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DNA EXTRACTION AND ANATOMIC CHARACTERIZATION IN DRIED HEARTWOOD FROM FABACEAE SPECIES

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ABSTRACT

The botanical family Fabaceae is the most representative in terms of diversity in Brazil, and it contains species of great importance to the domestic and international timber trade. In spite of numerous attempts to combat illegal logging, this practice is still common in Brazil, making it necessary to seek more accurate techniques for identifying wood species. This study aimed to test different protocols for extracting DNA from dried heartwood of *Amburana cearensis* (Fr. Allem) A. C. Smith (Cerejeira), *Dypterix odorata* and *Peltogyne confertiflora*. Additionally, these species were characterised through visual inspection and scanning electron microscopy. Five DNA extraction protocols were evaluated with six replicates. DNA amplification was conducted for the *rbcL* gene, a molecular marker of conserved regions in plants. It was possible to extract and amplify DNA from the dried heartwood of the tested species, with Protocol 2 (QIAGEN kit) being the most efficient.

KEYWORDS: *Wood DNA, kit Qiagen, rbc L.*

INTRODUCTION

Illegal logging accounts for approximately USD 100 billion in trade annually. The United Nations Environment Programme (UNEP) estimates that timber from suspect sources makes up 30% of the worldwide trade, with this figure reaching 90% of the total trade in some countries (Avancini 2014).

Timber logged illegally in the Amazon is marketed in the large urban centres of Brazil. In spite of inspection checkpoints along the nation's roadways, this illegal timber moves freely due largely to the difficulty of identifying it.

This difficulty can be minimized using scientific methods involving genetic identification of trees through molecular techniques. However, to achieve such identification, the first step is to obtain DNA of sufficient quality and quantity to develop these techniques.

Extracting DNA from dried heartwood is a complex technique because some of the DNA is in a degraded state (Jiao et al. 2012). Some work has been conducted with the aim of extracting high-quality DNA from dry wood; however, this work is still in its early stages in Brazil, especially with regard to native tropical woods. In this case, the applied protocols should be adapted to achieve greater efficiency in extracting DNA from the wood, minimizing or eliminating contamination of the genetic material by phenols, polysaccharides and proteins (Chiari et al. 2009).

The efficiency of protocols for extracting high-quality DNA is related to the composition of the extraction buffer, which usually consists of a buffering agent to stabilize the pH at approximately 8, a salt to dissociate the DNA-associated proteins, a detergent to solubilise the membranes and help inactivate some enzymes, and a DNase inhibitor to protect the DNA (Chiari et al. 2009).

Obtaining high-quality genetic material is an important step in the search for a marker that allows the identification and differentiation of species. In this type of investigation, chloroplast DNA (cpDNA) is generally used because it is highly conserved in terms of size, content, structure and gene order (JUDD et al. 2002). The analysis of cpDNA allows primers specific to the chloroplast genome to be applied almost universally throughout the plant kingdom, making it easier to perform comparisons across different taxa (Pinto 2015).

Among the chloroplast genes, it is worth noting the *rbcL* gene, which encodes the largest subunit of the enzyme ribulose 1,5-biphosphate carboxylase/oxygenase (RuBisCo), a catalyst in the first step of carbon fixation during photosynthesis. This gene has been proven efficient in identifying arboreal species in various studies (Chase et al. 2005).

Thus, DNA extraction-the first step in various molecular analyses-must be optimised to ensure a large quantity of high-quality material. Therefore, it is necessary to test various extraction protocols because heartwood tissue is rich in polysaccharides, which are substances that interfere with the yield and quality obtained at the end of the extraction process.

The presence of these substances in the wood highlights the need to test and adapt protocols to match the different objectives and species being studied.

Taking into consideration the widespread presence and commercial importance of the *Fabaceae* family - the third most common family in tropical regions (Feng et al. 2015) - three native species of this family that produce hardwoods of great economic value to the Brazilian timber trade were chosen: *Amburana cearensis* (Fr. Allem) A. C. Smith (Cerejeira), *Dypterix odorata* (Aubl.) Willd.and *Peltogyne confertiflora* (Hayne) Benth.

This study aimed to identify the main characteristics of *cearensis* (Fr. Allem) A. C. Smith (Cerejeira), *Dypterix odorata* and *Peltogyne confertiflora* timber through visual inspection and scanning electron microscopy and to determine an efficient protocol for extracting DNA from dried heartwood samples and amplifying the obtained DNA.

MATERIALS AND METHODS

The experiments were conducted at the Universidade Federal Rural do Rio de Janeiro – UFRRJ – Brazil in the Wood Biotechnology Laboratory, the Wood Anatomy Laboratory and the Plant Mineral Nutrition Laboratory. Samples of *Amburana cearensis* (Fr. Allem) A. C. Smith (Cerejeira), *Dypterix odorata* and *Peltogyne confertiflora* wood, dried at room temperature, were

provided by the Nova Canãa sawmill (Rio Branco / Acre) and stored in the wood collection of the Wood Anatomy Laboratory at the UFRRJ with their respective codes: 7610, 7611 and 7612.

Wood anatomy

The structures of the samples were analysed and identified using a magnifying lens (DiagTech) for macroscopic features and a scanning electron microscope (Hitachi Tabletop Microscope TM3000 – Ibaraki / Japan) for microscopic features. The surfaces of the samples were polished with #400 sandpaper to highlight their anatomical characteristics before they were examined with the magnifying glass and the scanning electron microscope.

Macroscopic structures were observed with the naked eye and using a 10x magnifying lens, while the ultrastructure was analysed using a scanning electron microscope (SEM) at 200 μm and 300 μm. For the microscopic analysis, the wood samples were pre-heated with distilled water at 100ºC and cut with a microtome into 2 μm thick slices.

DNA extraction

Wood chips were obtained with a razor blade and stored at -80ºC. To avoid contamination, each wood sample was prepared, from slicing up to the final DNA extraction, using a set of materials sterilised in an autoclave for each species. Five protocols for extracting DNA from dry wood were tested, with six replicates for each test, as described below:

Protocol 1 – adapted from Doyle (1990)

Extraction buffer: 2% w/v CTAB, 2.5% PVP, 2 M NaCl, 100 mMTris-HCl (pH 8.0), 25 mM EDTA (pH 8.0), 40 μL of ß-mercaptoethanol (2% v/v).

Samples containing 200 mg of wood were macerated in liquid nitrogen using a porcelain mortar and pestle. Then, 2 mL of the extraction buffer, preheated to 65ºC, was added to the macerated sample. The resulting solution was transferred to 2mL microtubes, to which 40 μL of ß-mercaptoethanol (2% v/v) was then added. Next, the samples were heated in an Eppendorf Thermomixer at 65ºC for 40 minutes with agitation (±800 rpm) every 10 minutes. After heating, the material was cooled on ice at 5ºC for five minutes and then centrifuged at 11,000 rpm for 10 minutes. The aqueous phase was subsequently collected and transferred to a new 2mL tube, to which 600 μL of chloroform:isoamyl alcohol (24:1) was added, and the mixture was lightly homogenised for 30 seconds. The samples were then centrifuged at 11,000 rpm for 10 minutes, and the aqueous phase was transferred to a new 1.5mL tube, to which 4 μL of RNaseA (100 μg/mL) was added. The samples were subsequently incubated for 30 minutes at 37ºC in the Thermomixer. Next, 240 μL of isopropyl alcohol was added to the samples, and the material was homogenised at a slow speed and incubated at -20ºC for 2 hours. The samples were then centrifuged at 11,000 rpm for 30 minutes, after which the supernatant was discarded, and the precipitate was rinsed with 400 μL of absolute ethanol and centrifuged again at 11,000 rpm for 3 minutes. Finally, the ethanol was discarded, and the precipitate was dried for 5 minutes at 30ºC in a vacuum concentrator (Eppendorf Concentrator plus), after which the pellet was re-suspended in 20 μL of autoclaved ultrapure water and stored in a freezer at -80ºC.

Protocol 2 – DNeasy Plant Mini Kit (Qiagen)

Samples containing 100 mg of dry wood were macerated separately in liquid nitrogen using a porcelain mortar and pestle. Genomic DNA was extracted using the QIAGEN DNeasy Plant Mini Kit, following the manufacturer's instructions. After extraction, the DNA was stored at -80ºC.

Protocol 3 – Adapted from Swetha et al. (2014)

Extraction buffer: 100 mMTris-HCl, mM EDTA, 3 M NaCl, 5% CTAB, 1% PVP, 0.3% v/v ß-mercaptoethanol.

Samples containing 200 mg of dry wood were macerated in liquid nitrogen using a porcelain mortar and pestle. Then, 2 mL of the extraction buffer, pre-heated to 65°C, was added. The resulting solution was transferred to 2mL microtubes, and 3 µL of ß-mercaptoethanol (0.3% v/v) was added. The samples were subsequent heated to 65°C for 2 hours in an Eppendorf Thermomixer with agitation (± 200 rpm). Next, the samples were cooled on ice for 5 minutes at 5°C, and an equal volume of chloroform:isoamyl alcohol (24:1) was added. The mixture was then stirred slowly and centrifuged at 1,118 rpm for 15 minutes at 4°C, after which the aqueous phase was collected and transferred to a new 2mL tube. To the aqueous phase, 1/3 volume of 3 M sodium acetate (pH 5.2) and 2/3 volume of chloroform:isoamyl alcohol (24:1) were added. The mixture was slowly homogenised and centrifuged again at 1,118 rpm for 15 minutes at 4°C. The aqueous phase was subsequently transferred to a new tube, and an equal volume of ice-cold isopropanol was added. The tubes were then incubated overnight at -80°C, and the DNA was precipitated via centrifugation at 4,472 rpm for 20 minutes at 4°C, after which the supernatant was discarded. The pellet was subsequently rinsed with 400 µL of 70% ethanol, followed by centrifugation for 10 minutes at 4,472 rpm and 4°C. Finally, the ethanol was discarded, and the precipitate was dried for 5 minutes at 30°C in a vacuum concentrator (Eppendorf Concentrator plus), re-suspended in autoclaved ultrapure water and stored at -80°C.

Protocol 4 – adapted from Doyle (1990).

Extraction buffer: 2% w/v CTAB, 2.5% PVP-40, 2 M NaCl, 100 mMTris-HCl (pH 8.0), 25 mM EDTA (pH 8.0), 2% (v/v) ß-mercaptoethanol.

Samples containing 200 mg of wood were macerated in liquid nitrogen using aporcelain mortar and pestle. The macerated mixture was transferred to a 2mL microtube, to which 1.4 mL of extraction buffer was added. The samples were then homogenised by inverting the microtube every 10 minutes, followed by incubation in a water bath at 65°C for 40 minutes. After being removed from the water bath, the samples were cooled on ice for 5 minutes and then immediately centrifuged for 10 minutes at 11,000 rpm. The aqueous phase was collected and transferred to a new 2mL tube, to which 800 µL of chloroform:isoamyl alcohol (24:1) was added. The mixture was subsequently homogenised by inverting the tube every 10 minutes, after which the samples were centrifuged for 10 minutes at 11,000 rpm, and the supernatant was collected and transferred to a new 1.5mL tube. Next, 600 µL of ice-cold isopropyl alcohol was added, and the material was homogenised by inverting the tube, followed by incubation for 2 hours at -20°C. Subsequently, the tube was centrifuged for 20 minutes at 11,000 rpm, and the supernatant was discarded. The precipitate was rinsed with 500 µL of ice-cold 70% ethanol and then centrifuged for another 5 minutes at 11,000 rpm. Finally, the precipitate was dried for 5 minutes in a vacuum concentrator (Eppendorf Concentrator plus), and the pellet was re-suspended in 50 µL of 1x TE buffer (10 mMTris-HCl, pH 8.0, EDTA 1 mM, pH 8.0) containing RNase at a final concentration of 40 $\mu g \cdot mL^{-1}$ and then stored at -80°C.

Protocol 5 – adapted from Novaes et al. (2009).

Extraction buffer: 2% CTAB, 1.4 M NaCl, 100 mMTris-HCl (pH 8.0), 20 mM EDTA (pH 8.0), 2% PVP, 20 µL of 2% ß-mercaptoethanol, 35 µL of proteinase K (1 mg/mL).

Samples containing 200 mg of dry wood were macerated in liquid nitrogen until a fine powder was obtained. As soon as the samples thawed, 1 mL of extraction buffer was added. Once a homogenous solution was obtained after maceration, 35 µL of 20% SDS (v/v) was added,

and the mixture was transferred to 2mL tubes, which were then incubated for 60 minutes at 60°C with occasional agitation. Next, the tubes were cooled to room temperature, and 600 µL of chloroform:isoamyl alcohol (24:1) was added, after which the mixture was homogenised by being gently inverted for 5 minutes. The samples were subsequently centrifuged for 15 minutes at 18,000 rpm, and the supernatant was transferred to new 1.5 mL tubes. Next, 140 µL of 10% (w/v) CTAB and 280 µL of NaCl 5 M were added to these tubes, which were then gently inverted until they were homogenised. Another 140 µL of 10% (w/v) CTAB and 280 µL of NaCl 5 M were added, followed by 1 volume of ice-cold isopropanol, and the material was incubated overnight at -20°C. Next, the samples were centrifuged at 18,000 rpm for 10 minutes at 4°C, and the supernatant was discarded. The resultant pellet was rinsed 3 times with ice-cold 70% ethanol and dried at 37°C for 15 minutes. Finally, the pellet was re-suspended in 30 µL of 1x TE buffer (10 mMTris-HCl, pH 8.0, EDTA 1 mM, pH 8.0) containing 10 µg/mL of RNase A for 2 hours at 37°C.

The DNA extracted using each protocol was quantified by measuring the absorbance of the sample at 260 nm in a NanoDrop (Thermo Scientific), and the purity of each sample was determined according to the 260/280 and 260/230 ratios. This highly sensitive instrument can measure small quantities of sample DNA (2 ng/mL) (ASIF; CANNON, 2005). A 2µL aliquot of the DNA sample from each species was tested to assess the purity and quantity of the DNA present in each sample.

The values obtained through quantification based on absorbance for each protocol were subjected to analysis of variance and Tukey's test using Assistat software version 7.7 beta (2014).

DNA Amplification

The DNA was amplified through polymerase chain reaction (PCR) using the following primers: *rbcL*a-F 5' – 3' ATGTCACCACAAACAGAGACTAAAGC and rbcLajf634-R 5' – 3' GAAACGGTCTCTCCAACGCAT (Nithaniyal et al. 2014).

Tab. 1 shows the volumes and final concentrations of the reagents in the reaction mixtures. Negative and positive (DNA from a leaf of *Lophantheral actescens*) controls were employed for all reactions. Amplification was carried out over 35 cycles using the following temperatures and times: denaturation: 94°C – 30", annealing: 55°C – 3", and extension: 72°C – 1'20", with a final extension at 4°C.

Tab. 1: PCR mixture (25 µL), with the quantities and final concentrations of each reagent.

	Volume (µL)	Final concentration
Ultrapure H_2O	19.15	
Buffer 10x	2.5	1x
Mg 50 mM	0.75	1.5 mM
Mix dNTPs 10 mM	0.5	0.2 mM
Forward primer	0.5	0.2 mM
Reverse primer	0.5	0.2 mM
Platinum Taq DNA polymerase (Invitrogen)	0.1	2U/reaction
Sample	1	-

The efficiency of PCR amplification was determined through 1% agarose gel electrophoresis, run at 100 V for 30 minutes, using Gel Red™ (Biotium) for staining the DNA. The length of the amplified fragments was assessed with 1kb molecular weight markers (Avati). The gels were photographed using a ChemiDoc MP imaging system (BioRad).

RESULTS

Wood anatomy

The wood of *Amburana cearensis* (Fr. Allem.) A.C. Smith (Cerejeira) (Fig. 1A and B) was soft and easy to cut, and the heartwood had a light beige-yellow colour and a pleasant fragrance.

Regarding the macroscopic features of the wood, vessels arranged in no specific pattern, in multiples, with commonly short (2–3 vessels) radial rows were observed. The vessel outline was rounded, and two distinct classes of vessel diameter were absent. The average tangential vessel diameter was approximately 145,195 and 230 μm, and the vessel luminal diameter was large or very large. The average number of vessels/mm^2 was approximately 2–6; there were either very few or few vessels.mm^{-2}. Simple perforation plates were present. The inter vessel pits were alternate, small and vestured, with an average diameter (vertical) of 5–7 μm. The vessel-ray pits had distinct borders, similar to the inter vessel pits, and were of uniform size or type, with the same type in adjacent elements located throughout the ray. Helical thickenings were absent. Tyloses in the vessels were absent. Other deposits in the heartwood vessels were present (coloured dark yellowish or greenish brown). An axial parenchyma was present but not banded. The paratracheal axial parenchyma was ali form and confluent (distinctly confluent towards the growth ring boundary or forming irregular bands). The ali form parenchyma had an ovoid form. The axial parenchyma was fusi form or present as strands. The average number of cells per axial parenchymal strand has been reported to be approximately 2–4(–6), and there is nounlignified parenchyma (Richter and Dallwitz 2000).The cross-section exhibited diffuse porosity with single and multiple pores, sometimes obstructed by a yellowish substance. By SEM, it was possible to observe the axial parenchyma of the paratrachealali form type in greater detail at a higher resolution.

The wood of *Dipteryxodorata* (Fig. 1C and D) was hard to cut and exhibited dark heartwood that was nut-brown in colour with some darker stripes. Regarding the macroscopic features, it was possible to observe vessels in multiples arranged in no specific pattern, with commonly short (2–3 vessels) radial rows and radial rows of 4 or more vessels. The average tangential vessel diameter varied between 100–250 μm, and the vessel luminal diameter was large to very large. The average number of vessels·mm^{-2} was approximately 5–10; there were few vessels·mm^{-2}. The average vessel element length was approximately 300–550 μm, which is medium, and the perforation plates were simple. The inter vessel pits were alternate, vestured, and medium to large, with an average diameter (vertical) of9–13 μm. This wood did not have vessel-ray pits with distinct borders, similar to the inter vessel pits. Helical thickenings and tyloses were absent from the vessels. Other deposits in the heartwood vessels were present. An axial parenchyma was present but not banded. The axial parenchyma was paratracheal and ali form to confluent, but the ali form parenchyma had a lozeng form. The axial parenchyma was present as strands. The average number of cells per axial parenchyma strand is 2–4 (Richter and Dallwitz 2000). The cross-section exhibited diffuse porosity with single and multiple pores, sometimes obstructed by a light substance. By SEM, it was possible to observe the axial parenchyma of the paratrachealali form type.

The wood of *Peltogyneconfertiflora* (Fig. 1E and F) was hard to cut and had purple heartwood. Regarding the macroscopic features, using the 10x magnifying lens, it was possible to observe vessels arranged in no specific pattern, in multiples, with commonly short (2–3 vessels) radial rows. The average tangential vessel diameter was approximately 50, 80 - 110 and 150 μm; the vessel luminal diameter was medium to large, and there were few vessels·mm^{-2}. The average vessel element length was approximately 250–500 μm, which is short to medium. The perforation plates were simple. The inter vessel pits were alternate, small, and vestured and had an average diameter (vertical) that varied between 4–7 μm. The vessel-ray pits had distinct borders, similar

to the inter vessel pits. This wood had no helical thickenings or tyloses in the vessels. Other deposits in the heartwood vessels were present (coloured violet or sometimes also brown to almost black). The axial parenchyma bands were marginal (or apparently marginal), fine, and up to three cells wide. The paratracheal axial parenchyma was ali form, confluent, or unilateral, and the ali form parenchyma had a an ovoid form. The axial parenchyma was present as strands. The average number of cells per axial parenchyma strand was approximately (2–) 4. The type and amount of axial parenchyma varies considerably from species to species (Richter and Dallwitz 2000). The cross-section showed diffuse porosity with both single and multiple small pores, sometimes obstructed by resins. By SEM, it was possible to observe procumbent ray cells.

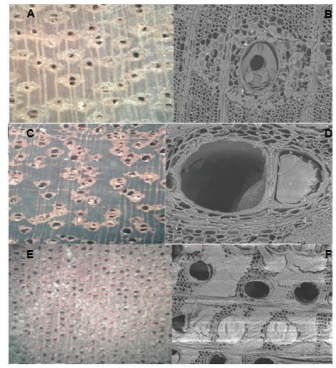

Fig. 1: (A) Amburana cearensis: cross section – 10x magnification; (B) Amburana cearensis: cross section – scanning electron microscope (SEM) – 250x magnification and 300-µm scale; (C) Dypterixodorata: cross section – 10x magnification; (D) Dypterixodorata: cross section – scanning electron microscope (SEM) – 250x magnification and 200-µm scale; (E) Peltogyneconfertiflora: cross section –10x magnification; (F) Peltogyneconfertiflora: cross section – scanning electron microscope (SEM) – 250x magnification and 300µm scale.

DNA extraction

It was possible to extract a considerable quantity of DNA from the dried heartwood of the three species of the Fabaceae family. Protocols 3 and 4 are adaptations of the CTAB method Doyle (1990) and provided the highest concentrations of DNA from the three species, with more than 200 ng/µL of DNA being extracted from the dried heartwood (Tab. 2).

Tab. 2: Concentration of DNA (ng/µL) obtained via the extraction of dried heartwood of three species of the Fabaceae family using five different protocols.

Species	Protocol 1	Protocol 2	Protocol 3	Protocol 4	Protocol 5
	---------- DNA Concentration (ng/µL) ----------				
Amburana cearensis	83.73 ab	11.23 b	**223.68 a**	88. 00 ab	52.10 ab
Dypteri xodorata	114.85 b	1.55 c	89.83 b	**400.40 a**	43.95 bc
Peltogynecon fertiflora	62.90 b	2.28 c	32.36 c	**257.06 a**	7.81 c

Measurements followed by the same letter in a column show no statistically significant difference using Tukey's test at a

With regard to purity, however, the best results were obtained using the QIAGEN Kit (Protocol 2), resulting in an A_{260}/A_{280} ratio higher than 1.8 (Tab. 3).

Tab. 3: Purity of the DNA obtained from three species of the Fabaceae family measured based on spectrophotometer readings of the A260/A280 absorbance ratio.

Species	Protocol 1	Protocol 2	Protocol 3	Protocol 4	Protocol 5
	---------- A_{260}/A_{280} Ratio ----------				
Amburana cearensis	1.18 ab	1.07 b	0.69 c	1.40 a	1.18 ab
Dypterix odorata	1.06 b	**2.08 a**	1.17 b	0.79 b	0.91 b
Peltogynecon fertiflora	0.86 bc	**2.06 a**	0.58 bc	0.56 c	1.11 b

Measurements followed by the same letters in a column show no statistically significant difference using Tukey's test at a 5% probability.

Protocols 2, 3 and 5 resulted in a greater quantity of DNA extracted from *Amburana cearensis* wood compared with the amount extracted from the other species. This result may be due to the anatomical characteristics of *Amburana cearensis*, which is soft to the cut, making the process of macerating the wood to extract genomic material easier and more efficient.

Regarding the A260/A230 ratio, which indicates the quality of DNA samples, the results confirmed what was expected for wood samples considering their chemical composition, with values far below 1.8 being obtained for all of the tested protocols (Tab. 4).

DNA Amplification

It was only possible to amplify the DNA of all three species from the genomic material extracted using Protocol 2 (Fig. 2), resulting in bands with a size of approximately 850 bp. This result demonstrates that in molecular biology studies involving DNA, the first step should be to fine-tune a protocol that enables high-quality genetic material to be obtained, as amplification was most effective for the protocol leading to the highest purity.

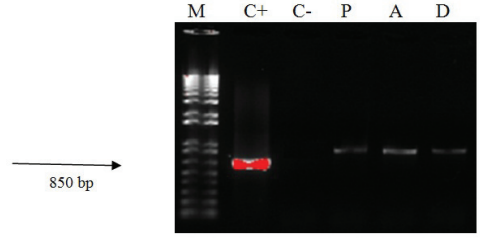

Fig. 2: Gel electrophoresis of the samples amplified using Protocol 2: M- marker 1-kb ladder (Avati), C+ positive control (DNA from a leaf of Lophantheral actescens), C- negative control, P – Peltogynecon fertiflora, A – Amburana cearensis, D – Dypterix odorata.

DISCUSSION

Wood anatomy

Among the main anatomical features of wood, the axial and radial parenchymas are most useful for distinguishing different species (Burger and Richter 1991). Nevertheless, it is difficult to differentiate the genera of certain families based solely on wood anatomy because their anatomical features are very similar (Lopes 2013). DNA identification presents a great advantage in overcoming this limitation because genetic material is unique to each species, allowing effective and highly accurate identification (Jiao et al. 2015).

DNA extraction

As if and Cannon (2005) report that the yield of DNA extracted from dry wood is excellent for quantities of 50 ng/μL or more, and an increase in the amount of wood is necessary to increase the concentration of extracted DNA. In this study, DNA was extracted from samples of dried heartwood, a tissue rich in carbohydrates (cellulose) and phenols (lignin) (LOWE 2008) that also contains dead cells and older cells (Klock et al. 2005).

These characteristics of heartwood lead to degradation of DNA and are directly responsible for the poor quality of the extracted material. Thus, even at concentrations higher than 50 ng/μL, it was not possible to clearly visualise the obtained DNA through gel electrophoresis because the resultant images showed a smear characteristic of degraded DNA. In spite of the highly degraded DNA, the high fidelity of the Taq polymerase enzyme made it possible to amplify the fragments using the PCR technique (Coley and Barone, 1996; Swetha et al. 2014).

The A_{260}/A_{280} ratio indicates the purity of a sample, as DNA shows maximum absorbance at 260 nm, while the other components present in wood, such as proteins, exhibit absorbance in the 280 nm region due to the diversity of amino acids (phenylalanine, cysteine, cystine, methionine, tryptophan, histidine and tyrosine) (Zaia et al., 1998). Thus, the 260/280 ratio represents the ratio of nucleic acids to contaminant proteins (Gallagher 2011). Other substances present in wood, such as phenols and carbohydrates, as well as the residual guanidine present in the columns of the DNA extraction kits, absorb in the 230 nm region (Wilfinger et al. 1997).

The A_{230}/A_{260} ratio must also be considered as a measure of the samples' purity. Ratios in the 1.6 to 1.9 range are considered sufficiently pure (Page 2010), while a ratio of less than 1.6 or greater than 1.9 indicates the presence of polysaccharides (Mello et al. 2015).

The analysis of A260/A230 ratios showed that the tested protocols were inefficient at removing polysaccharides and polyphenols from the samples, as the purity levels were much lower than 1.6, which was expected due to the characteristic chemical composition of the wood.

Mazza and Bitencourt (2000) noted that contaminants such as polyphenolic compounds and terpenoids, which are common in heartwood and are released during cell lysis, adhere irreversibly to DNA and can inhibit digestion by restriction endonucleases and/or amplification through PCR. Rupturing cells also release polysaccharides, which are difficult to separate from DNA (Lodhi et al. 1994).

However, as mentioned with regard to the 260/280 ratio, the impurities detected using the 260/230 ratio can be removed in the DNA amplification step.

According to Colpaert et al. (2005), extraction from dry wood can be challenging, especially if DNA is extracted from heartwood rather than sapwood, because of the presence of inhibitors such as carbohydrates (cellulose) and phenols (lignin) (Lowe 2008) due to the presence of dead and old cells in the heartwood, whereas new cells are found in the sapwood (Klock et al. 2005). In a study carried out by Jiao et al. (2013), the quantity of DNA extracted from fresh sapwood was 8.01ng/mg, while dried heartwood yielded only 4.73 ng/mg DNA, making it impossible to visualise through 1% agarose gel electrophoresis. This difference in the amount of DNA extracted from different radial positions can be explained by the fact that the majority of parenchyma cells are still alive in the sapwood, while the DNA in the heartwoodis gradually degraded during its formation by nucleases and the cell death process (Nakaba et al. 2012).

DNA amplification

The QIAGEN kit used in Protocol 2 has been cited in the literature as being able to generate high-quality DNA from wood extraction, in addition to guaranteeing close to a 100% rate of amplification of the genetic material. This has been confirmed in several studies, including in

the extraction of DNA from archaeological samples of *Populus euphratica* (Jiao et al. 2015); in the extraction of DNA from dry wood of *Cunninghami alanceolata* (Jiao et al. 2012); and in the extraction of DNA to characterise species of the *Dipterocarpaceae* family (Rachmayanti et al. 2006).

Protocol 3 was a modification of the CTAB method (Doyle and Doyle 1990) reported by Swetha et al. (2014), differing in the addition of 3 M sodium acetate (pH 5.2). The addition of sodium acetate together with chloroform and isoamyl alcohol was performed for the extraction of DNA of *Cinnamomum* sp., where sodium acetate functions to remove polysaccharides and proteins, thus directly increasing the quality of the amplification (Swetha et al. 2014). However, this modified protocol was not found to be efficient for the woods tested in the present study, indicating that the chemical composition of the wood affects whether high-quality genetic material is obtained.

CTAB-based protocols are cited in the literature as being efficient at extracting wood DNA. CTAB has been used successfully to extract DNA from *Prunus avium* bark (Cheng et al. 1997) and to extract genetic material for DNA barcoding of Indian tropical dry evergreen forest species (Nithaniyal et al. 2014). The choice of the *rbcL* gene marker for amplification was based on a suggestion from the CBOL – Plant working group (2009), a consortium for the generation of DNA barcodes for plant species, which recommends markers located in coding regions of the chloroplast genome.

Chloroplast DNA shows uniparental inheritance, being transmitted solely by the female gamete in most angiosperms (Demesure et al. 1995). In addition, it is highly conserved in terms of size, content, structure and gene order (Judd et al. 2002), thus allowing primers specific to this genome to be used almost universally throughout the plant kingdom, making it easier to perform comparisons across different taxa (Pinto 2015).

The data obtained from extracting and amplifying DNA from the species tested in this study can be employed in future DNA barcode projects. This identification technique is based on using a small standardised DNA fragment encoding a gene; this DNA sequence is considered a "barcode" stored inside the cells (Herbert et al. 2003). In addition, this technique has been used in phylogenetic studies in many plant species (Turktaş et al. 2012) and can aid in combating illegal logging by making it possible to compare extracted DNA sequences with those of other species by means of analyses with global databases (Jiao et al. 2013).

CONCLUSIONS

It is possible to extract DNA from dried heartwood of the species *Amburana cearensis*, *Dypterix odorata* and *Peltogyne confertiflora*, all of which are native to Brazil.

Considering the speed of the procedure and the usefulness of the data obtained, the QIAGEN kit (Protocol 2) is recommended for extracting DNA from the studied species.

The anatomical characteristic of wood known as "hardness to cut" may affect the maceration of the samples, making it difficult to obtain greater concentrations of the genetic material extracted for wood hard to cut.

DNA can be extracted from the wood of the studied species when it has been dried at room temperature in a lumber yard.

This study provides insights into the identification of native woods through molecular methods that may be used both to add value to commercial timber products and to help on the combat of illegal loggi.

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