

Notes and Comments

Low-Cost and rapid method for genomic DNA isolation from *Platonia insignis*: an endangered fruit tree species of the Brazilian Amazon

P. M. S. Rivas^{a*} , G. G. Santos^{b,c} , I. C. Albuquerque^a , J. M. A. Silva^d , N. L. Silva^d , V. K. O. Silva-Moraes^a , S. H. S. Felipe^a , J. R. G. Araújo^{a,d} , J. R. S. Barros^c  and T. R. Corrêa^{a,d} 

^aUniversidade Estadual do Maranhão – UEMA, Programa de Pós-graduação em Ciências Agrárias, São Luís, MA, Brasil

^bInstituto Nacional de Pesquisas da Amazônia – INPA, Programa de Pós-graduação em Genética, Conservação e Biologia Evolutiva, Manaus, AM, Brasil

^cUniversidade Estadual do Maranhão – UEMA, Departamento de Biologia, Laboratório de Genética e Biologia Molecular Warwick Estevam Kerr – LabWick, São Luís, MA, Brasil

^dUniversidade Estadual do Maranhão – UEMA, Centro de Ciências Agrárias, Departamento de Fitotecnia e Fitossanidade, São Luís, MA, Brasil

Platonia insignis is a fruit tree native to the Amazon, with enormous potential for food, cosmetic, and pharmaceutical industries (Lima et al., 2022; Rocha et al., 2024). It is an allogamous species with sporophytic self-incompatibility and the high heterozygosity in progenies results in considerable annual irregularity in fruit production (Saraiva et al., 2013). Recent loss of habitat (due to deforestation, changes in land use and urbanization) combined with long juvenile period's makes *P. insignis* an important species for conservation and genetic improvement efforts (Nascimento et al., 2021; Alves et al., 2025). In this context, the characterization of germplasm for the selection of more adapted, more productive and faster genotypes is essential, and molecular tools play a crucial role in accelerating breeding and conservation programs (Pontes et al., 2017). For that, high-quality DNA isolation is crucial. Also, the choice of an adequate tissue sampling method is a key step.

Obtaining leaf tissue from *P. insignis* trees requires complex tree climbing logistics, since productive trees reach up to 30 meters in height (Alves et al., 2025). Alternatively, leaf acquisition from seedlings is a slow process, since epicotyl emergence can last from 2 to 6 months, due to a dormancy in the seed plumule region (Carvalho and Nascimento, 2018). For that reason, we aimed to validate modifications on cetyltrimethylammonium bromide (CTAB) extraction protocol and compare its efficiency in leaf tissue preserved in four different forms. Also, we wanted to evaluate the efficiency of this method to isolate DNA from the meristematic tissue of the primary root, whose emergence is available approximately 30 days after sowing, which could contribute to early genotyping of *P. insignis* progenies.

The leaf tissues were used in four states: fresh leaves (FL), frozen leaves (FzL), previously stored at -20°C, in silica leaves (SiL), and dried leaves (DrL), from exsiccates. Root meristematic tissue (Frozen Root, FzR) was sampled 20 days post-sowing and stored at -20°C until DNA

extraction (Figure 1A). Approximately 200mg of tissues were subjected to the CTAB protocol with modifications, as described below: 1) Tissue were manually macerated with extraction buffer in porcelain mortars and pistils [CTAB 2%, NaCl 1.4M, EDTA 20mM, Tris-HCl pH 8.0 100mM, Polyvinylpyrrolidone 2%, Proteinase K 0.1mg/mL and β-mercaptoethanol 0.2%]; 2) The mixture was incubated in a water bath at 65°C for 45 minutes, 3) 700μL of Chloroform-isoamyl alcohol (CIA 24:1) was added, followed by homogenization by gentle inversions for 10 min; 4) First centrifugation was performed (13,200rpm, for 5 minutes) and the aqueous phase was transferred to new tubes; 5) 300μL of 10% CTAB were added to each tube and another 700 μL of CIA, followed by gentle inversions for 5 minutes; 6) Samples were centrifuged at 13,200rpm for 5 minutes, and the aqueous phase was removed to a new tube; 7) The aqueous phase was incubated with RNAase (40μg/μL) at 37°C for 30 min; 8) 500μL of isopropanol was added for DNA precipitation at -20°C; 9) Tubes were centrifuged at 13,200rpm for 10 min, at 4°C; 10) The isopropanol was discarded and the pellet was washed with 70% and 95% ethanol, and then allowed to dry; 11) DNA was resuspended in 50μL of TE (10mM Tris-HCl, 1mM EDTA pH 8.0). Modifications were adapted from Doyle and Doyle (1990) and Alzate-Marin et al. (2009).

DNA concentration (ng/μL) and purity (A260/A280 and A260/A230 ratios) were measured using a NanoDrop spectrophotometer (BioDrop UV/VIS, BioDrop Ltd.), based on 1μL of extracted DNA. DNA integrity was assessed by electrophoresis in a 1% agarose gel (100V, for 1 hour). To verify the efficiency of the method, ANOVA test followed by Tukey post-hoc test was used (p<0.05). Data were analyzed using GraphPadPrism v.8.0.1 software.

High concentrations of DNA were obtained in all tissues (Figure 1C). The DNA concentrations isolated in FzR were statistically lower than obtained in FzL, indicating that tissue influenced the amount of isolated DNA (Table 1, p<0.05).

*e-mail: priscila.sarivas@gmail.com

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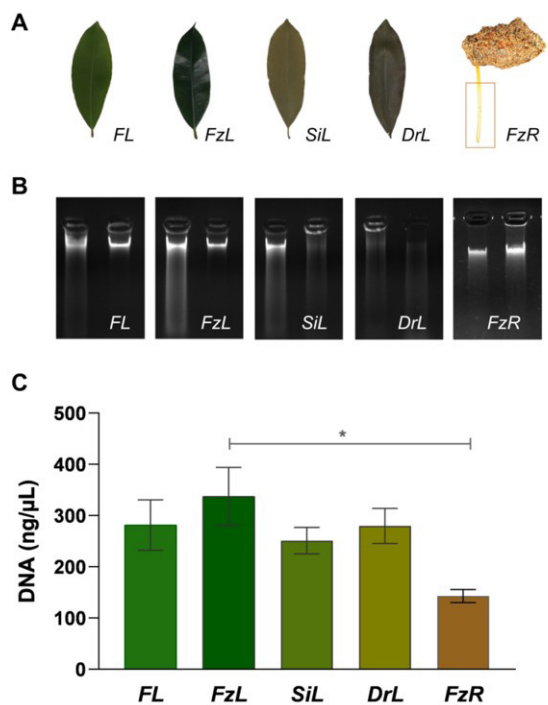


Figure 1. A) Plant tissues and preservation methods of *P. insignis*: fresh leaf (FL), frozen leaf (FzL), silica gel leaf (SiL), dried leaf (DrL) and frozen root (FzR); B) Agarose gel electrophoresis (1%) of extracted DNA from different tissues and preservation methods of *P. insignis*. C) DNA concentration (ng/μL) isolated from different tissues and preservation methods. Bars represent mean ± standard deviation. Asterisks indicate statistically significant differences after ANOVA test (* $p < 0.05$).

Table 1. DNA concentration (ng/μL) and absorbance ratio values obtained in *P. insignis* different tissues: fresh leaf (FL), frozen leaf (FzL), silica gel leaf (SiL), dried leaf (DrL) and frozen root (FzR).

Samples	DNA concentration (ng/μL)	A260/280	A260/230
FrL	281.60 ± 49.10 a	1.84 ± 0.01 a	1.72 ± 0.11 a
FzL	337.30 ± 56.62 ab	1.80 ± 0.01 a	1.73 ± 0.09 a
SiL	251.10 ± 25.80 ab	1.79 ± 0.11 a	1.05 ± 0.13 b
DrL	279.80 ± 34.02 ab	1.71 ± 0.09 a	1.48 ± 0.18 b
FrR	143.00 ± 12.77 b	1.83 ± 0.02 a	1.58 ± 0.11 ab
ANOVA			
F	3.385	0.658	4.344
P	< 0.05*	0.63 (ns)	< 0.01**

Data are presented as mean values ± standard error. ANOVA results are shown for each factor: ns: not-significant; *significant ($p \leq 0.05$); **significant ($p \leq 0.01$). Different letters indicate significant difference between the means of the experimental groups after Tukey post-hoc comparison test.

Although fresh leaves are considered the best tissue type for DNA isolation (Funk et al., 2017), our results indicate that root meristem tissue can also be isolated with our CTAB modified protocol, but with lower amounts. Some tissues and tree species require more complex and optimized extraction protocols than others (Alzate-Marin et al., 2009; Pratyusha, 2022; Schenk et al., 2023). For DNA isolation, *P. insignis* did not require big adaptations. The first adaptation made was the removal of liquid nitrogen for tissue maceration. Although liquid nitrogen is the most used for tissue maceration, since it paralyzes enzymatic

and cellular processes and prevents the degradation of DNA and RNA (Sahu et al., 2012), it requires a complex and high-cost infrastructure, which is not always available in molecular biology laboratories (Santos and Araújo, 2017; Hale et al., 2020). Here, we validated the manual maceration as equally effective for obtaining good quantity and high integrity of DNA (Figure 1B), with lower costs, similar to those reported by Quiñones et al. (2024). The modified protocol presented higher efficiency than observed in some commercial that are also expensive and not available for all labs (Abubakar et al., 2018).

The protocol was efficient to obtain DNA free of proteins or organic solvents (Table 1, xA260/280=1.8), but not free from phenolic compounds, especially on SiL and DrL samples, that significantly differed from FrL and FzL for A260/230 ratio (Table 1, $p < 0.05$). The A260/230 ratio ranged from 1.05 to 1.73 (Table 1), values below the ideal range (2.0–2.2), suggesting that additional washing steps of the pellet with 70% ethanol or an extended incubation period in the water bath could improve the removal of salts and phenolic residues, or increase their solubilization and consequent removal during contaminant centrifugation in the evaluated tissues, especially in SiL and DrL.

The A260/230 ratio ranged from 1.05 to 1.73 (Table 1), values below the ideal (2.0–2.2), suggesting that additional washing steps of the pellet wash with 70% ethanol could had improve the removal of salts and phenolic residues, other option would be increasing the incubation period on water bath, that could had enhance the contaminants solubilization and subsequent removal (on centrifugation) in the tissues, especially in SiL and DrL.

Long storage times can increased DNA fragmentation can negatively interfere with the detection limit of the molecular markers (Demeke and Jenkins, 2010). Our findings indicate that the storage in silica gel is not the best option for sampling *P. insignis* leaves if the goal is to isolate high-quality and pure DNA and that leaves freezing could be the best sampling method for genetic studies with *P. insignis*. Our data also evidenced a new method to isolate high-quality but in intermediate-quantity DNA from young roots meristematic tissue. In *P. insignis*, the emergence of the aerial part occurs slowly and unevenly, taking 2 to 5 months, due to a peculiar type of dormancy that occurs in the plumule region of bacuri seeds (Carvalho and Nascimento, 2018). But seeds undergo a period of vigorous growth of the primary root, whereas more meristematic root tissue can be sampled, without compromising the future seedling.

This research contributes to the optimization of DNA extraction techniques for *P. insignis*, supporting molecular marker-assisted selection in breeding, population genetics, and conservation programs for this threatened Amazonian species of nutritional, social, and economic significance in northern and northeastern Brazil

Data Availability Statement

The research data are only available upon request to the corresponding author.

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