GENETIC DIVERSITY OF *SOLANUM MAURITIANUM* SCOPOLI (SOLANACEAE) IN DIFFERENT SUCCESSIONAL STAGES

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INTRODUCTION

The environment degradation in southern Brazil reflects mainly the expansion of farming edges and also, natural land occupation. Deforestation leads to forest fragmentation and generally promotes reduction of population size as well as their isolation that may result in both endogamy and genetic drift. On this way, several approaches are being developed to restore forest patches and to connect them in order to promote genetic flow among them9.

In such approach, populations of pioneer species are quite relevant to start the successional process due to its wide ecological plasticity and direction on these processes. Also, genetic variability is a key factor on such methods because new established populations may need critical abiotic requirements, which will promote population growth or decline3,5.

Molecular markers have been known to be sharp into estimate genetic variability and, among several methods developed to measure molecular markers, RAPD (Random Amplified Polymorphic DNA) tecniches bring some advantages: simplicity, speed, low cost, low quantities of DNA to analyses, the possibility to study species with any kind of genetic information and species with low or none polymorphism in allozymic loci. On this way such analyses is quite interesting to perform plant genetic studies, due most to the restriction in obtaining large DNA quantities and qualities to amplification from many species10.

In Southern Brazil, a small native tree, popularly called fumo - brabo, *Solanum mauritianum* Scopoli (Solanaceae), spreads widely in recent left areas as edges of highways and tracks, forest gaps and borders, abandoned plantations and pasture, playing the role of pioneer and contributing to the forest expansion process. *S. mauritianum* is exceptionally abundant in those Seasonal Semideciduous Forests7 and shows both anatomical, physiological and morphological attributes that confer to this species high ecological value, as insect pollination, orthodox seeds, large seed bank12, stimulated germination by fire, rapid growth, longevity of up to fifteen years6 and wide dispersion area11. Also, many species of the genus *Solanum*, including *S. mauritianum* are recommended by many authors to plantation in degraded areas focusing on restoration. One example are the areas near the Itá hydroelectric reservoir in southern Brazil, where dense tree canopy (with predominance of *Solanum mauritianum*) promoted the establishment of pioneer and secondary species as bracatinga (*Mimosa scabrella*) and aroeirinha (*Schinus terebinthifolius*) which emerged over bush stratum starting a spontaneous restoration of that area.

However, the lack of information over seed technology and genetic diversity2 has hindered its use in environmental reforestation programs. Pioneer species like *S. mauritianum* presents own features dynamics to local extinction and recolonization, associated with soil seed bank. The persistent seed bank promotes generations' overlap that reduces the effect of founder, while this effect is stronger when the seed bank is narrow, which can contribute to gene flow by increasing or reducing genetic differentiation among populations13. The existence of seed banks that can act like gene buffer presents both genetic and ecological consequences to the species due to the ability to restore genetic variation lost by population before local extinction. Also, these species often have large pollen flow and seed dispersion which may decrease the genetic distance among populations geographically distant9,1. Related to this, the knowledge about genetic structure of pioneer species became important, which will contribute to better management planning, including seed collecting programs and restoration of corridors to gene flow9.

OBJECTIVES

The objective of this work was to investigate the genetic diversity of *S. mauritianum*, within and among four populations by RAPD molecular markers, in order to contribute to the knowledge about gene flow dynamics on this species, providing genetic diversity data about tree native pioneers

species that can be used into conservation strategies.

MATERIAL AND METHODS

Study areas and plant material

We sampled three natural populations of *Solanum mauritianum* in Rio Grande do Sul state (UCC and UCT-Conservation Unity Teixeira Soares in Marcelino Ramos; EE - Erechim), and a fourth population in Santa Catarina state (SC-Piratuba) including areas with different altitudinal (320, 480, 560 e 720m respectively) and spatial gradients (UCT-tracks borders; UCC-abandoned plantation; EE-anthropogenized area and SC-edge of highway. Indeed, the fourth population is separated from the others by the presence of the high order river Rio Uruguai, which we hypothesized that it could play a barrier role. We collected leaf samples from 12 randomly selected individuals within each population. We collected samples only from adults and kept 15 meters among individuals. All samples were identified and lodged in Styrofoam box with ice. In the Biotechnology Laboratory from URI, those samples were stored at deepfreezer (- 85^{0}C) to DNA extraction.

RAPD Analyses

Total genomic DNA was extracted using the protocol of Doyle; Doyle4 modified to optimize. The procedures consisted in maceration of equal quantities of fresh tissues in liquid nitrogen; addiction of extraction buffer (CTAB 2%); incubation in 65 ^{0}C for 30 min, desproteinization with chloroform - isoamylalcohol (24:1), precipitation with Absolute Ethanol; washing with Ethanol (70%) and the obtained nucleic acid pellet was dissolved in TE (trisma - EDTA). The DNA quantities were quantified by spectrophotometric UV, assuming an equivalence of 50 μg.ml 1 to one absorbance unity to 260 nm. Subsequently, we evaluated the DNA quality by the reason A260/A280 and viewing in agar gel electrophoresis (0.8%) to confirm DNA integrity.

To Polymerase Chain Reaction was used: reaction buffer (50 mM Tris - HCl pH 9.0; 50 mM KCl; 0.5% Triton - X 100); dNTPs (200 mM each), 0.2 microM primer, 3 mM MgCl2, 40 ng DNA and 1.5 U Taq DNA polymerase. The amplification was performed in a MJ Research INC thermalcycler, following the procedure: 3 minutes at 92 ºC (initial strand separation), 40 cycles of one minute at 92 ºC (denaturation), one minute at 36 ºC (annealing) and 2 minutes at 72 ºC (primer extension). After, 3 minutes at 72 ºC and cooling at 4 ºC until retreat of samples.

We made a sorting of primers that were able to detected polymorphism. For this, we tested primers from *Operon Technologies kits*: OPA, OPB, OPF, OPH, OPW, and OPY, evaluating the quantities, intensity and polymorphism that the bands generated. The bands were qualified in weak, medium and strong, being selected to results just medium and strong bands. We selected like polymorphics the *primers*: OPB18, OPB20, OPF6, OPF7, OPF13, OPH3, OPH5, OPH6, OPH15, OPH19, OPW10 and OPW17.

The amplified DNA was electrophoresed in a 1.4 % agar gel containing 0.5 mg.mL - 1 ethidium bromide, in TBE1X buffer (trisma, Boric Acid and EDTA), in horizontal electrophoresis system, with constant voltage (100 volts) during 240 minutes. The DNA size marker was Lambda DNA/EcoRI+HindIII. The visualization of fragments was released in UV transilluminator and photographed using Gel - Pro Imager.

Data Analyses

We constructed a binary matrix (1= presence; 0= absence) from the medium and strong band recordings which was submitted to similarity analyses aiming to detect the proximity or dissimilarity among both individuals and populations. We used the Jaccard's coefficient as the distance measure and UPGMA (Unweighted Pair Group Method - Arithmetic Averages) as the linkage measure to construct clusters by MVSP package8 in order to estimate diversity among and inside populations we applied the Shannon's diversity index. A loci was considered polymorphic when, at least, two individuals were different in relation to the others, considering presence or absence of bands.

RESULTS AND DISCUSSION

The twelve selected primers resulted in 129 bands, from these, 87 were polymorphics (67.45 %), in other words, making about 7,25 polymorphic loci by primer, while 32,55% presented monomorphics (presented bands in all analyzed individuals). The total bands by *primer* ranged from eight to 17.

Beyond usually nomenclature, was adopted a classification to the bands that followed this criterion: exclusive bands (polymorphics to one population and completely absents in other), specific bands (monomorphics to one population and completely absents in other). From the 129 considered bands, 6.2% were exclusive and 0.78% were specific. The *primers* that presented exclusive bands to populations of *S. mauritianum* sampled in UCT and UCC were OPF - 6, OPH15 and OPW - 17, with one band each. Already, to the population EE the *primers* OPB - 20 and OPH - 6 presented two exclusive bands and in SC, *primers* OPB - 20, OPH - 5 and OPH - 6 had one band. Only the *primer* OPW - 17 generated a specific band to SC population.

The estimated intrapopulation values to Shannon Index (Log10) were: UCT 1.86; UCC 1.90; EE 1.88 and to SC 1.92, indicating that polymorphic loci were similar to all four populations. The populations whole obtained a diversity of 1.89. These values showed high genetic diversity within and among *S. mauritianum* populations, that can be confirmed when comparing diversity values with other pioneer species, like *S. lycocarpum*14, that revealed variability within both populations by separating their individuals in sharp groups. The same pattern was detect by Ribas and Kageyama9 with *Trema micrantha*, a typically gap pioneer colonizer, that generally occurs associated with *S. mauritianum*. To that species, there was detected high heterozygosity and genetic diversity in fragmented populations. Studies with allozymic markers in *S. mauritianum* by Ruschel *et al.*, 11 indicated that large diversity (88.8%) occurs inside each population.

The Jaccard's coefficient presented values among 0.665 and 0.868 showing divergences among populations. Clus-

ter analyses (UPGMA) separated the individuals in three groups from similarity 0.7. The first covered plants majority from EE (54%); the second group encompassed SC individuals and the third group presented UCT, UCC and the remaining EE (46%) individuals. These results indicated that both geographic distribution and environmental conditions influenced genetic diversity of *Solanum mauritianum*. Indeed, the study showed that geographic distance and altitude are barriers less sensitive to group separation than the Rio Uruguay, which separated the SC population from the others (UCT, UCC and EE).

CONCLUSION

This paper showed that RAPD markers were powerful in detecting genetic diversity among genotypes of *Solanum mauritianum*. On this way, we found considerable polymorphism (67.45%) within and among all populations, which reflected in three sharp groups by Cluster Analysis. The number of bands from *S. mauritianum* populations, sampled in two states may represent species genetic diversity, as it is an allogamous plant.

There are high levels of genetic variability within and among natural populations and the presence of exclusive alleles by population suggests most individuals are used to species strategy conservation. Although most genetic variation is distributed within populations, population genetic diversity was moderate but sharp, so the sampling for conservations purposes should retain both levels of variation.

The low correlation between genetic and geographic distance suggest this factor was not decisive for the genetic distance patterns observed. The genetic description of *S. mauritianum* is an important step to design in situ or ex situ conservation strategies, forests resources valuation and degraded environment restoration in southern Brazil.

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