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DNA extraction in dendrobium

Article

Comparative study of DNA extraction methods for *Dendrobium bantimurung* orchids using commercial kits and a modified CTAB method

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Abstract

Genomic DNA extraction is a critical step in plant molecular studies, particularly in species rich in secondary metabolites that may interfere with DNA isolation and downstream analyses. *Dendrobium bantimurung* is an orchid species containing high levels of phenolic compounds; therefore, it requires an effective DNA extraction method. This study aimed to compare the effectiveness of a modified CTAB method with two commercial DNA extraction kits, Geneaid and Vazyme, for genomic DNA isolation from D. bantimurung plantlets. DNA was extracted from the leaf and root tissues of six-month-old plantlets. DNA quality and quantity were evaluated by DNA concentration, purity ratios (A260/280 and A260/230), agarose gel electrophoresis, and PCR amplification with ITS primers. The results showed that the modified CTAB method produced the highest DNA quality and the most successful PCR amplification compared with the commercial kits. However, the kit-based methods offered advantages in terms of speed and practicality. These findings indicate that the modified CTAB method is more suitable for genomic DNA extraction from *D. bantimurung*, whereas commercial kits require further optimization to improve their performance.

Keywords: *Dendrobium bantimurung*, DNA extraction, modified CTAB method

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1. Introduction

DNA extraction is a fundamental step in plant molecular genetics research that determines the quality and quantity of the obtained genomic DNA (Sari and Restanto, 2022). DNA quality plays a crucial role in the success of plant molecular genetics studies, as most molecular techniques, particularly Polymerase Chain Reaction (PCR), require genomic DNA with adequate purity and concentration (Aboul-Maaty and Oraby, 2019). The quality of extracted DNA is influenced by the biochemical characteristics of plant tissues. Each plant species contains different levels of polysaccharides, polyphenols, and secondary metabolites, which may bind to nucleic acids and reduce DNA purity (Abdel-Latif and Osman, 2017). DNA purity is generally evaluated using the A260/280 and A260/230 absorption ratios. Genomic DNA is considered pure when the A260/280 ratio is approximately 1.8 (1.85–1.88), and the A260/230 ratio is 2.3-2.4 (Koetsier and Cantor, 2019). An A260/280 ratio ≤1.6 indicates contamination by proteins, phenols, or other compounds,

whereas ratios above the ideal value suggest possible RNA contamination (Lucena-Aguilar et al., 2016).

Various methods have been developed to obtain plant genomic DNA, including commercial kits and protocols based on cetyltrimethylammonium bromide (CTAB), as described by Doyle (1991). However, the specific characteristics of certain plant tissues often result in inconsistent DNA extraction outcomes, particularly in plants rich in polysaccharides and phenolic compounds (Mitchell et al., 2023). Therefore, several modifications of the CTAB method have been developed, including adding polyvinylpyrrolidone (PVP) during the lysis stage, adjusting CTAB and NaCl concentrations, and varying incubation temperature and duration (Schenk et al., 2023). In addition to the CTAB method, plant DNA extraction is commonly performed with commercial kits, which offer convenience and rapid processing. Nevertheless, several studies have reported that commercial kits do not consistently yield high-quality DNA from plants with high levels of secondary metabolites. Abubakar et al. (2018) reported that a modified CTAB method produced better DNA quality than three different commercial kits (NucleoSpin Plant II, DNeasy, and Wizard Resin) in the extraction of the medicinal plant Eurycoma longifolia. Similar findings were reported by Esfandani-Bozchaloyi et al. (2019), who demonstrated that a specific modified CTAB method yielded the best DNA quality compared to other methods in the genus Gera-nium.

The genus Dendrobium is widely recognized not only as an ornamental plant but also as a medicinal plant (Huda and Jahan, 2019). Various studies have reported high levels of phenolic compounds and secondary metabolites in the genus Dendrobium, including antimicrobial compounds, flavonoids with antidiabetic activity, and metabolites associated with antioxidant and anticancer activities (Cheun-Arom and Sritularak, 2023; Setyati et al., 2024; Swainson et al., 2023). The high concentration of secondary metabolites poses a major challenge for obtaining high-quality genomic DNA. These compounds may also interfere with downstream molecular processes such as PCR and other enzymatic reactions (Ghadia et al., 2016). Vilanova et al. (2020) further stated that low-quality DNA or DNA contaminated with secondary metabolites may inhibit PCR amplification and sequencing processes. Although numerous comparative studies on modified CTAB methods and commercial kits have been conducted, no report has yet been found comparing a simple modified CTAB method with the Geneaid and Vazyme commercial kits for Dendrobium bantimurung. This comparative study is important for determining the most effective, efficient, and economical DNA extraction method. Therefore, this study aimed to identify an effective, affordable DNA extraction method capable of producing high-quality genomic DNA from Dendrobium bantimurung. The success of each method was evaluated based on DNA concentration and purity, measured by A260/280 and A260/230 ratios, and on PCR-based DNA amplification. DNA quality was also assessed through agarose gel electrophoresis. The results of this study are expected to improve the efficiency and quality of molecular research on orchids, particularly the genus Dendrobium, which is used both as an ornamental and a medicinal plant.

2. Materials and Methods

2.1. Research location, materials, and equipment

The study was conducted at the Biotechnology Laboratory, Faculty of Animal and Agricultural Sciences, Universitas Diponegoro, from January to February 2026. The materi-als used in this study included six-month-old *Dendrobium bantimurung* orchid plantlets obtained from the Tissue Culture Laboratory, Faculty of Agriculture, Universitas Muria Kudus (Figure 1), 1 M Tris-HCl buffer (pH 8.0), 0.5 M EDTA (pH 8.0), cetyltrimethylammo-nium bromide (CTAB) buffer powder, polyvinylpyrrolidone (PVP), sterile distilled water, 1 M NaCl, chloroform: isoamyl alcohol (24:1), cold absolute ethanol, cold 70%

ethanol, 1 M Tris-EDTA (TE) buffer, ITS primers (Widya Life Science), 2× MyTaq HS Red Mix (Bioline), 1000 bp and 100 bp DNA ladders (Intron), DNA gel stain (Intron), agarose, and Tris-borate-EDTA (TBE) buffer. Two commercial DNA extraction kits were evaluated in this study: the FastPure Plant DNA Isolation Mini Kit (Vazyme, China) and the Geneaid Ge-nomic DNA Purification Kit (Geneaid, Taiwan). The Vazyme kit consisted of RNase A, Buffer A1, Buffer A2, Buffer A3, Buffer AW, Elution Buffer, gDNA Columns IV, and 2 mL collection tubes. The Geneaid kit consisted of GP1 Buffer, GPX1 Buffer, GP2 Buffer, GP3 Buffer supplemented with isopropanol, W1 Buffer, Wash Buffer supplemented with ethanol, Elution Buffer, Filter Columns, GD Columns, and 2 mL collection tubes. The equipment used included sterile 1.5 mL microtubes, mortar and pestle, vortex mixer, dry bath (Thermo Fisher Scientific), centrifuge (D'Lab), micropipettes (Eppendorf), freezer, microwave, thermal cycler (Applied Biosystems™ MiniAmp™), electrophoresis apparatus (Mupid), Gel Documentation System (Bio-Rad), and NanoDrop spectrophotometer (Thermo Fisher Scientific).

Figure 1. *Dendrobium bantimurung* planlet (scale bar = 1 cm).

2.2. Modified CTAB DNA extraction method based on Doyle (1991)

Genomic DNA was extracted using the modified CTAB method (Doyle, 1991), with several modifications. Approximately 100 mg of leaf and root tissues were ground using a mortar and pestle and mixed with 500 µL extraction buffer contain-ing 3% CTAB, 1.4 M NaCl, EDTA, Tris-HCl, and PVP (pH 8.0). Samples were incubated at 60°C for 30 min, then centrifuged at 10,000 rpm for 20 min. The supernatant was purified using chloroform: isoamyl alcohol (24:1) and centrifuged again at 10,000 rpm for 30 min. DNA precipitation was performed with cold absolute ethanol (1:1, v/v), and the mixture was incubated overnight at -20°C. The DNA pellet was collected by centrifugation, washed with cold 70% ethanol, air-dried, and resuspended in 35 µL warm TE buffer. The extracted DNA was stored at -20°C until further analysis.

2.3. Plant DNA Extraction Using the FastPure Plant DNA Isolation Mini Kit (Vazyme, China)

Genomic DNA extraction using the FastPure Plant DNA Isolation Mini Kit (Vazyme, China) was performed according to the manufacturer's protocol (https://www.vazymeglobal.com/product-center/genomic-dna-extraction/fastpure-plant-dna-isolation-mini-kit) with minor modifications to centrifugation speed and duration to accommodate the laboratory's equipment conditions. Approximately 100 mg of fresh leaf and root tissues were homogenized and mixed with Buffer A1, 1% PVP, and RNase A, followed by incubation at 65°C for 10 min. After the addition of Buffer A2 and centrifugation, the supernatant was mixed with Buffer A3 and transferred to the gDNA Column IV for DNA binding. The column was washed twice using Buffer AW and centrifuged to remove residual ethanol. DNA was eluted using 35 µL warm Elution Buffer and stored at -20°C until further analysis.

2.4. Plant DNA extraction using the genomic DNA purification kit (Geneaid, Taiwan)

Genomic DNA extraction using the Geneaid Genomic DNA Purification Kit (GP100) was performed according to the manufacturer's protocol (https://www.geneaid.com/Genomic-DNA-Purification/GP100) with minor modifications in centrifugation speed and duration to accommodate laboratory equipment conditions. Approximately 100 mg of leaf and root tissues were homogenized and mixed with GPX1 Buffer and RNase A, followed by incubation at 60°C for 10 min. After adding GP2 Buffer and centrifuging, the supernatant was mixed with GP3 Buffer and transferred to the GD Column for DNA binding. The column was sequentially washed using W1 Buffer and Wash Buffer, followed by a final centrifugation step to remove residual ethanol. DNA was eluted using 35 µL warm Elution Buffer and stored at -20°C until further analysis.

2.5. DNA quality and quantity assessment using agarose gel electrophoresis and NanoDrop

The quality of genomic DNA (gDNA) was evaluated using 1% agarose gel electrophoresis in 1× TBE buffer and visualized using a Gel Documentation System (Bio-Rad). DNA quantity and purity were measured using a NanoDrop spectrophotometer (Thermo Fisher Scientific) based on DNA concentration and absorbance ratios (A260/280 and A260/230).

2.6. PCR Amplification and Visualization)

DNA fragments were amplified using Internal Transcribed Spacer (ITS) primers (Table 1) through PCR based on the MyTaq™ HS Red Mix protocol. The PCR reaction composition is presented in Table 2, while the PCR cycling conditions are shown in Table 3.

Table 1. Primers used for PCR amplification.

No	Primer Name	Sequence
1	ITS Forward	5′-TCCGTAGGTGAACCTGCGG-3′
2	ITS Reverse	5′-TCCTCCGCTTATTGATATGC-3′

Table 2. PCR reaction composition for ITS gene amplification.

Component	Volume (µL)
DNA	1.00
MyTaq™ HS Red Mix	6.25
ITS_F Forward Primer (10 µM)	0.50
ITS_R Reverse Primer (10 µM)	0.50
ddH_2O	4.25
Total Volume	12.50

Table 3. PCR cycling conditions for ITS gene amplification.

Step	Temperature (°C)	Time	Volume (μL)
Pre-denaturation	95	5 min	34 cycles
Denaturation	95	30 sec	
Annealing	53	15 sec	
Extension	72	45 sec	
Post-extension	72	5 min	
Hold	4	∞	

Following amplification, PCR products were visualized using 1.5% agarose gel electrophoresis. A 100 bp DNA ladder was used as a molecular size marker. Electrophoresis was performed at 50 V for 55 min, and amplified DNA bands were visualized using a Gel Documentation System (GelDoc).

3. Results

3.1. Genomic DNA electrophoresis

The electrophoresis results demonstrated variations in the quality of genomic DNA (Figure 2). In the CTAB method, the DNA bands appeared relatively clearer and thicker, particularly in the leaf samples (lanes 4–6), compared with the root samples (lanes 1–3). This result indicates that the CTAB method produced genomic DNA with higher concentration and greater integrity. However, lane 2 showed slight smearing, indicating partial DNA degradation or contamination with RNA and/or proteins during the extraction process. DNA extraction using the Vazyme kit yielded thinner, less intense DNA bands than the CTAB method. This finding suggests that the DNA concentration obtained using the Vazyme kit was relatively lower. Nevertheless, the resulting bands appeared relatively clean, with minimal smearing, indicating a fairly good level of DNA purity. The lower band intensity may have been caused by the limited DNA-binding capacity of the silica column or DNA loss during the washing and elution steps. In the root samples, the DNA bands appeared very faint, suggesting that root tissues may contain inhibitors that reduced the extraction efficiency of this commercial kit. In the Geneaid method, the DNA bands appeared thin, suggesting a low DNA concentration or possibly suboptimal cell lysis efficiency in the plant samples. Although the bands were relatively faint, the genomic DNA electrophoresis results from the leaf tissues were more visible than those obtained from the root tissues.

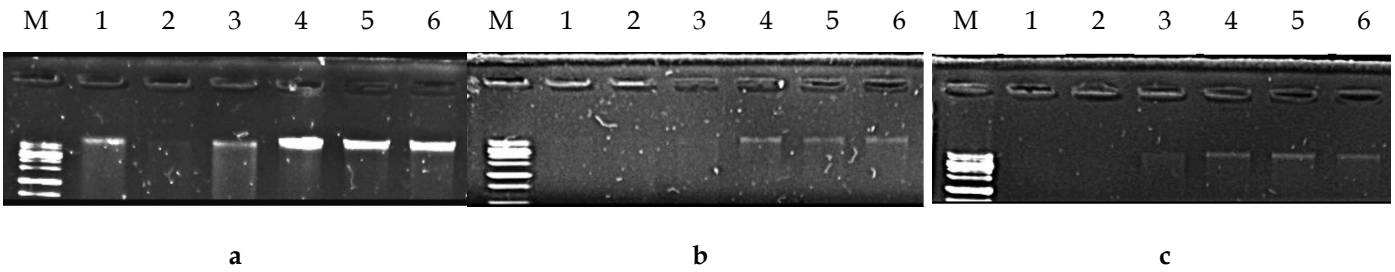

Figure 2. Comparison of genomic DNA electrophoresis results obtained using three extraction methods: (a) CTAB method; (b) Vazyme extraction kit; (c) Geneaid extraction kit. M: marker; lanes 1–3: root samples; lanes 4–6: leaf samples.

3.2. NanoDrop of genomic DNA extracted using the CTAB, Vazyme, and Geneaid protocols

The CTAB method produced the highest DNA concentration among the extraction methods (Table 4). The high DNA concentration indicates that the CTAB method was ef-

fective in lysing plant cells and isolating large amounts of genomic DNA. The A260/280 ratio in root samples ranged from 1.68 to 1.77, while that in leaf samples ranged from 1.51 to 1.79. Most values were within the standard range for pure DNA, although several leaf samples showed lower values, suggesting possible contamination with proteins or phenolic compounds. The A260/230 ratio in root samples ranged from 1.54 to 2.39, whereas that in leaf samples ranged from 0.74 to 3.22. This variation indicates that some samples still contained contaminants, such as polysaccharides, phenols, or residues from the extraction buffer. However, the CTAB method produced relatively good DNA quality with the highest DNA yield.

Table 4. NanoDrop of genomic DNA extracted using different protocols.

Protocols	Sample	Root			Leaf		
		Concentration	A260/A280	A260/230	Concentration	A260/A280	A260/230
CTAB	1	44.50	1.77	2.39	20.4	1.51	0.74
	2	22.00	1.68	2.23	28.3	1.79	3.22
	3	25.90	1.70	1.54	25.9	1.70	1.54
Vazyme	1	3.00	1.79	0.50	3.40	1.48	0.65
	2	2.90	1.61	0.60	3.40	1.63	0.58
	3	4.00	1.69	0.57	4.70	2.07	0.44
Geneaid	1	3.90	1.55	0.87	4.70	1.68	1.32
	2	3.30	1.33	0.30	3.50	1.83	1.75
	3	5.30	1.77	0.74	3.20	1.79	0.89

DNA extraction using the Vazyme kit resulted in lower DNA concentrations than the CTAB method. The lower DNA concentration may have been caused by limited DNA binding efficiency to the kit membrane or DNA loss during the washing process. The A260/280 ratio ranged from 1.61 to 1.79 in root samples and from 1.48 to 2.07 in leaf samples. Most values were close to the optimal range, although values above 2.0 may indicate RNA contamination. Meanwhile, the A260/230 ratios in all samples were relatively low, ranging from 0.44 to 0.65. These values indicate the presence of organic compounds, salts, or residues from the extraction buffer. Therefore, although the Vazyme kit produced DNA with relatively good purity (A260/280), the DNA quality (A260/230) remained suboptimal. The Geneaid method produced slightly higher DNA concentrations than the Vazyme kit, but still lower concentrations than those obtained using the CTAB method. The A260/280 ratio ranged from 1.33 to 1.77 in root samples and from 1.68 to 1.83 in leaf samples. Most values were within the standard range for pure DNA; however, the value of 1.33 observed in one root sample suggested possible protein contamination. The A260/230 ratio ranged from 0.30 to 0.87 in root samples and from 0.89 to 1.75 in leaf samples. These values remained below the ideal range, indicating the presence of contaminants, such as polysaccharides or phenolic compounds, that were not completely removed during the extraction process.

3.3. PCR results using ITS Primers

The DNA amplification results using ITS primers showed variations in PCR success among DNA samples extracted using the CTAB method, Vazyme kit, and Geneaid kit (Figure 3). PCR success was evaluated based on the presence of DNA bands on agarose gel electrophoresis after amplification. In the CTAB method, DNA bands appeared clearer and more consistent in both root (lanes 1–3) and leaf (lanes 4–6) samples. The relatively high band intensity indicates that the extracted DNA was of high quality and purity, enabling optimal amplification with ITS primers. The clear DNA bands also suggest that the DNA concentration obtained using the CTAB method was sufficiently high and relatively

free from PCR inhibitors, such as polysaccharides and plant phenolic compounds. In the Vazyme kit method, DNA bands were still observed; however, their intensity was weaker than that obtained using the CTAB method. Several samples showed faint bands, indicating that the amount of DNA template used was relatively low or that contaminants that inhibit PCR amplification were still present. These results are consistent with the previous NanoDrop analysis, which showed lower DNA concentrations and suboptimal A260/230 ratios. Meanwhile, in the Geneaid kit method, DNA bands were also detected, but their intensities varied among samples. Some bands appeared thin and less distinct, suggesting that the extracted DNA was not fully optimal for amplification. The low A260/230 ratios observed in several samples may indicate the presence of residual extraction contaminants that could affect PCR efficiency.

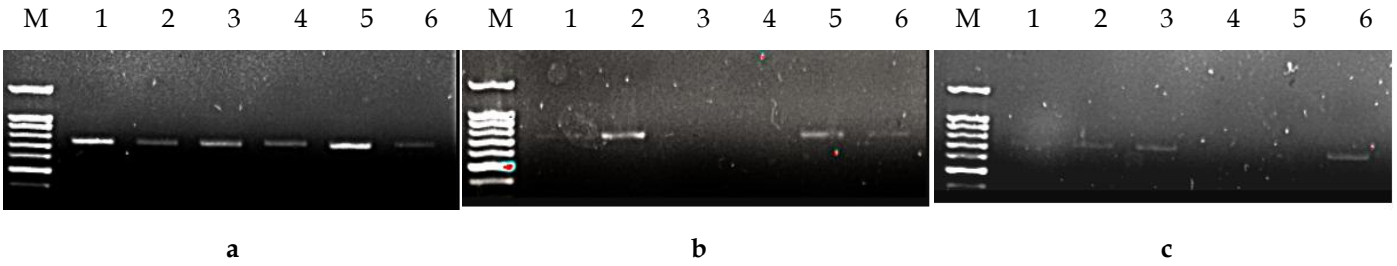

Figure 3. Comparison of PCR amplification results using ITS primers from genomic DNA obtained using three extraction methods: (a) CTAB method; (b) Vazyme extraction kit; (c) Geneaid extraction kit. M: marker; lanes 1–3: root samples; lanes 4–6: leaf samples.

4. Discussion

The present study demonstrated that the modified CTAB method produced superior genomic DNA quality and quantity compared to the Vazyme and Geneaid commercial kits. This superiority was evidenced by clearer, thicker DNA bands during electrophoresis, higher DNA concentrations measured with a NanoDrop, and more consistent PCR amplification with ITS primers. These findings indicate that the modified CTAB protocol was more effective for extracting plant genomic DNA, particularly from samples containing complex secondary metabolites. Similar findings have been reported in previous studies. Abubakar et al. (2018) showed that the modified CTAB method was the most efficient technique for extracting high-quality genomic DNA from Eurycoma longifolia leaves and roots, producing higher DNA yields, better purity, and successful ITS2 amplification compared to several commercial extraction kits. In addition, Esfandani-Bozchaloyi et al. (2019) reported that an optimized CTAB protocol generated high-quality genomic DNA from Geranium species, which are rich in polysaccharides, flavonoids, tannins, and other secondary metabolites that commonly interfere with DNA extraction. Their study demonstrated that the modified CTAB method yielded higher DNA quantity and quality than the commercial kit and conventional CTAB methods, while avoiding the use of expensive liquid nitrogen and toxic phenol compounds (Sahu et al., 2012). The CTAB protocol is effective for plant DNA extraction because it efficiently removes polysaccharides and phenolic compounds that commonly contaminate DNA in plants rich in secondary metabolites. The addition of compounds such as PVP and β-mercaptoethanol further helps prevent phenolic oxidation, resulting in high-quality DNA suitable for downstream molecular analyses (Schenk et al., 2023).

Although commercial DNA extraction kits are convenient and widely available, they may be less effective for plant species rich in secondary metabolites and polysaccharides, as these compounds can interfere with DNA isolation and reduce DNA quality (Sahu et al., 2012). DNA loss may also occur during the binding, washing, and elution steps of silica membrane-based purification, resulting in lower DNA recovery (Demeke & Jenkins,

2010). Differences between root and leaf tissues were also observed in this study. DNA extracted from leaf tissues generally showed higher concentrations and clearer electrophoresis bands. Similar findings were reported by Abubakar et al. (2018), who demonstrated that genomic DNA isolated from Eurycoma longifolia leaves produced higher DNA concentrations and greater purity than that from root tissues. The authors suggested that these differences were associated with the higher accumulation of secondary metabolites, polysaccharides, and phenolic compounds in root tissues, which can interfere with DNA isolation and reduce DNA quality.

PCR amplification using ITS primers confirmed that DNA quality significantly influences downstream molecular applications. Samples extracted using the modified CTAB method produced stronger, more consistent amplification bands, indicating that the extracted DNA was sufficiently intact and pure for PCR analysis. In contrast, the weaker amplification observed in samples extracted using commercial kits may have resulted from lower DNA template concentrations and residual inhibitory compounds. Similar findings were reported by Aboul-Maaty and Oraby (2019), who demonstrated that DNA extracted using a modified CTAB protocol produced clear, well-differentiated PCR amplification bands compared to DNA isolated using the conventional extraction method. Their study also showed that high-quality DNA obtained using the modified CTAB method was successfully amplified with RAPD and *nptII* primers, confirming that DNA purity and integrity strongly affect PCR performance and downstream molecular analyses.

5. Conclusions

The modified CTAB method proved more effective than the Vazyme and Geneaid commercial kits for extracting plant genomic DNA, yielding higher DNA quality, greater DNA concentration, and better PCR amplification success. Leaf tissues generally yielded better DNA quality than root tissues. The modified CTAB protocol is a reliable and cost-effective method for plant molecular analyses, particularly for samples rich in secondary metabolites.

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