Effects of Forest Management on the Genetic Diversity in a Population of *Araucaria angustifolia* (bert.) O. Kuntze

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Abstract

Araucaria angustifolia Kuntze, is a dioecious and wind pollinated species, which is found in the southern and southeastern regions of Brazil. Nowadays, *A. angustifolia* has become one of the most endangered Brazilian plant species. For this reason, some areas are kept intact to preserve the remained genetic resources of the species. In this study, the genetic variability of three subpopulations of *A. angustifolia* was compared by RAPD markers. The study includes a natural area with little human action, a managed area, where trees were selected and cut, and a progeny test, where the "mother trees" were sampled among those trees harvested in the managed area. The objective of this study was to verify the level of reduction in genetic variability of the managed area and the progeny test. The mean genetic diversity for the natural area, the managed area, and the progeny test were 0.26, 0.26, and 0.22, respectively. The polymorphism index showed a sharp reduction in the genetic variability among the three populations. The percentages of polymorphism were 82.0% for the natural area, 72.5% for the managed area and 59.7% for progeny test. The values of genetic diversity for the total and within populations were, 0.26 and 0.25, respectively. The coefficient of genetic differentiation among populations was 0.06. The results suggested that the management used in the area slightly reduce the genetic variability in the remained population. Furthermore, it is inferred that the sample used in the progeny test was unsuitable for *ex-situ* genetic conservation purposes.

Zusammenfassung

Araucaria angustifolia Kuntze, eine Dioecious durch den Wind bestäubte Baumart, wird im Süden und im Südosten Brasiliens vorgefunden. Heutzutage gilt *A. angustifolia* als eine vom Aussterben bedrohte brasilianische Baumart. Aus diesem Grund werden einige Bestände unberührt gehalten, mit dem Ziel, die genetischen Ressourcen dieser Art zu wahren. In der vorliegenden Studie wurde die genetische Variabilität von drei Subpopulationen mittels Anwendung von RAPD-Markern verglichen, ein natürliches vom Menschen kaum berührtes Gebiet; ein gepflegtes Gebiet, in dem Durchforstungen durchgeführt wurden; ein progeny-test, für den die Stichprobennahme von Matrizen anhand der im gepflegten Bestand abgeholzte Bäumen erfolgte. Ziel der Untersuchung war die Feststellung des Reduktionsniveaus der genetischen Variabilität bei dem gepflegten Bestand und dem progeny-test. Die durchschnittliche genetische Variabilität lag bei dem natürlichen Bestand, dem gepflegten Bestand und dem progeny-test bei 0,26, 0,26 bzw. 0,22. Der polymorphismus-Index zeigte eine Reduktion der genetischen Variabilität bei den drei Populationen. Die Prozentsätze des Polymorphismus entsprachen 82,0% bei dem natürlichen Bestand, 72,5% bei dem gepflegten Bestand und 59,7% bei dem progeny-test. Die Werte der gesamten genetischen Variabilität und innerhalb der verschiedenen Populationen betrugen Ht = 0,26 bzw. Hs = 0,25. Der genetische Differentiationskoeffizient der Bestände (Gst) betrug 0,06. Bei der Gesamtbetrachtung der Ergebnisse ließ sich feststellen, dass die angewandte Pflege zu einer geringen Reduktion der genetischen Variabilität führte. Es wurde ebenfalls deutlich, dass die angewandte Stichprobennahme für den progeny-test hinsichtlich der genetischen Erhaltung *ex-situ* nicht geeignet gewesen ist.

Key words: Araucaria angustifolia, management, population genetics, progeny test, RAPD markers.

Introduction

Araucaria angustifolia is a dioecious and wind pollinated species, which occurs naturally in the southern and southeastern regions of Brazil, in latitudes from 19°15'S to 31°30'S and longitudes from 41°10'W to 54°30'E (Carvalho, 1994). The original *A. angustifolia* forests covered an area of more than 200,000 square kilometers. However, uncontrolled deforestation has reduced the forest to less than 3% of the original area (Mazza, 1997).

Natural undisturbed populations are a unique opportunity to gather information on the level and distribution of genetic diversity that occurs in biological systems and for development of sustainable silvicultural practices (Ledig, 1992). Improper management of natural forest can lead to changes in the local genetic pool through various mechanisms, including directed selection, inbreeding and genetic drift (Barnes, 1989; Ledig, 1992). Because few studies have been developed to characterize the genetic structure of intact forests, it is not clear how management practices, which may remove many of the individuals from the genetic pool, may affect the genetic diversity (Buchert, 1994).

The existing methods of conservation should take into consideration the genetic variability of the population in question. The genetic characterization of *A. angustifolia* is important not only to establish adequate management practices that do not reduce the genetic variability of the population, but also for establishing natural reserves, that represent the genetic variation in the species throughout the area of distribution (Pitcher, 1976). If these reserves are properly characterized they will supply genetic material for *ex-situ* conservation (with formation of base populations) and sustainable management (Mello and Leite, 1987).

Recently, the interest in the use of DNA markers for a variety of applications such as population genetics of forest species, conservation, and breeding (Thomas et al., 1999) has considerably increased. Welsh and McClelland (1990) and Williams et al. (1990) developed a methodology that is based on the analysis of randomly amplified polymorphic DNA (RAPD) sequences. DNA markers generated by RAPD are neutral and highly variable (Wu et. al., 1999) and they are, therefore, appropriate for population analysis. The disadvantage of the use of RAPD polymorphism in population genetics is that most of the alleles (> 90%) segregate as dominant markers (Williams

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et al., 1990) and, therefore, it is not possible to distinguish whether they are homozygote or heterozygote (APOSTOL et al., 1996). To study the structure of population genetics by using RAPD, it is necessary to assume that RAPD products segregate as dominant alleles in a Mendelian fashion and that the genotypic frequencies of the RAPD loci are in Hardy-Weinberg equilibrium (APOSTOL et al., 1996). On basis of these assumptions, some analyses, which permit the characterization of the genetic structure of the population, can be applied. These analyses include the total genetic diversity and number of polymorphic loci for each population (NEI, 1973), and the F statistics (WRIGHT, 1965). In addition, it is possible to use the total gene diversity (Ht) partitioned into within (Hs) and among population (Dst) components, with the proportion of the total genetic diversity among population estimated as Gst = Dst/Ht (NEI, 1973). Besides heterozygosity, the percentage of polymorphic loci has also been used as a diversity index to characterize and compare the levels of genetic variation in natural populations.

In this study, the RAPD methodology was carried out to evaluate the effect of the management in maintaining the original characteristics of the natural population and to estimate the efficiency of the design used in the progeny test for the *ex-situ* conservation of the original genetic variability.

Material and Methods

Studied area and plant material

The paper manufacturing company, Irani Cellulose S. A., located at Vargem Bonita city, in Santa Catarina state, Brazil, carried out a management plan, in a mixed ombrophile forest area (mixed forest with *A. angustifolia* and Cfb type climate), to rationalize de use of the remaining natural populations and to supply raw material for sawn lumber and laminates. The management plan considered the maintenance of a percentage of the trees in all the diameter classes to reach these objectives. The possibilities of conserving the genetic variability were further guaranteed with a progeny test that was implanted from seeds of a sample of the felled trees to maintain an *ex-situ* conservation area of this population.

Three subpopulations, including a primitive area little affected by human action, a managed area, and a progeny test were used for this study. The managed area was submitted to one cutting cycle and 40% of the trees with diameters above 40 cm were removed. In order to preserve the maximum of genetic variability of the original population, the seeds used for setting up the progeny test were obtained from 100 mother trees that were cut down from the managed area. The intention, at the start of this test, was to collect seeds from one tree per hectare, throughout an area of 600 acres. However, because of the relative scarcity of mothers producing seeds at the time of felling, 100 trees belonging to a sub-area of 240 hectares were used. The progeny test was set up with one progeny of each mother tree in ten replications, totaling 1000 trees.

For the DNA analysis, 24 samples were collected from each area. In the natural and managed populations, the collected trees were sampled at a minimum distance of 100 meters one from another. For the progeny test, leaves with only one replication per mother were collected to maximize the quantity of genetic variability sampled.

DNA extraction, amplification and gel electrophoresis

Genomic DNA was isolated from frozen leaf following the CTAB method (DOYLE and DOYLE, 1987) with minor modifications. DNA concentration was estimated using a fluorometer (Dyna Quant 200, Höeffer, Pharmacia), according to the manufacture instructions. DNA samples of the twenty-four individuals of each population were diluted to a uniform concentration 10 ng/ul. The amplification reactions had a final volume of 15 ul consisting of 1x PCR buffer (75 mM Tris-/HCl, 50 mM KCl, 2.0 mM $MgCl_2$, and 20 mM $(NH_4)_2SO_4$); 0.2 mM each of dATP, dTTP, dCTTP, and dGTP; 0.4 µM of primer (Operon Technologies); 0.9U of Taq DNA polymerase (Biotools); and 20 ng template DNA. Samples were amplified in thermal cycler (PTC 200, MJ Research) programmed with a 3 min at 94 °C for initial denaturation, followed by 47 cycles of 1 min at 94 °C, 1 min 45 sec at 38 °C, and 2 min at 72 °C. The final cycle was followed by a 7 min extension at 72 °C. Amplified products were resolved in 1.4% agarose gels, stained with ethidium bromide, visualized in UV light, and stored in a PC computer for later analysis.

Statistical analysis

RAPD markers were scored for the presence (1) or absence (0) of homologous DNA bands among all individuals of each population studied. Only well-amplified fragments were considered. The data were analyzed using the POPGENE (YEH et al., 1996) software. The reproductive system of *Araucaria angustifolia* is dioecious anemophilous which favored a wide exchange of alleles among the close individuals and populations. Therefore, it was possible to assume that the RAPD loci are in Hardy-Weinberg equilibrium such as observed in several others conifer species, which exhibited only modest and transient departures from Hardy-Weinberg proportions (AAGAARD et al., 1998; WU et al., 1999). The frequencies of the markers were used to calculate the genetic diversity within population (Hs), the total gene diversity (Ht), the coefficient of genetic differentiation among populations (Gst) (NEI, 1987), and the genetic diversity of each population (NEI, 1973). Variation in RAPD patterns was analyzed by analysis of molecular variance (AMOVA) using the Arlequin, software, version 1.1 (SCHNEIDER et al., 1997). With the AMOVA we calculated the variance components and their significance levels for variance among plants within populations and among populations.

Results and Discussion

Dynamic of human occupation in the southern Brazilian regions and its influence on the genetic variability in the population analyzed

The occupation of the southern region of Brazil is relatively recent. Almost all forests covered with *A. angustifolia* of this region were kept untouched until the beginning of the twentieth century. Thus, the isolation of the populations that has occurred due to human action is recent when compared to the longevity of the forest species, whose trees live up to 300 years. The fact that the studied populations are only a few kilometers apart and that they previously formed a single continuous forest, favored the expectation of a low natural genetic divergence among them.

Primer selection and the use of the RAPD technique

For initial primer selection, 500 RAPD primers were amplified using DNA samples from ten plants. Thirty-five of these primers were then selected according to the repeatability of the amplification pattern and the number of polymorphic bands and used to amplify the DNA of all 24 individual plants of each population.

The primers were selected according to the repeatability of the amplification pattern and the number of polymorphic bands. DNA amplification of all 72 individuals resulted in 211 analyzable bands that were used to perform all the statistical parameters (*Tables 1, 2*). According to Fischer and MATTHIES

Table 1. – Gene diversity and percentage of polymorphic loci for three populations of *A. angustifolia.* Ht = total gene diversity. Hs = within population genetic diversity. Gst = coefficient of genetic differentiation.

Populations	Genetic diversity	Number of polymorphic loci	% of polymorphic loci	Difference of polymorphism between pop (%)
Natural	0.26 ± 0.19	173	82.0	11.58 (pop 1 and 2)
Managed	0.26 ± 0.20	153	72.5	27.43 (pop 1 and 3)
Progeny Test	0.22 ± 0.21	126	59.7	
Ht = 0.26		Hs = 0.25	Gst = 0.06	

(1998), because of the high resolution of the RAPD technique, significant genetic variation could be detected within and among populations with relatively small sample size, therefore, RAPD is especially suitable for analysis of genetic differentiation and to estimate the loss of genetic variation in natural populations.

Genetic analysis of the Natural, managed and progeny test populations

The mean genetic diversity obtained for the natural and the managed populations and for the progeny test was, 0.26, 0.26, and 0.22, respectively (*Table 1*). The estimative of the polymorphic index showed a sharp difference in the genetic variability among the three populations. The percentages of polymorphic loci were 82.0% for the natural area, 72.5% for the managed area, and 59.7% for the progeny test. The total gene diversity (Ht), the genetic diversity within population (Hs) and the coefficient of genetic differentiation among population (Gst) were 0.26, 0.25, and 0.06, respectively (Table 1). The genetic variation within and among populations was 94.6 and 5.4 (*Table 2*), respectively. The Fst, was 0.05 (*Table 2*), which agrees with the Gst value (0.06) obtained (*Table 1*).

Table 2. – Analysis of molecular variance (AMOVA) of three populations of *A. angustifolia.* Fst = Proportion of genetic diversity among populations.

Source of variation	d.f.	sum of squares	components of variance	variance percentage
between populations	2	95.83	1.15	5.36***
within populations	69	1401.46	20.31	94.64
Total	71	1497.29	21.46	

Fst: 0.05
*** $p < 0.001$

All results should be analyzed together, to more clearly establish the effect of management on the genetic diversity of *A. angustifolia.* Firstly, the total gene diversity obtained showed that these populations have high levels of total genetic variability (Ht = 0.26). According to ANTHONY et al. (2001), a total gene diversity index can be considered high if it is above 0.25. Further, we obtained a Hs value of 0.25 for the within genetic diversity, which is between medium to high (ANTHONY et al., 2001). The coefficient of genetic differentiation among groups (Gst = 0.06) and the Fst (0.05) showed moderate levels in *A. angustifolia*. These results become even more solid when compared with other studies. HAMRICK et al. (1992) reported that the mean gene diversity or Ht and the Gst values for gymnosperms were 0.17 and 0.07, respectively. Therefore, *A. angustifolia* with a Gst = 0.06 is within a general value expected for conifers. Isozyme markers applied in the study of several plants groups revealed mean values of Ht = 0.31, Hs = 0.23, and Gst = 0.22 (HAMRICK and GODT, 1990). The authors suggested that perennial long-lived cross-fertilized and wind pollinated species, at a final successional stage, present greater within genetic variation. RAPD markers evaluated by AMOVA in populations of *Digitalis minor,* (SALES et al., 2001) revealed that more of the genetic variation is distributed within the populations than among them, indicating a restricted population differentiation, which is expected for cross-pollinated species. The results obtained for *A. angustifolia* in this study showed a much greater within than among genetic variation, which is in line with the reproductive and ecological behavior of this species.

As already stated, the polymorphic loci indexes obtained revealed more marked differences among the three populations. There was an 11.58% fall in the polymorphism level in the managed population compared to the natural population (*Table 1*). The individual analysis of this variable indicated that the managed population acted to decrease the frequency of polymorphic markers. The progeny test revealed a more expressive fall in the polymorphic loci index (27.43%), which was accompanied by a loss in the genetic variability when compared with the natural population. This result showed that the sample used to set up the progeny test was insufficient to satisfactorily maintain all the possible genetic variability and part of the genetic resources of the managed *Araucaria angustifolia* population was lost.

Studies that examined the impact of management on genetic diversity in forests have produced contradictory results. KNOWLES (1985) reported that there was no differences in genetic diversity between primitive and regenerated areas of Jack Pine and Black Spruce. In contrast, GOMORY (1992) detected that reforested areas of Norwegian Spruce had a significantly lower quantity of genetic diversity than primitive areas. In the present study, it was found that the sampling used in setting up the progeny test as an *ex-situ* method was unsuitable to preserve the total level of genetic polymorphism detected in the original population of *A. angustifolia.*

Total level of genetic variability detected for the three populations and among population vs. within population genetic variability

As already discussed, the total level of gene diversity (Ht = 0.26) detected for the three *A. angustifolia* populations studied was high. It could be questioned that due to the high variability found in a few populations and because most of this variability is within populations, the preservation of a few populations would be sufficient to maintain the genetic resources of the species as a whole. This is not true, as genetic variability detection techniques based on molecular markers detected a greater within population genetic variability. On the other hand, other techniques based on morphological markers and adaptative characters show a greater among population genetic variability. According to YANG et al. (1996) and KARHU et al. (1996), adaptative and phenological features especially show much greater among population than within population variation patterns. The best-known examples are the variation in environment gradients such as altitude, longitude, and latitude. On the other hand, the within population genetic variability is greater when techniques based on DNA markers and biochemical characteristics (proteins, terpenes) are used. Further, according to these authors, this apparent contrast results from

the action of different evolutionary forces acting at molecular and phenotypic levels. While adaptative traits are frequently the result of adaptation to existing environmental conditions, most of the molecular markers remain neutral. The diversity in the first group reflects the pressures of natural selection while that of the second group reflects the history of the populations (migration effects, genetic drift). Therefore, it is pointed out that the genetic variability based on adaptative morphological traits cannot be disregarded as they are based on few alleles that are highly adaptative to specific environmental conditions. Thus, the definition of conservation strategies for *A. angustifolia* cannot be based on only one type of approach. Studies related to molecular, morphological, phenological, and adaptative markers should be analyzed together in the attempt to define management and species conservation strategies.

Proposals to improve the management design directed to the genetic conservation of A. angustifolia

The management used had little effect on the decrease of the genetic variability that originally existed. On the other hand, if the objective were to block even the small tendency to variability loss, as detected through the fall in the percentage of polymorphic loci, then some of the parameters would be reviewed. As mentioned, 40% of the trees were felled in all the diameter classes greater than 40 cm. A suggestion would be to decrease the thinning percentage to values lower than 40%, since this procedure could result in greater maintenance of the original genetic characteristics, because the percentage of trees preserved that might have single allele combinations would be increased. Nevertheless, the feasibility of such a modification should be establish in order to become economically attractive.

Regarding the progeny test, some suggestions could be implemented. As already stated, the intention was initially to set up the test from 600 mother samples well distributed in an area of 600 ha, which would have resulted in one tree per hectare. This was not accomplish because of the relative scarcity of trees carrying seeds at the time of management. Therefore, the design used was 100 mothers from a sub-area of 240 hectares with progenies from 1 tree and 10 replications that could have led to the loss of variability through the decreased number of mothers and because only part of the population of the area managed was represented.

The leaf collection for DNA extraction in the progeny test was done by sampling only one tree per mother. The sampling strategy was chosen because it samples a greater quantity of genetic variability than it would be maintained if more than one replication per progeny was collected, which would imply the maintenance of more similar materials as if they derived from the same mother. The genetic variability detected in this study therefore corresponds to the variability among mothers and thus, another methodology should be used to detect the variability within progenies that would enable the use of strategies for the preservation of genetic variability in the managed population based on the increase in the number of replications per progeny.

Thus, depending on the methodology used and availability of physical space and resources, both the strategies to increase the number of mothers and better distribution in the area such as increasing the number of replications per progeny could be implemented to representatively improve the gene pool obtained in the sampling and design of a progeny test.

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