Genetic diversity assessment of *Poincianella pyramidalis* (Tul.) L.P. Queiroz accessions using RAPD markers

J. S. Santos[1]; S. S. Mendes[1]; D. C. Conde[2]; R. C. Delmondez[3]; R. S.-Mann[2]; S. M. Thomazzi[1]

[1]*Department of Physiology, Federal University of Sergipe, 49100-000, São Cristóvão-Se, Brazil*

[2]*Department of Agronomic Engineering, Federal University of Sergipe, 49100-000, São Cristóvão-Se, Brazil*

[3]*Department of Biofunction, Federal University of Bahia, 40160-100, Salvador-Ba, Brazil*

sarathomazzi@ufs.br

Over the long term, the ability of a species to respond adaptively to environmental changes depends on its genetic variability. Catingueira (*Poincianella pyramidalis*) is a tree species that has been used by the population for treat some diseases in the folk medicine. Genetic relationships among 13 *Poincianella pyramidalis* genotypes collected from different places of Sergipe – Brazil were investigated by using Randomly Amplified Polymorphic DNA (RAPD), in order to provide some information about its genetic diversity for restore programs. A total of 10 RAPD primers were used to investigate the genetic diversity and genetic relationships among different germplasms of 13 accesses of catingueira. It was found a similarity (average) of 0.70 between the studied genotypes. The highest and lowest detected similarities were 0.89 and 0.55, respectively. The calculated Jaccard index was 99%, so all genotypes are considered genetically different between themselves and this is good specially because if we consider that restore programs that needs species with some genetic variability, the *P. pyramidalis* showed that although its was found in a site with antropical changes it can resist to the genetic erosion and keeps its variability.
Keywords: Catingueira; genetic diversity; *Poincianella pyramidalis*

A longo prazo, a capacidade de uma espécie em responder adaptativamente às mudanças ambientais depende da sua variabilidade genética. A catingueira (*Poincianella pyramidalis*) é uma espécie arbórea que tem sido utilizado pela população, na medicina popular, para tratar algumas doenças. Relações genéticas entre 13 genótipos da *Poincianella pyramidalis*, coletadas em diferentes locais de Sergipe – Brasil, foram investigadas usando DNA polimórfico amplificado ao acaso (RAPD), a fim de fornecer algumas informações sobre sua diversidade genética para programas de restauração. Um total de 10 primers aleatórios foram utilizados para investigar a diversidade genética e as relações genéticas entre diferentes germoplasmas de 13 acessos de catingueira. Foi encontrada uma similaridade (média) de 0,70 entre os genótipos estudados. A maior e a menor semelhanças detectadas foram de 0,89 e 0,55, respectivamente. O índice de Jaccard calculado foi de 99%, sendo portanto todos os genótipos considerados geneticamente diferentes entre si. Fato este positivo, especialmente se considerarmos que programas de restauração precisam de espécies com alguma variabilidade genética. A *P. pyramidalis* mostrou que mesmo sendo encontrada em um local com mudanças antrópicas, ela pode resistir à erosão genética e manter sua variabilidade.
Palavras-chave: Catingueira; diversidade genética; *Poincianella pyramidalys*

1.INTRODUCTION

The family Leguminosae is naturally present in the Caatinga environment, with approximately 293 species that are distributed into 3 subfamilies: Faboideae, Caesalpinioideae, and Mimosoideae [1]. The genus *Caesalpinia* (Caesalpinioideae, Fabaceae) comprised of tropical or subtropical trees or shrubs, contains more than 150 species worldwide [2]. Native Brazilian species such as *Caesalpinia echinata* "pau-brasil" had important economic value in the early colonial period of Brazil [3]. Previous studies of species of this genus report remarkable biological activities for its species such as antimicrobial [4], antidiabetic (*C. bonducella*) [5], antimalarial (*C. volkensii* and *C. pluviosa*) [6], and anti-inflammatory activities [7]. *Caesalpinia pyramidalis* Tul. is an endemic tree of northeastern region and one of the predominant species in the caatinga vegetation. This species is popularly known in Brazil as

"catingueira". It passes through a different classification and now it is called *Poincianella pyramidalis* (Tul.) L.P. Queiroz [8]. Its young leaves are used to feed animals [9]. The catingueira is also used in folk medicine as anti-inflammatory [10], and show good antioxidant activity [11]. Pharmacological studies of *Poincianella pyramidalis* have shown toxic effects on mollusks [12] and significant antifungal activity against candida [13].

It represents one of the main native species of the Caatinga and can be found in almost all Northeast of Brazil. Catingueira can support dry season and has a crescent economic potential [14]. Because of its medicinal and economical uses, this species from caatinga is under anthropic pressure and can become extinct; *P. pyramidalis* needs a good forest conservation program in order to obtain its genetic protection.

Apart from the fact that the maintenance of existing levels of genetic variability may be essential for the long-term survival of endangered species, it is also a pertinent factor when one considers reintroduction of plant populations in the wild. In addition, it can be useful to identify interesting genetic traits for future applied uses [15]. Genetic data may play a significant role in the formulation of appropriate management strategies direct toward the conservation of taxa, besides been useful in understanding the structure, evolutionary relationships, taxonomy, and demography of the species [16, 17]. Furthermore, knowledge of within and among population differentiation will help to develop efficient sampling strategies of genetic resources in rare and/or useful species [18].

The amount and distribution of variation among and within populations result from dynamic processes such as gene flow, selection, inbreeding, genetic drift, and mutation [19]. A species without enough genetic diversity is thought to be unable to survive in a changing environment or protect itself against evolving competitors and parasites. Therefore, investigations of genetic diversity and the genetic structure of populations within a species may not only illustrate the evolutionary process and mechanism, but also provide useful information for biological conservation and phylogenetic analysis [15].

Polymerase chain reaction (PCR) based DNA markers provides an opportunity to characterize genotypes and measure genetic relationships more precisely than other markers [20]. Random amplified polymorphic DNA (RAPD) [21] is an effective means of investigating genetic diversity within or among populations, used in many plant species [22, 23]. Once established, RAPD-PCR has the advantage of being quick and easy and requiring little genomic DNA as a template. Also, its amplified loci are randomly distributed on genomic DNA [24]. Furthermore, RAPD profiling appears to be a useful tool for population analysis as well as phylogenetic analysis [25].

This study assesses the portioning and the extent of genetic variation within 13 *P. pyramidalis* trees, using RAPD markers. The results obtained should help to provide a framework for the development of a sound *in situ* conservation programs for this endangered species.

2.MATERIAL AND METHODS

2.1 Plant material

The aerial parts of 13 specimens of *Poincianella pyramidalis* were collected during the wet season (flowering phase – August 2010) of the plants at the Nossa Senhora da Glória village at Sergipe State – Brazil all over the road called Rota do Sertão. The plant material was authenticated by Professor Ana Paula Prata, Department of Biology, Federal University of Sergipe, and voucher specimens were deposited in the Herbarium of the same university, as shown in Table 1. The material used in the DNA extraction was kept in the fridge until further analysis.

Table 1: Geographical origin of the different Poincianella pyramidalis individuals collected. Individuals were harvested on Nossa Senhora da Glória – Sergipe State - Brazil.

Genotype	Geografic Coordinate	Voucher specimens
C1	10º 13'39'' S and 37º 44'23'' W	17492
C2	10º 13'39'' S and 37º 23'08'' W	18063
C3	10º 13'41'' S and 37º 22'22'' W	18064
C4	10º 13'31'' S and 37º 22'18'' W	18065
C5	10º 13'57'' S and 37º 21'50'' W	17493
C6	10º 14'02'' S and 37º 21'38'' W	18066
C7	10º 14'01'' S and 37º 21'38'' W	18067
C8	10º 14'02'' S and 37º 21'37'' W	18068
C9	10º 14'03'' S and 37º 21'35'' W	18069
C10	10º 14'40'' S and 37º 20'20'' W	18070
C11	10º 14'40'' S and 37º 20'22'' W	18071
C12	10º 14'41'' S and 37º 20'20'' W	18072
C13	10º 14'48'' S and 37º 20'00'' W	18073

2.2 DNA extraction

Total DNA was extracted from leaves following the method previously described [26] with some slight modifications. 1.0 g leaves were ground in liquid nitrogen to a fine powder. 10 mL of extraction buffer CTAB (Cetil Trimetil Brometo de Amônio 2X - 2% w/v CTAB, 1.4 M NaCl, 20 mM EDTA, pH 8.0, 100 mM Tris–HCl, 1% PVP), and 20 µL de β-mercaptoethanol were added to the samples. The mixture was incubated at 65ºC for 60 min. After the incubation period, 1,000 µL of each sample were transferred into eppendorf tubes with 1,000 µL chloroform: isoamyl alcohol (24:1). DNA samples were stored at -20ºC during 12 hours. After, the samples were centrifuged in 14,000 rpm for 10 min. To the pellet were added 100 µL of ethanol during 10 min at room temperature. The material was centrifuged again at 4,000 rpm (10 min). The precipitate was washed with 10 mM ammonium acetate in 75% ethanol and the pellet was dissolved in TE buffer. Quality and concentration of total DNA was verified by spectrophotometry at 260 nm and 280 nm, using the following formula:

$$[DNA] = 50 \text{ mg/mL} \times D \times A_{260}$$

D is the dilution factor and A_{260} is the values obtained at the wave length of 260 nm.

2.3 RAPD amplification

The PCR protocol used was based on method previously described [21] with some slight modifications. Ten primers were used containing each 10 nucleotide. The primers used were acquired from Integrated DNA Technologies (IDT). Information about the primers can be seen in Table 2. PCR reactions were performed in a solution containing 2.92 µL H_2O; 1.30 µL PCR buffer 10X (10 mmol/L Tris-HCl, pH 8.0, 50 mmol/L KCl, 1.5 mmol/L $MgCl_2$); 1.04 µL dNTP; 1.04 µL bovine serum albumin (BSA); 0.20 µL Taq polimerase; 2.50 µL oligonucleotide, and 3.00 µL DNA. Amplifications were performed in a Thermal Cycler C1000, from Bio-Rad. The cycling program consisted of an initial denaturation of 40 s at 94°C, followed by 45 cycles of 60 s at 94°C, 1 min at 36°C, and a final extension at 72°C for 60 s.

Amplification products were separated on 1.2% agarose gels run in TBE buffer (Tris-borato 0.045 M, 5.4 g/L Tris-base, 2.75 g/L boric acid, 5 mL EDTA 0.20 M, and 1 L distilled water), detected by GoldView I dye staining, and photographed under ultraviolet light.

2.4 Data scoring and analysis

The gels were scored conservatively (i.e., only the most reliable and distinct bands were scored) as present (1) or absent (0). Staining intensity of bands was not considered as a difference. Only distinct, well-resolved fragments were scored as presence or absence for each of the RAPD marker in the 13 accessions. The polymorphic information content (PIC) was

calculated (Jaccard's coefficient) by applying the simplified formula: V= {nd (1-g)} / (n-1), where V = variance from the genetic between the genotypes pair; n = total number of polymorphic bands; g = genetic distance between the genotypes pair. The cluster analysis was conducted by the software NTSYS-pc version 2.01 [27]. Similarity matrices based on Jaccard's similarity coefficient was used to construct the unweight pair group method with arithmetic average (UPGMA) dendrogram.

3.RESULTS AND DISCUSSION

Studies of genetic diversity are important for the knowledge of genetic of populations [28]. This knowledge is important because can help the elaboration of some conservation and maintenance strategies of germoplasms [29].

PCR amplification of DNA, using 10 primers produced 56 bands. All the selected primers amplified DNA fragments across the 13 genotypes studied, with the number of amplified fragment varying from three (P-7 and 8) to 10 (P-6), with size ranging from 250 to 1000 bp. in the amplification tamboril (*Enterolobium contortisiliquum*) DNA using the same primers as used in this study were found 39 bands [30]. Probably these primers are better responsive for *P. pyramidalis* then for *E. contortiliquum*.

Of the 56 amplified bands, 53 were polymorphic, with an average of 5.3 polymorphic bands per primer. The highest number of polymorphism was observed with primer 6 (Table 2).

P. pyramidalis is a species that has sexual reproduction. For this reason, it was expect to find more polymorphyc band per primer. The sexual reproduction makes the genetic variability higher than asexual reproduction because the chromosome recombination in all its aspects [31]. According with this theory, some RAPD studies showed higher average then we found in our study: 7.21 for *Stylosanthes macrocephala* [32]; 11.14 for *Phaseolus vulgaris* [33] and 7.10 for *Pisum sativum* [34].

Environmental changes, as soil, temperature, polinization, etc, can interfere in the genetic variability, seeds dispersion, seedlings survival, among others characteristics [31].

Table 2: RAPD sequences used for screening of the 13 accessions, together with the scorable and polymorphic fragments obtained for each primer.

Primer	Sequence (5'-3')	Amplified product range (pb)	Total No. of bands	Polymorphic bands (No.)	Percent of polymorphim
1	TGA TCC CTG G	250 – 1000	6	6	100
2	GGA CCC AAC C	250 – 750	6	6	100
3	CCC AAG GTC C	250 – 750	6	6	100
4	GGT GCG GGA A	250 - 1000	7	7	100
5	GGC ACT GAG G	250 – 1000	5	5	100
6	GGT CGG AGA A	250 – 1000	10	9	90
7	TCG GAC GTG A	500 – 750	5	3	60
8	GGA GGA GAG G	250 – 750	3	3	100
9	CCC GGC ATA A	250 – 750	4	4	100
10	AAA GTT GGG A	250 – 500	4	4	100
TOTAL			**56**	**53**	

The Jaccard's coefficient is considered high 50% above [35]. In this study the calculation of similarity coefficient was based on 53 RAPD polymorphic bands and the mean similarity found among the groups was from 70%. The similarity matrix (Table 3) showed that the highest similarity (0.89) was for the genotype C8 and C10 and the lowest similarity (0.55) was found between the genotypes C12 and C1 or C5. The highest similarity found in this study was indicating that the studied population starts to lose some of their own genetic variability. This can be result of the environmental modification because the trees were located along the highway.

Table 3: Similarity coefficient values among 13 Poincianella pyramidalis samples based on data from 10 RAPD primers.

	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13
C1	1.00												
C2	0.66	1.00											
C3	0.58	0.81	1.00										
C4	0.64	0.79	0.75	1.00									
C5	0.70	0.81	0.74	0.72	1.00								
C6	0.64	0.68	0.68	0.66	0.57	1.00							
C7	0.70	0.66	0.66	0.64	0.70	0.64	1.00						
C8	0.70	0.81	0.81	0.83	0.74	0.79	0.74	1.00					
C9	0.72	0.68	0.64	0.66	0.68	0.70	0.72	0.79	1.00				
C10	0.70	0.70	0.70	0.75	0.66	0.83	0.70	0.89	0.72	1.00			
C11	0.62	0.77	0.66	0.75	0.70	0.79	0.62	0.81	0.68	0.85	1.00		
C12	0.55	0.62	0.58	0.68	0.55	0.60	0.58	0.66	0.57	0.66	0.66	1.00	
C13	0.58	0.77	0.77	0.79	0.70	0.72	0.74	0.85	0.68	0.77	0.77	0.74	1.00

The minimum value found by the similarity index, was 99%. According to this index, only the genotypes that show similarity higher than 99% could be considered as a unique group. The highest value found was 89%. According to our results, it was not possible to form any cluster using UPGMA parameter (Figure 1). This means that the present population showed a high genetic variability, although the environmental site is very different from the original one.

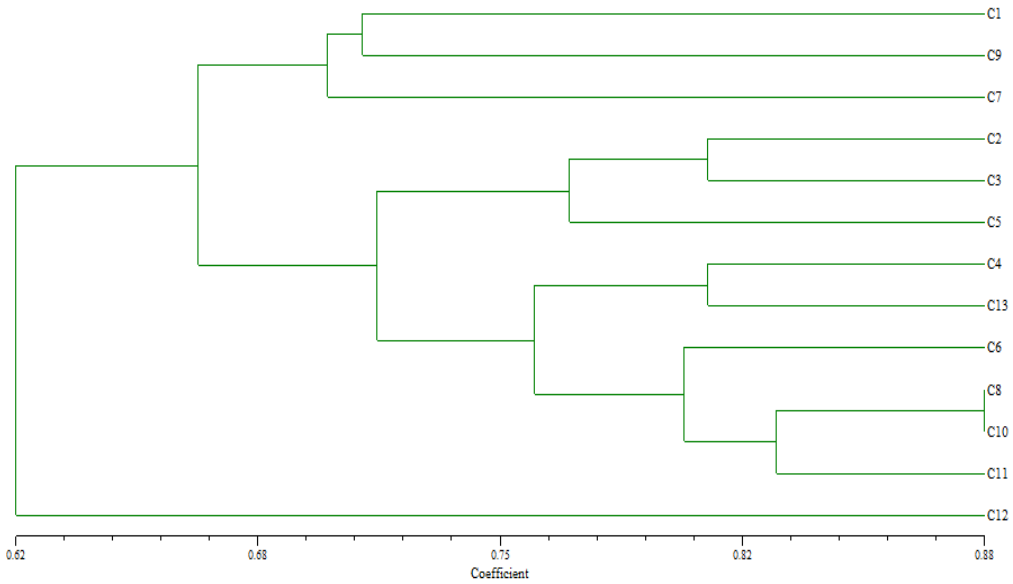

Figure 1: Dendrogram of 13 accessions of Poincianella pyramidalis derived from an UPGMA cluster analysis based on Jaccard's similarity coefficient matrix, using combined RAPD data.

By the evaluation of the *P. pyramidalis* fecundation, could be seen that it is auto-incompatible with the formation of fruits by the manual cross-pollination and natural pollination [36]. Individuals that have auto-pollination can decrease its genetic variability [37]. Probably, because of its reproduction, in spite of the high similarity index found in *P. pyramidalis*, we could see that the studied genotypes keep its wild variability. These founds appear to be a promise because the site of collect is full of anthropical changes and this species can be used in the restored programs.

4. CONCLUSION

The studied genotypes from *P. pyramidalis* showed genetic diversity that is very important form for its survival as well as for actions that can contribute with the Caatinga maintenance. However, some genotypes, as C10 and C8 showed high genetic similarity. Among the used primers, the 6 was the most efficient in the DNA amplification, generating 10 fragments in which 9 were showed polymorphism.

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