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Posted Date: 29 May 2024

doi: 10.20944/preprints202405.1944.v1

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## Article

# An Efficient Plant DNA Extraction Protocol of *Inga feuillei* (Pacae)

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**Abstract:** Plant DNA extraction techniques are well-established, featuring a variety of protocols tailored to the needs of individual laboratories or research goals. Currently, there is a strong demand for plant DNA extraction methods that are rapid, cost-effective, and uncomplicated. We have devised an optimized protocol for plant DNA extraction that eliminates the need for liquid nitrogen. DNA was extracted from leaves of *Inga feuillei* (pacae) in the Cañete Valley using a basic cetyltrimethylammonium bromide method. Subsequently, the extracted DNA was quantified and assessed for quality through agarose gel electrophoresis. This protocol holds the potential to significantly reduce costs in laboratories engaged in routine DNA extraction for genetic diversity and gene expression analyses. It addresses challenges encountered by other conventional methods, particularly in situations where they have proven ineffective due to issues related to explant, sample conditions, and the quantity and quality of nucleic acids. This is especially pertinent for numerous laboratories in developing countries where constraints related to the cost and availability of liquid nitrogen may be a limiting factor. Furthermore, this is the first study reported in this species for genomic studies.

**Keywords:** *Inga feuillei*; pacae; DNA; nucleic acids; plant

## 1. Introduction

*Inga feuillei*, also known as pacae, pacay, guaba, guamo or inga, is a legume and is a plant native to tropical areas and temperate areas of Latin America and the Caribbean. The Inga plant genus originated in the Amazon of Brazil, Ecuador, Colombia, Bolivia and Peru; this plant species is also introduced to a large part of Panama and Costa Rica [1].

Pacae has great nutritional value (vitamin A, B and C), its antioxidant, anti-inflammatory, antiseptic and healing properties stand out; in addition to having fiber, calcium, iron and phosphorus. The fruit, stem, and leaves have different types of metabolites, among them we find flavonoids and tannins; These metabolites could be responsible for the anti-inflammatory, analgesic and gastroprotective therapeutic activity. In addition, it is used to improve vision, reduce cholesterol and triglyceride levels, helps maintain the health of the circulatory system and heart diseases, strengthens bones and improves the immune system. It has a high fiber content, so it has detoxifying properties and helps maintain proper functioning of the system [2].

Many difficulties have been reported for isolating good-quality DNA from plants [3]. These difficulties were attributed to the fact that different plant species have varying levels of polysaccharides, polyphenols, and other secondary metabolites. These components are usually hindering the process of DNA purification and its further use in molecular studies. These plant components have a similar structure of nucleic acids that allow secondary metabolites and polysaccharides to interfere with total DNA isolation [4]. They strongly combine with nucleic acids during DNA isolation and affect the quality of the extracted DNA from higher plants. The present work describes a rapid CTAB-based method with modifications for the extraction of high-quality genomic DNA from *Inga feuillei*.

In this study

2. Experimental Design

A 3 x 2 factorial design was established with 15 repetitions, using 3 c.

Table 1. Factorial design 3 x 2.

Extraction method	Sample Type	
	Dry Sample (D)	Fresh Sample (F)
Cetyltrimethylammonium bromide (CTAB 2X) (Kit 1)	T1. CTAB-D	T2. CTAB-F
GeneJET Plant Genomic DNA Purification Kit (Kit 2)	T3. GenJET-D	T4. GenJET-F
EZ-10 Spin Column Genomic   DNA Minipreps Kit (Plant) (Kit 3)	T5. EZGen-D	T6. EZGen-F

2.1. Materials

- Fresh leaves sample of *Inga feuillei*
- Cetyltrimethylammonium bromide - CTAB (Promega Corporation, Madison, USA)
- β-mercaptoetanol (Central Drug House, New Delhi, India)
- Tris-HCl 1M (Promega Corporation, Madison, USA)
- EDTA 0.5M (Promega Corporation, Madison, USA)
- NaCl (Sigma-Milipore, Saint Louis, MO, USA).
- PVP-40 (Caisson Labs, Smithfield, UT, USA)
- Chloroform (J.T. Baker, Phillipsburg, NJ, USA)
- Iso amyl alcohol (Central Drug House, New Delhi, India)
- Isopropanol (Central Drug House, New Delhi, India)
- Absolute ethanol (Merck, Darmstadt, Germany)
- Rnase A solution (Thermo Fisher Scientific, Waltham, MA, USA)
- Buffer TE solution (Promega Corporation, Madison, USA)
- Agarose (Promega Corporation, Madison, USA)
- Loading dye 6X (Thermo Fisher Scientific, Waltham, MA, USA)
- GeneRuler 1 Kb DNA Ladder (Thermo Fisher Scientific, Waltham, MA, USA)

2.2. Equipment

- Biological safety cabinet (Labconco, Kansas, MO, USA)
- TissueLyser II (Qiagen, Hilden, Germany)
- Microcentrifuge air-cooled (Labnet International, Edison, NJ, USA)
- Thermomixer C (Eppendorf, Hamburg, Germany)
- Biospectrometer (Eppendorf, Hamburg, Germany)
- Enduro Gel documentation Systems (Labnet International, Edison, NJ, USA)

3. Procedure

3.1. Sample Collection

The samples leaf were collected in the district of Lunahuana, province of Cañete, Region Lima - Peru. And they were prepared in accordance with their respective sampling methodologies:

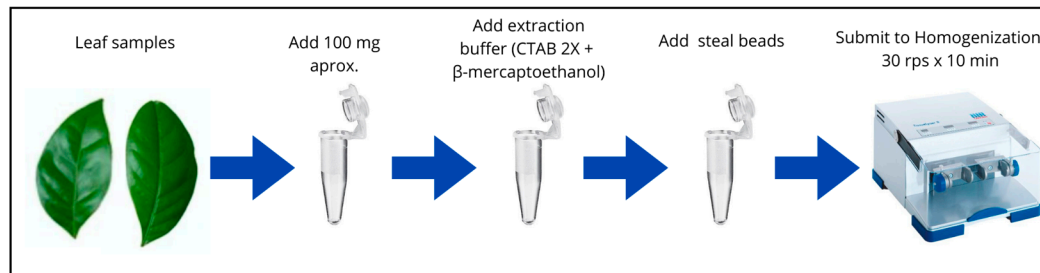
Fresh Sample: Utilizing sterilized forceps, foliar tissue was extracted from the plant and placed into a plastic bag containing moistened paper with distilled water. Subsequently, the samples were stored at 4°C. The viability for usage of these samples is restricted to a maximum duration of three to four days.

Dry Sample: Employing sterilized forceps, foliar tissue was carefully removed from the plant and placed into a plastic bag lined with completely dry paper, to which 100 grams of silica gel were added. This preparation protocol is implemented when the samples are to be processed subsequent to a period extending from four to eight weeks post-collection.

### 3.2. Sample Homogenization

Homogenization of the samples was carried out using a equipment TissueLyser II (Qiagen, Hilden, Germany) with steel beads. The process is described as follows:

1. The sample of plant material (leaves) is collected in a 2 mL tube with 750  $\mu$ L extraction buffer (2X CTAB extraction buffer and 2  $\mu$ L of  $\beta$ -mercaptoethanol).
2. The tubes are then opened, a steel bead is added to each, closed, and placed in the TissueLyser II disruptor kit.
3. The samples are ground for eight minutes at a speed of 30 rps. The tubes are then removed to continue the extraction.



**Figure 1.** Homogenization process.

### 3.3. DNA Extraction

1. Incubate samples at 65°C for 45 minutes to 1 hour. Shaking the tubes constantly (by inversion) every 20 minutes in a bain-marie or at 450 rpm in a Thermomixer C (Eppendorf, Hamburg, Germany).
2. Remove the samples and let them cool at room temperature for 4 min.
3. Add 750  $\mu$ L of Chloroform: Isoamyl Alcohol (24:1), shake the tubes slightly (by inversion approximately 40 times) until the content is homogenized (milky green).
4. Centrifuge at 14,000 rpm for 10 minutes.
5. Transfer 600  $\mu$ L of the supernatant to new 2 mL microtubes, previously labeled.
6. Add 150  $\mu$ L of CTAB 10X (preheated to 65°C), homogenize by inversion (10 times) and add 750  $\mu$ L of Chloroform: Isoamyl Alcohol (24:1) and homogenize (by inversion approximately 40 times).
7. Centrifuge at 14,000 rpm for 10 minutes.
8. Transfer 500  $\mu$ L of the supernatant to 1.5 mL microtubes, previously labeled.
9. Add approximately 2/3 volume of Isopropanol (350  $\mu$ L), shake gently by inversion (10 times) and incubate at -20°C for 30 minutes.
10. Centrifuge at 14,000 rpm for 10 minutes.
11. Decant the Isopropanol being careful not to lose the pellet.
12. Add 500  $\mu$ L of 70% ethanol. Remove the pellet from the bottom of the tube and gently shake the tube.
13. Centrifuge at 14,000 rpm for 10 minutes.
14. Decant the 70% ethanol, taking care not to lose the pellet.
15. Add 500  $\mu$ L of 90% Ethanol. Remove the pellet from the bottom of the tube and gently shake the tube.
16. Centrifuge at 14,000 rpm for 10 minutes.
17. Decant the 90% ethanol, taking care not to lose the pellet.
18. Let the ethanol evaporate until the next day or leave it upside down for 2 to 3 hours to ensure that there is no ethanol left in the tube.
19. Add 100  $\mu$ L T10E1. Gently homogenize the DNA sample.
20. Add 3  $\mu$ L of RNase A and incubate in the thermomixer at 37°C. for 60 minutes at 300 rpm.

### 3.4. Quantification of DNA

1. The ratio of absorbances  $A_{260}/A_{280}$  helps determine if the obtained DNA is contaminated by the presence of aromatic compounds, as these absorb at a wavelength of 280 nm.
2. DNA is considered of optimal quality when the 260/280 ratio is greater than 1.8. A ratio of  $A_{260}/A_{280} > 2.1$  could indicate the presence of RNA in the sample. Conversely, if this ratio is low ( $A_{260}/A_{280} < 1.6$ ), it could be indicative of contamination by proteins or phenols.
3. A second assessment of nucleic acid purity is the 260/230 ratio, as at 230 nm, the maximum absorbance of salts present in the solution, carbohydrates, or other possible contaminants is detected.
4. Accepted values fall within the range of 1.5 to 2.2; if the ratio is less than 1.5, it indicates the presence of contaminants in the sample.

It is crucial to assess the integrity or quality of the obtained DNA, and for this purpose, the agarose gel electrophoresis technique is employed. This technique relies on the utilization of a support matrix created by the polymerization of agarose substances, enabling the selective passage of molecules based on their size [8].

### Sample preparation and electrophoretic migration

1. Dispense 1  $\mu$ L of 6X loading buffer (Loading dye 6X, Thermo scientific) per sample to migrate on a parafilm sheet.
2. Add 2  $\mu$ L of the DNA sample. Mingle.
3. Add 1X TAE buffer to the electrophoretic chamber and place the mold with the already polymerized agarose gel.
4. From the second well, load the samples previously prepared with the 6X loading buffer.



5. Place 2-3  $\mu\text{L}$  of a 1 Kb DNA molecular weight marker (1 kb MP) in the first well of each row of the 1% agarose gel.
6. Connect the electrodes correctly and migrate to 100 V for 30 minutes
7. See the results with the Enduro Gel documentation Systems (Labnet International, Edison, NJ, USA).

### 3.6. DNA Amplification by Polymerase Chain Reaction (PCR)

Samples with higher concentrations and better quality of DNA were amplified using the PCR technique in a thermocycler (Mastercycler X50S, Eppendorf, Hamburg, Germany), using the primers *Inga03* F, 5'- TTCCAAGCTTATACAAACCTCC -3' and R, 5'- AGATCCGTACGTGTGATGGT-3' (Invitrogen, Waltham, MA, USA). The reaction was prepared in a final volume of 50  $\mu\text{L}$ , which contained 25  $\mu\text{L}$  of DreamTaq PCR Master Mix (2X) (Thermo Fisher Scientific Waltham, MA, USA), 1  $\mu\text{L}$  of each 10 mM primer, 2  $\mu\text{L}$  of DNA (~120 ng/ $\mu\text{L}$ ) and sufficient molecular grade water to reach the desired volume. The PCR products were evaluated by electrophoresis on 1% agarose gels in a horizontal electrophoresis chamber and then visually analyzed in a photodocumenter (Enduro GDS Labnet, Tewksbury, MA, USA). The size of the fragments was determined by comparison with a GeneRuler 1 Kb DNA Ladder (Thermo Fisher Scientific, Waltham, MA, USA).

## 4. Expected Results

### 4.1. Reagents Setup

For this activity, glassware and consumables were prepared through sterilization, glass jars, microcentrifuge tubes, and micropipette tips. Additionally, the preparation of extraction buffers was carried out according to the CTAB method, which was previously recommended based on previous research. For this activity, the buffers were prepared: CTAB 10X, CTAB 2X,  $T_{10}E_1$ , 1M Tris pH 8.0 and 0.5 M EDTA pH 8.0, which are required in the different stages of plant DNA isolation [11,12].

#### Buffer CTAB 10X

- Tris-HCl 1M, pH 8.0 100 mM
- EDTA 0.5M, pH 8.0 20 mM
- NaCl 0.7 M
- CTAB 10 %

#### Buffer de Lisis CTAB 2X

- Tris-HCl 1M, pH 8.0 100 mM
- EDTA 0.5M, pH 8.0 20 mM
- NaCl 1.4 M
- PVP-40 1 % (w/v)
- CTAB 2 %

#### $T_{10}E_1$

- Tris- pH=8.0 1M 10 mM
- EDTA-8.0 0,5 M 1 mM
- 1M TRIS -pH 8.0 (adjust pH 8.0 with HCl)
- Tris base pH=8.0 1 M
- 0.5M EDTA -pH 8.0 (adjust pH with NaOH)

## 5. Conclusions

This protocol provides a new guide for more investigations into the *Inga feuillei* (paca). Furthermore, no reports of DNA extraction protocols have been found in this crop, therefore, this study will serve to give other researchers a starting point to carry out new research at the molecular and genomic level.

**Funding:** This research was fully funded by National University of Cañete from Peru.

**Acknowledgments:** Thanks to the authorities of the National University of Cañete for allowing the development of this research and to the students who collaborated in the collection of this species.

**Conflicts of Interest:** The author declare no conflicts of interest.

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