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Optimization of Genomic DNA Extraction Method on Nutmeg (*Myristica fragrans* Houtt.)

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Abstract

Nutmeg (*Myristica fragrans*) is one of the commodities in the Myristicaceae family that has numerous benefits and high phenolic compounds. Elevated amounts of secondary metabolites in the Myristica genus pose significant challenges for obtaining high-quality DNA. This complexity often results in reduced DNA recovery and suboptimal absorbance ratios, attributed to contamination by RNA and proteins in DNA extraction yields. The results of DNA extraction that have been obtained through several variations of optimized methods are compared. The DNA extraction method using a modified Geneaid Genomic DNA Mini Kit was determined to produce the highest quality and quantity of DNA. Compared to other protocols such as Doyle and Doyle, modified Doyle and Doyle, and the basic protocol of the Geneaid kit without improvement, the modified Geneaid Genomic DNA Mini Kit had a high mean range purity of 1.83, and bands appeared.

Keywords: DNA extraction; Myristica fragrans; Nutmeg; Optimization.

INTRODUCTION

Nutmeg (*Myristica fragrans*) is a commodity from the Myristicaceae family that possesses numerous applications in culinary as food flavoring and medicinal as an essential oil and pharmaceutical ingredient (Naeem, 2016; Gupta, 2020). The study of the character of a plant is not only seen in its morphological character but can also be analyzed in the molecular area such as genetic material (DNA, RNA), proteins, and secondary metabolites (Wijayanti, 2018). With advancements in molecular genetic techniques, DNA analysis now enables the precise identification of genetic variations, offering deeper insights into plant biology (Gupta et al., 2020).

DNA extraction is crucial for obtaining high-quality genomic DNA. Due to the differential concentrations of secondary metabolites in plant cells, each species requires a specific extraction protocol to produce genomic DNA of sufficient quality for molecular analyses. Impure DNA can be caused by secondary compounds such as polysaccharides, phenolic compounds, or other contaminants (Pharmawati, 2009). Nutmeg has high levels of various types of phenolic compounds (Periasamy et al., 2016).

These metabolites negatively impact subsequent procedures such as DNA extraction, restriction, amplification, and cloning (Ashokkumar et al., 2022). The high levels of secondary metabolites present in leaves of the Myristica genus pose significant challenges to obtaining high-quality DNA (Diyasree, 2014). The problems in DNA extraction remain significant issues that need to be solved by optimization.

Procedure optimization can be attained by modifying the lysis buffer composition or employing specialized physical handling techniques to effectively separate genomic DNA from other components. The primary aim of these optimization strategies is to prevent degradation of genomic DNA caused by secondary compounds released during cellular lysis and subsequent handling (Milligan, 1992). Several techniques and procedures have been published, but these are not applicable because treatment for every genus or plant species is very specific. Modification of the conventional DNA extraction protocol is necessary for extracting DNA from plant leaves with high concentrations of polysaccharides or secondary metabolites (Sahu, 2012). Therefore, it is necessary to select and optimize the extraction method that allows the genomic DNA of the nutmeg plants to be obtained. Studying plant DNA isolation methods, especially nutmeg, is the first step that will be used as a reference for further research. This study aimed to obtain optimal DNA extraction methods and produce high-quality DNA genomes so they could be used for genetic diversity analysis in nutmeg plants.

MATERIALS AND METHODS

Sampling and preservation

Following Sudhamayee's (2010) recommendation, the third leaf from the shoot tip was collected from Sangihe Island. Samples were cleaned using alcohol and quickly put into an envelope, then wrapped in a zip-lock plastic with silica gel. The samples were transported to the laboratory in ice-filled containers and stored at -20 °C until utilized for DNA extraction.

Procedures

Doyle and Doyle (1987)

In accordance with the Doyle and Doyle (1987) protocol, 0.2 g of clean leaf tissue was homogenized with an extraction buffer consisting of 2% hexadecyltrimethylammonium bromide (CTAB), 1.4 M NaCl, 20 mM EDTA, 100 mM Tris-HCl (pH 8.0), and 0.2% β-mercaptoethanol, as specified in the original method (Doyle & Doyle, 1987).

Modified Doyle and Doyle (1987)

Genomic DNA was isolated using the original Doyle and Doyle (1987) protocols, with a modified protocol by adding PVP (Polyvinylpyrrolidone).

Genomic DNA Mini Kit (Plant)

Tissue Dissociation, a sample from 0.35 g of fresh leaf tissue was placed in a mortar, frozen with liquid nitrogen, and subsequently ground. The homogenized sample was transferred to a pre-labeled 1.5 mL microcentrifuge tube. During the lysis stage, 400 µL of GPX1 buffer and 5 µL of RNase were added to the sample, which was then vortexed and incubated at 60°C for 20 minutes. The tube was inverted every 5 minutes. Following incubation, GP2 solution was added, the mixture was vortexed, and the sample was incubated on ice for 3 minutes. The sample was then transferred to a column filter placed in a 2 mL collection tube and centrifuged at 1000 × g for 1 minute. The filter column was discarded, and the supernatant was transferred from the 2 mL collection tube to a new, pre-labeled 1.5 mL microcentrifuge tube.

Then, in **the binding stage**, the supernatant was added with 1.5 of the volume of GP3 (to which isopropanol has been added) and vortexed for 5 seconds, Subsequently, 700 µL of the mixture was aliquoted into the GD column, which was then inserted into a 2 mL collection tube and centrifuged at 15,000 × g for 2 minutes. The eluate was discarded, and the GD column was returned to the 2 mL collection tube. The remaining mixture was added to the GD column and centrifuged again at 15,000 × g for 2 minutes. The resultant liquid was discarded, and the GD column was repositioned in the 2 mL collection tube.

The next step is **washing**; this stage serves to remove the remnants of the extraction buffer and residual pigment. 400 µL of W1 buffer was added to the GD column, which was then centrifuged at 15,000 × g for 30 seconds. The eluate was discarded, and the GD column was returned to the 2 mL collection tube. Next, 600 µL of wash buffer was added to the GD column, followed by centrifugation at 15,000 × g for 30 seconds. The liquid was discarded, and the GD column was placed back into the 2 mL collection tube. The column was then centrifuged at 15,000 × g for 3 minutes to ensure complete drying of the column matrix.

In the last step, **DNA elution**, the dried GD column was transferred to a new 1.5 mL microcentrifuge tube, and 80 µL of preheated elution buffer was carefully added to the center of the column matrix. The column was allowed to stand for 10 minutes to ensure complete absorption of the elution buffer. Subsequently, the column was centrifuged at 15,000 × g for 30 seconds to elute the DNA.

Modified Genomic DNA Mini Kit (Plant)

Genomic DNA was isolated using the GENEAID brand extraction kit with a modified protocol by adding PVP at the **tissue dissociation stage**, incubation for 20 minutes at the original lysis stage (protocol: 10 minutes), and also incubation for 10 minutes in the **DNA elution stage** after adding the elution buffer (protocol: 2-3 minutes), and the centrifugation temperature adjustment to 4 ˚C.

DNA Concentration Measurement

The concentration of the extracted DNA samples was determined by measuring 50 µL of the sample at each stage. The sample is pipetted onto a spectrophotometer plate to obtain OD (Optical Density). The spectrophotometer used a single-wavelength method, and the OD was measured at a wavelength of 260 nm. The results from each method were compared.

Agarose Gel Electrophoresis

The extraction results were electrophoresed with a 100-bp DNA marker (DNA ladder) on a 1% agarose gel with florosafe DNA staining in TBE buffer (Tris/Borate/EDTA) for 40 minutes at 100 volts. Then the gel was observed under a UV lamp using the gel documentation system.

RESULTS AND DISCUSSION

Based on the results of quantitative tests using a spectrophotometer, the average λ of DNA produced in four methods was 1.17, 1.54, 0.00, and 1.83 (Table 1). Based on the comparison of the absorbance values of $\lambda_{260}/\lambda_{280}$ from the four methods, the 4th method obtained a high level of purity, and the DNA produced is quite clean. Theoretically, DNA samples that are considered quite pure have a ratio of $\lambda_{260}/\lambda_{280}$= 1.80-2.0. According to Watson et al. (2024), this range of numbers has met the requirements needed in molecular analysis.

Table 1. Quantitative and qualitative test results from nutmeg DNA extraction.

No	Isolation method	Mean $\lambda_{260}/\lambda_{280}$	Remarks
1	Doyle & Doyle (1987)	1,17	Low DNA content. The pellet was brown in color and difficult to dissolve in TE (Tris-HCl and EDTA) buffer. High protein contamination, no bands appeared.
2	Modified Doyle & Doyle (1987)	1,54	DNA yield was higher when compared to the original protocol of Doyle & Doyle (1987), but the concentration was still low. The pellet was cleaner and still difficult to dissolve, and no bands appeared.
3	Genomic DNA Mini Kit (Plant)	0,00	No evidence of DNA, no bands appeared.
4	Modified Genomic DNA Mini Kit (Plant)	1,83	Bands were obtained with the highest concentration of DNA.

The method using additional PVP resulted in a higher average DNA concentration. Phenolic compounds interact with proteins and DNA through hydrogen bonding immediately after cell lysis, which can alter their properties and impede effective extraction. Consequently, the incorporation of phenolic inhibitors is essential. Polyvinylpyrrolidone (PVP), or polyvidone or povidone, is a water-soluble polymer synthesized from N-vinylpyrrolidone. PVP binds to polyphenols and is available in different molecular weights, with lower molecular weight forms particularly effective at binding proteins (Li et al., 2021). Using a modified kit also results in better DNA quality because the kit is provided with a filter that is used specifically to filter macromolecules such as protein, so protein contaminants are lower than others.

The extraction results in methods 1, 2, and 3 did not show any DNA bands, respectively (Figure 1). This shows that the three methods cannot produce DNA extracts properly. Good-quality DNA extraction products are indicated by DNA bands that look thick and clean and DNA bands that glow. Thus, the three methods are not suitable for use in the isolation of nutmeg genomic DNA.

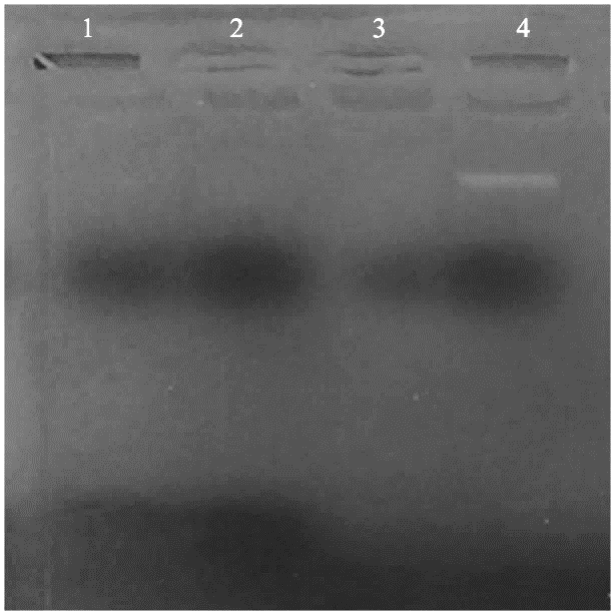

Figure 1. Visualization of qualitative test results from nutmeg DNA extraction.

The addition of reducing compounds such as merchaptoethanol (ß-ME) in the DNA extraction process can prevent the oxidation of phenolic compounds, thereby inhibiting the activity of free radicals generated by the oxidation of phenol to nucleic acids (Drygin, 2021). The use of CTAB in the extraction buffer is helpful for eliminating polysaccharides. Furthermore, Valizadeh et al. (2021) and Ali et al. (2019) stated that adding NaCl with a concentration above 1 M can increase the solubility of polysaccharides so that they are easier to remove. Thus, the CTAB buffer is qualified to be used in DNA extraction from plants containing high amounts of carbohydrates and phenols because it does not damage DNA. CTAB buffer with high salt content can separate polysaccharides from cell walls, while PVP can reduce browning due to phenol content in young leaves (Gill et al., 2025; Surzycki, 2000).

CONCLUSION

Based on the results of DNA quantitative and qualitative tests on nutmeg samples, the best DNA produced is using the Modified Genomic DNA Mini Kit (Plant).

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Competing Interests: The authors declare that there are no competing interests.

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