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EXTRACTION OF HIGH-QUALITY GENOMIC DNA FROM *Mansonia altissima* FOR MICROSATELLITE ANALYSIS

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ABSTRACT

This study was carried out to compare the deoxyribonucleic acid (DNA) extracted from fresh and dry leaves of Mansonia altissima with DNA extracted from fresh and dry cambium of the same species. Genomic DNA was extracted using Cetyltrimethylammonium bromide (CTAB) and DNA quantification was done using a UV Spectrometer. From the results obtained, fresh leaf and dried cambium samples had the highest DNA purity of 1.18 and 1.19 (at an absorbance ratio of A260/280) and 1.15 and 1.11 (at an absorbance ratio of $A_{260/230}$), respectively. DNA obtained from dried leaf samples had the lowest purity. The samples from fresh leaf and dried cambium had the highest concentrations of 56.77µg/ml and 55.27µg/ml respectively, while samples from fresh cambium had the lowest concentration of 13.47µg/ml. Subsequently, samples from fresh leaf and dried cambium exhibited the highest yields of 2.84µg and 2.77µg respectively, with samples from fresh cambium having the lowest yield of 0.67µg. This research therefore showed that samples from dried cambium exhibited considerably high DNA concentration and yield, and hence recommended for use for further molecular studies of the high-canopy trees of Mansonia altissima.

Keywords: Genomic DNA, Cambium, *Mansonia altissima* and Microsatellite Analysis

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INTRODUCTION

Sustainable management of forest genetic resources requires good knowledge of the genetic diversity of species (Nowakowska, 2016). Because of their longevity and wide geographic distribution, forest tree species have developed a high level of genomic heterogeneity as a genetic potential through which they adapt to the specific environmental factors of a given habitat (Hamrick, 2004; Ahuja and Neale, 2005). Human activities and changing environmental conditions have exposed many species to the threat of extinction, and many governments are aware of the need for forest management to maintain the biodiversity of locally adapted species (Nowakowska, 2016). Equally, not only endangered forest tree species but also economically important ones should be protected in a specific conservation program based on valuable genetic data (Lexer *et al.*, 2004).

Mansonia altissima (A. Chev.) is a tree species that belongs to the family of Sterculiaceae. The wood of *Mansonia altissima* is used for general and high-class joinery, cabinet work, furniture, turnery, decorative veneer, and handicrafts. It is also used in the construction of doors and windows (Osunlaja *et al.*, 2017). According to research by Jaiyeola (2019), *Mansonia altissima* was among the trees heavily exploited for their timber and has become relatively scarce in the South Western region of Nigeria.

The International Union for Conservation of Nature's (IUCN, 1998) red list of threatened

species included *Mansonia altissima* as one of the endangered valuable timber species (IUCN, 1998). To conserve its essential genetic resources, however, there is a need to understand its molecular mechanism. Microsatellite PCR offers a reliable method to assess the DNA polymorphism (genetic diversity) of numerous individuals within a species and among species of the same genus. However, all these molecular techniques require the availability of DNA in a sufficient quantity and of good quality and purity (Francois and Gerard, 2011).

The most common tissue sampled for genetic studies of tree populations has been leaf material. However, the collection of tropical tree leaf samples for molecular analysis is problematic as tropical tree species commonly reach a height of greater than 40 metres. Therefore, this study was designed to assess and compare the quality, concentration, and yield of DNA extracted from the leaves and cambium of *Mansonia altissima* in Queen's plot, Akure Forest Reserve, Ondo State.

MATERIALS AND METHODS

Sampling Procedure and Data Collection

Leaves and cambium of *Mansonia altissima* used for this experiment were collected from Queens's plot, Akure Forest Reserve, Ondo State, Nigeria. The collected samples were divided into two. Only one portion was preserved with silica gel in sealed nylon, and the other portion was kept in sealed nylon without silica gel. They were subsequently taken to the Biosafety laboratory for DNA extraction.

DNA Extraction Protocal

The extraction buffer (3x CTAB) was preheated in a water bath at 65°C, and 0.3% 2-mercaptoethanol was added to the buffer immediately before use. While the plant was still in the mortar, 800l of the 3x CTAB extraction buffer was added. The sample was transferred to a 2-ml micro-centrifuge tube, incubated in the water bath at 65^0C for 1 hour, mixed gently every 20 minutes by inverting the tube, and then cooled down to room temperature. An equal volume of chloroform: isoamyl alcohol (24:1 v/v) was added and mixed by slight inversion. The mixture was then centrifuged at 13,000rpm for 15 minutes at room temperature. The upper aqueous phase, which contained the DNA, was then transferred carefully, using a wide-bore pipette, to a new 1.5ml eppendorf tube. The volume of the aqueous phase was estimated (approximately 500µl), then half the volume (250µl) of 6M NaCl was added to the aqueous phase and mixed well. 50µl (1/10 volume of the aqueous phase) of 3M potassium acetate was added and simultaneously mixed with 300µl ice-cold 100% isopropyl alcohol (approximately 2/3 of the aqueous phase). The mixture was inverted gently to precipitate the formation of DNA threads. The mixture was then incubated at a temperature of -20^0C for 30 minutes, after which it was centrifuged at 13,000rpm for 5 minutes. The supernatant was discarded. The tube containing the DNA pellet was inverted on tissue paper to complete the draining off of the supernatant. The DNA pellet was washed with 500µl of 70% ethanol, inverted once (to dissolve residual salts and to increase the purity of DNA) and centrifuged at 13,000rpm for five minutes. The 70% ethanol was discarded from the tube. The tube containing the DNA pellet was inverted on a filter paper and allowed to air dry at room temperature for 15 minutes. The DNA pellet was re-suspended in 50µl 1x TE buffer and then incubated at 50^0C for 2 hours to ensure complete re-suspension. The DNA pellet was stored at a temperature of -20^0C for further use.

Quantification of DNA using UV Spectrometer

A 50µl of the extracted DNA sample was taken into a 1ml corvette. Readings were taken for all the samples at the absorption of 230 (A230), 260nm (A260), and 280 (A280) wavelengths, respectively in the UV Spectrophometer. The purity of the DNA samples was computed using the relationship below:

$$\text{Purity ratio } A_{260/280} = \frac{Readings\ taken\ at\ 260\ wavelength\ (A260)}{Readings\ taken\ at\ 280\ wavelength\ (A280)} \quad \ldots\ldots [1]$$

$$\text{Purity ratio } A_{260/230} = \frac{Readings\ taken\ at\ 260\ wavelength\ (A260)}{Readings\ taken\ at\ 230\ wavelength\ (A230)} \quad \ldots\ldots [2]$$

The DNA concentration was computed as:

Concentration of DNA (µg/ml) = Absorbent at 260nm (A_{260}) × Dilution factor × Constant … [3]
Dilution Factor = $\frac{Total\ volume}{Initial\ volume}$ ……[4]
$Total\ Volume = 2(Volume\ of\ TE\ (Tris\ EDTA)\ pH8 + Volume\ of\ DNA)$ ……. [5]

Initial Volume = Volume of DNA used
Constant = 50µg/mL
Yield of, DNA sample was also computed as:
Yield of DNA (µg) = DNA concentration × total sample volume (ml)

RESULTS

The analysis of variance (ANOVA) for comparing the quality, concentration, and yield of DNA extracted from fresh and silica-dried leaves with the quality, concentration, and yield of DNA extracted from fresh and silica dried cambium of *mansonia altissima* is presented in Table 1. In this study, there was a significant difference (P<0.05) in the DNA purity, DNA concentration, and yield among the plant materials at an absorbance ratio of $A_{260/280}$ and A260/230, respectively. The purity ratio obtained was relatively low compared to the generally accepted ratio of 1.6–1.7. However, samples from fresh leaves and dried cambium exhibited the highest purity ratio, as shown in Table 2. Also, samples from fresh leaf and dried cambium had the highest DNA concentrations of 56.77µg/ml and 55.26µg/ml respectively (Table 3). Moreover, samples from fresh leaf and dried cambium also recorded the highest yields of 2.84µg and 2.76µg respectively (Table 4).

Table 1: Analysis of Variance (ANOVA) for comparing DNA purity, concentration, and yield of Leaves (dried and fresh) and cambium (dried and fresh) of *Mansonia altissima*

Sources of Variation	Sum of Squares	df	Mean Square	F	Sig.
Purity 260/280	1.522	3	0.507	12.01	0.002
Error	0.338	8	0.042		
Total	**1.859**	**11**			
Purity 260/230	1.581	3	0.527	14.24	0.001
Error	.296	8	0.037		
Total	**1.877**	**11**			
Concentration	3701.820	3	1233.940	16.41	0.001
Error	601.647	8	75.206		
Total	**4303.467**	**11**			
Yield	9.275	3	3.092	16.50	0.001
Error	1.500	8	0.187		
Total	**10.775**	**11**			

Table 2: DNA Purity for leaves (fresh and dried) and cambium (fresh and dried) of *Mansonia* at an absorbance ratio of $A_{260/280}$ and $A2_{60/230}$

Plant materials	Absorbance ($A_{260/280}$)	Absorbance ($A_{260/230}$)
Fresh leaf	1.18^a	1.15^a
Dry leaf	0.42^b	0.53^b
Fresh cambium	0.53^b	0.32^b
Dry cambium	1.19^a	1.11^a

Table 3: DNA concentration (µg/ml) of *Mansonia altissima* leaves (fresh and dried) and cambium (fresh and dried)

Plant materials	Mean
Fresh leaf	56.77^a
Dry leaf	35.96^b
Fresh cambium	13.47^c
Dry cambium	55.26^a

Table 4: DNA Yield (µg) for leaves (fresh and dried) and cambium (fresh and dried) of *Mansonia altissim*

Plant materials	Mean
Fresh leaf	2.84[a]
Dry leaf	1.80[b]
Fresh cambium	0.67[c]
Dry cambium	2.76[a]

DISCUSSION

Knowledge of the genetic diversity of species in a biological community is useful for assessing the ecological and evolutionary processes that define the structure and dynamics of that community (Barbara *et al.*, 2021). Current genetic diversity is the result of selection and random evolutionary processes experienced by previous generations; this genetic variability determines the evolutionary potential of species within the prevailing ecological conditions at a given time. The recognition of tree species diversity represents basic knowledge for managing and conserving local species or their plant communities (Lowe *et al.*, 2018). Determination of DNA purity and yield is important for downstream applications, such as polymerase chain reaction (PCR) and multiplex PCR (Kelly *et al.,* 2012). The DNA purity ratio for all the samples was relatively low compared to the generally accepted ratio of 1.6 – 1.7 (Pich and Schubert, 1993). This may be largely due to the presence of protein, phenol or other contaminants that absorb strongly at or near 280nm. Anti-predation agents found in most tropical plant tissues include alkaloids, cyanides, polyphenols, and terpenes (Turner, 2001).The screening phytochemicals carried out on *Mansonia altissima* extracts from bark were described by the presence of active compounds such as alkaloids, tannins, saponins, polyphenols, terpenes, flavonoids, and cardiac glycosides (Adeoti *et al.,* 2016). Such compounds may inhibit downstream PCR applications (Colpaert *et al.,* 2005). These contaminants could also be the reagents used in the isolation protocol, which might have been added up during the course of extraction due to the inadequate precipitation of the DNA samples. In general, the quantity and quality of isolated DNA depend on precipitation, temperature, and duration (Michiels *et al.*, 2013).

We discovered that fresh leaves and dried cambium exhibited the highest purity ratio. Also, fresh leaves and dried cambium samples had the highest concentration of DNA and the highest DNA yield. Generally, young leaves are believed to be best for isolating the genomic DNA because the number of cells per unit area will be greater and it may contain fewer unwanted secondary metabolites and phenolic compounds. This explained why fresh leaves had the highest concentration of DNA and the highest DNA yield. The higher concentration in the dried cambium tallied with the research by Mangraravite *et al.* (2020), who reported high concentrations in dried cambium samples of *Anadenanthera peregrina*, *Cedrela fissilis,* and *Ceiba speciose*, using a similar DNA extraction protocol. Colpaert *et al.* (2005) also recorded positive yield in the DNA of dried cambium for *Ceiba pentandra, Maranthes panamensis, Eschweilera costaricensis, Lecythisampla, Goethalsia meiantha and Laetia procera*

In this study, dried cambia samples were allowed to dry for a period of time, but the concentration of DNA and yield did not decrease when compared to DNA extracted from fresh Cambium. This is a clear indication that the storage time does not decrease the overall quality of the genomic DNA. This finding was corroborated by Mangaravite *et al.* (2020). In their study, DNA extractions were performed after four storage periods: extraction performed on the same day of harvest; and extractions performed 7, 14, and 21 days after harvest, respectively. They discovered that there was no significant difference in the genomic DNA yield or quality among the four storage periods, and none significantly impaired the use of the samples for PCR. This suggests that cambium tissue can be harvested and kept for some weeks prior to DNA extraction, which is frequently convenient in fieldwork schedules (Mangaravite *et al.* (2020)

Conclusion and Recommendation

This study evaluated the DNA purity, concentration, and yield extracted from *Mansonia altissima* leaf (fresh and dried) and Cambium (fresh and dried). The highest DNA purity, concentration, and yield were recorded in the fresh leaf and dried cambium of *Mansonia altissima*, while the lowest concentration and yield were found in the fresh cambium sample. The DNA purity for both the leaf and cambium was relatively low compared to the generally accepted ratio. The purity of the DNA can, however, be improved by optimizing the DNA extraction protocol and including more washing steps to elute as many reagents as possible. The DNA pellets can also be washed with 70% ethanol to re-purify them.

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