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UNIVERSIDADE ESTADUAL PAULISTA "JÚLIO DE MESQUITA FILHO"
FACULDADE DE ENGENHARIA
CAMPUS DE ILHA SOLTEIRA

LORENA FRIGINI MORO CAPO

TIMBER TRACKING OF *Jacaranda copaia* (Aubl.) D. Don. (BIGNONACEAE) FROM AMAZON FOREST USING DNA FINGERPRINT

Ilha Solteira
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LORENA FRIGINI MORO CAPO

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Doctoral thesis presented to the Engineering Faculty of Ilha Solteira – UNESP as part of the requirements to obtain the title of PhD in Agronomy.

Supervisor
Prof. Dr. Alexandre Magno Sebbenn

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TÍTULO DA TESE: TIMBER TRACKING OF Jacaranda copaia (Aubl.) D. Don. (BIGNONACEAE) FROM AMAZON FOREST USING DNA FINGERPRINT

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DEDICATION

I dedicate this thesis to my father, Jorge Luiz Moro Capo (in memory) who supported me and made it all possible.

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RESUMO

A floresta Amazônica e outras florestas tropicais ao redor do mundo estão atualmente sendo intensivamente desmatadas para e abertura de áreas para uso na agropecuaria intensiva e extração de madeira, o que em geral ocorre ilegalmente em áreas protegidas como reservas e áreas indigenas, resultando em problemas ecológicos, ambientais e econômicos. Com o objetivo de parar o desmatamento e a comercialização de madeira de corte ilegal de florestas tropicais, novas leis foram introduzidas em muitos países. Aqui investigamos a utilidade da impressão digital de DNA de marcadores SNPs nucleares e citoplasmáticos para rastrear a origem da madeira extraida e comercializada da árvore neotropical *Jacaranda copaia*. Amostras de 832 indivíduos de 43 populações da Bolívia, Brasil, Guiana Francesa e Peru foram utilizadas para investigar o poder de marcadores SNPs, sendo 113 nucleares (nSNPs), 11 cloroplastidiais (CpSNPs) e quatro mitocondriais (MtSNPs) para determinar corretamente o país, população e região dentro do Brasil e Peru de origem. A diferenciação genética (G'_{ST}) entre todas as populações, entre populações de diferentes países e entre regiões dentro dos países foi alta (0,506-0,698), especialmente para locos CpMtSNP (> 0,9), mostrando um forte padrão genético de isolamento por distância entre populações, o que é favoravel à determinação correta do local de origem de amostras de mandeira de indivíduos de *J. copaia*. Para testes de auto-atribuição foi possivel determinar corretamente, com 100% de precisão, o país, população e região de origem de todas as amostras quando foram utilizados todos os locos SNPs ou apenas os nSNPs. Os resultados mostraram que o uso de todos os marcadores SNPs ou nSNPs é uma ferramenta precisa e útil para alfândegas e policias nacionais e internacionais averiguarem se o local de extração declarado na documentação de exportação de madeira de *J. copaia* da Floresta Amazônica tem origem legal ou ilegal.

Palavras-chave: comércio de madeira; desenvolvimento de marcadores moleculares; impressão digital de DNA; *Jacaranda copaia*; polimorfismo de nucleotídeo único; rastreamento de madeira.

ABSTRACT

Amazon and other tropical forest are actually subject to strong deforestation, generally originated from illegal logging, resulting in ecological, environmental and economic problems. Aiming stop deforestation and timber commercialization of illegal logging of tropical forest, new laws has been introduced in many countries. Here we investigated the utility of DNA fingerprinting of nuclear and cytoplasmatic SNPs markers to timber tracking the intensive logged and commercialized of the Amazonian Neotropical tree *Jacaranda copaia*. Samples of 832 individuals from 43 populations from French Guiana, Brazil, Peru, and Bolivia were used to investigate the power of 113 nuclear SNPs, 11 CpSNPs and four MtSNPs loci to determine the country, population and region within Brazil and Peru origin. The genetic differentiation (G'_{ST}) among all populations, contries, and regions within coutries was generaly high (0.506-0.698), specialy for CpMtSNP (> 0.9) loci, and there is a strong isolation by distance pathern among populations, favoring the group or individual samples tracking to correct site. For self-assignment tests, we were able to 100% correct determine country, population and region site origin of all samples using all SNPs and nSNPs. Our results show that the use of all SNP or nSNP markers are suitable to correct determination of country and population site of *J. copaia* timber origin and very useful tool for customs and local and international policies.

Keywords: DNA fingerprinting, *Jacaranda copaia*; marker development; single nucleotide polymorphism; timber tracking; timber trade.

SUMMARY

1 INTRODUCTION

Much of the world's commercial logging from natural forests is illegally sourced, and even the legal source has come from unsustainable logging. This is especially true for tropical forests, where most of the world's plant and animal biodiversity lives. Both illegal and legal harvesting contribute to the loss of biodiversity, diminishing the potential for human resources to find medicinal and future sources of wood. Illegal logging is also an economic problem for the legal market, as extremely low-priced timber competes with legal logging, where costs are higher (GRAY, 2002; DEGEN *et al*., 2013). Because of this, many international casualties were established in countries around the world to prevent illegal timber imports.

To trace the origin of wood, many methods have been tested, such as chemical differences between wood species (PAREDES-VILLANUEVA *et al*., 2018), transformation cyclotron resonance mass spectrometry (DEKLERCK *et al*., 2017), wood anatomy and DNA fingerprinting (JOLIVET; DEGEN 2012; DEGEN *et al*., 2013; LOWE *et al*., 2016; CHAVES *et al*., 2018; SEBBENN *et al*., 2019). Results from different methods showed strong potential for determining species, country, and site origin, in particular using DNA fingerprinting (LOWE *et al*., 2016; CHAVES *et al*., 2018)

The current work investigated the use of the DNA fingerprint method to track the intensive and high value wood of the Neotropical pioneer tree *Jacaranda copaia* (Aubl.) D. Don. (Bignonaceae). The wood is light and used for furniture (LOUREIRO *et al*., 1979). This is a fast-growing species, with an average annual increase in diameter at breast height (DBH) of 2.05 cm and height of 1.98 m, with a great capacity for regeneration in gabs (SAMPAIO *et al*., 1989). The trees have a straight stem, reaching 106 cm in DBH and 45 m in height (VINSON *et al*., 2015a). The species occurs from northern to western South America, from Belize to Bolivia and Brazil, French Guiana, Peru (GENTRY, 1992). In Brazil, the species is found in the states of Acre, Amapá, Amazonas, Maranhão, Rondônia, Roraima, Mato Grosso and Pará and populations generally have more than one tree per hectare (VINSON *et al*., 2015a). The species is hermaphroditic, self-incompatible and about 40 species of bees, butterflies and hummingbird wasps were detected as potential pollinators, although Euglossa spp. and Centris spp. bees were detected as the main pollinators in the Tapajós National Forest, Brazil (MAUES *et al*., 2008). The fruits can have up to 250 seeds and the winged seeds are dispersed by the wind (MAUES *et al*., 2008).

3.5 CONCLUSION

The genetic differentiation (G'_{ST}) among all populations, contries, and regions within coutries is generaly high, specialy for CpMtSNP loci, and there is a strong isolation by distance pathern among populations, favoring the group or individual samples tracking to correct site. For self-assignment tests, we were able to 100% correct determine country, population and region site origin of all samples using all SNPs and nSNPs. Our results show that the use of all SNP or nSNP markers are suitable to correct determination of country and population site of *J. copaia* timber origin and very useful tool for customs and local and international policies. The *J. copaia* reference database of our study represents a robust assignment tool available to timber companies or governmental agencies to test and validate origin declarations. It is recommended to use the method described here for other tropical native species, since it presents high efficiency when it comes to showing the origin of the wood, thus helping the police and the competent bodies in the delimitation of illegally deforested areas, as well as unsustainable extraction.

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