Annual Research & Review in Biology

Volume 39, Issue 11, Page 51-60, 2024; Article no.ARRB.125866
ISSN: 2347-565X, NLM ID: 101632869
(Past name: Annual Review & Research in Biology, Past ISSN: 2231-4776)

Extraction of Genomic DNA from Different Plant Tissues through Phenol-chloroform Method

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/arrb/2024/v39i112155

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/125866

Original Research Article

Received: 02/09/2024
Accepted: 05/11/2024
Published: 18/11/2024

ABSTRACT

Background: DNA extraction is a fundamental technique in molecular biology that involves isolating DNA from biological samples for various downstream applications, including PCR and sequencing. Phenol-chloroform DNA extraction is an alternate method of the CTAB method for genomic DNA extraction. This method typically yields more DNA than the CTAB with high purity. It is useful for high molecular weight/long fragment DNA determinations. This study aims to extract DNA from plant tissues using the phenol-chloroform method and compare the response of different plant leaves to this method.
Methods: In this study, we provide a step-by-step guide to DNA extraction from five different plant species, including *Ficus capensis* (Cape fig or Cape banyan), *Ficus exaspirata* (Forest Sandpaper), *Mangifera indica* (Mango), *Gmelina arborea* (Gamhar), and *Bauhinia purpurea* (Butterfly tree). The DNA isolation protocol involved several steps, such as grinding the plant tissue, adding NaCl

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Cite as: *Dawodu, Olufunke, and Quawiy Erinle. 2024. "Extraction of Genomic DNA from Different Plant Tissues through Phenol-Chloroform Method". Annual Research & Review in Biology 39 (11):51-60. https://doi.org/10.9734/arrb/2024/v39i112155.*

solution, using a chloroform-isoamyl alcohol mixture to extract DNA, and further purification with phenol-chloroform-isoamyl alcohol. Isopropanol was used to precipitate the DNA, and ethanol was used to wash the DNA pellet to remove any remaining contaminants. Finally, the DNA pellet was dissolved in TE buffer for storage.

Results: The results showed the successful implementation of the phenol chloroform method for isolating DNA from selected plant tissues. Overall, this study provides a comprehensive protocol for DNA extraction from plant tissue using readily available laboratory reagents and equipment.

Conclusion: The protocol can be modified to accommodate different plant species and sample types and can facilitate further research in plant genetics and biotechnology.

Keywords: DNA isolation; plant tissue; phenol chloroform method; centrifugation.

1. INTRODUCTION

DNA isolation is the process of separating DNA molecules from other cellular components, such as proteins, lipids, and carbohydrates. The extracted DNA can be used for various downstream applications, such as PCR, sequencing, and genetic engineering.

DNA isolation from plant tissue is important for various genetic and molecular studies. Plants have complex genomes with a large number of repetitive sequences, making DNA isolation from plant tissue challenging. However, the extracted DNA can be used for a variety of purposes, such as mapping of genomes, identification of gene sequences, and analysis of genetic diversity (Zhang et al. 2019).

DNA extraction is a fundamental procedure in molecular biology and biotechnology. DNA (Deoxyribonucleic Acid) is the genetic material that carries all the hereditary information in cells. The extraction of DNA from different sources even microbes (Raymond & Wilkins 1952) is critical for a wide range of applications, including gene cloning, genetic engineering, plant breeding, diagnostics, and forensic analysis.

Plants are a rich source of DNA and play an important role in the study of molecular biology and biotechnology. They contain high amounts of DNA in their cells, and the extraction of high-quality DNA from plant tissue is essential for various downstream applications. The extraction of DNA from plant tissue is a complex process, and the quality and yield of the extracted DNA can be influenced by many factors, including the type of tissue, the plant species, and the extraction conditions. Pandian et al (2018), successfully isolated genomic DNA from a medicinal plant *Gymnena slyvestre*.

The phenol-chloroform method (Ahmed et al., 2018) used a combination of phenol and chloroform to separate DNA from other cellular components. It is one of the most commonly used methods for DNA isolation in molecular biology (Gautam 2022). The method starts by homogenizing the plant tissue in a lysis buffer, followed by the addition of phenol and chloroform. After centrifugation, the DNA is recovered from the aqueous phase, and further purified by precipitation with ethanol. Dieki et al. (2022) compared six different DNA extraction methods in which the phenol-chlorofom extraction method was one of them.

The phenol-chloroform method is a widely used and well-established protocol for DNA extraction from various sources, including plant tissue (Choudhary et al. 2020). The method is based on the principle of differential solubility of DNA, proteins, and lipids. Phenol is a strong organic solvent that is used to dissolve proteins and lipids, while DNA remains in the aqueous phase. The DNA is then precipitated by the addition of an equal volume of chloroform, which separates the DNA from the aqueous and organic phases. The DNA is then recovered by centrifugation and purified by ethanol precipitation. The selection of DNA extraction methods for plant tissue is challenging, as plants have a complex cell wall structure, which can make it difficult to extract DNA efficiently. While there are several DNA extraction methods available e.g. (Sanger & Coulson 1975 and Dawodu et al. 2020), each method has its advantages and disadvantages. Some methods may be time-consuming or require expensive equipment, while others may yield low-quality DNA or be ineffective for certain plant species.

Although several DNA extraction methods are available, there is a need to evaluate the efficiency and reliability of the phenol chloroform method for isolating DNA from specific plant tissue samples. The phenol chloroform method is a widely used DNA extraction method that has

been shown to be effective for a variety of sample types, including plant tissue. The affordability of this method makes it a sought after procedure for low income countries who cannot afford the relatively new CTAB procedure. With its wide availability and applicability, it makes DNA extraction possible for a vast range of living tissues whether plant or animal and also makes most labs capable of performing one of the most unique and useful experiments known to man (Gautam 2022). There is no selectivity involved as the simplest of labs can carry out these procedures. The experimental protocol is quite easy to follow and the whole procedure can be done within the shortest possible time!

The aim of this study is to isolate DNA from selected plant tissues using phenol chloroform method.

2. MATERIALS AND METHODS

2.1 Sample Preparation

The plant tissues used for this experiment were leaves of the plants. Five different plant leaves were picked from the surroundings of Biochemistry lab, Federal Polytechnic Ede, and used for the DNA isolation.

2.2 Materials, Apparatus and Reagents

Plant tissue (fresh or frozen), Liquid nitrogen (if using fresh tissue), Mortar and pestle (or a blender), Extraction buffer (TE buffer {1M Tris-HCl, 0.5M EDTA, pH 8.0}, 2% SDS, 2M NaCl, 1M $MgCl_2$), Phenol-chloroform-isoamyl alcohol mixture (25:24:1), Chloroform-isoamyl alcohol (24:1), Isopropanol, Ethanol (70%), centrifuge tubes, Vortex mixer or hand-held vortexer (optional), Centrifuge, Salt solution (2M NaCl).

2.3 Procedure

The phenol chloroform method for DNA isolation of plant tissue involves several steps that are critical for successful extraction of high-quality DNA. The following is a broad overview of the steps involved in the phenol chloroform method.

- The plant tissue was grounded to fine powder using a mortar and pestle.
- 2ml of 2M NaCl or 1M $MgCl_2$ solution was added per gram of plant tissue to the ground tissue and mixed well.
- An equal volume of 24:1 chloroform: isoamyl alcohol was added to the mixture and shaken vigorously.
- The mixture was transferred to a test tube and the layers allowed to separate.
- The aqueous layer (supernatant) containing the DNA was collected and transferred to a new container.
- an equal volume of phenol: chloroform: isoamyl alcohol (25:24:1) was added and shaken vigorously.
- the mixture was transferred to a test tube and the layers allowed to separate.
- the aqueous layer containing the DNA was collected and transferred to a new container.
- 1/10th volume of isopropanol was added and mixed well.
- the mixture was transferred to a centrifuge tube and spun at 10000-12000 rpm for 15 minutes at 4^0C.
- The supernatant was discarded and add 1ml of 70% ethanol per 1g of plant tissue was added and mixed well.
- The resulting mixture was transferred to a centrifuge tube and spun at 10000-12000 rpm for 15 minutes at 4^0C.
- The supernatant was again discarded and add 1ml of TE buffer per 1g of plant tissue was added and shaken well.
- 0.1ml of 2% SDS was added and mixed well.
- The mixture was transferred to a centrifuge tube and spun at 10000-12000 rpm for 15 minutes at 4^0C.
- The supernatant as discarded and the pellet washed with 70% ethanol.
- The mixture was tansferred to a centrifuge tube and spun at 10000-12000 rpm for 15 minutes at 4^0C
- The supernatant was discarded and the pellet dissolved in TE buffer.
- The purified DNA is now ready for downstream applications.

3. RESULTS

Fig. 2 showed the first stage of the DNA isolation process: The plant tissue was grounded to a fine powder using a mortar and pestle. 2 ml of 2M

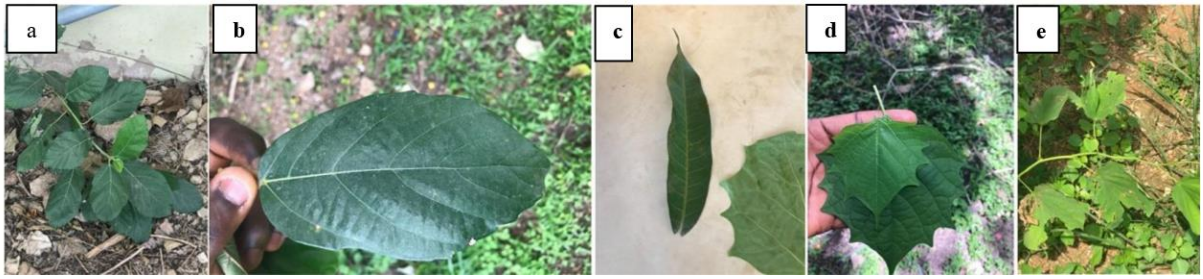

Fig. 1. Selected plants used for study: (a) *Ficus capensis* (Cape fig or Cape banyan), (b) *Ficus exaspirata* (Forest Sandpaper Fig), (c) *Mangifera indica* (Mango), (d) *Gmelina arborea* (Gamhar), and (e) *Bauhinia purpurea* (Butterfly tree)

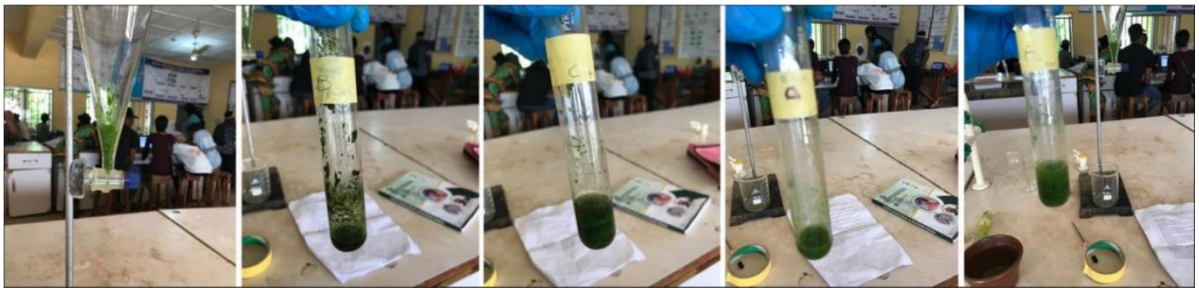

Fig. 2. First stage of the DNA isolation process grounding of plant tissue into powder and mixture with NaCl and chloroform:isoamyl alcohol

Fig. 3. Separation of organic from aqueous phase in mixture after addition of chloroform:isoamyl alcohol

NaCl was added per gram of plant tissue to the grounded tissue, and the mixture was mixed well. The addition of the NaCl salt solution is to stabilize the DNA and facilitate its binding to other molecules later in the extraction process. An equal volume of (24:1) Chloroform:Isoamyl alcohol was added to the mixture and shaken vigorously. The addition of Chloroform:isoamyl alcohol (24:1) mixture to the plant tissue homogenate is to extract DNA from the cell lysate. Chloroform denatures proteins and disrupts the cell membranes, while isoamyl alcohol helps prevent foaming.

In Fig. 3 the mixture was transferred to a centrifuge tube and spun the layers allowed to separate. The use of the centrifuge is to separate the aqueous and organic phases in the mixture after the addition of chloroform:isoamyl alcohol. The aqueous layer contains DNA, while the organic layer contains proteins and other contaminants. The aqueous layer containing the DNA was collected and transferred to a new container.

In Fig. 4, an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1) was added and shaken vigorously. The addition of phenol:chloroform:isoamyl alcohol (25:24:1) mixture to the aqueous layer is to further purify the DNA by removing any remaining proteins and other contaminants. The mixture was hen transferred to a separatory funnel and the layers allowed to separate. The aqueous layer

containing the DNA was again collected and transferred to a new container.

In Fig. 5, 1/10th volume of isopropanol was added to the mixture and mixed well. The inclusion of Isopropanol is to precipitate the DNA out of solution by binding to it and causing it to clump together. The mixture was transferred to a centrifuge tube and centrifuged at 10,000-12,000 rpm for 15 minutes at 4°C. The centrifuge was used to separate the precipitated DNA from the solution by applying a high force of gravity, with the heavier DNA molecules settling at the bottom of the tube.

Fig. 4. Further purification of DNA using phenol:chloroform:isoamyl alcohol and separation of organic from aqueous phase in mixture after addition of chloroform:isoamyl alcohol

Fig. 5. Precipitation of DNA using isopropanol and centrifugation at 10,000-12,000 rpm for 15 minutes at 4°C

Fig. 6. Washing of DNA pellet with ethanol and centrifugation at 10,000-12,000 rpm for 15 minutes at 4°C

Fig. 7. Final step of DNA isolation process pellet was dissolved with TE buffer, purified with SDS, centrifuged, washed with ethanol, centrifuged and finally dissolved in TE buffer

In Fig. 6, the supernatant was discarded, and 1 ml of 70% ethanol per 1 g of plant tissue was added and mixed well. The addition of ethanol was used to wash the DNA pellet to remove any remaining salts or contaminants that may have co-precipitated with the DNA. Then the mixture was transferred to a centrifuge tube and centrifuged at 10,000-12,000 rpm for 15 minutes at 4°C.

In Fig. 7, the supernatant was discarded, and 1 ml of TE buffer per 1 g of plant tissue was added and mixed well. The TE buffer is a buffer solution used to dissolve the DNA pellet, stabilizing the DNA in a pH-controlled solution. 0.1% of 2% SDS was added and mixed well. SDS is a detergent used to break down any remaining proteins that may still be attached to the DNA, further purifying it. The mixture was transferred to a centrifuge tube and centrifuged at 10,000-12,000 rpm for 15 minutes at 4°C.The centrifugation helped to remove any residual insoluble material that may still be present in the sample. The supernatant was discarded, and the pellet was washed with 70% ethanol. This step is repeated to remove any residual salts and contaminants that may still be present in the sample. The mixture was transferred to a centrifuge tube and spun at 10000-12000 rpm for 15 minutes at 4°C. This centrifugation step helped to remove any residual ethanol from the sample. The supernatant was discarded and the pellet dissolved in TE buffer.

4. DISCUSSION

The aim of this study was to isolate DNA from different plant species using the phenol chloroform method. The selected plant species were (a) *Ficus capensis* (Cape fig or Cape banyan), (b) *Ficus exaspirata* (Forest Sandpaper Fig), (c) *Mangifera indica* (Mango), (d) *Gmelina arborea* (Gamhar), and (e) *Bauhinia purpurea* (Butterfly tree) as shown in Fig. 1.

Fig. 2 illustrates the first stage of the DNA isolation process, which involved grinding the plant tissue to a fine powder and adding 2M NaCl to stabilize the DNA and facilitate its binding to other molecules later in the extraction process. The addition of chloroform:isoamyl alcohol (24:1) mixture helps to extract DNA from the cell lysate by denaturing proteins and disrupting cell membranes. The aqueous layer containing the DNA was collected and transferred to a new centrifuge tube.

Fig. 3 shows the separation of organic from aqueous phase after the addition of chloroform:isoamyl alcohol. The aqueous layer contained DNA, while the organic layer contains proteins and other contaminants. The aqueous layer containing the DNA was collected and transferred to a new container.

Further purification of the DNA was done using phenol:chloroform:isoamyl alcohol (25:24:1) mixture, as shown in Fig. 4. This step removes any remaining proteins and other contaminants from the DNA. The DNA was then precipitated using isopropanol and collected through centrifugation, as shown in Fig. 5.

After washing the DNA pellet with 70% ethanol, as shown in Fig. 6, the pellet was dissolved in TE buffer containing SDS to further purify the DNA and remove any remaining proteins attached to it. The centrifugation step helped to remove any residual insoluble material that may still be present in the sample. This process was repeated to ensure that all salts and contaminants were removed, and the DNA was finally dissolved in TE buffer.

The first successful isolation of DNA was achieved by Friedrich Miescher (1971) in 1869, who extracted a viscous substance from the nuclei of white blood cells that he called "nuclein".

Some of the early methods (Avery et al. 1944) involved the use of organic solvents such as phenol and chloroform, which were found to be effective in separating DNA from other cellular components.

The phenol chloroform method is one of the most widely used techniques for DNA isolation from plant tissues. The method involves the use of phenol and chloroform, which are organic solvents that denature proteins and disrupt lipid membranes, respectively but it has some disadvantages, such as the use of toxic and hazardous chemicals and the time-consuming nature of the process. Other DNA isolation methods have been developed to overcome these limitations, including the CTAB (cetyltrimethylammonium bromide) method, the silica-based method, and the magnetic bead-based method (De Silva et al. 2024). Infact another study used the phenol-chloroform free method to extract nucleic acids from recalcitrant woody species for gene expression and sequencing (Barbier et al. 2019).

The CTAB method is a popular alternative to the phenol chloroform method for DNA isolation from plant tissues. The CTAB method has the advantage of using non-toxic reagents and is less time-consuming than the phenol chloroform method.

Several studies have employed the phenol chloroform method for DNA isolation from various plant tissues. For instance, in a study by Pandian et al. (2018), the authors used the phenol chloroform method to extract DNA from leaves of the medicinal plant *Gymnema sylvestre*. The extracted DNA was used for PCR amplification of the ITS region, and the results showed that the phenol chloroform method yielded high-quality DNA that was suitable for downstream applications. However, it should be noted that the quality and yield of the extracted DNA can vary depending on the type of tissue and the protocol used for extraction.

In another study, Ravi et al. (2017) used the phenol chloroform method to isolate DNA from the leaves of the bamboo species *Bambusa vulgaris.* The authors compared the phenol chloroform method with the CTAB method and found that the former yielded higher quality DNA with better purity and yield. The extracted DNA was used for PCR amplification of the rbcL gene, and the results showed successful amplification.

Similarly, in a study by Elangbam et al. (2014) the authors used the phenol chloroform method

to isolate DNA from the leaves of the medicinal plant *Andrographis paniculata.* The extracted DNA was used for PCR amplification of the ITS region, and the results showed that the phenol chloroform method yielded high-quality DNA that was suitable for downstream applications.

These studies demonstrate the efficacy of the phenol chloroform method for DNA isolation from various plant tissues and its suitability for downstream applications such as PCR amplification. However, it should be noted that the quality and yield of the extracted DNA can vary depending on the type of tissue and the protocol used for extraction.

Furthermore, while the phenol chloroform method has been widely used for DNA isolation, it has some limitations that need to be considered. For example, the use of toxic and hazardous chemicals such as phenol and chloroform raises safety concerns, and their disposal can have environmental impacts. Also, the method is time-consuming and requires multiple steps, including the removal of protein and polysaccharides, which can lead to low yield and purity of DNA. In contrast, the CTAB method has been reported to be more efficient and less time-consuming[16], as it involves fewer steps and uses non-toxic reagents. Several studies have compared the phenol chloroform method with the CTAB method and found that the latter can yield high-quality DNA with better purity and yield in some cases [6]. For instance, in a study by Sahoo et al. (2019), the authors compared the phenol chloroform method and the CTAB method for DNA isolation from the leaves of the medicinal plant *Boerhaavia diffusa*. The results showed that the CTAB method yielded higher quality DNA with better purity and yield compared to the phenol chloroform method. Moreover, silica-based methods and magnetic bead-based methods[12] have been developed as alternatives to the phenol chloroform method, as they are faster, easier, and more reproducible (Kiss et al. 2024). These methods use solid-phase extraction techniques, where DNA is selectively adsorbed onto silica particles or magnetic beads and then eluted with a buffer solution. The silica-based method has been reported to be highly efficient for DNA isolation from plant tissues, including recalcitrant ones such as woody tissues, and is less time-consuming than the phenol chloroform method. In a study by Fang et al. (2019) the authors used the silica-based method to extract DNA from the leaves of the tea plant *Camellia sinensis.* The extracted DNA was used for PCR amplification of the rbcL gene, and the results showed successful amplification with high yield and purity. In summary, the phenol chloroform method has been widely used for DNA isolation from various plant tissues and has been reported to yield high-quality DNA suitable for downstream applications (Ahmed et al. 2018). However, the method has some limitations such as the use of toxic and hazardous chemicals and its time-consuming nature. Alternative methods such as the CTAB method, silica-based method (Lai et al. 2012), and magnetic bead-based method (Kawasaki et al. 2010) have been developed to overcome these limitations and have been reported to be more efficient, less time-consuming, and use non-toxic reagents. The choice of method should depend on the type of tissue, the downstream application, and safety considerations. Even though the phenol-chloroform method has its limitations, it is still relevant to present day and even futuristic researches as confirmed by Dieki et al., (2022) in their study 6 possible methods for DNA extraction was considered and the phenol-chloroform method was as good as the CTAB method in terms of percentage purity, DNA concentration and even gel electrophoresis as it gave clear bands. Till today it's a method worthy of note as expertise usage is not a criteria as confirmed by Gautam (2022).

5. CONCLUSION

In this study, the experiment showing phenol chloroform method for isolating DNA from selected plant tissue was carried out successfully. The results showed that the phenol chloroform method is an effective DNA extraction method for various plant tissue samples.

6. RECOMMENDATIONS

Based on the findings of this study, the following recommendations are made:

1. It is recommended that researchers should carefully select the appropriate DNA extraction method depending on the plant species and tissue type being used. The phenol chloroform method is effective for various plant tissue samples, but its efficiency and reliability can be affected by factors such as tissue type and quality of the starting material.
2. To obtain high-quality DNA using the phenol chloroform method, it is

recommended that researchers optimize the protocol by varying the extraction conditions such as incubation time, temperature, and the ratio of the phenol-chloroform mixture to the aqueous phase.

3. It is recommended that researchers should carefully select the plant tissue samples, taking into account the plant species and tissue type, as this can significantly affect the yield and quality of the extracted DNA.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

The authors hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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