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Desenvolvimento de marcadores moleculares espécie-específicos para
a identificação de *Eucalyptus*

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**Botucatu-SP
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UNIVERSIDADE ESTADUAL PAULISTA
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INSTITUTO DE BIOCÊNCIAS DE BOTUCATU

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a identificação de *Eucalyptus*

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Orientador: Prof. Dr. Celso Luís Marino

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PREFACE

The forest-breeding program in Brazil has the general objective of providing most adapted plants to different environments for various Brazilian regions, for fulfilling timber demands meant for multiple uses in the country. One of the main problems found in different forest breeding programs are the difficulty to identify the different species and hybrids. The use of molecular biology techniques in plant breeding programs is found very effective in the optimization of the time and the direction of these programs, particularly among those plants of the same subgenus. The process of selection and hybrid plants selected for planting in most cases; significantly increase the gain in terms of production and adaptability. The use of molecular markers to characterize the molecular variability of forest species has revolutionized genetic analysis in recent years. The bulk segregant analysis (BSA) is a technique used to identify molecular markers linked to monogenic, dominant or recessive characters. BSA technique in combination with Amplified Fragment Length Polymorphisms (AFLP) technique is an efficient methodology for the detection of polymorphism from genomic restriction fragments through PCR amplification; which helps in analyzing large number of loci for testing without the need for previous information of their sequence in respect to their dominance and reproducibility. The most recent and promising applications of molecular biological methods for the detection of small DNA fragments as identification tool and constituency of species in plants and animals is called DNA barcode. The use of barcode DNA sequence is useful for grouping data and analyzing jointly in order with ease of amplification and sequencing and quality of sequence discriminatory power of the marker.

The results obtained during the development of this study are presented in a scientific article format. The first paper was submitted to the *Silvae Genetics* journal, entitled “**Development of molecular markers for the eucalyptus species identification**”, which showed the analysis of AFLP markers and BSA that were associated with the identification of five species of *Eucalyptus* (*E. saligna*, *E. tereticornis*, *E. urophylla*, *E. grandis* and *E. brassiana*). The second article will be submitted to the *Plos One* journal with the title “**Evaluating the capacity of plant DNA barcodes to discriminate species of *Eucalypts***”, we are presenting our results for evaluating of the discriminatory power of the DNA barcode label based on the use of internal transcribed spacer *ITS1*, *ITS2* and plastid genomes *rpoCl*, *matK*, *rbcL* and *ycf1*, enabling the genetic separation of 14 species of *Eucalyptus*.

Objectives

The general objective of this study was to evaluate the applicability of molecular markers associated with the identification of species of eucalypts.

They are also specific objectives:

Identifying by applying Bulk Segregant Analysis (BSA) and Amplified Fragment Length Polymorphism (AFLP) markers associated with specific species of *Eucalyptus*.

Evaluating and quantifying the DNA-barcode efficiency to discriminate species of *Eucalyptus*, based on internal transcribed spacer *ITS1*, *ITS2* and plastid genomes *rpoC1*, *matK*, *rbcL* and *ycf1*.

Chapter I

- Article submitted for journal *Silvae Genetica* (under revision).

Title: Development of molecular markers for the eucalypts species identification

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