EnPress Publisher

Sustainable Forestry 2024, 7(2), 10776.
https://doi.org/10.24294/sf10776

Article

Development of ten species-specific primers for sustainable management of *Pterygota macrocarpa* K.Schum in Nigeria using MatK and RuBisCo genes

Amadu Lawal

Department of Forestry and Wood Technology, Federal University of Technology Akure, Akure 340110, Nigeria; alawal@futa.edu.ng

CITATION

Lawal.A. Development of ten species-specific primers for sustainable management of *Pterygota macrocarpa* K.Schum in Nigeria using MatK and RuBisCo genes. Sustainable Forestry. 2024; 7(2): 10776.
https://doi.org/10.24294/sf10776

ARTICLE INFO

Received: 8 November 2024
Accepted: 6 December 2024
Available online: 13 December 2024

COPYRIGHT

Copyright © 2024 by author(s). *Sustainable Forestry* is published by EnPress Publisher, LLC. This work is licensed under the Creative Commons Attribution (CC BY) license.
https://creativecommons.org/licenses/by/4.0/

Abstract: In Nigeria, deforestation has led to an unimaginable loss of genetic variation within tree populations. Regrettably, little is known about the genetic variation of many important indigenous timber species in Nigeria. More so, the specific tools to evaluate the genetic diversity of these timber species are scarce. Therefore, this study developed species-specific markers for *Pterygota macrocarpa* using state-of-the-art equipment. Leaf samples were collected from Akure Forest Reserve, Ondo State, Nigeria. DNA isolation, quantification, PCR amplification, gel electrophoresis, post-PCR purification, and sequencing were done following a standardized protocol. The melting temperatures (TM) of the DNA fragments range from 57.5 °Cto 60.1 °C for primers developed from the MatK gene and 58.7 °C to 60.5 °C for primers developed from the RuBisCo gene. The characteristics of the ten primers developed are within the range appropriate for genetic diversity assessment. These species-specific primers are therefore recommended for population evaluation of *Pterygota macrocarpa* in Nigeria.

Keywords: primers; genetic diversity; *Pterygota macrocarpa*; MatK and RuBisCo genes

1. Introduction

For populations of forest trees to be able to adapt and show resilience to changes in the environment and stresses from human activity, their genetic diversity must be maintained. Tropical rainforests contain a lot of biological diversity that provide important ecosystem services, but they are facing anthropogenic and climate threats. Lawal et al. [1] reveal that the world forest is disappearing at an alarming rate, leading to the extinction of important timber species. FAO [2] discovered that between 2010 and 2020, the continent lost 3.9 million hectares of forest area per year, compared to 3.4 million hectares between 2000 and 2010. Anthropogenic activities in forest ecosystems do not only lead to the destruction of mature trees [1]; they also negatively impact the regeneration potential of such ecosystems [3]. In a study, Lawal et al. [4] compared the regeneration potential of a primary forest with degraded and enrichment forests. They revealed that the primary forest had a very good regeneration potential (seedlings > wildlings > overstory), enrichment forest had a fair regeneration potential (seedlings > wildlings < overstory), while regeneration potential was poor in degraded forest areas (seedling = 0, wildlings < overstory).

Lawal et al. [3] reported that the mean estimate of total gene diversity (Ht), gene diversity due to variation within populations (Hs), and gene diversity due to differences among populations (Gst) of Khaya species indicated that their populations have been negatively impacted by anthropogenic activities. Generally, researchers link deforestation and forest degradation to the loss of tree species. At

times, the genetic makeup of individual trees in forest ecosystems is scarcely considered. For instance, Barnes at al. [5] defined species diversity as the number of various species found in an ecosystem and the relative abundance of each of these species. This information is also used by the International Union for Conservation of Nature (IUCN) to classify species as "Not Evaluated, Data Deficient, Least Concern, Near Threatened, Vulnerable, Endangered, Critically Endangered, Extinct in the Wild, and Extinct". Researchers should note that the population of a particular tree species in a forest ecosystem may be considered intact, while the few removed in the course of illegal logging may be those required to maintain such a tree population in perpetuity [6].

Pterygota macrocarpa is a species of tree commonly known as the large-fruited *Pterygota.* It belongs to the family *Sterculiaceae* and is native to tropical Africa. It is commonly found in the rainforest, gallery forest, and savanna woodland habitats. The tree can grow up to 50 m tall and has large, distinctive fruit capsules that can reach up to 20 cm in length. The wood of *Pterygota macrocarpa* is highly priced and is commonly used for construction purposes. The population of this species is declining with habitat loss, and it has since been classified by the IUCN Red List of Threatened Species as vulnerable [7].

Ismail et al. [8] pointed out that gene markers are specific genes that distinguish one species from another. Ribulose-1,5-bisphosphate carboxylase-oxygenase (RuBisCo) is the enzyme that plays a crucial role in the process of photosynthesis. It is responsible for fixing carbon dioxide (CO_2) from the atmosphere into organic compounds that can be used by plants for growth and development. While Maturase K (MatK) is a plant plastidial gene, the protein it encodes is an organelle intron maturase, an organelle that splices group II introns. The maturase K (MatK) gene marker has a total sequence length of 1500 bp, and ribulose-1,5-bisphosphate carboxylase/oxygenase (rbcL) has approximately 1400 bp, which has high accuracy for DNA barcoding [9].

The rate of deforestation and forest degradation in Africa, particularly in Nigeria, is worse. The annual rate of deforestation in Nigeria has since been estimated to be 3.5% [10]. Many researchers have cited rapid population growth, clearing of forested land for agricultural purposes, and selective logging as major drivers of deforestation in Nigeria [11,6]. This annual rate of deforestation in Nigeria may not have only led to a reduction in forest areas; it would have also brought about an unimaginable loss of genetic variation within tree populations. Unfortunately, little is known about the genetic variation within the population of important timber species in Nigeria. To worsen the situation, the specific tools to evaluate withi-population genetic diversity for many important tropical timber species are scarce. Hence, the need to develop primers for genetic diversity assessment of important timber species in Nigeria.

2. Method of data collection

Leaf samples of four (4) *Pterygota macrocarpa* trees and three (3) wildings were collected from Akure Forest Reserve, Ondo State, Nigeria. The samples collected were cleaned with tissue paper and stored in a zip-lock bag containing

silica gel, then taken to the Biosafety Postgraduate Research Laboratory of the Federal University of Technology Akure for DNA extraction. The coordinate of each sampled tree was recorded with the aid of a global positioning system (GPS).

2.1. DNA extraction

Doyle and Doyle's [12] DNA extraction protocol was followed. A 50 mg leaf sample of *Pterygota macrocarpa* was ground to a fine powder with a mortar and pestle. The powder was then placed in 1.5-mL microtubes containing 700 µL of 2% CTAB extraction buffer [20 mM EDTA, 0.1 M Tris-HCl pH 8.0, 1.4 M NaCl, 2% CTAB, plus 0.4% b-mercaptoethanol added just before use]. The solution was incubated at 65°C for 45 min, gently mixing by inversion every 15 min; 500µL of chloroform-isoamylalcohol (24:1) was added to the tubes and gently mixed for 1 min; samples were centrifuged for 10 min. at 12,000 rpm; 500µL of the supernatant was transferred to a fresh tube with 700µL of cold isopropanol (−200°C); samples were gently mixed by inversion and centrifuged at 12,000 rpm for 10 min, and so it was possible to visualize the DNA adhered to the bottom of the tube.

The liquid solution was released, and the DNA pellet was washed with 700µL of 70% ethanol to eliminate salt residues adhered to the DNA and set to dry for approximately 12 h with the tubes inverted over a filter paper at room temperature. The pellet was re-suspended in 100µL TE buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA pH 8.0) plus 5µL ribonuclease (RNAse 10 mg μL^{-1}) in each tube; this solution was incubated at 37 °C for 1h and then stored at −20 °C.

2.2. DNA quantification

DNA absorbent value was obtained from the GS-UV32PCS spectrophotometer using a 1 mLcuvette. The formula below was used to get the concentration of the DNA.

$$\text{Concentration } ofDNA = Absorbent\ @260 \times Dilutionfactor \times Constant$$

$$Dilusion\ Factor = \frac{TotalVolume}{InitialVolume}$$

$$Total\ Volume = (VolumeofTE(TrisEDTA)pH8 + VolumeofDNA)$$

Initial Volume = Volume of DNA used

Constant = 50µg/mL

2.3. DNA normalization

Concentrated DNA was normalized using the formula below:

$$C_1V_1 = C_2V_2$$

where:

- C_1= Concentration of DNA to be normalized (known).
  V_1= Volume of DNA taken (known).
- C_2 = final concentration (known).
  V_2= Volume of distilled water to be added (unknown).

2.4. PCR amplification

DNA amplification of the target regions was carried out with OneTaq® Quick-Load® 2X Master Mixin a total volume of 12 µL (**Table 1**) using MaturaseK (MatK) and RuBisCo (rbcL) genes, following the conditions presented in **Table 2**. The samples were subjected to the following thermal cycling conditions using the Eppendorf MasterCycler (Nexus gradient 230, Germany) as presented in **Table 3**.

Table 1. Reagent for PCR.

COMPONENT	Volumes for a 12.5µL reaction
Template DNA	2 µL
10µM Forward Primer	0.25µL
10µM Reverse Primer	0.25µL
One Taq Quick Load 2X Master Mix with Standard Buffer	6.25µL
Nuclease free water	3.75 µL

Table 2. Target genes for polymerase chain reaction.

Primers		Primer sequence (5′ to 3′)	Length (bp)
MaturaseK gene (MatK)	390F	5′-CGATCTATTCATTCAATATTTC-3′	22
	1326R	5′-TCTAGCACACGAAAGTCGAAGT-3′	22
RuBisCo gene (rbcL)	cF	5′-TGAAAACGTGAATTCCCAACCGTTTATGCG-3′	30
	cR	5′-GCAGCAGCTAGTTCCGGGCTCCA-3′	23

Table 3. Thermal cycling protocol.

Step	Stage of PCR	Temp (°C)	Time (min: s)
1.	Initial Denaturation	94	5:00
2.	Denaturation	94	0:30
3.	Annealing	52	1:00
4.	Extension	68	1:30
5.	Final Extension	68	10:00
6.	Hold	4	Hold

2.5. Gel electrophoresis

After PCR amplification, 2 ul of each PCR product was run on 1% agarose gel, stained with SafeView Red (5 ul), and photographed using a gel documentation system (E-BOX, VilberLourmat, Italy).

2.6. Post-PCR purification

PCR products were cleaned usingan enzymatic method (ExoSAP) as follows:The ExoSAP master mixture was prepared by adding 50µL of 20U/µL Exonuclease I (Catalogue No. NEB M0293L) with 200µL of 1U/µL of Shrimp Alkaline Phosphatase (Catalogue No. NEB M0371) to a 0.6mL microcentrifuge tube. The reaction mixture was prepared by mixing 10 µL of amplified PCR product with 2.5 µL of ExoSAP Mix (step 1), and the resulting mixture was incubated at 37 °C for 15 min and at 80 °C for 15 min.

2.7. Sequencing

The fragments were sequenced using the Nimagen, Brilliant Dye Terminator Cycle Sequencing Kit V3.1, and BRD3-100/1000 according to the manufacturer's instructions: The labelled products were cleaned with the ZR-96 DNA Sequencing Clean-up Kit. The cleaned products were injected into the Applied Biosystems ABI 3500XL Genetic Analyzer with a 50cm array using POP7, and their sequence data were collected.

2.8. Data analysis

GPS data were used to reveal the spatial distribution of the sampled species in the study area using ArcGIS 10.7. The sequence data collected were analyzed using BioEdit software for sequence data trimming and Primer3Plus for the development of the primer pairs.

3. Results

The spatial distribution of the sampled *Pterygota macrocarpa* is presented in **Figure 1**. In this study, a total of seven *Pterygota macrocarpa*, comprising five (5) mature trees and three (3) wildlings, were sampled as presented in **Figure 1**. More so, the chromatograms for the MatK gene (**Figure 2**) and the RuBisCo gene (**Figure 3**) reveal good-quality sequence results with clear peaks and are devoid of noise.

Developed primers for *Pterygota macrocarpa using* MatK and RuBisCo genes are presented in **Table 4**. The size of each primer varied from 20bp to 24bp for both genes. The melting temperatures (TM) of the DNA fragments varied slightly among the primer pairs, with values ranging from 57.5 °C to 60.1 °C for primers developed from the MatK gene and 58.7 °C to 60.5°C for primers developed from the RuBisCo gene. More so, the percentage of GC content ranged from 41.7% to 52.4% for primers developed from the MatK gene and 45.8% to 60.0% for primers developed from the RuBisCo gene, respectively.

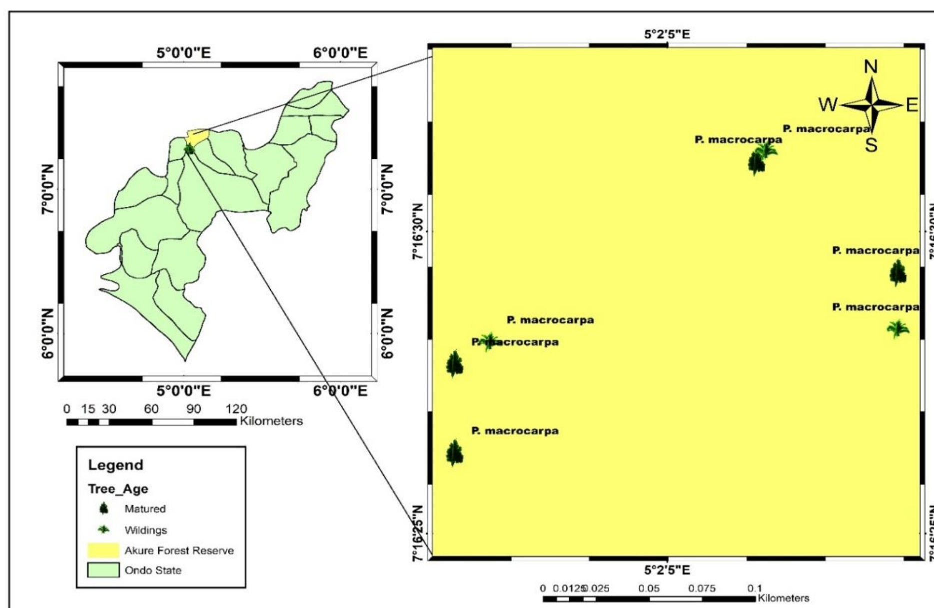

Figure 1. Spatial distribution of the sampled *Pterygota macrocarpa* in the study area.

Figure 2. A trimmed portion of the chromatogram for the MatK gene.

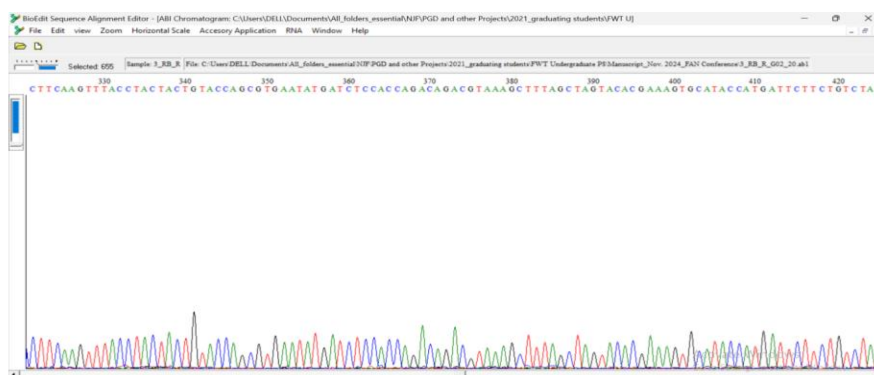

Figure 3. A trimmed portion of the chromatogram for the RuBisCo gene.

Table 4. Developed primers for *Pterygota macrocarpa using* MatKand RuBisCo genes.

S/N	NAME	SEQUENCE	BASE(bp)	Tm(°C)	GC (%)
Primers developed using MatK gene					
1	Ptm1 F	5′-CGCTACTGGGTAAAAGATGCC-3′	21	59.1	52.4
	Ptm1 R	5′-GCCCAGAAAGTCGAAGGAA-3′	20	58.9	50.0
2	Ptm2 F	5′-GCTACTGGGTAAAAGATGCCTCT-3′	23	60.1	47.8
	Ptm2 R	5′-CGCACACTTGAAAGATAGCCC-3′	21	59.6	52.4
3	Ptm3 F	5′-ACTGGGTAAAAGATGCCTCTTCT-3′	23	59.4	43.5
	Ptm3 R	5′-AGGGTTTAGTCGCACACTTGA-3′	21	59.6	47.6
4	Ptm4 F	5′-GGTAAAAGATGCCTCTTCTTTGCA-3′	24	59.8	41.7
	Ptm4 R	5′-TGAAGGGTTTAGTCGCACACT-3′	21	59.6	47.5
5	Ptm5 F	5′-CTCTTCGCTACTGGGTAAAAGA-3′	22	57.5	45.5
	Ptm5 R	5′-TGACTCCGTACCATTGAAGGG-3′	21	59.4	52.4
Primers developed using RuBisCo gene					
1	Ptr1 F	5′-AGGGTGTCCTAAAGTTCCTCC-3′	21	58.7	52.4
	Ptr1 R	5′-GCGGGTACATGTGAAGAAATGATG-3′	24	60.5	45.8
2	Ptr2 F	5′-CGGTCAAAGCAGGCATATGC-3′	20	60.0	55.0
	Ptr2 R	5′-TGTGTGCCAGAGAATTGGGA-3′	20	59.2	50.0
3	Ptr3 F	5′-CTCGATTAGCTACGGCACCC-3′	20	60.3	60.0
	Ptr3 R	5′-TGCTACTGCGGGTACATGTG-3′	20	60.1	55.0
4	Ptr4 F	5′-TGCATTTCCCCAAGGGTGTC-3′	20	60.5	55.0
	Ptr4 R	5′-ACTTGAATGCTACTGCGGGT-3′	20	59.7	50.0
5	Ptr5 F	5′-GCTACTCGATTAGCTACGGCA-3′	21	59.7	52.4
	Ptr5 R	5′-ATGAAAAGGGCCATGTGTGC-3′	20	59.4	50.0

4. Discussion

The current rate of deforestation and forest degradation in Nigeria is alarming, and their devastating effects on the genetic variation of important timber species like *Pterygota macrocarpa* are unknown. No doubt, demand for agricultural land is not the only factor responsible for deforestation in Nigeria.

Koko et al. [13] projected LULC geospatial maps for 2050 in Zaria City, Nigeria, indicating that urbanization, deforestation, and the expansion of agricultural activities will likely continue to transform forested areas into built-up land over the next 30 years. Debnath et al. [14] reported that the amount of agricultural land in the Koch Bihar urban agglomeration would decline significantly by 2050, while the built-up area would grow dramatically. If reasonable measures to curb urban development are not put in place, the ongoing expansion will drastically decimate our forest estates, hindering progress toward achieving Sustainable Development Goals (SDGs) 11 (sustainable cities and communities), 13 (climate action), and 15 (life on land) [15].

The development of species-specific primers could allow researchers to identify and characterize genetic variation within and between populations of forest trees. For instance, Utama et al. [9] designed MatK and rbcL region-specific primers for DNA barcoding *Nepenthesspathulata* (pitcher plant). Antil et al. [16] pointed out that a short standardized sequence can distinguish individuals of a species because genetic variation between species exceeds that within species. The markers developed in this study could be used to better understand the adaptive potential of *Pterygota macrocarpa*. Genetic markers can provide insights into the genetic structure and diversity of a population, which can help in developing strategies for the conservation and management of the species [17].

Both MatK and rbcL genes have specific base sequences that can differentiate between one species and another in plants systematically [18,19]. Lawal et al. [20] successfully used these genes to characterize the populationof *Chrysophyllumalbidum* (G. Don) in a Nigerian forest reserve. If the authors had used species-specific markers, the population of *Chrysophyllumalbidum* could have revealed more genetic diversity. Although there are limited studies on the development of species-specific markers for accurate and reliable identification of taxa, their use has been recommended. For instance, Alexandrov et al. [21] developed some species-specific molecular markers for Elaeagnus plants in botanical, dendrological, and genetic research as well as in breeding and hybridization experiments. Also, Devi et al. [22] used developed species-specific markers based on *tef1* and *rpb2* gene sequences to detect and discriminate *T. aserellum* and *T. harzianum*. *Pterygotamacrocarpa* is an important timber species, and the creation of species-specific markers for it will therefore contribute significantly to ensuring its survival in the face of present climate change.

The TM indicates the temperature at which half of the DNA strands in a double-stranded DNA molecule are denatured or melted, which is a key step in PCR amplification [23]. The primers developed in this study are appropriate considering their characteristics. Generally, the criteria of a molecular marker suitable for use include that the primers should be designed in 5′ to 3′ directions. The optimal length

for a primer is 18 to 25 base pairs. Primer should have a GC content of 40% to 60%; the melting temperature (TM) should be between 55% and 65%; the difference between the melting points should not be more than 5 degrees Celsius. There should be at least 2G or C bases (GC clamp) in the last 5 bases at the 3′ end of the primer. According to Kumar et al. [24], GC content should not be too high because of the potential for dimer formation in the amplification process. Bases G and C also affect Tm, G, and C, resulting in higher temperatures than A and T [25].

5. Conclusion and recommendation

Pterygota macrocarpa is one of the most important timber species that are selectively logged. This action has significantly decimated the population of *Pterygota macrocarpa*. Using Matk and RuBisCo gene sequences from *Pterygotamacrocarpa*, this study successfully developed ten species-specific primer pairs for assessing genetic variation. The characteristics of the developed primers are within the range appropriate for genetic diversity assessment. These species-specific primers are therefore recommended for population evaluation of *Pterygota macrocarpa* in Nigerian forest reserves. More so, further studies should be carried out to develop species-specific primers for other important timber species to ensure their continuous existence in the face of current climate change and other anthropogenic activities.

Conflict of interest: The author declares no conflict of interest.

References

1. Lawal A, Adekunle VAJ, Oghenekome UO. Molecular Characterization of the Genus Khaya in South West Nigeria using CPSSR Markers. Nigerian Journal of Genetics. 2023; 37(1): 87–98.
2. FAO. Global Forest Resources Assessment 2020: Main report. FAO; 2020.
3. Lawal A, Ariyo RO. Edge-Interior Disparities in Tree Species Diversity and Soil Physicochemical Properties in a Nigerian Strict Nature Reserve. Journal of Research in Forestry, Wildlife & Environment. 2023; 15(2): 268–280.
4. Lawal A, Adeyemi OA, Jimoh B. Does forest cover type affect the regeneration potential of tree species? A case study of Triplochiton scleroxylon in Akure forest reserve. Journal of Research in Forestry, Wildlife & Environment. 2024; 16(1): 62–67.
5. Barnes AD, Scherber C, Brose U, et al. Biodiversity enhances the multitrophic control of arthro pod herbivory. Sci. Adv. 2020; 6(45).
6. Lawal A, Adekunle VAJ,Onokpise OU. Phytotherapy and Polycyclic Logging: Implication on Genetic Multiplicity and Diversity of African Mahogany in Tropical Rainforest. Not. Sci. Biol. 2019; 11(1):21–25.
7. Borokini TI. A Systematic Compilation of IUCN Red-listed Threatened Plant Species in Nigeria. International Journal of Environmental Sciences Vol. 2014; 3: 104–133.
8. Ismail M, Ahmad A, Nadeem M, et al. Development of DNA barcodes for selected Acacia species by using rbcL and matK DNA markers. Saudi. J. Biol. Sci. 2020; 27(12): 3735–3742. doi: 10.1016/j.sjbs.2020.08.020
9. Utama MN, Etikawati NS, Susilowati A. New specific primer matK and rbcL region for DNA barcode pitcher plant Nepenthes spathulata. Biodiversitas. 2024; 25: 2515–2523.
10. Batta H, Ashong CA, Bashir AS. Press Coverage of Climate Change Issues in Nigeria and Implications for Public Participation Opportunities. J. Sustain. Develop. 2013; 6(2): 56–62.
11. Curtis PG, Slay CM, Harris NL, et al. Classifying drivers of global forest loss. For. Ecol. 2018; 1111: 108–1111.
12. Doyle JJ, Doyle JL. Isolation of plant DNA from fresh tissue. Focus. 1990; 12: 13–15.

13. Koko AF, Yue W, Abubakar GA, et al. Monitoring and Predicting Spatio-Temporal Land Use/Land Cover Changes in Zaria City, Nigeria, through an Integrated Cellular Automata and Markov Chain Model (CA-Markov). Sustainability. 2020; 12: 10452–10473.
14. Debnath M, Islam N, Gayen SK, et al. Prediction spatiotemporal (2030 and 2050) land-use and land-cover changes in Koch Bihar urban agglomeration (West Bengal), India, using artificial neural network-based Markov chain model. Modeling Earth Systems and Environment. 2023; 1–22.
15. Amaechi CF, Ugwu VC, Okoduwa AK. Geospatial Assessment of Land Use/Land Cover of the Federal Capital Territory: A Case Study for the year 2050. Ethiopian Journal of Environmental Studies and Management. 2024; 17(1): 95–109.
16. Antil S, Abraham JS, Sripoorna S, et al. DNA barcoding, an effective tool for species identification: A review. Mol. Biol. Rep. 2023; 50(1): 761–775.
17. Duan W, Fan G, Wang L, et al. Genetic diversity and population structure of Magnolia officinalis subsp. biloba revealed by EST-SSR markers. Journal of Genetics. 2014; 93(2): 467–471.
18. Ahmed SS. DNA barcoding in plants and animals: A critical review. Plant Sciences. 2022. doi: 10.20944/preprints202201.0310.v1
19. Chac LD, Thinh BB. Species identification through DNA barcoding and its applications: A review. Biol. Bull. 2023; 50(6): 1143–1156. doi: 10.1134/S106235902360229X
20. Lawal A, Sale FA, Arogundade JC. Characterization of MatK and RuBisCo Genes within the Population of Chrysophyllum albidum (G. Don) in Akure Forest Reserve, Ondo State, Nigeria. Biological and Environmental Sciences Journal for the Tropics. 2024; 21(1): 178–188.
21. Alexandrov OS, Karlov GI. The Development of New Species-Specific Molecular Markers Based on 5S rDNA in Elaeagnus L. Species. Plants. 2021; 10: 2–10.
22. Devi T, Prabhakaran N, Kamil D. Development of species specific markers for the identification of *Trichoderma asperellum* and *Trichoderma harzianum*. Vegetos. 2017; 30: 94–100.
23. Pierce KE, Wangh LJ. Effect of mismatches on the fluorescent signature of a DNA probe. Biophysical Journal. 2007; 93(2): 546–554.
24. Kumar P, Chatli MK, Mehta N, et al. Meat analogues: Health promising sustainable meat substitutes. Crit. Rev. Food Sci.Nutr. 2017; 57: 923–932.
25. Delghandi M, Delghandi MP, Goddard S. The significance of PCR primer design in genetic diversity studies: Exemplified by recent research into the genetic structure of marine species. Methods Mol. Biol. 2022; 2392: 3–15.