Hevea genus, 18S rRNA, *trnL-F*, ISSR, RAPD.

Introduction. Rubber tree (*Hevea brasiliensis* [Willd. ex A. Juss.] Müll. Arg.) is the most important economic crop, timber and source of natural rubber production. It is a perennial and economic viability in the 30 – 35 years (Chudnoff 1980). The demand for natural and synthetic rubber has increased steadily over the past century (Rahman et al 2013). In Thailand, a total area of 7,299,815 acres of rubber plantations was in 2011 and production of the country reached 3,778,010 tons in 2012 (Rubber Research Institute of Thailand 2013) as a result of promotion by the Government of Thailand and the steadily rise of the rubber price.

Rubber tree genome research has attracted much attention recently (Rahman et al 2013; Mantello et al 2012; Pootakham et al 2012). Genetic diversity is not the only basis for conservation but also breeding and commercial production (Souza et al 2009). Quality of DNA affects the success of modern genotyping platforms (Bayes & Gut 2011). Polyphenols and polysaccharides in plant tissues can interfere DNA amplification reactions (Khan et al 2004). Many protocols for extracting DNA from plant species diversity has been published (Osmundson et al 2013), but none is effective universally (Varma & Padh 2007). Plant secondary compounds can interfere with a variety of methods to extract DNA (Doyle & Doyle 1987). DNA extracted by a modified method of Doyle & Doyle (1987) (Gouvêa et al 2010; Mantello et al 2012; Souza et al 2009) and the commercial kit Qiagen (Pootakham et al 2012) have been used in genetic studies of rubber trees. A few effective DNA extraction protocols developed previously for rubber (An et al 2012; Huang et al 2013), but the materials used to extract DNA are soft and tenderness (Mantello et al 2012; Rahman et al 2013).

On the availability of young leaves and expansion has become necessary to limit the time of collection. Therefore, the comparison between different modified protocols and modifications can lead to the ideal method suitable for the isolation of high quality and high molecular weight DNA from mature leaves of the rubber tree.

Our main objectives were to conduct a comparative evaluation of eight methods to extract DNA from mature leaf of rubber tree to get the most suitable protocol to obtain high yield and purity of DNA from mature leaf tissue of rubber tree for molecular application.

Material and Method

Plant Materials. The bulk of the mature leaf tissue collected from a 26-year-old single individual rubber tree grown in an outdoor field at the Department of Biology, Faculty of Science, Khon Kaen University was used for comparison. Mature leaves sampled from nine accessions of rubber tree from Sakon Nakhon, Loei and Khon Kaen provinces and seventeen species of tropical plant plants from Khon Kaen province were also conducted to confirm the effectiveness of the protocol.

DNA extraction from rubber tree. The genomic DNA was isolated following eight extraction methods described below. Each procedure was done with five independent replications. Mature leaf tissue (50 mg) was ground with mortar and pestle in the presence of liquid nitrogen for each replication.

Method 1 modified from Doyle & Doyle (1987). Protocol:

1. Add 700 µL extraction buffer (2% w/v CTAB [Hexadecyltrimethyl-ammonium bromide], 100 mM Tris-HCl pH 8.0, 50 mM EDTA pH 8.0, 0.5 M NaCl) to the fine powder before some more grinding.
2. Transfer the CTAB/plant extract mixture to a microcentrifuge and add 5 µL (10 mg/mL) RNaseA and mix.
3. Incubate at 65 ºC for 15 min. Add 600 µL of chloroform:isoamyl alcohol (24:1) into the mixture.
4. Centrifuge the sample at 8,800 rpm in a microfuge for 3 min. Transfer the supernatant to a clean microcentrifuge, and re-purify with the chloroform:isoamyl alcohol again.
5. Recover the DNA by adding 500 µL of isopropanol. Mix thoroughly and recover the DNA pellet by centrifugation at 10,000 rpm for 3 min. Rinse with 70 % ethanol and re-suspended in 150 µL of TE buffer, and store the DNA solution at -20 ºC.

Method 2 modified from Porebski et al (1997). Protocol:

1. Add 700 µL extraction buffer (100 mM Tris-HCl pH 8.0, 1.4 M NaCl, 20 mM EDTA pH 8.0, 2 % w/v CTAB, 0.05 % w/v Polyvinylpyrrolidone [PVP]) to the fine powder before some more grinding, and transferred to a microcentrifuge.
2. Add 1.4 µL (0.2 %) β-mercaptoetanol and 5 µL (10 mg/mL) RNaseA, mix and incubate at 65 ºC for 15 min.
3. Remove the protein contaminant by adding 500 µL of chloroform:isoamyl alcohol (24:1), mix well, and centrifuge at 8,800 rpm for 3 min. Transfer the supernatant to a new tube.
4. Recover DNA by adding 250 µL of 5 M NaCl and 2 volumes of ice-cold ethanol. Centrifuge at 10,000 rpm for 3 min. Rinse the pellet with 70 % ethanol and re-suspend in 150 µL of TE. Store the DNA at -20 ºC.

Method 3 modified from Štorchová et al (2000). This protocol was modified from Štorchová et al (2000), with the addition of manitol instead of sorbitol in the extraction buffer.

1. Add 700 µL of extraction buffer (100 mM Tris-HCl pH 7.5, 50 mM EDTA pH 8.0, 0.35 M mannitol and 0.2% β-mercaptoethanol (just before use) to the fine powder before some more grinding.

2. Transfer the suspension to a microcentrifuge, and incubate at room temperature for 15 min. Centrifuge at 10,000 rpm for 3 min.
3. Pour off the supernatant. Re-suspend the pellet in 300 µL lysis buffer (200 mM Tris-HCl pH 7.5, 50 mM EDTA pH 8.0, 2 M NaCl, 2 % w/v CTAB). Add 5 µL RNaseA (10 mg/mL) before incubation at 65 ºC for 15 min.
4. Add 500 µL of chloroform:isoamyl alcohol (24:1) to the mixture and mix. Centrifuge at 8,800 rpm for 3 min and transfer the supernatant to a new microfuge tube.
5. Add 500 µL of isopropanol, mix and centrifuge at 10,000 rpm for 3 min to precipitate the DNA precipitation. Rinse the pellet with 70 % ethanol and then re-suspend in 150 µL of TE. Store the DNA solution at -20 ºC.

Method 4 modified from Novaes et al (2009). Protocol:

1. Add 700 µL extraction buffer (100 mM Tris-HCl, pH 8.0, 20 mM EDTA, pH 8.0, 2% (w/v) CTAB, 1.4 M NaCl, 2 % PVP) to the fine powder before some more grinding, and transfer to a microcentrifuge.
2. Add 1.4 µL (0.2 %) β-mercaptoetanol and 5 µL RNaseA (10 mg/mL) and mix until getting a homogeneous mixture.
3. Add 35 µL of 20 % SDS, mix well and incubate at 65 °C for 15 min with occasional swirling.
4. Add 600 µL chloroform:isoamyl alcohol (24:1) to the tube and inverse gently before centrifuge at 8,800 rpm for 3 min in a microcentrifuge.
5. Transfer the supernatant carefully to new tubes. Add 140 µL 10 % (w/v) CTAB and 280 µL 5 M NaCl, mix well and then incubate at 65 °C for 5 min.
6. Repeat Step CIA.
7. Precipitate the DNA by adding a 0.67 volume of isopropanol, mix well, and centrifuge at 12,000 rpm for 3 min.
8. Pour off the supernatant, and rinse the pellet with 70 % ethanol. Add 150 µL TE, and store the DNA solution at -20 °C.

Method 5 modified from Moreira & Oliveira (2011). Protocol:

1. Add 700 µL extraction buffer (100 mM Tris-HCl, pH 8.0, 20 mM EDTA, pH 8.0, 2.8 % (w/v) CTAB, 1.3 M NaCl and 1 % PVP), and grind until getting suspension. Transfer to a micro centrifuge.
2. Add 1.4 µL (0.2 %) β-mercaptoetanol and 5 µL RNaseA (10 mg/mL), mix and incubate each sample at 65 °C for 15 min with occasional swirling.
3. Add 500 µL chloroform:isoamyl alcohol (24:1) to the tube and homogenize by gentle inversion. Centrifuge samples at 8,800 rpm for 3 min in a micro centrifuge at room temperature.
4. Transfer the supernatant carefully to new tubes, and incubate at 65 °C for 5 min.
5. Repeat Step CIA.
6. Precipitate the DNA by adding a 0.7 volume of isopropanol, mix well, and centrifuge at 12,000 rpm for 3 min. Pour of the supernatant and rinse the pellet with 70 % ethanol.
7. Add 150 µL TE. Store the DNA solution at -20 °C.

Method 6 modified from Tai & Tanksley (1990). Protocol:

1. Add 700 µL extraction buffer (100 mM Tris-HCl pH 8.0, 50 mM EDTA pH 8.0, 0.5 M NaCl, 1.25 % SDS, 8.3 mM NaOH, and 0.38 % Na bisulfate) before some more grinding, and transfer to a micro centrifuge.
2. Add 1.4 µL (0.2 %) β-mercaptoetanol and 5 µL RNaseA (10 mg/mL), mixed until getting a homogeneous mixture. Incubate at 65 ºC for 15 min and add 0.22 ml of 5 M potassium acetate, and mix. Place the tube at -20 ºC for 10 min, and then centrifuge at 10,000 rpm for 3 min in a micro centrifuge.
3. Transfer the supernatant to a new tube. Precipitate the DNA by adding a 0.7 volume of isopropanol, mix well, and centrifuge at 10,000 rpm for 3 min.

4. Pour of the supernatant and rinse the pellet with 70 % ethanol. Resuspend the pellet in 300 µL of T5E (50 mM Tris-HCl pH 8.0, 10 mM EDTA) by briefly vortexing, incubating at 65 ºC for 5 min, and re-vortexing.
5. Add 150 µL of 7.4 M ammonium acetate and mix well before centrifugation for 3 min and removal of the supernatant to a new tube.
6. Precipitate the DNA by mixing with 330 µL of isopropanol and centrifuge for 3 min.
7. Rinse the pellet with 70 % ethanol and re-suspend in 100 µL of T5E by vortexing, incubating at 65 ºC for 5 min, and re-vortexing.
8. Add 10 µL of 3 M sodium acetate and 75 µL of isopropanol, mix well followed by centrifugation for 3 min to re-precipitate the DNA.
9. Wash the pellet with 70 % ethanol, and add 150 µL of TE to dissolve the DNA. Store the DNA solution at -20 ºC.

Method 7 based on DNeasy Plant Mini Kit (Qiagen, Germany). Protocol: the DNA was extracted as per manufacturer's instructions (Qiagen, Germany), except for the increase of AE buffer to 150 µL per elution.

Method 8 based on E.N.Z.A. Plant DNA Kit (Omega Bio-tek, USA). Protocol: the DNA was extracted as per manufacturer's instructions (Omega Bio-tek, USA), except for the increase of E buffer to 150 µL per elution.

DNA extraction from mature leaves of other plant species. DNA extraction from mature leaves of seventeen other plant species was done according to method 5 which proved to be the most efficient method selected from the methods described above. After the DNA precipitation with isopropanol, the pellet was dissolved in 150 µL of TE.

DNA evaluation. The analysis of DNA concentration and quality were based on the 260 nm/280 nm and 260/230 absorbance ratios using the spectrophotometer NanoDrop™ (Thermo Scientific) according to manufacturer's instructions. The yield of total DNA extracted from 50 mg fresh weight was reported. The variation in the efficiency of DNA extractions was analyzed using Statistix 8 (Analytical software 2003). Genomic DNA integrity was evaluated from the bands from 5 µL of total DNA on 1.5 % agarose gel electrophoresis.

PCR amplification. The quality of extracted DNA was also assessed with the ISSR, RAPD markers, *trnL-F* and 18S rRNA synthesized by Bio Basic Inc. (Canada) (Table 1).

Table 1

List of primers used to amplify DNA

Primer name	*Sequence (5' - 3')*	*Origin of primers*
trnL-F (F)	GGTTCAAGTCCCTCTATCCC	Taberlet et al (1991)
trnL-F (R)	ATTTGAACTGGTGACACGA	Taberlet et al (1991)
18S rRNA (F)	TAATCAAGAACGAAGTTGGG	This study
18S rRNA (R)	TTTCAGCCTTGCGACCATA	This study
(AC)8T	ACACACACACACACACT	Bayraktar & Dolar (2009)
(AC)8YA	ACACACACACACACACYA	Bayraktar & Dolar (2009)
(ATG)6	ATGATGATGATGATGATG	Bayraktar & Dolar (2009)
UBC835	AGAGAGAGAGAGAGAGYC	Nghia et al (2008)
OPB-04	GGACTGGAGT	Operon Biotechnology GmbH
OPC-16	CACACTCCAG	Operon Biotechnology GmbH

10 µL of a PCR reaction contained 1 µL of 2.0 mM dNTPs (Vivantis), 0.4 unit Taq DNA polymerase (Vivantis), 1 µL of 10X PCR buffer (160 mM $[NH4]_2SO_4$, 500 mM Tris-HCl, pH 9.1, 17.5 mM $MgCl_2$, 0.1 % Triton x-100, Vivantis), 0.5 µM of each forward and reverse

primers and sterile water. The PCR condition included 94 °C for 1 min, 40 cycles at 94 °C for 1 min, 50 °C (for ISSR, *trnL-F* and 18S rRNA) or 35°C (for RAPD) for 1 min and 72 °C for 2 min, subsequently with a final extension at 72 °C for 5 min. The PCR reaction was performed in an Agilent Technologies Sure Cycler 8800 (Germany). Storage of the PCR products were at 4 ºC and analyzed on 1 - 1.5 % agarose electrophoresis, stain with ethidium bromide, and visualized under UV light. The photograph was taken using *Vilber Lourmat* (France). The images were inverted in Adobe Photoshop.

Results and Discussion. Molecular genetic analysis such as DNA fingerprinting, High Resolution Melting (HRM) and High throughput sequencing (HTS) require high-quality and high molecular weight DNA (Lutz et al 2011). Although the basic idea behind the DNA extraction is relatively simple, it is increasingly difficult to deliver a reliable and purity of DNA for molecular analysis of leaf as adults than from etiolated leaf tissue (Michiels et al 2003) because of the cell wall thickness and their high content of secondary metabolites (Zhang et al 2013; Moreira & Oliveira 2011) that influence the performance of isolated DNA. In our work in the genetic analysis of population, we often encounter a situation where the young and tender leaves will not be available at all times or many samples need to be managed simultaneously. Method with high yield and quality of DNA is required to reduce the time, to cut cost without compromising the accuracy of downstream processes. We compared the eight modified protocols based on Doyle & Doyle (1987), Porebski et al (1997), Štorchová et al (2000), Novaes et al (2009), Moreira & Oliveira (2011), Tai & Tanksley (1990), and two commercial kits.

Yields of DNA from mature leaf of rubber tree obtained from all protocols studied were analyzed using NanoDrop™ spectrophotometer and were summarized in table 2.

Table 2

Comparison in quality and quantity of DNA extracted from mature leaves of rubber tree among eight DNA extraction methods

Extraction methods	*Concentration (ng/µL)*[(1)]	*Yield (µg/50 mg fresh weight)*[(1)]	*Absorption ratio (260nm/280nm)*[(1)]	*Absorption ratio (260nm/230nm)*[(1)]
1. Doyle & Doyle (1987)	138.64^{c}	20.79^{c}	1.82d^{e}	2.18^{e}
2. Porebski et al (1997)	202.07^{b}	30.31^{b}	1.91^{a}	2.45^{d}
3. Tai & Tanksley (1990)	140.26^{c}	21.03^{c}	1.88^{b}	2.86^{a}
4. Štorchová et al (2000)	98.24^{ef}	14.74^{e}	1.82^{de}	2.87^{a}
5. Novaes et al (2009)	113.83^{de}	17.08^{de}	1.84^{c}	2.91^{a}
6. Moreira & Oliveira (2011)	229.94^{a}	34.78^{a}	1.83^{cd}	2.08^{f}
7. DNeasy Plant Mini Kit	129.52^{cd}	19.43^{cd}	1.88^{b}	2.70^{b}
8. E.N.Z.A Plant DNA Kit	32.59^{f}	4.89^{f}	1.80^{e}	1.93^{g}
LSD[(2)]	16.48*	2.45*	0.02*	0.08*

[(1)] Values with different letters within column are significantly different at $p \leq 0.05$ by LSD, [(2)] * Significant at the $p \leq 0.05$ probability level.

The variability of the method for extraction DNA caused the differences in the yield of DNA from 4.89 µg / 50 mg tissue to 34.78 µg / 50 mg tissue. DNA extraction with the addition of PVP to the CTAB solution helped to get rid of the polysaccharides from nucleic acid (Fang et al 1992). All of the protocols used gave high-quality genomic DNA according to the range of A260 nm / A280 nm, and A260 nm / A230 nm values (Sambrook et al 1989). This may be the result of incubation at room temperature with short incubation time (less than 5 min) during the precipitation of DNA (Haque et al 2008). The modified method of Moreira & Oliveira (2011), which could be performed within approximately half an hour, provided the highest DNA yield. The E.N.Z.A. Plant DNA Kit showed the lowest DNA yield (Table 2, Figures 1 & 2A). Commercial kits were

not designed for extracting DNA from plant tissues with a high concentration of polyphenols, polysaccharides and other secondary compounds.

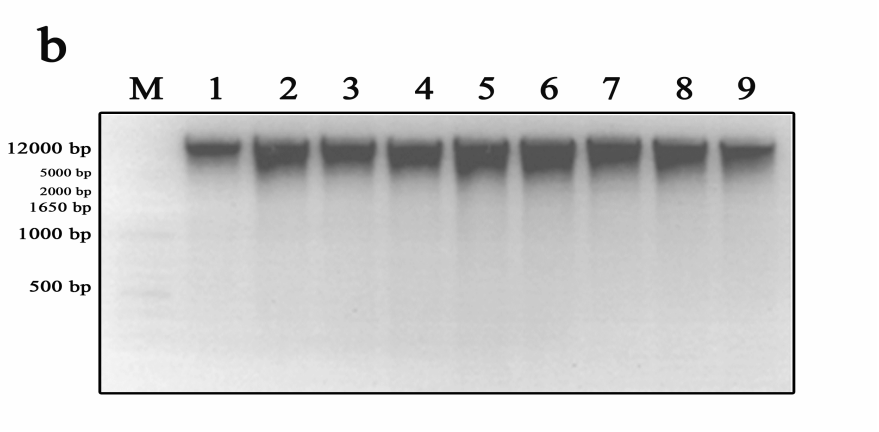

Figure 1. Gel image of DNA extracted by modified protocol of Moreira & Oliveira (2011) from mature leaves of nine rubber tree genotypes (number 1 to 10). 5 µL of the extracts was separated on 1.5 % agarose gel.

The changes in modified procedure of Moreira & Oliveira (2011) were (1) a short grinding of leaf powder in mortar with extraction buffer after blending the leaf tissue in the presence of liquid nitrogen; (2) the optimization of the ratio of tissue (weight) to buffer (volume); (3) CTAB was added only once and (4) reduction in the incubation time of the macerated tissue in an extraction buffer including RNase at 65 °C for 15 minutes to remove RNA contamination. We tested the effect of the ratio of buffer to leaf tissue. Using 700 µL of extraction buffer yielded the highest amount of DNA with the same quality compared to that of 500 or 900 µL according to the ratios of 260/280 and 260/230 (data not show). It was observed that the ratio of buffer to leaves which is high in phenolic content should always be 4:1 v/w or greater to obtain sufficient amount of clean DNA (Puchooa 2004; John 1992).

According to Li et al (2007), the CTAB is used as an effective method to extract DNA from mature leaves of sunflower as compared to the SDS-based method. CTAB is a cationic detergent that dissolves the cells and solubilizes protein and lipid contamination in the mixture. Under conditions of high salt, CTAB binds polysaccharides, removing them from the solution. Nucleic acids could be selectively precipitated.

To evaluate the performance of the protocol, we also assayed a series of fresh mature leaves from 9 clones of rubber tree from Sakon Nakhon, Loei and Khon Kaen provinces. The Results are encouraging and proved that it can be applied to all mature leaves of rubber tree (Figure 1). The yields ranged from 31.21 to 37.11 µg DNA / 50 mg fresh weight. These results demonstrated the benefits of the modified method for the rapid isolation of DNA from small quantities compared to other protocols for DNA extraction (Doyle & Doyle 1987; Porebski et al 1997; Štorchová et al 2000; Novaes et al 2009; Moreira & Oliveira 2011; Tai & Tanksley 1990); the latter mostly used 0.1 – 1 g leaf material. The original protocol of Moreira & Oliveira (2011) used 1 gram of tissue and added high concentration of CTAB twice during DNA isolation. It was unable to separate the DNA from old leaves of *Dimorphandra mollis*. Novaes et al (2009) reported problems in expanding the DNA extracted from leaves of *D. mollis* to yield good quality DNA.

Presence of unusual compounds might hinder DNA extraction and downstream analysis through the inhibition of the enzyme. To access the integrity of the extracted DNA from mature leaves of rubber tree using our modified method, ISSR and RAPD markers, and also 18S rRNA and *trnL-F* regions were amplified. The PCR patterns after agarose gel electrophoresis were consistent with the obvious bands (Figures 2 & 3). The DNA samples have been used successfully to analyze genotyping analysis of High Resolution Melting (HRM) and restriction digest in our laboratory (results not shown).

These results confirm that the modified Moreira & Oliveira (2011) approach is effective to extract DNA for molecular analysis in mature leaves of rubber tree.

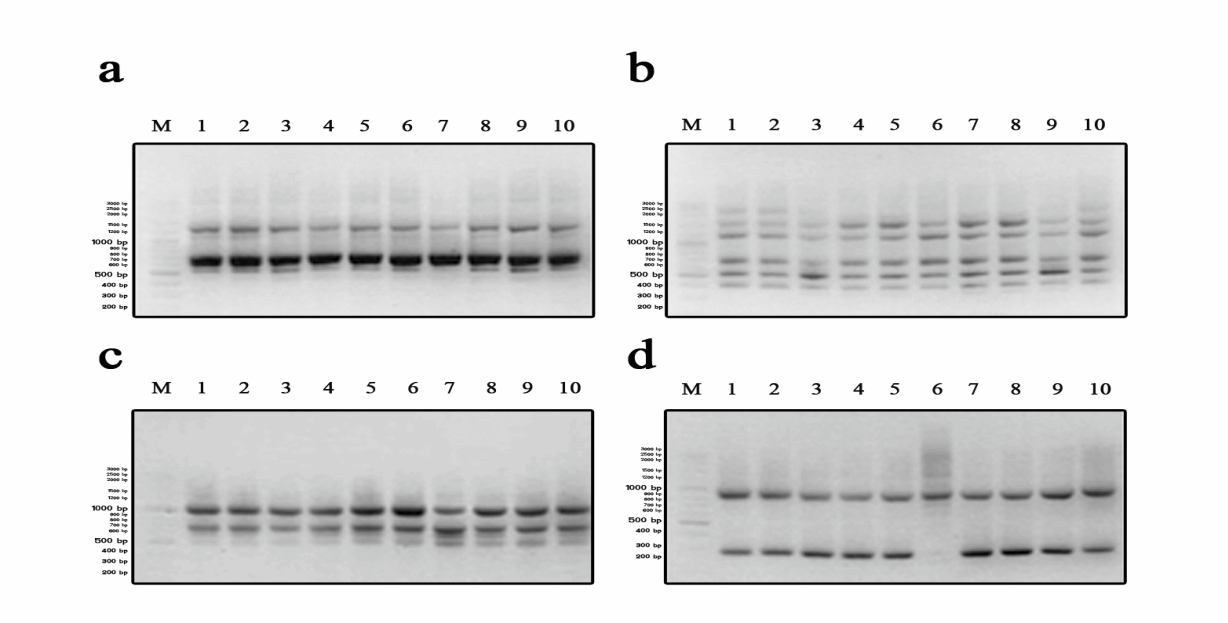

Figure 2. Agarose gel electrophoresis of PCR amplicons using ISSR primer (AC)8T (a), and UBC835 (b), Amplification of ISSR (a & b) and RAPD primer OPB-04 (c), and OPC-16 (d) from DNA extracted from mature leaves of 10 rubber tree genotypes. The first lane was standard 100 bp ladder Plus.

We also evaluated the applicability of the modified MM method to extract DNA from mature leaf of seventeen other tropical plant species (Table 3). The yield of DNA isolated from these leaves ranged between 4.44 – 19.19 µg / 50 mg fresh weight (Table 3). DNA absorbance (A260/280) values of *Annona squamosa* L. range from 1.43 to 1.66 showing the impurity of protein (Table 3).

To further assess the integrity of the DNA extracted from the analysis of these species, ISSR, RAPD, 18S rRNA and *trnL-F* amplifications were performed. Figure 3 showed that the amplifications were successfully in all species. Obviously, the modified method based on Moreira & Oliveira (2011) was effective in extracting DNA of quality and quantity needs from a small amount of mature leaves of several plant species studied here.

Table 3

Details of the mature leaf samples tested in the study

Scientific name	*Family*	*DNA concentration*	*Range*	*Yield*
		(ng/µL)	*A260/A280*	*(µg/50 mg fresh weight)*
Bouae burmanica Griff.	Anacardiaceae	29.60 ± 4.42	1.85 - 1.98	4.44 ± 0.66
Mangifera indica L. cv. Raet	Anacardiaceae	51.80 ± 6.94	1.86 - 1.89	7.77 ± 1.04
Mangifera indica L. cv. Mahachanok	Anacardiaceae	70.76 ± 4.81	1.86 - 1.90	10.61 ± 0.72
Annona squamosa L. cv. Phet Ban Lat	Annonaceae	44.50 ± 9.44	1.43 - 1.66	6.68 ± 1.42
Annona squamosa L. cv. Nang	Annonaceae	47.86 ± 7.79	1.43 - 1.66	7.18 ± 1.16
Hevea brasiliensis (Willd. ex A. Juss.) Müll. Arg.	Euphorbiaceae	227.73 ± 19.69	1.84 - 1.88	34.16 ± 2.95
Manihot esculenta Crantz cv. Huay Bong 60	Euphorbiaceae	127.90 ± 23.56	1.78 - 1.92	19.19 ± 3.53
Manihot esculenta Crantz cv. Huay Bong 80	Euphorbiaceae	141.97 ± 25.92	1.81 - 1.90	21.30 ± 3.89
Psidium guajava L.	Myrtaceae	43.53 ± 1.88	1.80 - 1.90	6.53 ± 0.28
Syzygium cumini (L.) Skeels	Myrtaceae	35.46 ± 3.30	1.89 - 1.95	5.32 ± 0.49
Eugenia javanica Lam.	Myrtaceae	33.60 ± 5.89	1.77 - 1.79	5.04 ± 0.88
Artocarpus heterophyllus Lam.	Moraceae	90.00 ± 12.96	1.93 - 2.03	13.50 ± 1.94
Sandoricum koetjape (Burm.f.) Merr.	Meliaceae	26.62 ± 5.13	1.75 - 1.85	3.97 ± 0.94
Citrus maxima (Burm.) Merr.	Rutaceae	46.00 ± 2.69	1.81 - 1.83	6.90 ± 0.403
Citrus aurantiifolia (Christm.) Swingle	Rutaceae	91.86 ± 14.17	1.81 - 1.91	13.78 ± 2.12
Morinda citrifolia L.	Rubiaceae	40.90 ± 1.55	1.95 - 1.99	6.135 ± 0.23
Dimocarpus longan Lour. cv. Edor	Sapindaceae	36.20 ± 3.74	1.73 - 1.81	5.43 ± 0.56
Dimocarpus longan Lour. cv. Srichompoo	Sapindaceae	49.63 ± 8.39	1.73 - 1.81	7.45 ± 1.25
Litchi chinensis Sonn.	Sapindaceae	50.40 ± 7.10	1.84 - 1.91	7.56 ± 1.06
Averrhoa carambola L.	Oxalidaceae	51.00 ± 10.25	1.82 - 1.85	7.65 ± 1.53
Nymphaea lotus L.	Nymphaeaceae	41.37 ± 7.98	1.99 - 2.04	6.205 ± 1.19
Garcinia mangostana L.	Guttiferae	32.57 ± 9.47	1.81 - 1.84	4.88 ± 1.42

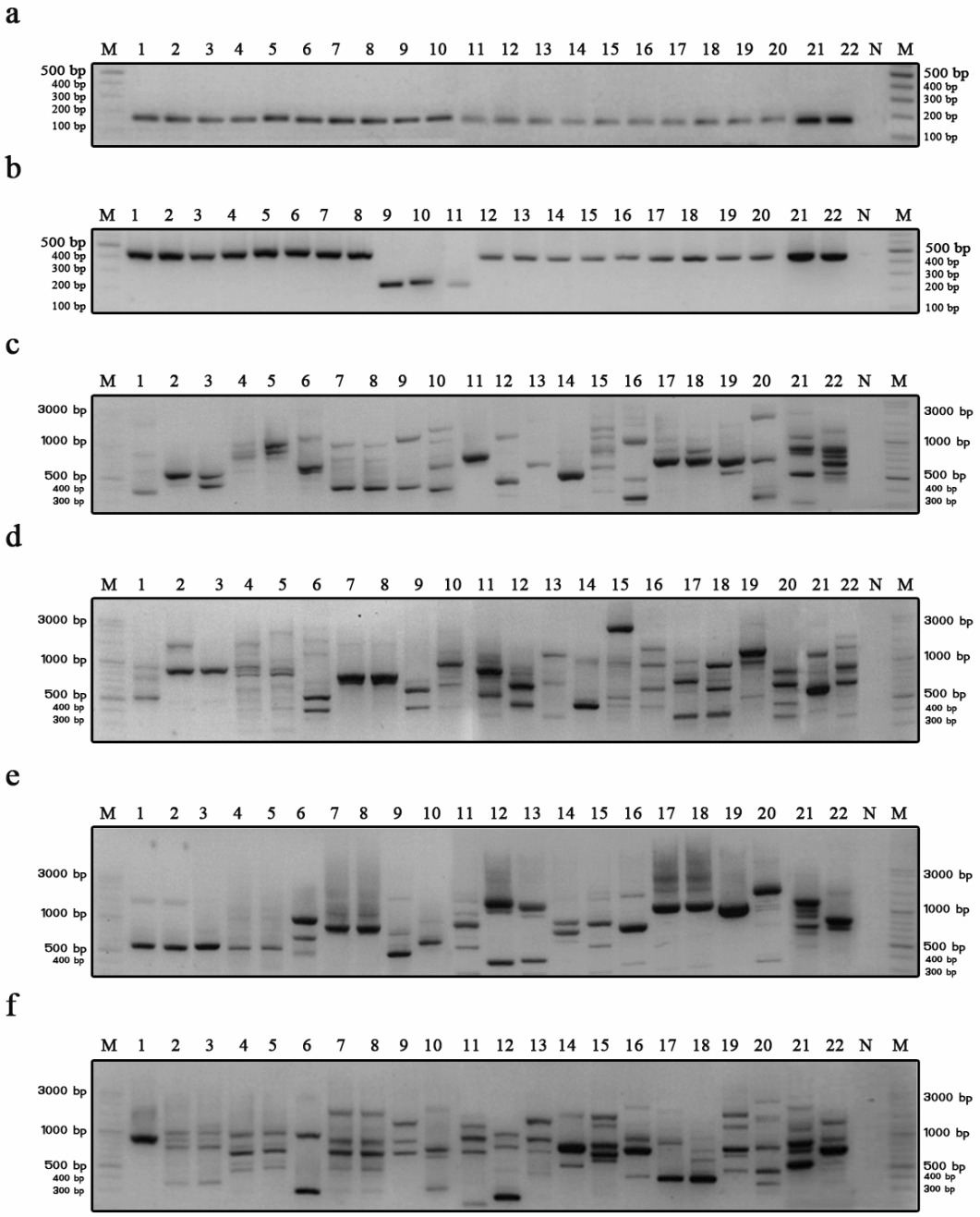

Figure 3. PCR amplification on agarose gel with the 18S rRNA (a), *trnL-F* (b), ISSR primer (AC)8T (c), ISSR primer UBC835 (d), RAPD primer OPB-04 (e), and RAPD primer OPC-16 (f) of mature leaf DNA extracted using our modified protocol. Lane M = 100-bp DNA ladder plus. The numbers indicate plant species, 1: *Bouae burmanica* Griff.; 2: *Mangifera indica* L. cv. Raet; 3: *Mangifera indica* L. cv. Mahachanok; 4: *Annona squamosa* L. cv. Phet Ban Lat.; 5: *Annona squamosa* L. cv. Nang; 6: *Hevea brasiliensis* (Willd. ex A. Juss.) Müll. Arg.; 7: *Manihot esculenta* Crantz cv. Huay Bong 60; 8: *Manihot esculenta* Crantz cv. Huay Bong 80; 9: *Psidium guajava* L.; 10: *Syzygium cumini* (L.) Skeels; 11: *Eugenia javanica* Lam.; 12: *Artocarpus heterophyllus* Lam.; 13: *Sandoricum koetjape* (Burm.f.) Merr.; 14: *Citrus maxima* (Burm.) Merr.; 15: *Citrus aurantiifolia* (Christm.) Swingle; 16: *Morinda citrifolia* L.; 17: *Dimocarpus longan* Lour. cv. Edor.; 18: *Dimocarpus longan* Lour. cv. Srichompoo.; 19: *Litchi chinensis* Sonn.; 20: *Averrhoa carambola* L.; 21: *Nymphaea lotus* L.; 22: *Garcinia mangostana* L. Lane N = Negative control.

Conclusions. A simple, fast, inexpensive and effective protocol that can be adapted for routine use to obtain high-quantity and -quality DNA from mature leaves of rubber tree suitable for further genome analysis is provided. The superiority of the modified method also confirmed empirically about the efficiency in the use extensively in our laboratory to extract DNA from many plant species. This will provides choices of different sampling. The results presented here have potential to be an effective protocol for extraction of DNA of other latex-containing plants, and perhaps for plant species rich in secondary compounds in general.

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