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Full Length Research

Effects of Preservation Methods on Nucleic Acid Yield and Integrity from African Yam Bean (*Sphenostylis Stenocarpahochst. Ex A.* Rich.) Tissues for Sustainable Molecular Research

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ABSTRACT

The African yam bean (AYB) (*Sphenostylis stenocarpa* Hochst. Ex A. Rich.) is an underutilized legume native to sub-Saharan Africa, valued for its edible seeds and tubers rich in proteins, carbohydrates, and essential micronutrients. This study aimed to optimize cost-effective preservation methods for African Yam Bean leaf tissues to ensure high-quality Deoxyribonucleic acid extraction, critical for molecular biology applications. African Yam Bean leaves were subjected to various preservation conditions, including chemical buffers (1× CTAB, 1× TAE, and 70% ethanol) and temperature settings (-20°C, 4°C, 25°C, and >25°C). Results indicated that freezing at -20°C yielded the highest Deoxyribonucleic acid (DNA) purity and stability, with consistent A_{260}/A_{280} ratios (1.7–2.0). Ethanol preservation also demonstrated strong performance, providing a viable alternative for resource-constrained environments. The DNA preserved with cetyltrimethylammonium bromide and Tris-acetate-ethylenediaminetetraacetic acid buffers exhibited lower stability and increased contamination over time. These findings highlight practical and scalable methods to enhance DNA integrity from plant tissues, advancing the utility of AYB in molecular biology research and sustainable agriculture.

Keywords: African yam bean, DNA extraction, molecular biology, preservation methods, sustainable agriculture.

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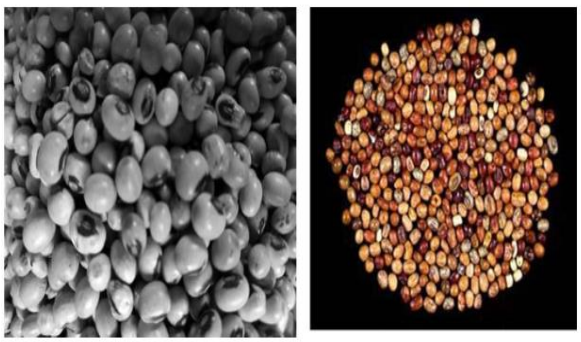
INTRODUCTION

The African yam bean (*Sphenostylis stenocarpa*) is a nutritionally rich but underutilized legume native to sub-Saharan Africa. The African yam bean belongs to the family Leguminosae (Fabaceae), an annual, climbing or prostrate vine (Plate 1). This resilient crop produces both edible seeds and tubers and can be found in different varieties as shown in Plate 2. The crop is a valuable source of protein, carbohydrates, and micronutrients such as iron and zinc. Despite its high nutritional potential, the crop remains marginalized due to limited awareness, inadequate breeding programs, and its association with traditional, subsistence-level farming (Gbenga-Fabusiwa, 2021: Baiyeri et al., 2018). The African yam bean is highly adaptable, thriving in diverse and often marginal soils with minimal inputs, making it a promising candidate for improving food security in resource-constrained regions (Oagile et al., 2012). Its seeds are comparable to other legumes in nutritional content, while the tubers provide additional dietary energy, creating a dual-purpose crop with significant potential for food diversification (Adewale & Nnamani, 2022). This crop is also recognized for its contributions to sustainable agriculture. African yam bean nodulates with rhizobia, enhancing nitrogen fixation and reducing dependency on synthetic fertilizers (Assefa & Kleiner, 1997). However, its broader adoption is hindered by challenges such as limited processing knowledge, anti-nutritional factors, and insufficient research into its genetic diversity and agronomic practices (Gbenga-Fabusiwa, 2021).

In light of global efforts to combat malnutrition and diversify food systems, the African yam bean offers a unique opportunity to integrate an overlooked crop into modern agricultural and nutritional strategies. Its potential for genetic improvement, coupled with its adaptability and nutritional value, makes it a promising candidate for addressing the dual challenges of food security and sustainable farming in the face of climate change (Shitta et al., 2021).

Plate. 1. African Yam Bean



A **B**

Plate. 2. Varieties of African Yam Bean seeds (Gbenga-Fabusiwa, 2021)

An oval shaped cream colour variety with black eye colour (A) A reddish brown variety with black eye colour (B)

Nucleic acid (NA) extraction is a cornerstone of molecular biology, enabling a wide array of applications in research, diagnostics, and therapeutic development. The quality and purity of extracted Deoxyribonucleic acid (DNA) and Ribonucleic acid (RNA) are critical for the accuracy of downstream molecular techniques, including polymerase chain reaction (PCR), next-generation sequencing (NGS), and gene expression analysis (Widen & Silbert, 2016). Emerging technologies, such as automated and portable systems, are making nucleic acid extraction more accessible. These innovations are critical for point-of-care diagnostics, enabling rapid, efficient preparation of nucleic acids even in resource-limited settings (Paul et al., 2020). Extraction of high-quality DNA in terms of quality and quantity is necessary for molecular biology studies. Generally, samples are obtained fresh and their DNA extracted for research purposes, but in a situation where the place of sample collection is far from the laboratory, the need for preservation therefore arises. Preservation methods are complicated and expensive and its apparatus is hard to come by, as well as the possibility of health hazards issues developing from them. For instance -80^{0}C freezer is very expensive and not readily found in institutions in developing countries like Nigeria. Naphthalene, a preservative is both carcinogenic and dangerous to the eyes; liquid nitrogen which can be used to freeze-dry plant materials is volatile and needs optimal direction when used. There is therefore a need for alternative preservation methods. Works of literature have shown that silica gel desiccation of leaf samples and immersion in Nacl-Cetyltrimethylammonium bromide (CTAB) solutions have been effective. Quality DNA extracts were obtained from tissues preserved for over a month using these methods (Chase & Hills, 1991; Bhattacharjee et al., 2009). Recently Johnson et al., 2023 reported that leaf samples preserved in ethanol, particularly 96% ethanol, demonstrate superior DNA quality but the procedure was combined with proteinase digestion.

This research aimed to develop cost-effective preservation methods for plant samples before DNA extraction, focusing on alternatives to those commonly reported in the literature. By addressing the challenges of implementing sophisticated and expensive techniques in developing countries, this study seeks to offer a broader range of practical options for researchers in molecular biology.

MATERIALS AND METHODS

Source of Plant Materials: African yam bean (*Sphenostylis stenocarpa* Hochst. Ex A. Rich.) leaves were collected from the Botanical Garden of the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Edo State, Nigeria. They were authenticated at the departmental herbarium by the curator and assigned an ID number of UNIBEN/PSBBG/010624.

Chemicals and Reagents: The preservation buffers used were obtained from the Nigerian Institute for Oil Palm Research (NIFOR), Nigeria. They include 1× CTAB, 1× Tris-acetate- ethylenediaminetetraacetic acid, and 70% ethanol. The DNA extraction

buffer was prepared as 10× CTAB, comprising 0.5 M Tris, 5 M NaCl, 0.3 M ethylenediaminetetraacetic acid, 10% CTAB, and 20 g Polyvinylpyrrolidone (PVP) . Additional reagents included chloroform: phenol (1:1), ice-cold 100% ethanol and 70% ethanol as precipitation buffers, sterile water for dissolving DNA, and TAE buffer (0.89 M Tris, 0.89 M acetic acid, 25 mM EDTA, pH 8.3) as a loading buffer. Ethidium bromide was used as the staining dye, and agarose was utilized for gel electrophoresis.

Sample Preservation: After collection, the leaves were preserved under various conditions to study their effects on DNA integrity. Specifically, preservation was carried out at four temperature settings (-20°C, 4°C, 25°C, and above 25°C in sunlight) and in three chemical buffers (1× CTAB, 1× TAE, and 70% ethanol). The samples were stored for durations of 3, 6, 9, and 12 days. Leaves from each condition were retrieved at these intervals and prepared for DNA extraction.

DNA Extraction: DNA extraction was performed using a CTAB-based protocol adapted from NACGRAB. Preserved leaf tissue of 0.3g was ground with 2 mL of preheated 10× CTAB buffer and 20 mg of acid-washed sand using a mortar and pestle, which were preheated at 65°C. The resulting homogenate was transferred into microfuge tubes and incubated at 65°C for 15 minutes in a water bath. The suspension was centrifuged at 12,500 rpm for 5 minutes at 4°C, and the supernatant was carefully transferred into a fresh tube. Chloroform: phenol (1:1) was added to the supernatant, mixed thoroughly, and centrifuged at 14,000 rpm for 5 minutes at 4°C. The aqueous phase was transferred to a new tube, and DNA was precipitated by adding 0.8 mL of ice-cold 100% ethanol and incubating the mixture at -20°C for 1 hour. After centrifugation at 13,000 rpm for 20 minutes at 4°C, the pellet was washed three times with 500 µL of 70% ethanol, followed by centrifugation at 1,000 rpm for 3 minutes at 4°C. The DNA pellet was air-dried and dissolved in 100 µL of sterile water.

Spectrophotometric Analysis: DNA purity and yield were determined using Ultraviolet Spectrophotometry. To blank the spectrophotometer, 1.5 mL of sterile water was used in a cuvette. For analysis, 20 µL of each DNA extract was diluted in 1.48 mL of sterile water, and absorbance readings were taken at 260 nm and 280 nm. Triplicate readings were recorded for each sample. DNA purity was assessed using the A_{260}/A_{280} ratio, with values between 1.7 and 2.0 considered pure. The applied purity keys were: 1.7–2.0 for pure DNA, <1.7 for protein contamination, and >2.0 for RNA contamination.

DNA concentration was calculated using the formula: Concentration (ng/µL) = A_{260} × Dilution Factor × 50-

Where A_{260} represents absorbance at 260 nm, the Dilution Factor accounts for sample dilution, and 50 is a standard conversion factor for double-stranded DNA.

The DNA yield was determined by multiplying the concentration by the total

volume of the extract and dividing by 1000 to convert from nanograms (ng) to micrograms (µg).

Agarose Gel Electrophoresis: To verify the integrity of the extracted DNA, agarose gel electrophoresis was performed. A 0.8% agarose gel was prepared by dissolving 0.8g of agarose in 100mL TAE buffer, followed by the addition of 1 µL of ethidium bromide. The gel was carefully poured into a tray with a well comb and allowed to set and be cooled at room temperature for 20 minutes. DNA samples (8 µL) were mixed with 2 µL of loading dye, loaded into the gel wells, and subjected to electrophoresis under an electric current at 80V for 1 hour. DNA bands were visualized under UV light, and their clarity and consistency were assessed to determine DNA integrity.

Statistical Analysis: Significant differences between groups were assessed using Analysis of Variance (ANOVA) with a significance threshold of $p < 0.05$. The yields were averages calculated from triplicate experiments, with error margins reflecting the standard error of the mean (SEM).

RESULTS

Nucleic Acid Yield from Different Preservation Methods

Chemical Preservation

The nucleic acid yield was monitored across twelve (12) days using three chemical preservation methods, which were the preservation of African yam bean leaf tissues in 70% Ethanol, 1× TAE, and 1× CTAB. Nucleic acid yield under chemical preservation varied significantly across the methods with a threshold of $p < 0.05$.

Table 1 shows the nucleic acid yield in µg/g from the chemical preservation method while Figure 1 presents a graphical representation of DNA yields obtained from the same preservation process. The 70% Ethanol preservation method showed consistent yields initially, but a marked decline on Day 12. The 1× TAE preservation method maintained stable yields until Day 9, followed by a decline, while 1× CTAB showed fluctuating yields, peaking significantly on Day 9.

Table 1. Nucleic Acid Yield from Chemical Preservation Method

Time (Day)	70% Ethanol (µg/g)	1× TAE (µg/g)	1× CTAB (µg/g)
3	20 ± 1.5	44 ± 3.2	13 ± 0.9
6	33 ± 2.1	47 ± 2.8	8 ± 0.6
9	59 ± 3.8	28 ± 2.0	126 ± 6.4
12	-2 ± 0.2	-3 ± 0.3	12 ± 0.8

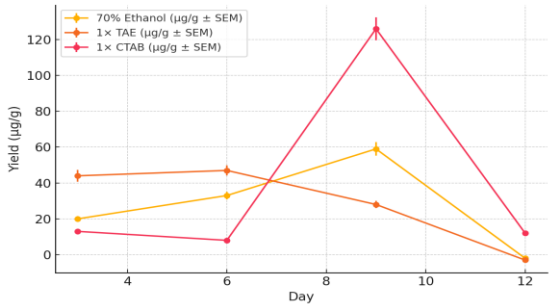


Fig. 1. Trends in Nucleic Acid Yield (Chemical Preservation).

Temperature Preservation: Temperature preservation revealed that -20°C consistently maintained the highest yield, though there was variability on Day 6. Preservation at higher temperatures (>25°C) and 4°C showed reduced yields and lower stability over time. Table 2 shows the results obtained for nucleic acid yield isolated from AYB leaf tissues preserved in different temperature conditions for 3, 6, 9 and 12 days while Figure 2 is a graphical visualization of the various nucleic acid yields obtained from tissues preserved in the same temperature preservation methods and days.

Table 2. Nucleic Acid Yield from Temperature Preservation Method

Time (Day)	-20°C (µg/g)	4°C (µg/g)	25°C (µg/g)	>25°C (µg/g)
3	87 ± 5.4	4 ± 0.3	58± 3.8	31 ± 2.1
6	5 ± 0.5	22 ± 1.7	38 ± 2.9	58 ± 3.2
9	70 ± 4.2	55 ± 3.3	170 ± 8.5	125 ± 6.2
12	45 ± 3.2	40 ± 2.8	20 ± 1.4	16 ± 0.9

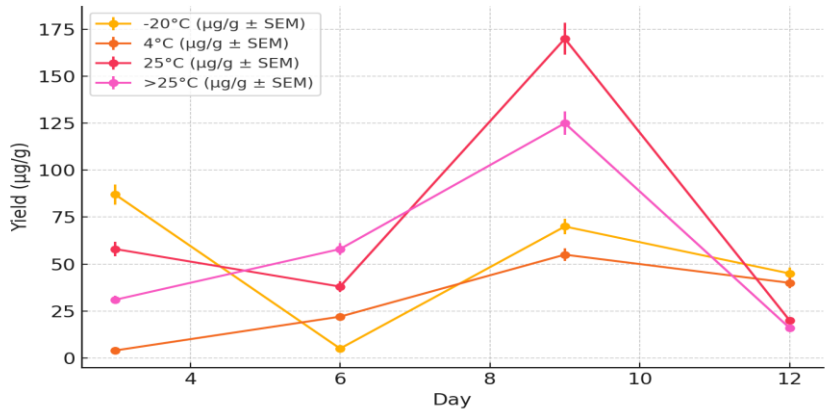


Fig. 2. Trends in Nucleic Acid Yield (Temperature Preservation)

Nucleic Acid Purity across Preservation Methods: The purity of DNA was evaluated using the A_{260}/A_{280} ratio, which indicated contamination by proteins or RNA.

Chemical Preservation: DNA purity varied across different preservation methods, with a noticeable decline by Day 12. The use of 1× TAE demonstrated inconsistent purity levels, with evidence of both protein contamination (ratio <1.7) and RNA contamination (ratio >2.0) over the observation period. Table 3 shows the effect of three chemical treatments on nucleic acid purity for 3, 6, 9, and 12 days.

Table 3. Nucleic Acid Purity Ratios for Chemical Preservation

Time (Day)	70% Ethanol	1× TAE	1× CTAB
3	2.0 (Pure)	0.2 (<1.7, Protein)	1.7 (Pure)
6	2.4 (>2.0, RNA)	0.6 (<1.7, Protein)	2.1 (>2.0, RNA)
9	1.8 (Pure)	1.8 (Pure)	1.4 (<1.7, Protein)
12	- (No Data)	- (No Data)	2.8 (>2.0, RNA)

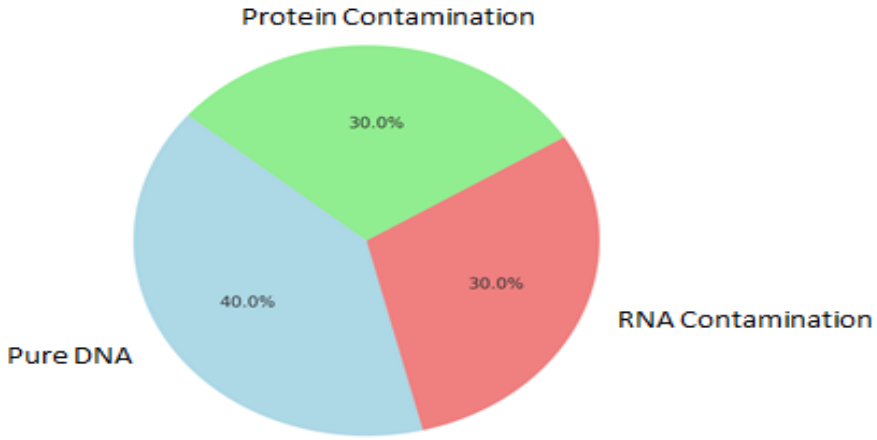


Fig. 3. Purity Trends in Chemical Preservation Method

Figure 3 shows that pure DNA accounts for the largest proportion of samples, followed by RNA and protein contamination in nearly equal amounts. RNA contamination represents a significant fraction, indicating frequent co-extraction of RNA alongside DNA.

Temperature Preservation

DNA stored at -20°C consistently retained high purity across all time points, with A_{260}/A_{280} ratios remaining within the optimal range of 1.7–2.0. Conversely, elevated temperatures, especially >25°C, led to increased RNA contamination, as indicated by ratios exceeding 2.0. Table 4 shows the effect of the temperature preservation method on nucleic acid purity for varying numbers of days.

Table 4. DNA Purity Ratios for Temperature Preservation

Time (Day)	-20°C	4°C	25°C	>25°C
3	1.8 (Pure)	1.6 (<1.7, Protein)	1.9 (Pure)	1.4 (<1.7, Protein)
6	1.9 (Pure)	2.3 (>2.0, RNA)	1.8 (Pure)	2.2 (>2.0, RNA)
9	2.0 (Pure)	1.9 (Pure)	1.5(<1.7, Protein)	1.8 (Pure)
12	2.0 (Pure)	1.9 (Pure)	2.8 (>2.0, RNA)	3.0 (>2.0, RNA)

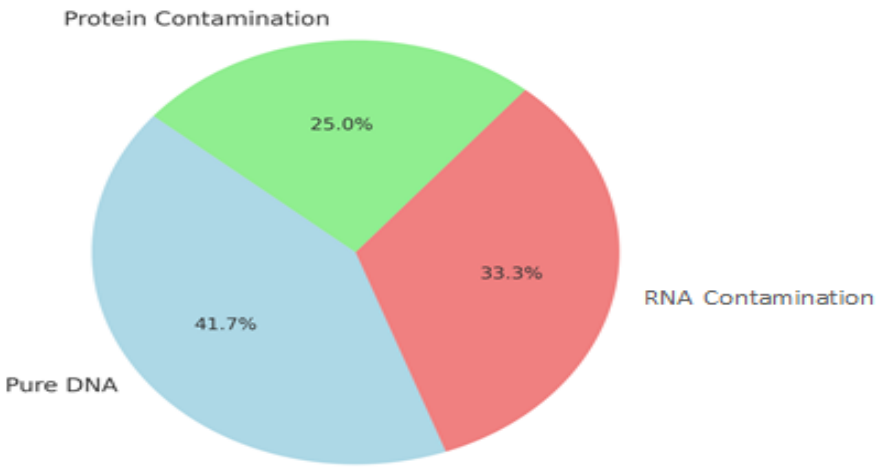


Fig. 4. Purity Trends in Temperature Preservation Method.

Figure 4 shows that pure DNA is the most prevalent, indicating that optimal storage temperature conditions can effectively maintain nucleic acid integrity.

DNA Integrity Check by Gel Electrophoresis: This method was selected due to the specificity of ethidium bromide staining for DNA, enabling clear visualization of nucleic acid bands. Figure 5 below illustrates the DNA profiles obtained from samples preserved under different conditions at intervals of 3, 6, 9, and 12 days. Each lane represents one of the four temperature conditions (arranged in the order: -20°C, 4°C, 25°C, and >25°C) and one of the three chemical preservation methods (arranged in the order: 1× CTAB, 1× TAE, and 70% ethanol).

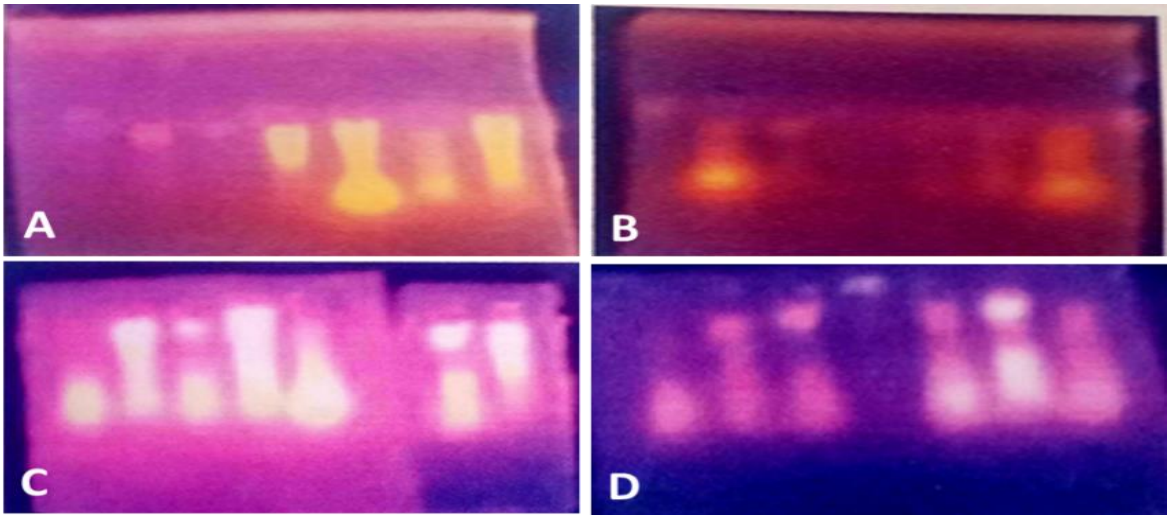


Fig. 5. *Agarose electrophoresis gel images of nucleic acid extracts from preserved tissues of African yam bean (Sphenostylis stenocarpa). Samples preserved for 3 days (A) 6 days (B) 9 days (C) and 12 days (D) providing acomparative view of nucleic acid integrity over time under different preservation conditions.*

DISCUSSION

This study provides an in-depth evaluation of preservation methods for African yam bean (AYB) leaf tissues, contributing to a growing body of knowledge on sample preparation for molecular biology. The results confirmed that both the preservation method and duration had a significant impact on nucleic acid yield and purity. The findings indicate that chemical preservation methods demonstrated varying effectiveness in maintaining DNA yield and purity over time. Ethanol preservation proved highly effective initially, maintaining DNA integrity with minimal contamination. However, DNA yield declined significantly by Day 12. This result aligns with Johnson et al. (2023) and Bressan et al. (2014) who highlighted the effectiveness of ethanol for preserving recalcitrant plant species, though their methodology incorporated additional proteinase digestion, suggesting a potential refinement for ethanol-based protocols. The 1× TAE buffer demonstrated stable yields until Day 9, after which a decline was observed. Tris-based buffers like TAE have been reported to interact with DNA, altering its migration properties and potentially leading to instability (Stellwagen et al., 2000). The 1× CTAB method exhibited fluctuations, peaking significantly on Day 9. This transient peak observed in CTAB-preserved samples on Day 9 might reflect initial stabilization by its detergent properties, which degraded over time, leading to renewed enzymatic activity and reduced DNA stability. This aligns with findings that CTAB enhances

DNA integrity initially by binding to nucleic acids and reducing nuclease activity but can later cause DNA shearing or contamination due to residual chemicals (Guertler et al., 2013). The decline in DNA yield with CTAB and TAE buffers could be linked to their inability to adequately inhibit enzymatic activity over extended durations. Additionally, these variations suggest that buffer selection is critical for maintaining nucleic acid integrity (Carey et al., 2023).

Temperature preservation results showed that -20°C consistently maintained the highest yield and DNA stability over time, with some variability on Day 6 which may be due to differences in sample handling, pipetting errors, or variations in extraction efficiency on that particular day. In addition, samples frozen over time may experience phase separation or ice layering, leading to uneven distribution of nucleic acids within the sample. This could make DNA extraction inconsistent on different days (Cordsmeier & Hahn, 2022). The superior performance of -20°C freezing in preserving DNA integrity likely comes from its ability to arrest enzymatic activities and stabilize macromolecular structures, thereby minimizing degradation. Studies have shown that freezing at -20°C maintains DNA yield and quality over extended periods, comparable to fresh samples, by preventing enzymatic hydrolysis and oxidative damage (Wood & Wang, 2024).

However, repeated freeze-thaw cycles have been reported to cause progressive DNA degradation, particularly for high-molecular-weight fragments (Shao et al., 2012). Furthermore, Bainard et al. (2010) reported that freezing at -20°C effectively preserved DNA integrity across diverse plant tissues. Preservation at higher temperatures (>25°C) and 4°C resulted in reduced yields and increasing contamination. These observations align with Paul et al. (2020), who demonstrated that high temperatures accelerate nucleic acid degradation through enzymatic and oxidative pathways.

The work of Michaud and Foran (2011) also supports these findings for preservation in lower and higher temperatures, showing that refrigerated storage was intermediate in effectiveness while desiccation was least effective for long-term preservation. DNA purity evaluation showed that the chemical preservation methods exhibited varying DNA purity levels. Ethanol generally maintained purity but showed a noticeable decline by Day 12. Ethanol's efficacy in DNA preservation may be attributed to its ability to dehydrate the cellular matrix, effectively halting enzymatic functions

responsible for DNA breakdown. Ethanol has been demonstrated to maintain DNA stability by preventing hydrolytic degradation and protein-DNA interactions, particularly in plant and insect specimens (Marquina et al., 2021). The 1× TAE method exhibited inconsistent purity levels, with evidence of protein contamination ($A_{260}/A_{280} < 1.7$) and RNA contamination ($A_{260}/A_{280} > 2.0$) over time. The 1× CTAB method also showed fluctuations, with protein and RNA contaminations becoming evident at later time points. The results suggest that the peak in terms of DNA yield observed on Day 9 from samples preserved in 1× CTAB were contaminated by protein and therefore not fit for downstream experiments (Guertler et al., 2013).

DNA stored at -20°C consistently retained high purity, with A_{260}/A_{280} ratios remaining within the optimal range of 1.7–2.0 across all time points. In contrast, elevated temperatures (>25°C) resulted in increased RNA contamination, as indicated by ratios exceeding 2.0. This is in line with Sadler & Khodavirdi (2015), who demonstrated that viable RNA could be extracted from tissue samples stored at room temperature for up to three months, challenging the assumption that RNA rapidly degrades at high temperatures. The results further suggest that higher temperatures may promote RNA retention rather than degradation under certain conditions. Protein contamination, though slightly less frequent, remained a concern, implying that some preservation methods may not sufficiently inhibit protein carryover (McNevin, 2016).

Overall, Figures 3 and 4 showed that the temperature preservation method yielded purer DNA (41.1%) compared to chemical preservation (40%). The distribution highlights the influence of temperature on nucleic acid purity and the need for precise storage conditions to minimize contamination. This study also highlights that preservation methods influence not only the quantity of extracted DNA but also its usability for downstream applications. Freezing and ethanol preservation produced DNA with high purity, suitable for sensitive molecular techniques like PCR and sequencing, whereas DNA preserved with CTAB and TAE exhibited contamination, potentially limiting its use.

While the study establishes the efficacy of specific preservation methods, some limitations require consideration. The influence of secondary metabolites in AYB leaves on DNA extraction efficiency remains an open question. These metabolites might interact with preservation agents, influencing yield and purity. Additionally, the variability

introduced by manual handling during preservation and extraction, though minimized through standard protocols, could be further refined with automated processes.

Future research should explore testing these methods across other underutilized and recalcitrant species to validate their efficacy. Another area should be in investigating the interactions between plant metabolites and preservation chemicals to improve DNA yield and purity.

CONCLUSION

This study identifies freezing at -20°C as the most effective preservation method for African yam bean leaf tissues in terms of DNA yield and purity, consistently producing DNA suitable for molecular applications. Ethanol preservation also demonstrated excellent performance, providing a viable, cost-effective alternative for resource-limited environments. In contrast, preservation using CTAB and TAE buffers showed limitations, with reduced DNA stability over time. These findings provide a practical framework for enhancing molecular biology preservative research in developing countries and contribute to the broader goal of integrating underutilized crops into sustainable agricultural systems.

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