DEVELOPMENT OF 13 MICROSATELLITE LOCI TO *Cariniana legalis* MART. O. KUNTZE FROM AN ENRICHED GENOMIC LIBRARY.

Evandro Vagner Tambarussi, Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo (Esalq/USP), Departamento de Genética, Piracicaba, SP. evtambarussi@yahoo.com.br.; Alexandre Magno Sebbenn, Estação Experimental de Tupi, Instituto Florestal de São Paulo, Piracicaba, SP. Maria Andréia Moreno, Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo (Esalq/USP), Departamento de Ciências Florestais, Piracicaba, SP. Elza Martins Ferraz, Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo (Esalq/USP), Departamento de Ciências Florestais, Piracicaba, SP.Paulo Yoshio Kageyama, Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo (Esalq/USP), Departamento de Ciências Florestais, Piracicaba, SP. Roland Vencovsky, Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo (Esalq/USP), Departamento de Genética, Piracicaba,SP.

INTRODUÇÃO

Jequitibá-rosa (*Cariniana legalis* Mart. O. Kuntze), the symbol tree of São Paulo state, is one of the largest trees of the Atlantic Forest. This hermaphroditic species is pollinated by bees and dispersed by wind. The specie is endemic from Brazil and occur in low population density (< 1 tree/ha). Because of intensive exploration and high deforestation rates in native populations, C. legalis were reduced to small forest fragments. Here we report the development of a range of thirteen nuclear microsatellite markers for C. legalis.

OBJETIVOS

Thus, the aim of this work was develop microsatellite loci for C. legalis species to facilitate some studies of genetic diversity and structure, gene flow, and mating system.

MATERIAL E MÉTODOS

The enriched genomic library was obtained using a protocol of biotin-labeled microsatellite oligoprobe and streptavidin-coated magnetic beads (Billotte *et al*., 1999). Total genomic DNA was extracted from an individual tree of *C. legalis* (Ibicati-SP). 1µg from total DNA extracted was digested with RsaI enzyme restriction. The fragments were then linked to adapters Rsa21 (5'CTCTTGCTTACGCGTGGACTA3') and Rsa25 (5' AGTCCACGCGTAAGCAAGAGCACA3'). Fragments containing CTn/GTn repeats were selected by hybridization to biotinylated oligonucleotides complementary to the repetitive sequence, and were recovered by magnetic beads linked to streptavidine. cloned in plasmid vector pGEM-T (Promega) and transformed into chemically competent Escherichia coli cells. Transformed cells were cultivated on agar plates containing 100 g ml.1-1 ampicillin and 50 g ml.1-1 X-galactosidase and Isopropyl b-D-1-thiogalactopyranoside (IPTG). Single white colonies were selected and storage at -80°C.

RESULTADOS

We identified 96 clones containing 82 repeat motifs from a genomic library enriched for (CT)8 and (GT)8. Using universal primers, DNA inserts were sequenced on ABI 3730 automated DNA sequencer (Applied Biosystems,

CA) using dye-terminator fluorescent chemistry. Primers pairs flanking the repetitive sequences were designed. Thirteen pairs of primers were designed. Of these, 10 (12.2%) were mononucleotide, 62 (75.6%) di, 2 (2.4%) tri, 6 (7.3), tetra and 2 (2.4) were hexanucleotides. Pentanucleotides were not found.

DISCUSSÃO

We are scanning and optimizing these loci for the species under study and in addition to being tested also in *C. estrellensis*. We plan to use these markers to estimate mating system and gene flow patterns in natural populations of *C. legalis* and *C. estrellensis*.

CONCLUSÃO

In conclusion, this study developed 13 pairs of primers that will allow their use to understand the genetic status of current forest fragments, as well as remnant populations in preserved areas.

REFERÊNCIAS BIBLIOGRÁFICAS

BILLOTTE N; P.J.L.LAGODA; A.M.RISTERUCCI; F.C. BAURENS (1999). Microsatellite-enriched libraries: Applied methodology for the development of SSR markers in tropical crops. Fruits 54: 277– 288.

Agradecimento

O autor gostaria de agradecer à Fundação de Amparo a Pesquisa - FAPESP pelo apoio financeiro concedido na forma de bolsa de estudos de Doutorado (Fapesp 2010/12354-3).