Standardization of Protocol for DNA Extraction from camu-camu (*Myrciaria dubia*).

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INTRODUCTION

The camu-camu (*Myrciaria dubia* (HBK) McVaugh 1963) species of the family *Myrtaceae*, popularly known as caçari, araçá d'água or sarão, are found naturally on the border of rivers, lakes and flooded forests throughout the Amazon region, predominantly in the black waters, alluvial substrates, loamy in texture, clay, silt-loam, sandy loam, and drained soils. Also occurs naturally on the border of the muddy and white waters and white. Its geographical distribution extends from the central region of the state of Pará, through the middle and high Amazon River to the western part of Peru and the extreme north brazilian state of Roraima, where is found in the Casiquiare river and much of the high and middle basin Orinoco. In Rondonia state, it can be found along the rivers Ji-Parana, Candeias and Madeira. The consumer market of camu-camu is growing, due to the nutritional value of fruit that has a high concentration of vitamin C (ascorbic acid) with a wide variation between 0.845 g to 6.112 g ascorbic acid /100g of pulp higher than in acerola (a , 79g/100g of pulp). The objective of this study was to establish a protocol for pure DNA extraction, intact, in high concentration, suitable for analysis of genetic diversity.

MATERIAL AND METHODS

The biological material used for gDNA extraction were fresh and dried leaves which were a overall of 30 accessions of plant camu-camu, found along the border of the Madeira river in the region of Porto Velho / RO, around the Santo Antonio Hydroelectric Mill (UHSA.)

Fig 01 **A**: Fruits of camu-camu, **B:** Seeds of camu-camu benefited and **C:** Seedlings of camu-camu selected for gDNA extraction and study of genetic variability.

To standardize the protocol for DNA extraction were tested three different methods, Doyle & Doyle (1987), Plant DNAzol (Invitrogen) and Dellaporta et al. (1983). The quality of extracted gDNA was tested with three microsatellite markers via PCR.

RESULTS AND DISCUSSION

Among the three methods of gDNA extraction from camu-camu tested the one who presented the best result was the method of Doyle & Doyle (1987) (CTAB 2%) and modified the conditions of the laboratory (fig. 2) .

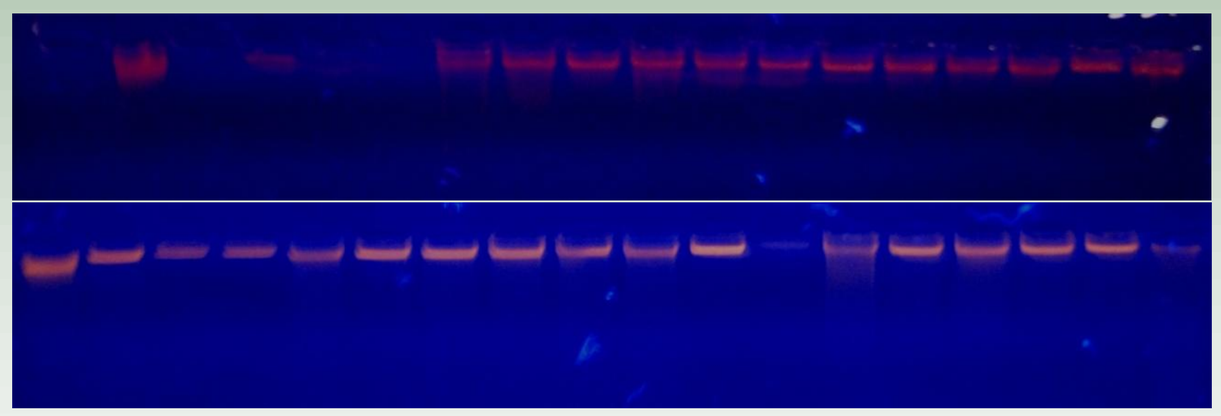

Fig. 02: Electrophoresis profiles of DNA camu-camu extracted with CTAB 2% protocol in agarose gel and stained with ethidium bromide.

The analysis results via PCR with three microsatellite markers, amplification fragments were obtained from 30 samples (fig. 03).

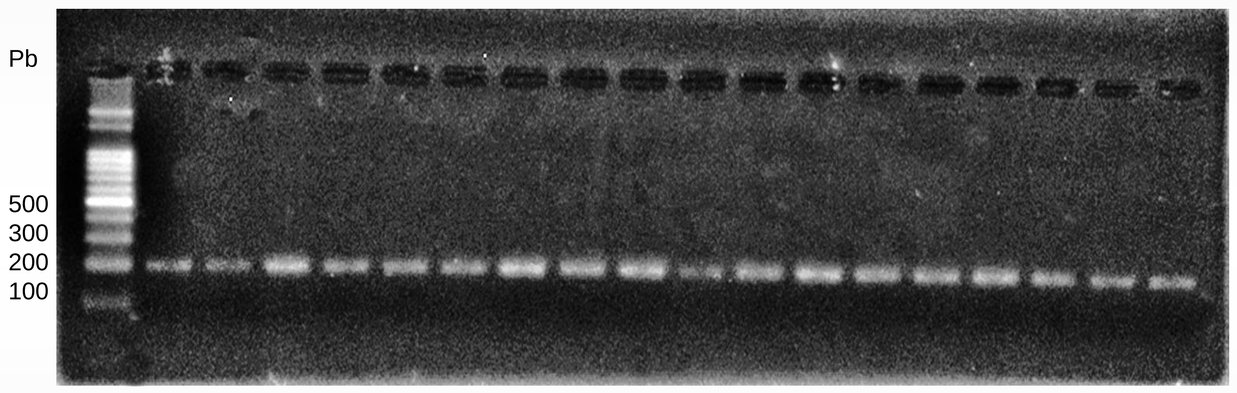

Fig.03 Agarose gel with the profile of the fragments amplified with primer MDI010 DNA samples extracted camu-camu protocol established with the method of Doyle & Doyle (1987) with adaptations to the conditions of the laboratory.

CONCLUSION

Of the three methods of gDNA extraction plants tested in this study, the method showed that the efficacy of gDNA extraction from leaf samples of camu-camu was to Doyle & Doyle (1987).

The amplification fragments obtained with primers of microsatellite revealed that the DNA extracted are of high quality, i.e., has no inhibitory compounds of the PCR reaction.

REFERENCES

DOYLE, J. J.; DOYLE, J. L. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. **Phytochemical Bulletin**, v. 19, p. 11-15, 1987.

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